UNIVERSITY OF CAPE COAST

ANALYSIS OF LEVELS OF HEAVY METALS, ESSENTIAL ELEMENTS AND PERSISTENT ORGANIC POLLUTANTS (POPs) IN HUMAN BREAST MILK: A STUDY AT THE HO TEACHING HOSPITAL

JUSTICE WISTON AMSTRONG JONATHAN

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BY

JUSTICE WISTON AMSTRONG JONATHAN

Thesis submitted to the Department of Chemistry of the School of Physical Sciences, College of Agriculture and Natural Science, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Chemistry

FEBRUARY, 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or anywhere.

Candidate's Signature…… Date……

Name: JUSTICE WISTON AMSTRONG JONATHAN

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast

Principal Supervisors Signature……. Date: …….

Name: Prof. David Kofi Essumang

Co-Supervisors Signature: ……. Date:……

Name: Prof. John Kwesi Bentum

ABSTRACT

Human breast milk is, by far, the richest source of nutrition. However, breast milk is not pristine. The study aimed to analyse breast milk at lactational stages for persistent organic pollutants (OCPs, PCBs and PFAS), five heavy metals and essential elements. Participants for the study were healthy lactating mothers (aged 18 – 42 years) from first to third week postpartum. Forty-seven participants were recruited for the study. Forty millilitres (40 mL) of colostrum, transitional milk and mature milk were collected from each participant, making a total of 150 samples. Besides, each participant completed a comprehensive questionnaire to elicit information on biodata, place of residence and dietary pattern. Ten millilitres (10 mL) aliquot of each breast milk sample was prepared, extracted and analysed for PFAS using UPLC–MS/MS. Another 10 mL aliquot sample was extracted using QUECHERS and cleaned up and analysed for OCPs and PCBs using GC – ECD and GC–MS respectively A further 10 mL aliquots sample were acid digested employing EPA Method 3010A and analysed using ICP-OES. Statistical analyses were performed using IBM SPSS (Version 24), Excel Tool Pak and XLSTAT 2022.4.1.1377 and the results summarised in tables and figures. The mean Levels and ranges of PFAS detected in breast milk ranged from 2.65 ± 3.31 ng/L (PFHxA) – 83.14 ± 38.61 ng/L (PFOS). Both OCPs and PCBs analyzed were all below limits of detection. Mean levels of heavy metals in colostrum, transitional milk and mature milk respectively ranged from 0.002 (Cd) – 0.872 (Al) µg/L; 0.002 (Cd) – 0.997 (Pb) µg/L and 0.002 (Cd) – 0.564 (Al). The mean levels of essential elements ranged from 0.07 (Se) – 815.00 (K); 0.07 (Se) – 1008,00 (Na) and 0.07 (Se) – 596.00 (K) respectively during the stages of lactation.

KEY WORDS

Colostrum

Lactation

Mature milk

Organochlorine pesticides

Polychlorinated biphenyls

Transitional milk

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DEDICATION

To my beloved wife: Mrs. Andriana Jonathan and my sons: Valentine, Roderick, Manfred and Esmond.

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LIST OF ABBREVIATIONS

3M	The Minnesota Mining and Manufacturing Company
ADD_{Ing}	Average daily dose via ingestion
AFFF	Aqueous film forming foam
AI	Adequate Intake
ASTSWMO	Association of State and Territorial Solid Waste Management Officials
ATP	Adenosine Triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BMI	Body mass index
BO	Both sea water and fresh water
CAA	Chromated copper arsenate
CAAA	The Clean Air Act Amendment
CDC	Centre for Disease Control
CM	Colostrum milk
CONCAWE	Conservation of Clean Air and Water in Europe
CPSC	Consumer Product Safety Commission
CR	Cancer risk
CRCCARE	The Cooperative Research Centre for Contamination Assessment and Remediation of the Environment
CV	Coefficient of variation
CVS	Calibration verification standard
DDE	Dichlorodiphenyldichloroethylene

DDT	Dichlorodiphenyltrichloroethane
DEPA	Danish Environmental Protection Agency
DHHS	Department of Human Health Services
DoD-SERD	Department of Défense Support Equipment Requirements Document
DWS	Drinking water source
ECD	Electron capture detector
EDI	Estimated daily intake
EFA	Exploratory factor analysis
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FDA	Food and Drug Authority
FHSA	The Federal Hazardous Substances Act
FW	Fresh water
GC	Gas chromatogram
HBM	Human breast milk
HMO	Human milk oligosaccharides
HQ	Hazard quotient
HTH	Ho Teaching Hospital
IARC	International Agency for Research on Cancer
ICP – MS	inductively coupled plasma mass spectrometer
ICP-OES	Inductively coupled optical emission spectroscopy
ID	Iron deficiency

IDA	Iron deficiency anaemia
IUPAC	International Union of Pure and Applied Chemistry
JMPR	The Joint Meeting on Pesticide Residues
LDL	Low density lipoprotein
LOD	Limit of detection
LOQ	Limit of quantitation
LRB	Laboratory reagent blank
Max	Maximum
MCL	Maximum Contaminant Level
MEEIR	Medical evaluation and environmental investigation and remediation
MM	Mature breast milk
MS	Mass Spectrometer
MP	Multiparous
NIOSH	National Institute for Occupational Safety and Health
OCPs	Organochlorine pesticides
OSHA	Occupational Health and Safety Administration
OT	Other
PCA	Principal component analysis
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-para-dioxins
PCDFs	Polychlorinated dibenzofurans
PEL	Permissible Exposure Limit

PFAS	Perfluoroalkyl substances
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
POPs	Persistent organic pollutants
PP	Primiparous
QC	Quality control
QuEChERS	Quick Easy Cheap Effective Rugged Safe Sample preparation method
RDA	Recommended Dietary Allowance
SAAs	Sulphur-containing amino acids
TBM	Total breast milk – it consists of colostrum, transitional milk and mature milk combined
TDI	Tolerable daily intake
TM	Transitional milk
TWI	Tolerable weekly intake
TW	Tap water
UL	Tolerable Upper Intake Level
UNICEF	United Nations International Children's Emergency Fund
UPLC	Ultrahigh performance liquid chromatography
US EPA	United States Environmental Protection Agency
WFPHA	World Federation of Public Health Associations

LIST OF CHEMICAL SYMBOLS

Al	Aluminium
As	Arsenic
Cd	Cadmium
Ca	Calcium
Cu	Copper
Fe	Iron
HCl	hydrochloric acid
Hg	Mercury
K	Potassium
MeOH	Methanol
Mg	Magnesium
$MgSO_4$	Magnesium sulphate
Na	sodium
NH4OH	Ammonia solution
NHO_3	Trioxonitrate (V) acid
P	Phosphorus
Pb	Lead
S	Sulphur
Se	Selenium
Zn	Zinc

CHAPTER ONE

INTRODUCTION

We live in a polluted world. With most of our food and water sources contaminated with environmental chemicals, human breast is envisaged to be the cleanest source of nutrition for neonates. But the vital question is: How clean is human breast milk? The study therefore, took a look at the levels of some environmental chemicals in human breast milk and the possible adverse health effects they may pose to breastfeeding infants. The chapter introduced the study topic and discussed the background of the study, the problem statement, objectives and significance of the study as well as the delimitations and limitation of the study.

Bckground to the Study

There is considerable doubt that human breast milk is an essential source of sustenance for neonates, particularly in the early months following birth and maybe for as long as two more years. This is because breast milk from a mother contains not only the antibodies, immunity, and growth factors that the infant requires, but also all the nutritional components necessary for the baby's optimal development and growth. Studies have adequately documented the various health benefits associated with breastfeeding such as significantly minimal risks of infection, diabetes, arthritis, heart-related diseases, obesity, a number of cancers, allergy and asthma in both childhood and adult life (Elbeltagi et al., 2023; Horta & Cesar, 2013; Muro-Valdez et al., 2023). Babies who are breastfed have a higher IQ than babies who are not breastfed (Werts et al., 2007). Other research findings indicate that breast milk

provides enzymes and antibodies that protect the health of the baby until the body's system grows strong (Landrigan et al., 2002).

Additionally, studies have shown that, generally, breastfed babies around the world tend to be stronger and healthier than those fed with milk substitutes (American Public Health Association, 2007). Besides, breastfeeding is known to foster a deep-rooted relationship rich in caring and trust building between the child and the mother (Liu et al., 2014; Modak et al., 2023). Based on this understanding, the United Nations Children's Fund (UNICEF), the World Health Organization (WHO), and the Ghana Health Service vigorously promote, encourage, support, and safeguard breastfeeding.

Despite the fundamental nature of human breast milk as the prime source of food for the new-born, maternal breastmilk can be contaminated with hazardous chemicals which when transferred through breastfeeding to the new-born can impact negatively on the health of the nursing infant at any stage of growth and development. Basically, contaminants that can limit breastfeeding include occupational chemicals, medications, and persistent environmental toxins. Among the environmental pollutants that may contaminate human breastmilk and thereby negatively affect the health of babies are harmful heavy metals and long-lasting toxic organic contaminant (POPs) and perfluoroalkyl substances such as perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA). Numerous nations throughout the world have reported finding toxic heavy metals in human breast milk: Slovakia , Sweden (Lignell et al., 2013), Canada (Ryan & Rawn, 2014), Tunisia (Hassine et al., 2012), Poland (Szyrwinska & Lulek, 2007), Croatia (Letinić et al., 2016), Russia (Polder et al., 2008), Ireland (Pratt et al., 2012),

Hungary (Vigh et al., 2013), Turkey (Altun et al., 2018) and many others. Under the Priority List of Hazardous Chemicals, the Agency for Toxic Substances and Disease Registry has classed substances including arsenic, cadmium, lead, mercury, and aluminium as dangerous heavy metals (ATSDR, 2007a). Pregnant women exposed to arsenic may lead to placental insufficiencies, leading to intrauterine growth retardation through oxidative stress induction (Vahter, 2007).

In a review (Quansah et al., 2015) acknowledged a correlation between exposure to arsenic and negative pregnancy outcomes, including increased rates of miscarriage, stillbirth, moderate risk of infant death, and reduced birth weight. It has been reported that lead exposure during foetal development is connected to low birth weight, premature birth, abortus, or stillbirth (ATSDR, 2007b; Hu, 1991). Pregnancy-related lead exposure hinders the development of the brain by inhibiting cell differentiation and proliferation, synaptic growth, and apoptosis (Dribben et al., 2011; Lidsky & Schneider, 2003). Prolonged intrauterine lead exposure may result in cognitive impairment, growth retardation, lower IQ score, attention deficits hyperactivity disorder, lower academic performance, lower scores in ability tests and behavioural changes in later stages of life (ATSDR, 2007b). The negative consequences of mercury exposure on children are extensively recognised in the literature. According to epidemiological studies on mercury poisoning, prolonged exposure to high mercury concentrations can harm the brain system (Davidson et al., 2004). Low levels of mercury exposure in pregnancy have been linked to a variety of adult concerns, including decreased motor learning abilities, growth and developmental disorders, attention deficit hyperactivity disorder

(ADHD), memory and learning challenges, and speech disorders, according to study (ATSDR, 1999b; Davidson et al., 2004; Grandjean et al., 1997). High doses of mercury at the prenatal stage may, however, lead to conditions like cerebral palsy, vision problems, mental retardation, movement disorders, defective speech and hearing (Amin-Zaki et al., 1979; Counter & Buchanan, 2004). Research findings indicate that exposure to cadmium in the womb are linked to deficiencies in motor and perceptual skills, as well as mental retardation during childhood (ATSDR, 2012; Counter & Buchanan, 2004; Schoeters et al., 2006) whereas aluminium exposure has been connected to senility, Alzheimer's disease, presenile dementia and Parkinson's disease (ATSDR, 2008a).

Moreover, there is a significant amount of evidence showing widespread contamination of human blood and breast milk with POPs globally. (Solomon & Weiss, 2002). Exposure to POPs can have a wide range of health effects, including developmental issues, chronic illnesses, cancer, endocrine disruption, damage to the immune system, nervous system, reproductive system and in some cases even death. (WHO, 2010b). Many POPs have been found to disrupt the normal functioning of the endocrine system in humans and other animals. The occurrence of xenobiotic compounds in human breast milk raises serious problems in the fields of paediatrics, public health, and environmental health studies. Furthermore, it emphasises knowledge gaps such as limited information on the types and concentrations of contaminants in breast milk, the absence of standardised protocols for collecting and analysing breast milk samples, a lack of toxicokinetic data, and a scarcity of data on the health effects of chemicals in

breast milk on infants (Landrigan et al., 2002). A major public health concern for children regarding these environmental chemicals in maternal breast milk is the increased susceptibility of children and developing foetuses to these chemicals. The issue is exacerbated by negative environmental and societal factors such as poverty, poor nutrition, and others.

Besides heavy metals and POPs, another chemical contaminants of emerging concern are PFOS and PFOA. PFOA and PFOS are synthetic fluorinated organic compounds that are included in a broader category of chemicals known as perfluoroalkyl substances (PFAS) (US EPA, 2017). Though PFOS and PFOA are essentially synthetic organic chemicals, PFAS can also be created as a result of environmental damage or through the metabolic processes in organisms from a wide range of related precursor compounds (ATSDR, 2018). These substances have found their way into the environment due to industrial production and the use and disposal of products that contain PFAS (Liu & Mejia, 2013). Studies show that Perfluoroalkyls have been identified in every type of environment, including the groundwater, surface water, air, including drinking water, food, and sediment organisms (Abunada et al., 2020; Cao et al., 2015; Giesy & Kannan, 2001)soil.

All of these sources have the potential to expose humans to these chemicals. As a result of poisoned drinking water, several communities near fluoropolymer production sites have been exposed to high levels of PFOA, PFOS, and other perfluoroalkyls (Emmett et al., 2006; Steenland et al., 2009). PFOS and PFOA are persistent in the environment because to their resistance to breakdown by usual environmental processes. As a result, PFOS and PFOA can be found in the soil, air, and groundwater of the United States, as well as

at all points along the food chain (US EPA, 2014). The environment and human health may be negatively impacted by their toxicity, mobility, and propensity to accumulate in organisms(US EPA, 2012a). Food packaging, clothing, furniture fabrics, kitchenware, and other items made of water, grease, or stain-resistant materials all contain PFAS (US EPA & ATSDR, 2018) which offer easy means of exposure which may impact on human health..

Additionally, they are employed in a number of industrial procedures including firefighting on airfields(ATSDR, 2018; US EPA, 2017). Both the environment and the human body retain PFOA and PFOS for a very long time. Over time, they have become widespread in the environment and have built up in the blood of humans, wildlife, and fish. Researchers have discovered that perfluoroalkyls can be identified in breastmilk and umbilical cord blood, with the highest amounts of PFOA and PFOS recorded as 0.210–0.490 ng/mL and 0.360–0.639 ng/mL, respectively (Kärrman et al., 2007; Volkel et al., 2008). According to research, exposure to PFOA and PFOS above a particular level may have a significant effect on one's health, including having an adverse effect on an unborn child's development during pregnancy or an infant who is breastfed. These effects may include low birth weight, early onset of puberty, changes in bone structure; cancer such as testicular and kidney cancer; damage to the liver; changes in the immune system such as decreased antibody production and immunity and changes in cholesterol levels. (ATSDR, 2018; US EPA, 2017).

Besides toxic heavy metals, some essential elements (also known as mineral elements) are present in human breast milk. An essential element refers to any of the chemical elements vital for the successful growth,

development and the maintenance of healthy functions of an organism (Callaban et al., 2020; MedlinePlus, 2016; Zoroddu et al., 2019). Considering the quantity required in the body, essential elements can be categorised into major or macro-elements and trace or micro-elements. Major or macro-elements are those elements needed in relatively large amounts for normal growth and development e.g. calcium (Ca), potassium (K), magnesium (Mg) and sodium (Na) whereas trace or microelements are needed in small quantities e.g. iron (Fe), zinc (Zn), selenium (Se) and copper (Cu) (Callaban et al., 2020; MedlinePlus, 2016). The vital elements in human breast milk are needed for a baby's healthy growth and development. Research indicates that deficiencies of essential elements correlate with chronic diseases and increased infection rates. Moreover, excess amounts of essential elements may also pose harm to human life (Klein et al., 2017).

Statement of the Problem

We are confronted with a growing number of disease burdens in recent times such as kidney probles, cancers, diabetes, cardiovascular diseases, neurological disorders and many more, in both children and adults. Some studies have shown that environmental chemicals stressors are responsible for low IQ scores, cerebral palsy, endocrine disruption, cancers, Type 2 Diabetes, immune and neurological disorders (Ameen & Keizer, 2023; Aronson et al., 2000; Gorini et al., 2014; Massart et al., 2008; Quansah et al., 2015). With most of our water and food sources polluted, breast milk is supposed to be the cleanest food for babies, hence the concept of exclusive breastfeeding. However, breast milk is not pristine. The persistent drive for industrialization and urbanisation has resulted in the release of harmful compounds into the

environment (Landrigan et al., 2002). These harmful chemicals find their way into ground water and food chains, the human body and ultimately into breast milk (Landrigan et al., 2002; Massart et al., 2005, 2008). Neonates are exposed to these hazardous chemicals during intrauterine life and through breast feeding. Heavy metals like mercury, lead, cadmium, arsenic, and aluminium have no beneficial purpose in infant nutrition and can only cause harm.

Furthermore, during the past seventy years, persistent organic pollutants (POPs) have been discovered in human milk in significant concentrations despite the barn ontheir use and prohibition for years, PFAS have been introduced into the environment as a result of industrial manufacturing, as well as the use and disposal of PFAS-containing items (Liu & Mejia, 2013; US EPA, 2012a). Perfluoroalkyls have been found in all environmental media, including air, surface water, groundwater (including drinking water), soil, and food, according to research (Cao et al., 2015).

Examining human breast milk allows for the evaluation of the mother and baby's exposure. This is a non-intrusive way to monitor environmental contamination (Lopes et al., 2016; Rebelo & Caldas, 2016) and it is recommended by the World Health Organization (WHO).

Several studies have detected heavy metals and POPs in the breast milk of nursing mothers, raising worries about the contamination and pollution of breast milk and the potential consequences it may provide to the infants and the mother. Unfortunately, in Ghana, this field of study has received minimal attention. Minimal investigations have been conducted to determine heavy metals and POPs (i.e. OCPs, PCBs and PFAS) present in the breast milk of

nursing women in Ghana. According to the published literature, there are currently no studies on PFAS in human breast milk in Antarctica, Australia, or South America (Macheka-Tendenguwo et al., 2018) and only one recent study on PFAS in Africa (Macheka et al., 2022) In the case of heavy metals and some POPs (e.g., PCBs & OCPs)), where some studies have been carried out in Ghana, these studies did not consider lactational stages. Colostrum, transitional milk, and mature milk are the three types of breast milk that nursing mothers produce. Based on these observations in the literature, I proposed that Ghana, which is rapidly becoming industrialized, motorized and urbanized, may be contaminated with high levels of these heavy metals and POPs, Therefore, I hypothesized that there is significant difference between the levels of these contaminants in the three types of breast milk produced by the nursing mother at levels exceeding the permissible safety levels set by the WHO.

It's also imperative to remember that infants' proper growth and development depend on essential elements found in human breast milk. Research has demonstrated that deficiencies in essential elements are linked to chronic diseases and higher rates of infection. On the other hand, excess amounts of these elements may also pose harm to life (Klein et al., 2017). This is despite the fact that much research on critical element concentrations in different parts of the world have been conducted.

Objectives of the Study

This study, therefore, seeks to determine the levels of toxic heavy metals, persistent organic pollutants (POPs) and essential elements (EEs) in human breast milk (HBM) and assess the extent to which infants are exposed to these toxicants.

Specific Objectives

To determine the levels of:

1) Four (4) PFAS (PFHxA, PFHpA, PFOS and PFOA) in HBM;
2) Fourteen (14) PCB congeners at three lactational stages;
3) Six (6) OCPs across lactational stages;
4) Five (5) toxic heavy metals (Al, As, Cd, Hg & Pb) across lactational stages;
5) Ten (10) essential mineral elements (Ca, Cu, Fe, Mg, P, Na, K, S, Se and Zn) across lactational stages;
6) Compare levels of Toxic Heavy Metals (THMs) in the Colostrum Milk, Transitional Milk & Mature Milk;
7) Compare the levels of Essential Elements in human milk at 3 lactational stages;
8) Compare levels of POPs and THMs in human milk (HM) with recommended allowable safety levels.
9) Evaluate and assess the extent of how infants are exposed to these pollutants through breastfeeding.

Significance of the Study

This research is essential because it will serve as a baseline for future studies to be conducted in other regions of the nation since, to the best of the researchers' knowledge, no such extensive study on human breast milk has been conducted in Ghana. This will guarantee that when it comes to encouraging women to nurse their new-borns, the potential health risks linked with breastfeeding are taken into account in the larger public health evaluation frameworks.

The study's results will also enable the researchers to produce and disseminate educational materials that will inform the public about ways to minimise exposure to these toxins. The study's overall impact will be to provide details on the levels and related health risks of harmful heavy metals, essential elements, POPs, PFOS, and PFOA in breast milk, to make the research's findings more widely known in order to improve maternal and neonatal health, and finally to contribute to the development of a productive Ghanaian population.

Delimitations of the Study

The study focused on healthy mothers aged 18 – 48 years who had just delivered at the Maternity Ward of the Ho Teaching Hospital in the Ho Municipality during the time of the study. The study specifically examined the concentrations of three groups of persistent organic pollutants (POPs), five heavy metals and ten essential elements in human breast milk at the Ho Teaching Hospital and compared them with the recommended safety limits. The specific POPs are:

- Four types of per-and-polyfluoroalkyl substances (PFAS) namely, perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA) and perfluooctane sulphonic acid (PFOS);
- Six organochlorine pesticides – Aldrin, dieldrin, dichloro-diphenyl-trichloroethane (DDT), endrin, and gama chlordane; and
- Fourteen congeners of polychlorinated biphenyls.

The specific heavy metals are aluminium (Al), arsenic (As), cadmium (Cd) lead (Pb) and mercury (Hg) while the specific essential elements are

calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), phosphorous (P), sodium (Na), potassium (K), Sulphur (S), selenium (Se) and zinc (Zn).

The organochlorine pesticides, polychlorinated biphenyls, heavy metals and the essential elements were examined across three lactational stages (colostrum, transitional milk and mature milk). The PFAS were, however, not examined at lactational stages due to lack instrumentation in

Limitations of the Study

Just a few mothers who gave birth at the hospital's maternity ward during the study's time period participated in the research, which was conducted at the Ho Teaching Hospital. The outcome of the research is, therefore, only representative of the Ho Teaching Hospital and cannot be generalized.

Definition of Key Terms

Human Breast Milk: It refers to the fluid that a human female's mammary glands, which are situated in her breast, make to nourish her unborn children. This fluid can be yellowish, white, clear, creamy, or bluish.

Colostrum, CM: It is the first milk that the breasts synthesize after birth.

Transitional Milk, TM: Transitional milk is essentially the breast milk produced between colostrum and mature milk

Mature Milk, MM: Breast milk produced after two weeks postpartum

Heavy Metal: Any chemical element (metallic or metalloid) with a high density and the potential to be hazardous or fatal at even low concentrations is referred to as a heavy metal.

Persistent Organic Pollutants (POPs): They are chemical compounds of global concern (also known as the Dirty Dozen) owing to their persistence in

the environment, long-range transport, ability to bioaccumulate and biomagnify in the ecosystems as well as their potential negative effects on human health and the environment.

Xenobiotics: They refer to foreign chemical substances found within the human body which are not supposed to be there.

PFOS/ PFOA: perfluorooctane sulphonic acid and Perfluorooctanoic acid are two of the newly emerging POPs that belong to the general group known as PFAS (perfluoroalkyl substances) or forever chemicals. They are two mostly produced man-made fluorinated organic chemicals.

TBM: It refers to total breast milk. It is the average of mother's milk during the three stages of lactation namely; CM, TM, and MM.

IQ: Intelligence quotient

Organization of the Study

There are five chapters in this thesis. The study's background is covered in Chapter One, which also discusses how environmental contaminants can contaminate breast milk and the subsequent health impacts on breastfed infants. The problem statement in Chapter One states the issue of heavy metal and POP pollution of breast milk and also emphasises the significance of vital elements. The general purpose, specific objectives, significance of the study, study limitations, and key word definitions are elements included in this chapter.

The review of pertinent literature is covered in Chapter 2. It talks about human breast milk, its components, how it helps babies, and how anthropogenic pollutants can contaminate it. The chapter also discusses toxic heavy metals, which are of particular concern, how they enter breast milk, and how they affect infants who are breastfed. POPs including polychlorinated

biphenyls (PCBs), PFAS (also known as everlasting chemicals), and organochlorine pesticides (OCPs), as well as their origin, how they infect human milk, and their effects on people, are also covered.

The study's third chapter outlines the research technique. The investigation was carried out at the Ho Teaching Hospital using a cross-sectional design. The study site, population, sample size calculation, sampling, and ethical clearance are covered in sections. Others include methods for extracting various contaminants, storing samples, collecting samples, quality assurance procedures, and health risk assessment methods.

The explanation of the study's findings is covered in Chapter Four. It compares and examines aspects related to levels of toxic heavy metals in colostrum, transitional milk, and mature milk, as well as levels of harmful heavy metals and essential mineral elements in human breast milk during the three stages of lactation. The chapter also discusses the relationships between dangerous heavy metal concentrations in breast milk and permitted safety limits established by the WHO, as well as exploratory factor analysis of heavy metals and essential elements at the three lactational stages. In order to establish whether amounts of toxic heavy metals found in breast milk could provide any major health hazards to nursing infants, the chapter concludes by examining the health risks evaluation.

The study's summary findings, conclusion, and recommendations are covered in Chapter 5. A conclusion is drawn based on the study's specific goals, indicating the degree to which they were met. The final section of the chapter offers suggestions on how to make the system better.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter contains the review of the research literature that is relevant to the study: "Toxic Heavy Metals, Persistent Organic Pollutants (POPs) and Essential Elements in Human Breast Milk at the Ho Municipality in Ghana". For the purpose of this study, the literature will be reviewed under the following thematic areas:

- Human breast milk, composition and its benefits to neonates
- Contamination of breast milk by anthropogenic chemicals
- The range of heavy metals levels in breast milk monitoring studies
- Previous studies on the determination of heavy metals in human breast milk
- Toxic heavy metals of prime concern in breast milk – aluminium, arsenic, cadmium, lead and mercury; sources, uses, modes of exposure, how heavy metals enter and leave the body, effects on human health, effects on children and recommended levels in air, water and food.
- Essential element in human breast milk
- Overview of studies on determination of heavy metals in human breast milk
- Persistent Organic Pollutants (POPs) in human breast milk
- Health effects of POPs on children
- Review on PFAS and their health impacts on humans and the environment

What is Breast Milk?

Breast milk, commonly known as mother's milk, is the yellowish, white, clear, creamy, or bluish liquid that a human female's mammary glands make to nourish her neonates. The mother's breasts produce a liquid source of food, known as milk, for her neonates. This milk is produced by the woman's body in response to pregnancy and the act of breastfeeding. Nevertheless, women who are not pregnant could also be induced to breastfeed with the help of hormones, medications and stimulations (Farhadi & Roy, 2017). As breastfeeding progresses, changes in breast milk's colour and composition may occur (Andreas et al., 2015). Before they can consume and digest other kinds of food, new-borns are mostly dependent on breast milk as their source of sustenance. As infants and toddlers get older, they may continue to breastfeed while also incorporating other types of food into their diet, starting at around six months of age and gradually increasing the amount of solid food they eat (Victora et al., 2016; WHO, 2023).

Within few minutes after birth, secretion of breast milk and for that matter, breastfeeding begins.

Colostrum, transitional milk, and mature milk are the three types of milk that the human breast generally produces throughout the various lactation periods. Mature milk is made up of foremilk and hind milk. The synthesis and secretion of breastmilk can be categorized into the following segments: Milk secreted right after birth to day four is called colostrum; milk secreted from day five or more onwards is known as transitional milk, while the milk secreted from two to six weeks onwards is known as mature milk (Turan et al., 2001). The breast produces a limited quantity of colostrum, which is highly

nutritious and concentrated. Colostrum can vary in consistency, appearing either clear and thin or yellow or orange and thick. The presence of beta-carotene gives it a distinct dark yellow or orange colour (ABA, 2023; Patton et al., 1990).

After the initial colostrum secretion in the first few days, the production of breast milk increases and the body begins to produce transitional milk. This transition period causes the colour of the breast milk to change from yellow to white around two weeks' post-birth, the body reaches the mature milk stage, in which the appearance of the breast milk can vary depending on the amount of fat it contains. Generally, when a breastfeeding or pumping session starts, the first milk to be expressed is thinner, clear and blue in colour, and contains less fat. This is referred to as foremilk. As breastfeeding continues, the fat contents of the milk increases and the colour of the breast milk turns into creamy thicker milk with yellow appearance called hind milk (Kent et al., 2016). Breast milk is described as creamy and sweet. It derives its creamy nature from the amount of fat it contains and its sweetness from lactose. Mother's daily intake of food as part of her breastfeeding diet can also influence the flavour of her breast milk (Mastorakou et al., 2019).

Composition of Breast Milk

Over 200 different molecules, including a range of carbohydrates, proteins, lipids, vitamins, minerals, other nutrients, hormones, bioactive compounds, and enzymes make up the intricate makeup of breast milk (Bode et al., 2014; Moukarzel & Bode, 2016). Breast milk's composition is dynamic and changes over time during each feeding throughout the day during each

feeding and during the entire lactation period to meet the needs of the growing child. Its dynamic nature helps provide optimal nutrition for the growing child.

The brain, digestive system, and immune systems of the infant are all developed with the help of the bioactive substances found in breast milk. Breast milk is made up primarily of water (87%) followed by lactose (7%), fat (4%), and protein (1%) (Guo, 2014; Martin et al., 2016). The carbohydrates profile of breast milk is diverse but the predominate one is lactose. Lactose concentration is fairly constant around three weeks postpartum onward. Besides providing energy for the developing brain, it also ensures milk consistency and absorption of minerals like calcium needed for development of bones and teeth. Additionally, breast milk contains a recently discovered category of carbohydrates known as human milk oligosaccharides (HMOs). It is useful in promoting the development of helpful bacteria in the gut, reducing the incidence of infant diarrhoea and respiratory tract infections, and promoting a healthy gut microbiota, HMOs serve as prebiotics (Hegar et al., 2019; Ray et al., 2019). Fats are very important constituents of breast milk. The fats content of breast milk is quite varied. Breast milk contains more than 200 different types of fatty acids, each serving a specific purpose for promoting optimal health and growth (Moukarzel & Bode, 2016). Figure 1 illustrates the various constituents of breast milk.

Figure 1: Composition of Breast Milk. Source: Shenker (2013)

Colostrum

Colostrum, the first milk secreted by the breasts, is a viscous, yellowish liquid thick, sticky, and includes all the nutrients needed for a new-born to leave the womb and enter the world. It may sometimes leak from the breasts during pregnancy. It could also be thin and white or orange in colour. Within the first three- or four-days following birth, it is created. Colostrum, high in antibody and protein that provide the child immune protection, supports the development and proper functioning of the neonate's digestive system. Colostrum differs significantly from the typical human breast milk released by the mammary glands because it contains more lactoprotein, lactalbumin, and antibodies that give the new-born a passive immunity (Godhia & Patel, 2013; Sood & Gupta, 2017). Colostrum, referred to as "foremilk", "liquid gold", or "Golden Milk", is a vital component of breast milk and plays crucial roles in the immune system of mammals (Godhia & Patel, 2013).

Due to the significant amounts of antibodies and white blood cells it carries, colostrum is frequently referred to as a "natural immunization", The

nutrient profile and immunological composition of colostrum is entirely different from mature milk in that colostrum contains macronutrients, micronutrients, anti-microbial agents, growth factors and immune regulating components all of which may either not be present in mature milk or present in markedly reduced concentrations (Godhia & Patel, 2013).The macronutrients composition in colostrum include carbohydrates, proteins, oligosaccharides, fats, while the micronutrients include a range of vitamins and minerals.

Therefore, based on the above, colostrum's constituents may be categorized into three: nutritional factors, immune factors and growth factors that help build lean muscles and growth hormone (GH). This has nutritional components: proteins, carbohydrates, energy, fat, minerals, and vitamins. Generally, proteins and fats concentrations in colostrum are markedly higher than in milk. The digestive system of neonates is immature, and thus colostrum, with its nutrients-rich contents in a highly concentrated form, is delivered to the baby in low volumes. It possesses a mild laxative effect, enabling the neonates to pass its first stool known as meconium. It is crucial for the baby's well-being as it eliminates excessive bilirubin, which is a by-product of deceased red blood cells produced in abundance at birth and helps prevent jaundice in new-borns (Sood & Gupta, 2017).

Colostrum comprises of several growth factors like Insulin-like Growth Factor I (IGF-1) and II (18 mg/ml), transforming growth factors alpha (2.2-7.2 (µg/L), beta 1 and beta 2 (20-40 mg/L), fibroblast growth factors, platelet-derived growth factor, granulocyte-macrophage-stimulating growth factor, epidermal growth factor (200 µg/L) and many others. Cell

development, differentiation, and maturity are all induced and facilitated by growth factors. The Epidermal Growth Factors (EGF) cohort of growth factors influence the development of the mammary glands, epidermis and the gut. The fibroblast growth factors facilitate the healing of wounds and growth of new blood vessels. Insulin-like growth factors play significant role during childhood growth whereas in adults, it has anabolic effects. Colostrum contains platelet-derived growth factors, which are protein-like in nature and are responsible for the regulation of cell division and growth, and also plays an important role in the formation of blood vessels (Andrae et al., 2008; Godhia & Patel, 2013).

The immune factors in colostrum include antibodies such as immunoglobulins A, (IgA), immunoglobulin G, (IgG), immunoglobulin M, (IgM), Immunoglobulin F, (IgE) and immunoglobulin D, (IgD), cytokines, lactoferrin, lactoperoxidase, lysosomes, and proline-rich polypeptides. (Godhia &Patel, 2013). immunoglobulin F (IgE) and immunoglobulin D, (IgD), cytokines, lactoferrin), lactoperoxidase, lysosomes, and proline-rich polypeptides. The immune system uses antibodies to look for, find, attack, and neutralize foreign organisms (pathogens), such as viruses and bacteria. Thus, the development of passive immunity after birth depends on the presence of immunoglobulins in the neonate. The first to react when a pathogen enters the body is IgM, which fights microorganisms and renders them inactive. In addition to providing a significant amount of immunity against invasive infections, IgG also triggers a series of other immunological reactions (Donovan & Odie, 1994). It is well known that IgE plays a significant role in allergic reactions and reacts to parasites in the gastrointestinal system. On the

other hand, IgA positions itself in advantageous areas such as the respiratory, gastrointestinal, and urogenital tracts where they perform a crucial role in mucosal immunity by preventing certain pathogens from colonizing as well as supporting the protection of the mucous membranes in the new-borns' lungs, throat, ear, and intestine (Sood & Gupta, 2017). Immunoglobulin-D collaborates closely with immunoglobulin-M by signalling and activating B cells. IgD also works in conjunction with other immunoglobulins to strengthen or enhance the body's immune system.

Lysosomes are a category of enzymes which are anti-microbial in nature. Through the destruction of dangerous bacterial cell walls, they aid the immune system. Also, lysosomes can uniquely collaborate with other colostrum constituents to combat germs. For instance, it has been shown that lysosomes work synergistically with IgA, lactoperoxidase and lactoferrin to combat bacteria. Lysosomes partly activate lactoperoxidase by forming a complex, while they work in synergy with IgA to fight E. coli. According to some research, lactoferrin also improves the antibacterial abilities of lysosomes (Gruden & Ulrih, 2021; Niaz et al., 2019).

Lactalbumin is a water-soluble protein found in milk and is an important nutrient, providing essential amino acids required for growth and development of neonates (Layman et al., 2018).

Cytokines in colostrum contain many biological response modifiers which could be peptide, glycoprotein or protein signalling used in cell communication, allowing the immune system and the host tissue to exchange information. They perform a unique role as regulators of inflammatory diseases, epithelial development and growth, and epithelial restoration in cases

of mucosal damage. They also act as mediators in controlling immunological and inflammatory responses (Pajewska-szmyt et al., 2019). Recent research indicates that cytokines accelerate tendon repair.

Lactoperoxidase is a haeme peroxidase enzyme that is secreted by the breast, salivary, and other mucous glands. Hydrogen peroxidase catalyses the oxidation of many organic and inorganic compounds. By eliminating germs in milk and mucosal secretions, the lactoperoxidase system aids the innate immune system. Lactoperoxidase protects the breastfeeding mammary glands from infection. It occasionally works in concert with lactoferrin to provide antibacterial characteristics. Proline residues are present in proline-rich peptides, which are a diverse set of tiny to medium-sized peptides and give rise to certain sequences. They are capable of initiating and balancing immunological responses, modulating the immune system, acting as molecular signalling devices, promoting B cell development and differentiation, promoting the activity of Natural Killer cells, and increasing the production of white blood cells (Pajewska-szmyt et al., 2019).

Benefits of Colostrum to Neonates

Despite not being secreted in large amounts colostrum has a number of advantages for the new-born. It has a low-fat content and is quite abundant in carbs, proteins, and antibodies. Additionally, it is loaded with vitamins A, B6, B12, D, and K, as well as minerals like calcium and zinc that are crucial for the baby's overall growth and development. Colostrum nourishes the human body in two ways and is a natural source of vital development and healing components (Godhia & Patel, 2013; Playford et al., 2000). First, its numerous immunity factors and natural antibiotics offer the immune system significant

support, and second, its numerous growth factors give the body a broad-spectrum boost to promote maximum health and repair. Growth factors, antibodies, and immunoglobulins all function to prevent infection (Playford et al., 2000; Sood & Gupta, 2017). Colostrum is probably, the most important natural food substance. The presence of immune factors in colostrum can help regulate immune responses, and growth factors, leading to repair of damaged cells and anti-inflammatory substances to minimize inflammation and thereby helping to prevent autoimmune diseases. Studies have also shown that lactoferrin in colostrum helps to prevent or shrink cancerous cells. It also contains milk fats possessing anti-carcinogenic properties and therefore, can prevent colon, bladder, tongue, oesophagus and lung cancers (Godhia & Patel, 2013).

General Benefits of Breast Milk to Neonates

Breastfeeding has a wide range of benefits to neonates. Research indicates that, babies who have been breastfed exclusively for the first 6 months without any formula have been observed to have minimal ear infections, respiratory illnesses, and bouts of diarrhoea as well as fewer hospitalizations and trips to the doctor (Frank et al., 2019; Hossain & Mihrshahi, 2022). Other benefits include higher intelligence quotient (IQ) scores as reported in some studies, prevention of sudden infant death syndrome (SIDS), lower risk of caners, obesity, both forms of diabetes and certain cancers during later life(Mead, 2008; Motee & Jeewon, 2014; US CDC, 2023), minimal risk of middle-ear infection, cold and flu, a tiny reduction in the risk of childhood leukaemia, a decreased risk of asthma and eczema and decreased risk of development of psychological disorders (Motee & Jeewon, 2014; US CDC, 2023).

Besides, Breast feeding also provides numerous advantages for the mother, including reducing the time it takes for the uterus to return to its normal size after pregnancy, limiting postnatal bleeding, and aiding in weight loss. Furthermore, breastfeeding can lower the likelihood of developing breast cancer later on and protect both the mother and infant from both forms of diabetes. Indeed, nursing has many positive effects, and a recent study has shown how breastfeeding can reduce moms' chance of developing heart disease, adding yet another significant benefit to the list. At a recent meeting of the European Society of Endocrinology, researchers from the University of Athens in Greece presented their findings, which suggested that breastfeeding may reduce the risk of heart disease. They looked at 283 postmenopausal women and compared their blood vessel and heart health to their experience of nursing. The breastfeeding duration of the women in the study ranged from one month to 80 months. They found that women who had breastfed had decreased artery stiffness and atherosclerosis after controlling for other risk factors such age, weight, smoking status, and cholesterol levels. The increased prolactin levels in breastfeeding moms, which can also lower the incidence of diabetes, another significant risk factor for heart disease, are thought to be responsible for this benefit, according to the researchers. Another study including nearly 300,000 Chinese women discovered that breastfeeding lowers heart disease risk by 10% and that breastfeeding for at least two years lowers risk by 18%. According to research from the University of Pittsburgh, women who do not breastfeed are more likely to develop heart disease.

Contamination of Breast Milk: A Cause for Concern

While nursing is the best and most natural way to nurture and care for infants, the recent environmental contamination of breast milk with a variety of hazardous chemicals is a serious problem. This is due to the fact that breast milk can expose new-borns to these harmful substances as well (Yurdakök, 2015). It has been well documented that breast milk is contaminated with toxic heavy metals and persistent organic pollutants (Mead, 2008). This is as a consequence of decades of globalization and industrialization, economic development and adoption of different agricultural practices which rely heavily on the use of chemicals to improve crops yield in order to feed the world's teaming population. One of the most widespread environmental problems plaguing the world today is the contamination of ground water and among the myriad of pollutants affecting water resources are heavy metals, persistent organic pollutants (POPs), as well as per- and polyfluoroalkyl compounds. Aquatic ecosystems, agriculture, and public health are seriously threatened by the contamination of ground water and soil habitats. In aquatic ecosystems, heavy metals build up in the silt at the water's bottom and in the tissues of living things. Heavy metals are absorbed by aquatic plants from surface water and water columns and are then incorporated into food chains. The concentrations of these chemicals increase along the food chain through the process of biological magnification (Veschasit et al., 2012). Whereas some toxic sediments kill some bottom-feeding organisms thereby decreasing the quantity of food available to larger fish, other benthic organisms bioaccumulate these toxic chemicals in their bodies. When animals at the top of the food chain consume organisms that have been contaminated with toxins,

the toxins accumulate in their bodies, becoming more concentrated as they move up the food chain through a process known as biomagnification (Khamis et al., 2017). Consequently, fish, shellfish, waterfowl, freshwater and marine mammals may consume toxic chemicals in hazardous concentrations, resulting in the accumulation of these chemicals in their bodies. (Begum et al., 2009). In this regard, humans, who are on top of the food chain, when they consume fish, shellfish, waterfowls, freshwater and marine mammals, they end up accumulating these toxic chemicals in their tissues in even higher concentrations. These chemicals later enter the bloodstream and eventually find their way into different human body fluids such as blood, breast milk, saliva, semen and urine.

In a review (Landrigan et al., 2002), it was confirmed that human milk is widely contaminated due to decades of poor regulation of toxic chemical pollution in the environment. Some of the most commonly found toxic chemicals in breast milk include Polychlorinated biphenyls (PCBs), DDT, its byproducts, dioxins, dibenzofurans, polybrominated diphenyl ethers (PBDEs), and heavy metals (Hooper et al., 1999; Sonawane, 1995). In both industrialized and undeveloped nations, women accumulate these chemicals to differing degrees. In underdeveloped countries where pesticides are widely used, women in agricultural areas have some of the highest levels of pollutants (Hooper et al., 1999). Among rural women such as the Canadian Innuit who consume diets rich in seals, whales, and other species high up in the marine food chain tend to carry significant burdens of persistent organic pollutants (Dewailly et al., 1993).

Heavy metals such as arsenic, cadmium, lead, and mercury have been found in human breast milk in several nations across the world. According to the World Health Organization (WHO), the average and range of hazardous metals identified in breast milk worldwide are: Lead at 5.0 ppb (0.0-41.1 ppb), Mercury at 2.7 ppb (0.64-257.1 ppb), and Cadmium at 0.1 ppb (0.1-3.8 ppb) (NRDC, 2001).

These hazardous metals are ingested by mothers throughout their lifetimes. Unlike persistent organic pollutants (POPs), these metals do not accumulate in maternal adipose tissue, therefore their quantities in breast milk are often lower than in the mother's blood. Heavy metals with high binding capacities to plasma proteins or red blood cells are unlikely to transfer into breast milk by passive diffusion, resulting in lower levels of these metals in breast milk than in cord blood. As a result, babies are more likely than nursing mothers to be exposed to higher levels of these metals before to birth through the placenta and cord blood (Yurdakök, 2015).

According to scientific research, cadmium, lead, and mercury have no nutritional benefits and only have harmful effects on the human body (Jaishankar et al., 2014; Mitra et al., 2022; Sears et al., 2012) . Regrettably, these heavy metals may be present in breast milk and infant formulas, which can be detrimental to neonates and nursing infants. For hazardous metals, the FAO/WHO has created a provisional tolerable weekly intake (PTWI) value. Studies have shown that infants are more vulnerable to the effects of contaminated food due to factors such as high gastrointestinal absorption, decreased effectiveness of the blood-brain barrier against toxic metals, rapid organ growth, immature detoxification systems, and immature development of

the central nervous system and immune system (Mielech et al., 2021). The estimated PTWI for lead in infants and children is 25 g per kilogramme of body weight (WHO, 1993). The corresponding value for cadmium is 7 µg/kg, and for total Hg it is 5 µg/kg (< 3.3 µg/kg for methyl-Hg) (FAO/WHO, 2004a).

According to the World Health Organization, the acceptable levels of mercury and lead in breast milk are 1.4-1.7 ng/g and 2-5 ng/g, respectively (WHO/IAEA, 1989). Research on babies who have been exposed to chemicals in breast milk has not provided clear proof of negative health impacts on the nursing child. Additionally, it can be challenging to determine if any negative effects observed are a result of exposure before or after birth. However, since no life studies have been carried out on children whose mothers have been exposed to these heavy metals, it is difficult to draw any definite conclusions.

How Xenobiotics (Heavy Metals and POPs) Cause Health Effects in Humans

According to research, taking large levels of heavy metals can cause serious health problems with a wide range of symptoms depending on the type and amount of metal taken. Heavy metal toxicity occurs when metals bind to functional groups in proteins such as carboxylic acid (-COOH), amine (-NH2), and thiol (–SH) (Igiri et al., 2018; Khamis et al., 2017). Heavy metals form bonds with these functional groups leading to formation of compounds which are not biologically important in the human body. When biomolecules are altered by the binding of heavy metals, they lose their normal functionality and can lead to cell malfunction or death. In addition, when metals bind to these functional groups, they can inactivate important enzyme systems or

change the structure of proteins, which can affect the enzymes' ability to catalyse reactions (Khamis et al., 2017). This type of toxin may also cause the formation of radicals which are dangerous chemical entities that cause the oxidation of biological molecules.

The heavy metals that humans are most commonly exposed to are aluminium, arsenic, cadmium, lead, and mercury. Aluminium has been linked to diseases such as Alzheimer's and Parkinson's, as well as conditions such as senility and presenile dementia. Arsenic exposure can cause cancer, abdominal pains, and skin lesions. Cadmium can lead to kidney damage and high blood pressure. Lead is a toxic substance that may also be a human carcinogen. Exposure to mercury can cause mental disturbances and impairments in speech, hearing, vision, and movement (Khamis et al., 2017). In addition, lead and mercury may lead to the development of autoimmunity, which can result in joint diseases, kidney problems, and issues with the circulatory system and neurons. Research has established that elevated concentrations of lead and mercury have the potential to cause irreversible brain damage (Momodo & Anyakora, 2010).

According to the literature, ionic lead toxicity can have a significant impact on a variety of biological processes, including changes in cell adhesion, signalling within and between cells, protein folding and maturation, cell death (apoptosis), ion transport, enzyme regulation, and neurotransmitter release (Jaishankar et al., 2014)). In very small concentrations, lead can substitute for calcium, altering protein kinase C, an enzyme that regulates brain excitation and memory storage (Flora et al., 2012). While mercury is most commonly associated with the brain, it can impact any organ and cause

neurons, kidneys, and muscles to malfunction. It can disturb membrane potential and disrupt intracellular calcium homeostasis. Because of the high stability constants for these binding interactions, mercury easily attaches to thiols (Jaishankar et al., 2014). Mercury vapour exposure can result in bronchitis, asthma, and other temporary respiratory problems. Mercury has a significant impact on tertiary and quaternary protein structure, causing changes in cellular function by adhering to selenohydryl and sulfhydryl groups, which react with methyl mercury and disrupt cellular structure. Additionally, it impairs transcription and translation processes, resulting in ribosome loss, endoplasmic reticulum collapse, and a decrease in natural killer cell activity. Furthermore, it has the ability to disturb cellular integrity and contribute to free radical formation.

Recent studies show a strong connection between exposure to certain chemicals and a rise in various health issues in children, including immune system, neurological, behavioural, and developmental disorders like autism, ADHD, low IQ, and cerebral palsy (Aronson et al., 2000; Carroquino et al., 2012). Additionally, research has suggested a correlation between exposure to certain chemicals known as POPs and an increased risk of cancer, as some POPs have been identified as cancer-causing agents (Aronson et al., 2000). Chemicals that have a high affinity for fats, known as lipophilic chemicals, are more likely to accumulate in the mother's tissues, be released during breastfeeding, and transfer from the mother's blood to milk, thereby increasing the dose received by the infant. This is because these chemicals are able to penetrate through various barriers more easily. Heavy metals such as arsenic,

lead, mercury, and cadmium, as well as persistent organic pollutants, are considered to be particularly concerning in this regard (Khamis et al., 2017)

Strategies used for Quantifying Contaminants in Breast Milk

Studies show that analysing the levels of pollutants in breast milk not only reveals the mother's exposure to toxins, but also serves as an indicator of the foetus's exposure to these chemicals during pregnancy (Mead, 2008). Assessing the levels of pollutants in breast milk, particularly in the colostrum, is a way to measure exposure to these contaminants during pregnancy, similar to other methods such as analysing cord blood, infant hair, nails, or meconium (Yurdakök, 2015). It also takes into account the possibility of exposure risk for breastfed infants throughout the postnatal period. Additionally, the presence and concentration of specific harmful compounds in breast milk are used to biomonitor the level of environmental pollution and evaluate the efficacy of preventive measures (Yurdakök, 2015).

Reports of Heavy Metal Levels in Breast Milk Monitoring Studies

Many potentially harmful metals, including lead, mercury, and cadmium, have been discovered in breast milk research, and their amounts in human milk vary widely. Before 1973, the average lead concentration in breast milk ranged from 5 to 277 mg/ml (Dillon et al., 1974), while Iyengar & Woittiez (1988) proposed a breast milk reference value of 30 g/l.

Souad et al. (2006) detected 3.1-117.4 g/l lead in Moroccan women's breast milk, while (Al-Saleh et al., 2003) found 31.67 g/l lead in Saudi Arabia's breast milk.

A study conducted in Turkey found that the level of lead and cadmium present in breast milk was 14.6 micrograms per litre and 2.8 micrograms per

litre, respectively (Turan et al., 2001). Cadmium (0.14-0.19 g/l) and lead (0.15-0.48 g/l) ranges recently found in the Greek population (Leotsinidis et al., 2005) ranges, on the other hand, were among the lowest documented in the literature. Global mercury contaminations ranged from 0.03 ng/ml in Canada (Winfield et al., 1994) to 200 ng/ml in Iraq (Bakir et al., 1973; Dorea, 2004). The vast range of heavy metal levels shows a significant association with a number of factors, including the family's socioeconomic condition as well as dietary and behavioural habits. One study discovered that people who work in plants had higher levels of heavy metals such as lead, mercury, and manganese in their breast milk and blood samples than those who reside close or outside of industrial areas (Sharma & Pervez, 2005).

In his study (Leotsinidis et al., 2005), he discovered that the location of residence has a significant impact on the amount of lead present in breast milk. Mothers living in cities had considerably higher levels of lead in their breast milk than moms living in rural regions ($p=0.001$). This disparity is most likely related to city traffic density.

Several other studies have revealed that women who live in cities with a lot of traffic and industrial activity have more lead in their breast milk than women who live in rural areas (Frkovic et al., 1997; Huat et al., 1983; Saleh et al., 1996). Despite the fact that airborne particles are the primary source of lead exposure for women, these particles have no direct effects on the level of lead in breast milk (Dorea, 2004). Positive associations have been demonstrated between breast milk mercury and fish consumption (Oskarsson et al., 1995, 1996) and between breast milk mercury and amalgam fillings (Drasch et al., 1998; Oskarsson et al., 1995, 1996).

The link between heavy metals and transitional milk, on the other hand, was not statistically significant (Klemann et al., 1990). There is, however, a clear link between cadmium levels in breast milk and cigarette smoking (Hallen et al., 1995). One German study found a direct correlation between a mother's daily cigarette consumption and the level of cadmium in her breast milk (Radisch et al., 1987).

It's worth noting that some study suggests that infants' cadmium exposure from soy formula is roughly 20 times higher than the average levels reported in breast milk (Oskarsson et al., 1998). Heavy metals such as lead, mercury, and cadmium present in lower concentrations in human milk than liposoluble compounds, in contrast to other persistent organic pollutants. Generally, the concentration of these heavy metals in human milk is roughly 20% of the level seen in the same individual's blood (Golding, 1997). This is because these heavy metals have little lipid solubility and strongly bind to erythrocytes. As a result, infants are more likely to be exposed to heavy metals before birth than while breastfeeding (Needham & Wang, 2002). Even though hazardous metals in breast milk represent a secondary source of exposure, they are nevertheless worth considering since they can serve as an indicator of probable prenatal exposure (Cerná et al., 2012; Solomon & Weiss, 2002).

Studies on the Determination of Heavy Metals in Human Breast Milk

Numerous investigations have been undertaken to detect and report heavy metal levels in human breast milk using various analytical techniques such as Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and Graphite Furnace Absorption Spectroscopy (GFAS) (GFAS). The study used ICP-MS to investigate the quantities of

heavy metals such as arsenic, cadmium, lead, and aluminium in human breast milk during four different stages of lactation: colostrum, transitional milk, early mature milk, and mature milk in Taiwan (Chao et al., 2014). They discovered that the quantities of lead, cadmium, aluminium, and arsenic in colostrum were in the following order: 13.22 ± 3.58 ng/mL, 1.37 ± 0.94 ng/mL, 56.45 ± 22.77 ng/mL, and 1.50 ± 1.50 ng/mL, respectively. According to the findings, the concentrations of these microelements reduced as the breastfeeding stage advanced. Also, the study indicated that infants of smoking mothers were exposed to higher cadmium than infants of non-smoking moms ($p<0.05$).

The study evaluated lead contents in human milk at four different stages of lactation and found that the amounts varied between 22.36 ng/mL to 0.45 ng/mL. In each stage, the mean and standard deviation of lead concentration were: 13.22 ± 3.58 ng/mL (range: 6.70 - 22.36 ng/mL), 8.92 ± 2.60 ng/mL (range: 3.52 - 14.71 ng/mL), 11.72 ± 2.58 ng/mL (range: 0.76 - 11.72 ng/mL), and 2.93 ± 1.70 ng/mL (range: 0.45 - 7.80 ng/mL) (Chao et al., 2014). The study discovered that lead concentrations in colostrum were substantially greater ($p = 0.01$) than in transitional and mature milk. The medians for colostrum, transitional, early mature, and mature milk were 13.3 ng/mL, 9.09 ng/mL, 5.21 ng/mL, and 3.01 ng/mL respectively. The researchers discovered a pattern of substantial reduction ($p = 0.01$) in lead levels at each stage of lactation. Figure 2 illustrates the results of Pb concentrations at four lactational stages.

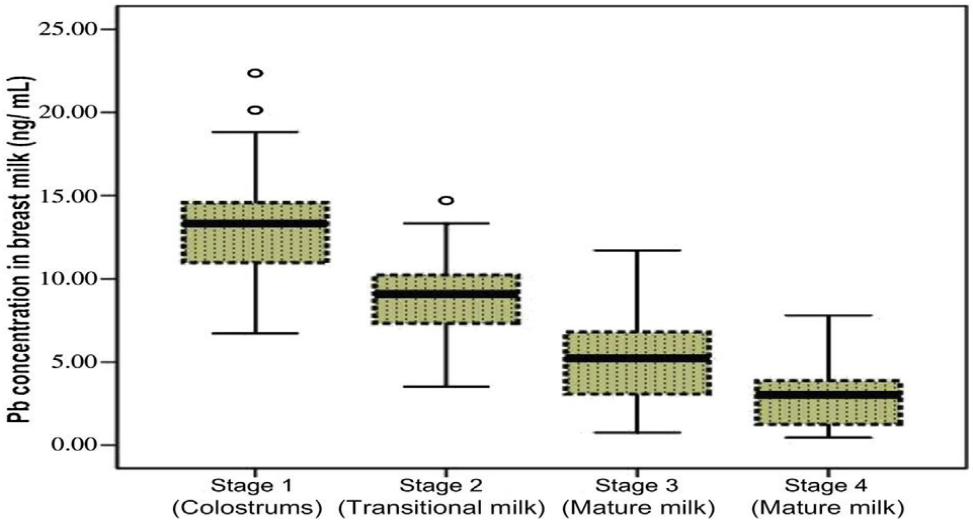

Figure 2: Box and Whisker Plots Displaying Levels of Pb in Breast Milk in Four Stages of Lactation. Source: Chao et al. (2014)

The study also discovered statistically significant changes in cadmium, aluminium, and arsenic levels between lactation phases. Colostrum had the highest quantities of cadmium, aluminium, and arsenic: 1.37 ± 0.94 ng/mL, 56.45 ± 22.77 ng/mL, and 1.50 ± 1.50 ng/mL, respectively (Chao et al., 2014). The amounts of these heavy metals in transitional milk were 0.65 ± 0.36 ng/mL, 36.57 ± 16.62 ng/mL, and 0.68 ± 1.09 ng/mL, respectively. The study discovered that the concentration of heavy metals in human milk reduced significantly ($p = 0.01$ for all elements) as lactation continued, reaching 0.49 ± 0.25 ng/mL, 18.11 8 ±.89 ng/mL, and 0.27 ± 1.26 ng/mL, respectively, at the level of early mature milk (Days 30–35 postpartum). Finally, at the mature milk stage (Days 60-65 postpartum), the concentrations of cadmium, aluminium, and arsenic were 0.34 ± 0.19 ng/mL, 13.44 ± 6.28 ng/mL, and 0.16 ± 0.24 ng/mL, respectively (Chao et al., 2014). Figure 3, 4 & 5 respectively display the concentrations of Cd, Al & As at four lactational stages.

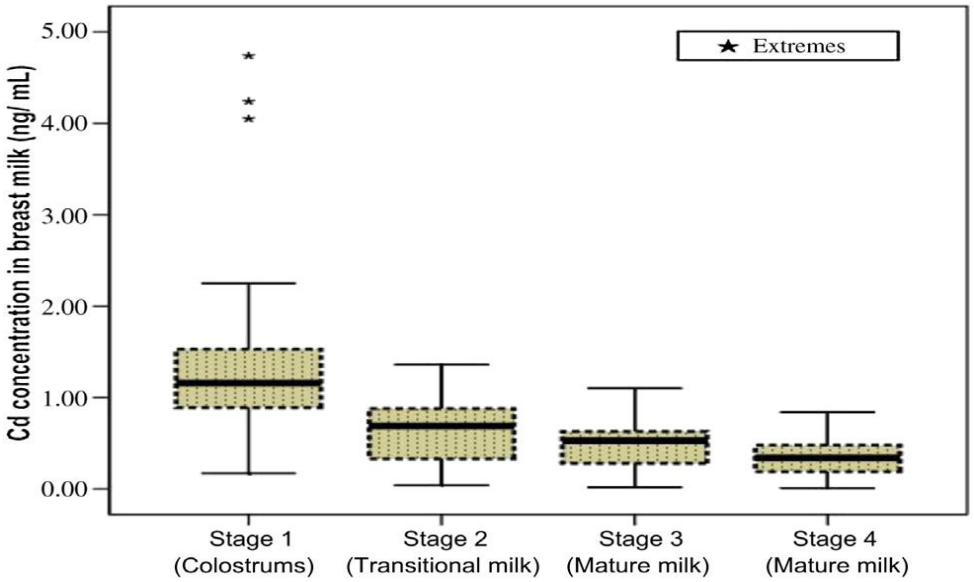

Figure 3: Box and Whisker Plots Displaying Levels of Cd in Breast Milk in Four Stages of Lactation. Source**:** Chao et al. (2014)

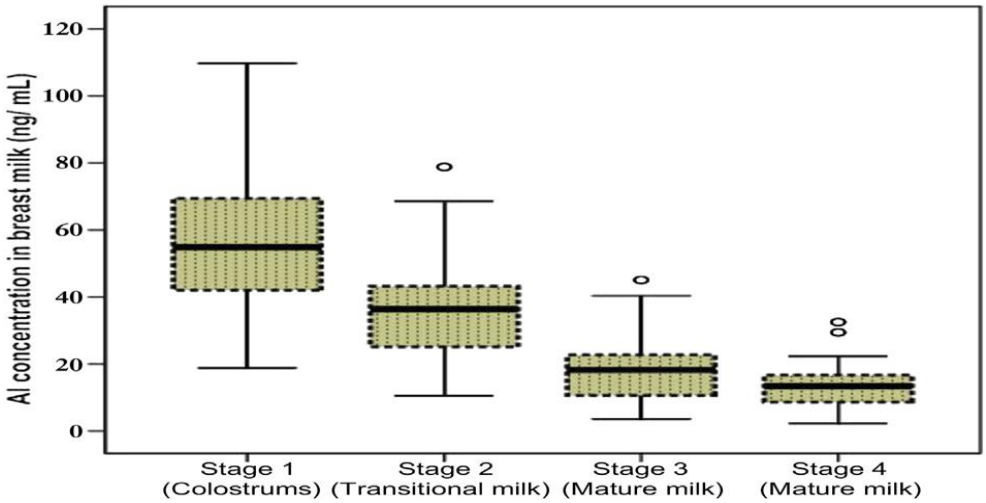

Figure 4: Box and Whisker Plots Displaying Levels of Al in Breast Milk in Four Stages of Lactation. Source: Chao et al. (2014)

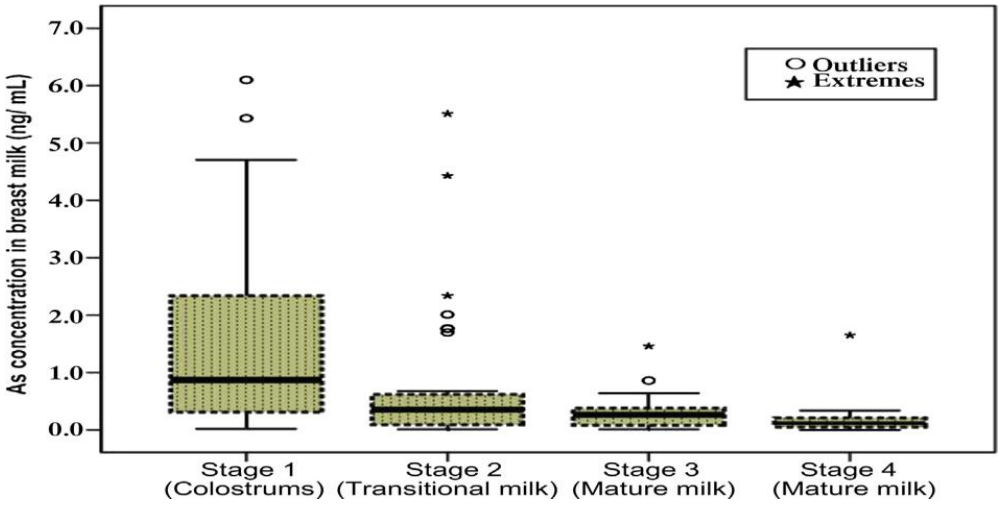

Figure 5: Box and Whisker Plots Displaying Levels of As in Breast Milk in Four Stages of Lactation. Source: Chao et al. (2014)

Factors Associated with Heavy Metals Levels in Breast Milk

In terms of the presence of heavy metals in human breast milk, (Chao et al., 2014) discovered that the age of the breastfeeding mother had a significant link with the lead level in colostrum ($r = 0.396$, $p = 0.007$). There was also a significant relationship between the mother's age and education level ($r = 0.311$, $p = 0.038$). Mothers with a college education or above had a mean age of 30.84 ± 4.93, whereas mothers with a high school education or less had a mean age of 26.60 ± 3.87 (Chao et al., 2014). Additionally, data from questionnaires completed by all 45 breastfeeding mothers, as well as levels of lead, cadmium, aluminium, and arsenic in breast milk at four distinct stages of lactation, revealed no significant link between cadmium levels in colostrum ($r = 0.285$, $p = 0.058$) (Chao et al., 2014). Nonetheless, there was a substantial positive connection ($r = 0.361$, $p = 0.015$) between cadmium levels in colostrum and the mother's cigarette intake, demonstrating that the mother's smoking behaviours influence the cadmium level in breast milk. Additional investigation indicated no significant relationship between cadmium levels in breast milk and mother cigarette smoking at any other stage of lactation,

including transitional milk (r = 0.015, p = 0.921), early mature milk (r = 0.031, p = 0.841), or mature milk (r = 0.132, p = 0.387).

Also, Chao's study looked into the impact of mother's smoking behaviours on the cadmium levels in breast milk. The results established a significant difference between heavy smokers and non-smokers (p = 0.019). The researchers discovered that levels of cadmium, lead, aluminium, and arsenic in breast milk decreased rapidly and drastically as lactation progressed. Some researchers have noticed a decline in the contents of other elements as well(Moser & Reynolds, 1993; Silvestre et al., 2001). However, the study did not explain why hazardous components were found in the highest concentrations during the colostrum period. The researchers noted that the statistical drop of dangerous metals in human milk across various lactational phases is difficult to explain. It has been argued, however, that new-borns require additional trace elements during their first few days of life, and that the proteins that bind to these metals change as a result of nursing. Additionally, breast milk is produced by the mammary gland's epithelial cells, and some milk elements such as lipids, proteins, and lactose are formed in the epithelial cells from blood precursors.

The amounts of lead, cadmium, and arsenic in human breast milk were detected in Lebanon (Bassil et al., 2017). The study discovered that 63.5% of breast milk samples contained arsenic contamination (M = 2.4 ± 1.9 g/L), whereas 40.5% and 67.6% of samples contained cadmium and lead contamination (M = of 0.87 ± 1.18 g/L and 18.18 ± 13.31 g/L, respectively). Consuming cereal and fish was associated with arsenic contamination (p = 0.013 and p = 0.042, respectively), while living near cultivation activities,

smoking status before pregnancy, potato consumption, and education level were associated with lead contamination ($p = 0.008$, $p = 0.046$, $p = 0.046$, and $p = 0.041$, respectively). Cadmium contamination was shown to be related to random smoke exposure ($p = 0.002$) (Bassil et al., 2017).

Bassil's study comprised 74 nursing mothers with a mean age of 26.8 (SD = 4.8) and a pre-pregnancy BMI of 22.8 (SD = 3.5). The majority of the participants were from Mount Lebanon (37.7%), the South (30.43%), and Beirut (15.9%). The Mount Lebanon governorate had the greatest mean arsenic levels (2.7 ± 1.8 g/L), whereas the South had the highest mean cadmium (1.3 ± 1.5 g/L) and lead (21.7 ± 11.1 g/L) values. The bulk of the mothers (72.5%) had university degrees, however only 48.5% worked in business or as professors/teachers (Bassil et al., 2017). In the study, mothers who had not completed a university education had higher mean arsenic, cadmium, and lead levels than those who had completed a university or technical education. 35.2% of the nursing mothers reported smoking prior to pregnancy, but only 14.5% smoked throughout pregnancy. When compared to those who did not smoke during pregnancy, the latter had much greater arsenic levels in their milk. Furthermore, 80.36% of participants reported being exposed to second-hand smoke in restaurants, their homes, or their workplace. When asked if they took supplements, 82% of the mothers said they did, including multivitamins, folic acid, iron, and vitamin D. The mothers were also asked about their eating habits, namely their consumption of fish/seafood, rice/cereals, potatoes, and fresh vegetables. Mothers who consumed fresh vegetables more than twice a week had substantially higher arsenic levels in their milk than those who consumed fresh vegetables less than or equal to

twice a week (p = 0.05). In terms of residence, 39.4% of the mothers lived near a garbage disposal site, whereas 23.6% lived near agricultural activities. These mothers exhibited significantly greater lead levels in their breast milk compared to individuals who did not live near a cultivation site (Bassil et al., 2017).

The study found that arsenic was present in 63.5 percent of the breast milk samples, with an average level of 2.4 ± 1.9 g/L. Also, 87.2% of the positive samples surpassed the World Health Organization's (1 g/L) standard. Lead contamination was identified in 67.6% of the breast milk samples, with 83.3% of the positive samples above WHO standards (5 g/L) and an average value of 21.3 ± 12.4 g/L. Cadmium contamination was identified in 40.5% of the samples (M = 0.9 ± 1.2 g/L), with 23.3% of the positive samples exceeding WHO guidelines (1 g/L). Consuming cereals was shown to be strongly connected with arsenic pollution, while education level, potato consumption, smoking status before pregnancy, and neighbouring farming activity were found to be significantly associated with lead in breast milk. Cadmium contamination was shown to be substantially associated to random smoke exposure. After controlling for other covariates, the multiple linear regression models revealed that regularly ingesting rice/cereals and fish/seafood were independently linked with the presence of arsenic in breast milk (p = 0.013 and p = 0.042, respectively). After controlling for other variables, residence near agriculture operations, smoking status before pregnancy, potato consumption, and lower education level all predicted higher levels of lead in breast milk (p = 0.008; p = 0.046, andp = 0.046, respectively). After

controlling for other covariates, random smoke exposure was the only independent predictor of cadmium in breast milk ($p = 0.002$).

In Hungary (Ecsedi-angyal et al., 2019) used ICP-MS in a study to ascertain the levels of arsenic, lead, cadmium, and mercury in the breast milk of lactating Hungarian mothers. Twenty-seven (27) healthy, non-smoking volunteers between the ages of 25 and 41 who resided in Budapest's residential neighbourhoods and its urban areas took part in the study. The participants delivered full-term newborns with a gestational age from 38 to 41 weeks between August and December 2017. None of the women reported having been exposed to specific sources of toxic metals, whether at home or at work. Their mean age and parity were 32.9 ± 4.4 years and 2.3 ± 1.3, respectively. None of the mothers included in the study gave birth to twins. Their educational level was very similar. Thus, one third graduated from vocational schools, one third had a bachelor degree and another one third had a university master degree. All infants were healthy, had normal birth weight and were exclusively breastfed. Summary results reported from the study are presented as follows:

Mercury concentration was greater 0.4 μg/L in all samples. The order of cadmium, arsenic and lead mean concentration in the samples were 0.188 ± 0.071 μg/L < 0.41± 0.20 μg/L < 1.74 ± 0.77 μg/L, respectively. Mean and median concentration of arsenic and lead data generally grouped into two-week intervals of lactation decreased by about 25–35% after two months. Moreover, statistically significant increase was observed for lead concentration with the age of the mothers according to paired sample t-test almost 20 years after the prohibition of the marketing of leaded gasoline in the

European Union and about 40 years after the ban on lead pipes for drinking water delivery in Hungary ($p < 0.05$). However, according to their dietary intake estimations, no other threat for newborns fed by exclusive breastfeeding was detected.

The researchers found that rice consumption was comparable in Budapest and the metropolitan region when it came to dietary risk factors linked to elevated levels of arsenic in breast milk. However, the metropolitan area's consumption of marine fish, broiler chicken, and mushrooms was higher than Budapest's by 113 %, 42 %, and 50%, respectively (Ecsedi-angyal et al., 2019). These food items mostly contain organic arsenic species, which are less harmful, as is widely known. According to the responses to their questionnaire, the mothers' exposure to mercury was also minimal because none of the new mothers from Budapest had dental amalgam fillings, compared to a percentage of less than 20% for those who lived in the city's metropolitan region. Additionally, consumption of marine seafood, which serves as a major dietary source of mercury, was also relatively low. The study also revealed that as lactation develops, the concentration of heavy metals gradually decreases.

In Zanzibar Khamis et al. (2017) did a study to quantify levels of heavy metals namely; cadmium, lead, aluminium and zinc in the breast milk of nursing moms using atomic absorption spectrometer (AAS, Model iCE 300 Series). Zinc concentrations ranged from 231 g/L to 1466 g/L of milk, with an average of 900 ± 457 g/L; cadmium concentrations ranged from 24.1 g/L to 35.9 g/L, with an average of 31.1 ± 3.5 g/L; and lead concentrations ranged from 32.4 to 1630 g/L, with an average of 707 ± 582 g/L. The concentration of

aluminium metal identified in a single sample was 0.91 g/L. The findings demonstrate that heavy metal contamination in breast milk must be eliminated prior to newborn feeding.

In Ankara, Turkey (Turan et al., 2001) conducted research to ascertain levels of lead, cadmium and some other heavy metals in colostrum from humans using Electrothermal Atomic Absorption Spectrophotometer, ETAAS, (Hitachi Model 180/80 flame and graphite furnace atomic spectrophotometer, equipped with Zeeman effect background corrector, an autosampler (P/N–70/0126) and an automatic data processor), with all of the operating settings set as the manufacturer has advised. The injected sample volume was 20.0 µL. The results obtained indicate that the concentration of cadmium and lead in human colostrum were 2.8 ± 1.7 µg/L and 14.6 ± 5.5 µg/L respectively. When compared to the lead levels found in mature breast milk and colostrum from other nations, the study discovered that the lead levels in the colostrum samples it examined were safe. Additionally, Smokers' cadmium levels did not significantly differ from non-smokers', indicating that the donors did not smoke frequently. The researchers came to the conclusion that the amounts of cadmium in the samples they examined were not expected to be hazardous to new-born development.

In Ghana (Bentum et al., 2010) carried out a study using Atomic Absorption Spectrometer (AAS) to measure the amounts of lead, cadmium, and arsenic in nursing mothers' breast milk in the Odumase-Atua Community in the Manya Krobo District in Eastern Ghana (Philips AAS 9200 Model). The analysis was performed on 20 lactating mothers aged below 25 years. According to the findings, the mean lead concentration was 4.33 g/L, with a

range of limit of detection, LOD-32.0 g/L. The mean level of cadmium was 1.34 µg/L and the range was < LOD -12.301 µg/L. Between LOD-6.22 g/L, the mean arsenic content was 1.54 g/L. In accordance with the findings, 60%, 60%, and 40% of the samples, respectively, had lead, cadmium, and arsenic levels below the detection limit. This suggests that exposure to arsenic was most prevalent among the three metals. All three metals were not found in any of the samples simultaneously. The samples varied in their metal content, with 10% being devoid of any metal, 15% containing both lead and arsenic, 15% containing both lead and cadmium, and 20% including both cadmium and arsenic. Cadmium > Lead > Arsenic was the order of the three sets of measurements' relative standard deviations, showing how variable each metal was in breast milk.

Also in another study in Ghana (Koka et al., 2011) measured the levels of cadmium and lead in the breast milk of healthy nursing women who had worked in a business setting for at least five years in Accra and Tema using atomic absorption spectrometry (Greater Accra). A total of 48 samples were analysed. They found that on average, metal levels were higher in Tema than in Accra. The study's findings showed that lead concentrations in Accra and Tema, respectively, varied from 0.0456 to 5.224 g/L with a mean concentration of 2.476 g/L and 1.375 to 5.890 g/L with a mean concentration of 3.367 g/L. It was discovered that infants in Accra are expected to eat roughly 1.93 g of lead daily, assuming they take 0.78 L of breast milk each day. In Accra, the daily consumption is 0.21 and 0.15 g/kg/day for babies aged 7 to 12 months and 1-3 years with average weights of 9 and 13 kg, respectively. Around 2.63 g of lead per day are probably consumed by infants

in Tema. The daily consumption is 0.29 and 0.20 g/kg/day for babies aged 7 to 12 months and 1 to 3 years, respectively, with typical weights of 9 and 13 kg. The researchers highlighted that none of the data they gleaned from their investigation exceeded the daily permitted intake (DPI) set by the World Health Organization at 5 g/kg/day. (Hallen et al.,1995).

The values of cadmium in Accra and Tema were 0.0085 to 0.0500 g/L and 0.0122 to 0.0644 g/L, respectively, with a mean concentration of 0.0329 g/L (Koka et al., 2011). Based on these results, the study calculated that a new-born in Tema would consume 0.026 g or 0.003 and 0.0020 g/kg/day for an average weight of 9 and 13 kg, respectively, while an infant in Accra would consume 0.019 g or 0.002 and 0.0015 g/kg/day. Based on these results, the study calculated that a new-born in Tema would consume 0.026 g or 0.003 and 0.0020 g/kg/day for an average weight of 9 and 13 kg, respectively, while an infant in Accra would consume 0.019 g or 0.002 and 0.0015 g/kg/day. These concentrations were considerably lower than the DPI for adults, which is 1 g/kg/day. The research also revealed a strong positive link between lead and cadmium concentrations in mothers' breast milk samples from both metropolises ($P<0.05$). Additionally, compared to non-exposed mothers, women who were passively exposed to smoking had significantly higher levels of these metals in their breast milk($p<0.05$). There was no work exposure to the metals for any of the study's female participants.

In Austria Gundacker et al. (2002) identified the lead and mercury concentrations in human breast milk, cow milk, and infant formula. The purpose of the study was to ascertain the amounts of lead and mercury present in nursing mothers' breast milk in three cities around Austria. While mercury

figured out using cold-vapor-AAS in conjunction with a Hitachi HFS-3 hydride formation system and a gold wire amalgamation trap, lead was measured using a Hitachi Z 8200 Polarized Zeeman Atomic Absorption Spectrophotometer (AAS) and a graphite furnace The purpose of the study was to ascertain the amounts of lead and mercury present in nursing mothers' breast milk in three cities around Austria. While mercury was determined using cold-vapor-AAS in conjunction with a Hitachi HFS-3 hydride formation system and a gold wire amalgamation trap, lead was measured using a Hitachi Z 8200 Polarized Zeeman Atomic Absorption Spectrophotometer (AAS) and a graphite furnace (Uwe Binninger Analytik, Schwa¨bisch-Gmund, Germany). The detection limit for mercury was 0.14 µg/L and for lead it was 0.10 µg/L.

The results showed that the mean concentration of mercury in breast milk (n =116) in the three Austrian cities was 1.59 ± 1.21 µg/L, and the mean lead content of breast milk was 1.63 ± 1.66 µg/L. The mercury content of breast milk and cow milk was significantly higher than in infant formulas. Infant formulae had a slightly higher lead concentration than milk samples. To compare differences in the mean values of the categories, such as the amount of metal in smokers versus non-smokers, the Kruskal-Wallis test was utilized. There were considerable differences in the amounts of metal in Vienna, Linz, and Tulln, with Vienna having the highest mercury levels in breast milk and Linz having the highest lead concentrations. Additionally, it was discovered that premature mothers and mothers who weighed less than 60 kg had higher mercury levels in their breast milk (Gundacker et al., 2002). Fish eating appeared to affect lead levels, and frequent cereal consumption was associated with higher mercury levels. Mercury levels appeared to rise after taking

vitamin pills. Breast milk from smokers contained considerably more lead than that of non-smokers.

In Saudi Arabia (Al-Saleh et al., 2003) measured the levels of cadmium, lead, and mercury in the breast milk of breastfeeding women from the Riyadh and Al-Ehssa regions who were not exposed at work. A GTA-100 electrothermal atomizer, a programmable sample dispenser, and a Varian AA-880 Zeeman atomic absorption spectrophotometer (Varian Techtron PTY, Ltd., Australia) was used. In the Riyadh region (n = 171) and the Al-Ehssa region (n = 201), healthy nursing women aged 15-51 years who attended the Primary Health Care Units (PHCU) for their child's immunization provided 372 breast milk samples at random. It was discovered that the mean concentrations of cadmium, lead, and mercury were 1.7 g/L, 31.7 g/L, and 3.1 g/L, respectively. Compared to mothers in the Riyadh region, mothers in the Al-Ehssa region had significantly higher cadmium and lead concentrations in their breast milk. The health of the breast-fed infants in this study is at risk since the estimated weekly intakes of cadmium, lead, and mercury were in certain cases greater than the Provisional Tolerance Weekly Intake (PTWI) advised by FAO/WHO.

The study discovered that all of the analysed samples of breast milk had average levels of cadmium, lead, and mercury to be 1.7 ± 1.691, 31.7 ± 45.7, and 3.1 ± 4.053 μg/L, respectively. The levels of lead, mercury and cadmium were above the detection thresholds of 0.12, 1.33, and 0.20 g/L in 95.1 %, 94.8 %, and 87.0 % of milk samples, respectively (Al-Saleh et al., 2003). The Wilcoxon rank sum test was used to examine the association between the concentrations of cadmium, lead, and mercury and the study area.

The two locations clearly differed from one another ($p < 0.0001$). The average levels of cadmium and lead in lactating mothers in the Al-Ehssa region were substantially higher than those in the Riyadh region. On the other hand, women who were nursing had significantly higher mercury levels in the Riyadh area than in the Al-Ehssa area. The quantities of cadmium, lead, and mercury in breast milk may vary depending on a variety of demographic, socioeconomic, and environmental factors, the researchers found. They performed numerous regression analyses after accounting for a range of socioeconomic and demographic factors. With regard to cadmium, lead, and mercury, only two factors—the study area and fish consumption—remained significant. Unexpectedly, moms who did not consume fish had greater levels of cadmium and lead in their breast milk. The average length of lactation for all new-borns was highly correlated with the lead model as well. Parity, the number of breastfeeds per day, the mother's age, and the father's degree of education were other factors that were effective predictors of elevated mercury levels in breast milk.

In Iran Goudarzi et al. (2013) the levels of cadmium, lead, and mercury in the breast milk of healthy nursing women living in Isfahan, Iran were determined. 37 milk samples obtained from the first to sixth postpartum weeks were analysed using Graphite Furnace Atomic Absorption Spectrometry (GFASS) to determine the concentrations of these heavy metals. With the aid of reference material, the analysis' correctness was verified. The mean ± SD of the concentrations of cadmium, lead and mercury in human breast milk were found to be 1.92 ± 1.04 mg/L (range: 0.45 – 5.87 mg/L), 7.11 ± 3.96 mg/L (range: 3.06 – 19.47 mg/L) and 0.92 ± 0.54 mg/L (range: 0.0 – 2.07 mg/L)

respectively. The findings revealed high levels of mercury, lead, and cadmium in the milk samples from nursing women in Isfahan, posing a serious risk to the health of the community, particularly the neonates and young children who live near industrial areas. The study suggested that safe ingestion levels of heavy metals in human milk should be established. The findings show that while background levels of mercury lead, and cadmium typically range in breastmilk between 0.6 and 0 mg/L, 5 and 20 mg/L, and 0.05 and 2 mg/L, respectively, in extremely polluted locations, they may be up to 20 times higher.

Also in Zarrinshahr, an industrial area in Iran Rahimi et al. (2009) ascertained the levels of cadmium and lead in the breast milk of healthy nursing mothers and to look into the impact of the mother's age, parity, and smoking habits in families who live in heavy metal-contaminated areas. 44 milk samples from healthy lactating women were obtained between the first and sixth postpartum week, and cadmium and lead contents were assessed using graphite furnace atomic absorption spectrometry. Utilizing reference material, the analysis' correctness was evaluated. The mean ± standard deviation of cadmium and lead concentrations in human milk were found to be 2.44 ± 1.47 µg/L (range 0.62 – 6.32 µg/L) and 10.39 ± 4.72 µg/L (range 3.18 – 24.67 µg/L), respectively. The study discovered a significant ($P < 0.05$) positive correlation between the lead levels in milk samples and the mother's age and parity. Cadmium levels in breast milk were similarly considerably higher in mothers who smoked actively or passively ($P<0.05$). The findings of this investigation demonstrated that milk samples from nursing women in Zarrinshahr had elevated quantities of lead and cadmium. In human milk,

average background concentrations of cadmium and lead are between 0.05-2 µg/L and 5-20 µg/L, respectively, while in heavily polluted areas they may be up to 20 times higher (Frkovic *et al*., 1997).

The researchers discovered that the high levels of cadmium, lead, and mercury present in the breast milk of breastfeeding women in this industrial region of Iran can pose a serious harm to the local population's health.

Furthermore, in Hamadan, Iran (Rahimi et al., 2009) the amounts of cadmium, lead, mercury, and barium in human breast milk were measured by inductively coupled plasma mass spectrometry (ICP-MS) and compared to other sociodemographic variables. The 100 milk samples' median concentrations of lead, mercury, and barium were 41.9, 2.8, and 1.95 g/L, respectively. The 100 milk samples' median concentrations of lead, mercury, and barium were 41.9, 2.8, and 1.95 g/L, respectively. They found that cadmium levels were < 1 µg/L in all samples. 94% of the samples had lead levels higher than the recommended limit of < 5 µg/L set by the World Health Organization (WHO) and 54% of the breast milk samples had mercury levels higher than the normal mean concentration (1.7 µg/L) suggested by WHO. They discovered no connection between sociodemographic characteristics and the amount of mercury in breast milk. They recommended additional research to determine the sources of exposure, define safe intake values, and develop preventive strategies due to the toxicity of these metals and the sensitivity of babies.

Also in another study in Tehran, Iran (Soleimani et al., 2014) measured the amount of lead present in the breast milk of mothers who were breastfeeding for an extended period. They used 43 milk samples collected

two months after delivery and analysed them using an atomic absorption spectrophotometer. They found that the average level of lead in the breast milk was 23.66 ± 22.43 µg/L. There was no connection between the amount of lead in breast milk and the mother's education level, age, parity, height, or weight, they also noticed, and they found that this concentration was higher than in other nations. They came to the conclusion that new-borns and children to live in industrial regions face a serious public health risk due to the high levels of lead in the milk samples.

In Poland Winiarska-Mieczan (2014) determined the average weekly consumption of these metals by breastfed infants from Poland. They measured the quantities of cadmium and lead present in human breast milk. The results showed that while the levels of these toxic metals were not above acceptable limits, they were still relatively high, particularly in infants who were 6 months old. The study proposed that women should get education on how to lower the amounts of these metals in breast milk, with a focus on banning cigarette use by breastfeeding mothers and those around them.

The researchers also found that the average concentration of cadmium in the breast milk was slightly above 2 µg/L, ranging from 0.215 to 7.355 (Winiarska-Mieczan, 2014). The breast milk of women between the ages of 31 and 35 had the greatest cadmium concentrations, with more than 3 µg/L. The highest levels of lead were found in the breast milk of women aged 36 to 40 and 26 to 30, with around 7-7.4 µg/L. The lowest levels of both cadmium and lead were found in the breast milk of women aged 20 to 25. The amount of cadmium and lead in breast milk was significantly influenced by the lactational stage. The first month's milk, often known as colostrum, had the

lowest levels of cadmium whereas the breast milk between months four and six of lactation had the greatest levels. The breast milk had the highest lead concentrations between 2 and 3 months of lactation and the lowest concentrations between 7 and 12 months of lactation.

What is a Heavy Metal?

The term "heavy metal" does not have a precise definition, but based on the literature, it refers to any element (whether metallic or metalloid) that has a high density and, at low concentrations, is dangerous or lethal (Järup, 2003; Ojovan & Lee, 2005). It is generally known that the environment is a major source of several hazardous heavy metals. If a heavy metal is discovered in an area where it is not intended or in a concentration that has a harmful impact on people or the environment, it is regarded as a pollution. Lead, mercury, copper, selenium, cadmium, arsenic, nickel, aluminium, and zinc are a few examples of heavy metals. Though heavy metals are naturally present in the earth's crust, their presence in the human body can be harmful even at low concentrations. For diverse physiological and metabolic functions, humans need 72 trace elements, including trace levels of heavy metals like tin, copper, manganese, molybdenum, chromium, vanadium, and cobalt.

While most metals can be harmful at high levels, some can cause harm even at very low concentrations. Studies show that certain heavy metals like lead, cadmium, arsenic, mercury and aluminium are particularly dangerous in our environment. (Järup, 2003). Due to their numerous uses by humans, these heavy metals also happen to be the most prevalent ones to which people are commonly exposed. There are several different heavy metal sources in the environment. Depending on the type of heavy metal under consideration, they

include both general sources and food sources. Below are few examples of some important heavy metals indicating their sources.

What is Aluminium?

Al is the chemical symbol for the naturally occurring element Aluminium, which has the atomic number 13 and the weight 26.9815385 (de Laeter et al., 2009; Prohaska et al., 2022). It is a lightweight, white, silvery metal that does not rust. The majority of this element, which comes in a variety of forms, is found in the earth's crust. The most common metallic element, Aluminium, makes up around 8% of the earth's crust. It is present in the environment as silicates, oxides, and hydroxides as well as complexes with organic materials and other elements like sodium and fluoride (WHO, 2010a). Aluminium is a highly reactive metal and is not found in its pure form in nature. It is usually found combined with other elements, mainly oxygen, silicon and fluorine. These compounds can be located in soil, rocks, especially volcanic rocks, and clays. They can also be found in minerals like sapphires, rubies, and turquoise. The main source of aluminium is bauxite. Some common Aluminium compounds found in the environment include Aluminium chloride ($AlCl_3$), Aluminium oxide (Al_2O_3), Aluminium hydroxide [$Al(OH)_3$], Aluminium trioxonitrate (V) nonahydrate [$Al(NO_3)_3.9H_2O$], Aluminium nitrate [$Al(NO_3)_3$], and Aluminium sulphate (VI) $Al_2(SO_4)_3$ (WHO, 2010a).

Uses of Aluminium in its Various Forms

As Aluminium Metal

Aluminium is used in a variety of manufacturing applications, such as making cooking pots and pans, beverage cans, pots, foils, siding, airplanes,

automobile parts, roofing and other structural components. Powdered aluminium is also commonly used in the production of explosives and fireworks (ATSDR, 2008b).

Aluminium Compounds

Alumina is utilized for abrasives and furnace linings, while Aluminium sulphates (sometimes known as "alums") is used in the manufacturing of many other Aluminium compounds (ATSDR, 2008b).

Consumer Products

Consumer goods include antacids, astringents, buffered aspirin, food additives, cosmetics, and antiperspirants contain Aluminium (ATSDR, 2008b).

Occurrence and Sources of Aluminium

Natural sources of Aluminium include air, water, and soil. Aluminium can be produced as metal, alloys, and compounds as well as mined and processed from Aluminium ores, which can lead to a rise in the amount of Aluminium in the environment. Additionally, coal-burning power stations and incinerators release minor amounts of Aluminium into the environment (ATSDR, 2008a).

General Sources

Natural sources of aluminium include air, water, and soil. Aluminium can be produced as metal, alloys, and compounds as well as mined and processed from aluminium ores, which can result in increased amounts of aluminium in the environment. Additionally, coal-burning power stations and incinerators release minor amounts of aluminium into the environment (WHO, 2010a).

Food Sources

This include drinking water, seasonings, colour additives, baking powder, salt, milk products, beer, treated water, vanilla powder tap water and bleached (ATSDR, 2008b; WHO, 2010a).

Fate of Aluminium in the Environment

Because it is a stable element, Aluminium can only change its physical properties, become separated from other particles, or bind itself to them. It cannot decompose in the environment. Aluminium flakes may fall to the ground or be removed from the atmosphere by rain. However, Aluminium dust has a lengthy half-life in the atmosphere. Most Aluminium compounds rarely dissolve in water quickly unless the water is quite alkaline (ATSDR, 2008b).

Modes of Exposure to Aluminium in the Environment

Aluminium can be ingested or inhaled from a variety of sources, including food, air, water/soil, and consumer goods. Food is the major way that people are exposed to Aluminium, however unprocessed foods like fresh fruit, vegetables, and meat have relatively little Aluminium content. Aluminium compounds, which can be found in flour, baking powder, colourings, and anti-caking agents, can be added to food during processing. The average adult in the United States is thought to consume 7-9 mg of Aluminium each day (ATSDR, 2008b).

Most individuals take in very little Aluminium through inhalation. The concentrations of Aluminium in the air typically range from 0.005 to 0.18 micrograms per cubic meter ($\mu g/m^3$), depending on weather conditions, location, and the type and level of industrial activity in the area (ATSDR,

2008b). Small suspended soil particles make up a significant fraction of the Aluminium in the air (dust). However, Aluminium concentrations in urban and industrial regions can range from 0.4 to 8.0 μg/m^3, making them potentially greater. Typically, people consume very little Aluminium from drinking water. The concentration of Aluminium in water from natural sources such as lakes, rivers, and streams are typically less than 0.10 micrograms per litre (0.10 μg/L). Aluminium salts are frequently used in the processing of water to make it safe for consumption. Even then, Aluminium concentrations often do not go above 0.10 mg/L. Aluminium concentrations in drinking water have been observed to reach as high as 0.4–1.0 mg/L in some cities (ATSDR, 2008b). Individuals may come into contact with Aluminium through certain consumer products such as cosmetics, antiperspirants, and pharmaceuticals like antacids and buffered aspirin. It's estimated that antacids contain 300-600 mg of Aluminium hydroxide (or approximately 104-208 mg of Aluminium) per tablet, capsule, or 5 millilitre (mL) liquid dose. Only small amounts of this form of Aluminium are absorbed into the bloodstream. Buffered aspirin may have 10-20 mg of Aluminium per tablet and vaccines may contain small amounts of Aluminium compounds, not exceeding 0.85 mg per dose.

Adult daily Aluminium intake has been measured in various countries including Australia, Finland, Germany, Japan, the Netherlands, Sweden, Switzerland, the United Kingdom, and the USA, with levels ranging from 1.9-2.4 mg/day in Australia to 13 mg/day in Sweden. Children aged 5-8 years old had intake levels of 0.8 mg/day in Germany and 6.5 mg/day in the USA. Infants in Canada, the United Kingdom and the USA had Aluminium intake levels between 0.03 and 0.7 mg/day (WHO, 2010a).

How Aluminium Enters and Leaves the Human Body

Through the skin, digestive system, and lungs, Aluminium can enter the body and leave through faeces or urine. Inhaled Aluminium enters through the lungs, ingested or topical Aluminium through the digestive tract or skin respectively. A lesser amount that reaches the bloodstream is swiftly excreted by urine, whereas the majority of ingested Aluminium in food, water, and medications is promptly excreted through faeces (ATSDR, 2008b).

How Aluminium Affect the Health of People

Inhalation of Aluminium by Workers

Studies have revealed that individuals exposed to high levels of Aluminium dust through their occupation may experience lung issues like coughing or changes visible on chest X-rays (ATSDR, 2008b). However, according to research, people who work in environments with high levels of Aluminium dust may develop lung problems including coughing or abnormalities that are obvious on chest X-rays. The use of protective equipment and regulations to limit dust exposure in workplaces has greatly reduced this risk. Some workers exposed to Aluminium dust or fumes may also have impaired cognitive function as measured by certain neurological tests (ATSDR, 2008b; WHO, 2010a).

Oral Ingestion of Aluminium by Humans

Despite the fact that eating metal is generally safe, some evidence suggests a link between prolonged exposure to Aluminium and the development of Alzheimer's disease (ATSDR, 2008a). However, other research has been unable to confirm this association. Alzheimer's disease and Aluminium have a complicated link that is still being investigated. As a result

of the accumulation of Aluminium, people with kidney illness may retain more Aluminium in their bodies and be more susceptible to bone or brain problems (ATSDR, 2008b). Aluminium-containing oral medications are regarded as safe when used as recommended, however some people have had ill effects from prolonged use. There is currently no proof that Aluminium causes cancer (WHO, 2010a).

How Aluminium Affects the Health of Children

Children with kidney disease may develop brain and bone problems as a result of excessive body Aluminium levels, according to research (ATSDR, 2008b). Additionally, children taking certain medications containing Aluminium have also been found to develop bone disease (ATSDR, 2008a). This is assumed to be caused by the stomach's Aluminium inhibiting the absorption of phosphate, which is essential for the development of healthy bones. Even while there is Aluminium in breast milk, infants are probably only going to absorb a little quantity of it. The concentration of Aluminium in breast milk is estimated to be between 0.0092 - 0.049 mg/L, while soy-based and milk-based infant formula contain higher levels at 0.46–0.93 mg/L and 0.058–0.15 mg/L respectively.

The effects of Aluminium on birth defects in humans are uncertain as it has not been observed in animals. However, research on animals that were pregnant or nursing and exposed to high levels of Aluminium revealed that the young animals were weaker and less energetic, with some movements appearing less coordinated, and that their memory was also impacted (ATSDR, 2008b).

How to Minimize the Risk of Exposure to Aluminium in the Environment

People are frequently receiving exposure to Aluminium in air, water and through consumption of food and consumer products. It is not possible to completely avoid exposure to Aluminium as it is found in many everyday items and environments. The levels of Aluminium that people are naturally exposed to through their diet and the environment are not harmful. A person may be exposed to higher levels of Aluminium than someone who only eats whole foods and uses pots made of other materials if they frequently prepare acidic foods in Aluminium pots or eat a lot of processed foods with Aluminium additives.

The levels of Aluminium found in processed foods and foods cooked in metal pots are usually believed to be safe, nevertheless (ATSDR, 2008b). The most effective way to limit exposure to aluminium from antacids and buffered aspirin is to take them in smaller quantities and use them as directed. To avoid unintentional consumption, these products should be stored away from children or equipped with child-proof caps.

Laboratory Reports on Animals Exposed to Aluminium Through Inhalation and Dermal Contact

Although animals exposed to Aluminium dust have shown signs of lung damage, it is not known whether these symptoms are brought on by the Aluminium itself or by breathing in a lot of dust. The neurological system is vulnerable to Aluminium poisoning, according to animal studies (ATSDR, 2008b, 2008a). Animals given high amounts of Aluminium orally showed reduced performance in tests that tested grip strength and mobility, but showing no obvious injury.

Recommendations and Regulations on Safe Aluminium Levels in the Human Body to Protect Human Health

The federal government of the United States has established laws and guidelines for permissible levels of dangerous substances in order to protect the public's health. These regulations are produced by organisations such as the US Environmental Protection Agency (US EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (US FDA). These institutions provide essential information for protecting the public's health, but they cannot be enforced through legislation. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) both develop dangerous substance recommendations.

These restrictions and guidelines are frequently given as "not-to-exceed" levels, which refer to the maximum amount of a harmful material that can be safely present in the air, water, soil, or food (ATSDR, 2008a). These values are often determined by research of how the chemical affects animals and then adjusted to be safe for people. The "not-to-exceed" criteria may range among federal agencies depending on factors such as exposure length (an 8-hour workday vs. a 24-hour day), animal studies, or other considerations (ATSDR, 2008b). As new information becomes available, the rules and suggestions are evaluated and revised. The following are some guidelines and regulations regarding aluminium:

Workplace Air

The allowable exposure limit for Aluminium in dusts set by OSHA is 15 mg/m^3 (total dust) and 5 mg/m^3 (respirable fraction), averaged over an 8-hour workday (ATSDR, 2008a).

Drinking Water

The US EPA has suggested a Secondary Maximum Contaminant Limit (SMCL) of 0.05 to 0.2 mg/L for aluminium in drinking water. The SMCL is determined by taste, smell, or colour rather than levels that could harm people or animals (ATSDR, 2008a).

Consumer Products

The Foods and Drugs Authority (US FDA) has determined that Aluminium is generally safe when used as a food additive and in pharmaceutical products like antacids. A 0.2 mg/L limit for Aluminium has been set by the US FDA for bottled water (ATSDR, 2008a).

What is Arsenic?

Arsenic, with the symbol As, atomic number 33, and atomic weight 74.9 (de Laeter et al., 2009; Prohaska et al., 2022), is a naturally occurring chemical element. It is a semi-metal that is fragile. Arsenic is harmful and ranks number one on the Agency for Toxic Substances and Disease Registry's revised 2019 Substance Priority List (ATSDR, 2019). Even though arsenic is a metalloid, it is often described as a metal and has a steel-grey appearance.

Occurrence and Sources of Arsenic

Arsenic, which is widely distributed in the Earth's crust and is present in the air, water, and land, pollutes the ecosystem(ATSDR, 2007c; WHO, 2018a, 2019). Arsenic is frequently found in nature in association with

substances like chlorine, sulphur, and oxygen. It comes in both inorganic and organic forms. Arsenic is said to as organic when it is combined with carbon and hydrogen and inorganic when it is associated with oxygen, chlorine, or sulphur. Arsenic that is not organic is quite poisonous. Both forms of arsenic are usually non-volatile, colourless or white powder compounds that have no taste or smell, making their presence in air, water and food difficult to detect (ATSDR, 2007c). Natural sources of inorganic arsenic include soil and a variety of rocks, particularly minerals and ores that contain lead and copper. These rocks can be smelted to produce arsenic trioxide, or As_2O_3, which can then be obtained. It's vital to remember that the United States no longer produces any arsenic, and all of the arsenic used there is imported(ATSDR, 2007c).

Uses of Arsenic

The lead-acid batteries used in autos are where arsenic in alloys is most commonly used. Arsenic compounds are used extensively in semiconductors and light-emitting diodes(ATSDR, 2007c). Currently, a preservative made of arsenic that is commonly coated with copper to form copper chromate arsenate (CCA) is used to preserve products in about 90% of all cases. This compound is used to treat wood to make it resilient to rotting and deterioration. For certain household uses, such as playsets, picnic tables, decks, fencing, and boardwalks, manufacturers of wood preservatives in the United States began converting from using CCA to other preservatives that do not contain arsenic in 2003. After the transition was completed on December 31, it is still possible to use previously treated wo and to build with CCA-treated wood. However, it

is unclear how much arsenic CCA-treated wood exposure may cause in humans (ATSDR, 2007c).

Previously, the primary application of inorganic arsenic compounds was as a pesticide, particularly in cotton fields and orchards. Inorganic arsenic compounds are no longer used in agriculture, with a few exceptions. Organic arsenic compounds such as cacodylic acid, disodium methylarsenate (DSMA), and monosodium methylarsenate (MSMA) are still used as insecticides, primarily on cotton crops. Tiny amounts of elemental arsenic are combined with other metals to create superior metal combinations or alloys. Various organic arsenic compounds are employed as animal feed additives (ATSDR, 2007c).

Fate of Arsenic in the Environment

Arsenic is a naturally occurring element present in soil and minerals that can enter the air, water, and land via wind-borne dust, runoff, and leaching. Arsenic emission from volcanic eruptions is a possibility. It is commonly found in ores containing metals such as lead and copper. Arsenic may be released into the environment during the mining and smelting of certain ores (ATSDR, 2007c). Furthermore, because coal and waste products often include arsenic, coal-fired power plants and incinerators may emit trace levels of arsenic into the atmosphere (ATSDR, 2007c). Arsenic is an intriguing element since it cannot be destroyed by the environment while still having the ability to modify its form by connecting with or detaching from particles. It could change shape by reacting with oxygen or other molecules in the air, water, or soil, or by the action of bacteria in soil or sediment (ATSDR, 2007c). Arsenic is normally present in bigger particles in wind-borne soil, but

it is also discovered in extremely minute particles when it is emitted from power plants and other combustion processes. These specks either fall to the ground or are washed away by rain. Particulate-bound arsenic has the ability to persist in the atmosphere for several days and travel long distances. Some common arsenic compounds can dissolve in water as well.

As a result, industrial waste and precipitation or snow melting can both release arsenic into the environment, allowing it to find its way into lakes, rivers, or subterranean water sources. Arsenic can be conveyed by water, adhere to water-borne particles, or settle on the bottom of lakes or rivers (ATSDR, 2007c). The majority of the arsenic eventually ends up in soil or sediment. Although some fish and shellfish may consume arsenic, which can accumulate in their tissues, the majority of this arsenic is in the safe organic form known as arsenobetaine (commonly referred to as "fish arsenic").

Modes of Exposure to Arsenic in the Environment

Contaminated groundwater is the primary source of arsenic's threat to public health. Groundwater in a variety of countries, including Argentina, Bangladesh, Chile, China, India, Mexico, and the United States, includes high levels of inorganic arsenic. Drinking water, crops irrigated with contaminated water, and food produced with contaminated water can all expose people to arsenic. Other food sources of arsenic include fish, shellfish, pork, chicken, dairy products, and cereals, but exposure from these sources is often much lower than exposure from contaminated groundwater. Arsenic is primarily present in seafood in its less hazardous form, organic arsenic (WHO, 2019).

Because arsenic is a naturally occurring component in the environment, it can enter the food chain via soil, water, and air (US FDA,

2019). Although environmental arsenic concentrations are normally modest, they can vary depending on the local geological features. Arsenic, for instance, can be exposed to the atmosphere during volcanic eruptions. Higher levels of arsenic may also be caused by pollution from coal-fired power plants, mining, fracking, lumber treated with arsenic, and herbicides containing arsenic (US FDA, 2019).

Therefore, drinking water, eating food, and inhaling the air expose them to some arsenic. Children, especially toddlers, may consume soil that contains arsenic (ATSDR, 2007c) while breastfeeding and placental transfer expose infants to it. The precise form of arsenic present is frequently not determined by the analytical procedures used by scientists to identify arsenic levels in the environment. As a result, the type of arsenic to which a person may be exposed is uncertain. It is frequently unknown what kinds of arsenic are present in hazardous waste sites, as it is with arsenic. Plants and animals cannot absorb some kinds of arsenic because they are tightly attached to particles or embedded in minerals.

Arsenic concentrations in soil can range from 1 to 40 parts per million of soil (ppm), with an average of 3 to 4 ppm. However, soils near geological features with high arsenic concentrations, some mining and smelting sites, or agricultural regions where arsenic pesticides have historically been used may have significantly higher arsenic concentrations (ATSDR, 2007c).

Natural surface and groundwater contain about 1 part per billion (ppb) of arsenic, however in contaminated areas or areas with high soil arsenic levels, this amount can exceed 1,000 ppb. Groundwater is more likely to contain large arsenic concentrations than surface water. According to studies

on drinking water sources in the United States, 80% of supplies had less than 2 ppb of arsenic, whereas 2% have more than 20 ppb (ATSDR, 2007c).

While the levels of inorganic arsenic, which is the most worrying, are much lower, they typically vary between 20 and 140 ppb in food. Depending on geography, weather conditions, and the level of industrial activity in the area, arsenic levels in the air normally range from less than 1 to roughly 2,000 nanograms (1 nanogram = a billionth of a gramme) per cubic metre of air (less than 1-2,000 ng/m^3). Yet, in metropolitan areas, the typical airborne arsenic content ranges from 20 to 30 ng/m^3. Arsenic is frequently ingested in trace amounts via food, water, and respiration. Yet, eating is the most common way for people to be exposed to arsenic. Fish is the most common source of arsenic in the diet, followed by rice and rice products, mushrooms, and poultry. Despite having the greatest levels of arsenic, the majority of it is in the less harmful organic form known as arsenobetaine in fish and shellfish (ATSDR, 2007c). Some seaweeds may contain inorganic arsenic which could be more harmful.

People typically take in 90 ng of arsenic daily from various sources, of which only 3.5 ng are the inorganic, extremely poisonous form. Playing on platforms or decks constructed of CCA-treated wood may expose kids to trace quantities of arsenic through hand-to-mouth activities. They often receive less exposure from this possible source than they would through food and water, though. After playing on such structures, children may be exposed to less arsenic if they wash their hands because water washes away the majority of the arsenic on their hands (ATSDR, 2007c). Other sources of arsenic include rocks which might increase the level in water, hazardous waste sites

containing improperly disposed arsenic, occupational sources, inhalation of arsenic-treated wood dust or smoke from burning of such materials (ATSDR, 2007c).

According to a report (WHO, 2019) People can be exposed to high quantities of inorganic arsenic in a variety of ways, including by drinking contaminated water, cooking with contaminated water, cultivating crops with contaminated water, engaging in industrial operations, eating contaminated food, and smoking cigarettes. The most common symptoms of chronic arsenic poisoning are skin lesions and skin cancer, which can be caused by long-term exposure to inorganic arsenic, typically through food and drink. The most common symptoms of chronic arsenic poisoning are skin lesions and skin cancer, which can be caused by long-term exposure to inorganic arsenic, typically through food and drink (ATSDR, 2007c).

How Arsenic Enters and Leaves the Human Body

When a person ingests arsenic through water, soil, or food, it can quickly enter the body, depending on the amount consumed and the form of arsenic. This is the most typical method that someone in close proximity to a garbage site is exposed to arsenic. The majority of dust particles inhaled when breathing arsenic-contaminated air settle in the pulmonary lining, where the majority of the arsenic in those particles is absorbed by the body. Arsenic exposure does not provide a major risk through skin contact. Urine is utilised to excrete arsenic, both inorganic and organic forms. While the majority of inorganic arsenic is eliminated from the body within a few days, some may remain for months or even years. When exposed to organic arsenic, the vast majority of it is eliminated from the body within a few days (ATSDR, 2007c).

How Arsenic Affects the Health of People

Since the dawn of civilization, inorganic arsenic has been known to be poisonous, and excessive doses can be lethal (for instance, more than 60,000 ppb in water, which is 10,000 times more than the highest level of arsenic found in 80% of U.S. drinking water) (ATSDR, 2007c). Smaller concentrations of inorganic arsenic (between 300 and 30,000 ppb in water, which is 100-10,000 times higher than the majority of drinking water in the United States) may irritate the stomach and intestinal tract, resulting in symptoms such as nausea, vomiting, diarrhoea, and stomach discomfort (ATSDR, 2007c). Ingestion of inorganic arsenic also causes aberrant heart rhythm, blood vessel damage that causes bruising, poor nerve activity that produces tingling in the hands and feet, and decreased production of red and white blood cells, which results in weariness (ATSDR, 2007c).

Long-term oral consumption of inorganic arsenic can cause a distinct pattern of skin alterations, including regions of black skin and the appearance of microscopic "corns" or "warts" on the palms, soles, and torso. These skin changes are typically accompanied by changes in the skin's blood vessels. Skin cancer could arise from this exposure. Arsenic use has also been linked to an increased risk of lung, liver, and bladder cancer. Both the Department of Health and Human Services and the International Agency for Research on Cancer have identified inorganic arsenic as a human carcinogen. The Environmental Protection Agency lists inorganic arsenic as a recognized human carcinogen (ATSDR, 2007c). Long-term arsenic exposure increases the risk of bladder and lung cancer, as well as skin cancer. The International Agency for Research on Cancer (IARC) has determined that eating arsenic

through drinking water, as well as arsenic and arsenic compounds, is carcinogenic to humans (WHO, 2018a). Figure 6 illustrates the effects of arsenic on the human skin.

Figure 6: Dermatological Effects of Arsenic. Source: Jaishankar et al. (2014)

How Arsenic Affect the Health of Children

Inorganic arsenic exposure can cause stomach and intestinal pain, blood vessel damage, skin changes, and reduced brain function in children, just as it does in adults (ATSDR, 2007c). As a result, all health problems observed in adults may be a source of concern for children. There is some evidence that long-term inorganic arsenic exposure in children may lower IQ (ATSDR, 2007c, 2011b). It does not appear that children absorb inorganic arsenic differently than adults. According to study, young adults who are exposed to arsenic in the womb or as a child are more likely to die.

Although studies are inconsistent, there is some evidence that inhaling or consuming inorganic arsenic may affect pregnant women or their unborn children. According to animal studies, large quantities of inorganic arsenic that

cause illness in pregnant women can also result in low birth weight, foetal deformities, and even foetal mortality. Arsenic has been found in foetal tissues and can cross the placenta, but it can also be found in minute amounts in breast milk. Animals exposed to organic arsenic compounds can have low birth weight, congenital abnormalities, and neonatal deaths. These symptoms are experienced by the mothers at the same dosage levels that produce them (ATSDR, 2007c, 2011b; US CDC, 2009).

How to Minimize the Risk of Exposure to Arsenic in the Environment

It is vital to evaluate for arsenic in wells that supply water for drinking and cooking. As of January 2006, the United States EPA's Maximum Contaminant Limit (MCL) for arsenic in drinking water was 10 ppb (ATSDR, 2007c, 2011b). If the level of arsenic in your drinking water exceeds the US EPA's MCL, consider utilising a different source of water for drinking and cooking. When working with arsenic-treated wood in your house, it is recommended that you wear personal protection equipment such as dust masks, gloves, and protective clothing to limit exposure to arsenic-containing sawdust. Wood that has been exposed to arsenic shouldn't be burned, composted, or used as mulch. After using CCA-treated wood play structures, children may be less likely to be exposed to arsenic by washing their hands.

Parents who work in businesses that use arsenic should be mindful of whether they may be bringing any mercury home on their person, their body, or their instruments in order to reduce their children's exposure to mercury. Their occupational health and safety officer should encourage them to take a shower and change before returning home, store their work clothes separately from other clothing, and wash them separately at home.

Parents have the right to file formal complaints with the Occupational Safety and Health Administration (OSHA) about health hazards at work and to request inspections without fear of repercussions.

Recommendations on Safe Arsenic Levels to Protect Human Health

The United States Environmental Protection Agency (US EPA) has established limits on the amount of arsenic that industrial sources are allowed to leak into the environment. Several of the applications of arsenic in pesticides are also being limited or abandoned, and the EPA is looking into more restrictions. The EPA reduced the acceptable exposure limit (PEL) for inorganic arsenic use in workplaces from 50 parts per billion to 10 parts per billion in January 2001. OSHA established a PEL of 10 g/m^3 for an 8-hour time-weighted average (ATSDR, 2007c, 2011b).

What is Cadmium?

With the chemical symbol Cd, atomic weight 112.414, and atomic number 48. It is a soft, pliable, ductile, silvery-white, divalent metal. Cadmium, a hazardous heavy metal that doesn't corrode and is frequently found with copper, lead, and zinc ores, is a component of the earth's crust. On the updated 2019 Substance Priority List published by the Agency for Toxic Substance and Disease Registry (ATSDR), it is ranked eighth (SPL) (ATSDR, 2019).

Uses of Cadmium

Due to its toxicity, cadmium only has a few applications. The majority of cadmium used in the US is obtained as a by-product of the manufacture of other metals including zinc, lead, and copper. Used batteries can also be used to recover cadmium metal. Additionally, cadmium can be found in a variety of

consumer goods, including batteries (83%), pigments (8%) coatings and plating's (7%) and plastic stabilizers (1.2%) in addition to nonferrous alloys, photovoltaics, and other uses (0.8 percent) (ATSDR, 2012). One of its few latest uses is found in cadmium telluride solar panels.

Sources and Fate of Cadmium in the Environment

Cadmium is present in the atmosphere primarily as oxide, sulfate, or chloride particles or vapours from high temperature activities. These particles or vapours can travel great distances in the atmosphere and deposit on soil or water surfaces (ATSDR, 2012).

The agility of cadmium and its compounds in soil depends on a number of factors, including the pH and the amount of organic matter, both of which are subject to environmental change. Generally speaking, cadmium has a great affinity for organic materials, where it will remain immobile in the soil, be ingested by plant life, and eventually enter the food chain. In water, cadmium coexists as a hydrated ion or ionic complex with other inorganic or organic molecules. While soluble forms float on water, insoluble forms do not (ATSDR, 2012).

Modes of Exposure to Cadmium in the Environment

The main sources of cadmium exposure in the environment are food, tobacco use, and employment. Water and the air are additional sources. In the US, the food supply is the main way that nonsmokers are exposed to cadmium (and may also apply to persons in other nations). Peanuts, soybeans, sunflower seeds, leafy vegetables like spinach and lettuce, cereals and potatoes, peanut butter, and sunflower seeds all often have high cadmium concentrations of between 0.05 and 0.12 mg/kg. High quantities of cadmium from the soil are

accumulated in tobacco leaves. Adult Americans' geometric mean blood cadmium levels are 0.38 μg/L nation wide. The geometric mean blood cadmium level for smokers in New York City, however, was observed to be 1.58 μg/L. How much cadmium was absorbed from (ATSDR, 2012).

In addition, exposure from heating cadmium-containing materials during procedures like smelting and electroplating has a substantial risk. Risk will, however, differ depending on the workplace. The primary means of exposure are inadvertent consumption from contaminated hands, food, or cigarettes, as well as inhalation of dust and fumes (ATSDR, 2012). Utilizing personal protective equipment, following appropriate industrial hygiene procedures, and managing and reducing cadmium emissions are all effective ways to reduce exposure. Furthermore, both historically and currently, elevated cadmium concentrations have been seen in water sources close to cadmium-emitting enterprises. Cadmium will build up in aquatic species and possibly infiltrate the food chain. People who eat fish from nearby waters should exercise caution and follow any recommendations regarding rules. If you don't already live near cadmium-emitting industry, inhaling (ATSDR, 2012).

How Cadmium Enters and Leave the Human Body

By inhaling it, consuming it, or letting it come into touch with the skin, cadmium can enter the body of a human. The amount of cadmium that is inhaled is thought to be absorbed through the lungs by 5-50 %, while the amount of cadmium that is in food and drink is thought to be absorbed by the gastrointestinal tract in much smaller amounts. It is possible to absorb more cadmium than usual when there is a deficiency in the diet in terms of iron or

other elements. The body cannot absorb cadmium via the skin, according to research.

According to research, the kidneys and liver are where the majority of cadmium that enters the body is stored for many years. Cadmium is also minimally eliminated in faeces and urine. When there is too much cadmium in the body, the liver and kidneys may not be able to completely transform it into a form that is not hazardous (ATSDR, 2012).

How Cadmium Affects the Health of People

According to research, breathing in air with exceptionally high levels of cadmium can cause serious lung damage and even death. In addition, exposure to low quantities of cadmium in the air over an extended length of time might cause cadmium to build up in the kidneys, perhaps resulting in renal disease (ATSDR, 2012). Studies on lab animals have demonstrated that cadmium exposure can harm the lungs and nasal passages. According to public health reports, eating or drinking things with a lot of cadmium can cause severe stomach irritation, which can cause vomiting, diarrhoea, and in rare circumstances, even death. The accumulation of cadmium in the kidneys as a result of prolonged exposure to low levels of cadmium through food or water can harm the kidneys. Bone brittleness and easy fracture are other effects of prolonged exposure to low levels of cadmium (osteoporosis) (ATSDR, 2012).

There is no data on the doses of cadmium that would cause these consequences, although some studies have revealed that swallowing cadmium can lead to anaemia, liver disease, and nerve or brain damage in animals.

In other studies, cadmium exposure has also been linked to lung cancer in workers and rodents. According to the U. S. Department of Health and Human Services, the International Agency for Research on Cancer, and the Environmental Protection Agency, cadmium is a known human carcinogen. A possible human carcinogen is cadmium.

How Cadmium Affect the Health of Children

According to public health statistics, children who are exposed to high amounts of cadmium are more likely to experience adult-level health issues such kidney and lung damage. Although there is no proof that cadmium exposure harms a child's development or behaviour, further research is required in this area. Younger animals may absorb more cadmium than adults and may be more vulnerable to bone loss and lower bone strength as a result of cadmium exposure, according to a few studies on animals. Human breast milk contains the contaminant cadmium, which may be transferred to nursing infants in trace amounts (ATSDR, 2012). The quantity of maternal exposure to cadmium determines the amount of cadmium that can be passed to the infant.

The potential impact of cadmium exposure on human foetal development is currently unknown. Studies on animals, though, have revealed negative effects on the foetus when high levels of cadmium were present during pregnancy. Exposure to cadmium seems to affect the neurological system the most. Studies on animals have demonstrated that prenatal cadmium exposure can have an impact on behaviour and learning, as well as body weight and bone development (ATSDR, 2012).

How to Minimize the Risk of Exposure to Cadmium

Avoiding tobacco use, maintaining good personal hygiene, avoiding cadmium-contaminated food and locations, making sure cadmium-containing items are handled properly, and disposing of cadmium-containing waste properly can all significantly reduce one's exposure to cadmium (ATSDR, 2012).

The ability of tobacco leaves to absorb cadmium is well recognised. The best way to limit your exposure to cadmium is to stop using tobacco and its derivatives and stop smoking. Reduced occupational exposure can be achieved by the use of personal protective equipment, efficient industrial hygiene practises, and—most importantly—the control and reduction of cadmium emissions to the environment (ATSDR, 2012). Children can be exposed to cadmium through parents who work in industries that emit cadmium. To minimize this exposure, parents should practice good personal hygiene such as washing and changing clothes before returning home. It is also important to avoid cadmium-contaminated food and areas by following local fishing advisories before consuming fish, shellfish, and other seafood and avoiding hazardous waste sites. Additionally, to prevent direct exposure to cadmium, parents should not let their children play with batteries in the home, as mishandled batteries can burst open and release cadmium, which can be ingested by children. Children may also accidentally swallow small nickel-cadmium batteries (ATSDR, 2012) if not handled properly.

Recommendations on Safe Cadmium Levels in the Human Body to Protect Human Health

The following are a few cadmium-related rules and recommendations:

Air in the Workplace

A 5.0 μg/m^3 of air allowable exposure limit for cadmium has been set by the Occupational Safety and Health Administration (OSHA), calculated over an eight-hour workday (ATSDR, 2012).

Drinking Water

The Environmental Protection Agency (EPA) has determined that children are not expected to be harmed by exposure to cadmium in drinking water at a concentration of 0.04 mg/L for up to 10 days. A lifetime exposure to 0.005 milligrams of cadmium per litre in drinking water, according to the EPA, is not anticipated to have any negative effects (ATSDR, 2012).

Consumer Products

The Food and Drug Administration (FDA) has determined that the maximum cadmium concentration in bottled water is 0.005 mg/L.

What is Lead?

A naturally occurring element with the symbol Pb, atomic weight 207.2, and atomic number 82 is called lead. It is a toxic, bluish-gray, heavy, low-melting metal that is present in the Earth's crust. Rarely is lead discovered in its pure state; instead, compounds made of lead and other elements are more common. Because of its resistance to corrosion, the metal produces thin coatings of compounds that shield it from further harm when exposed to water. Alloys can be created by combining lead with other elements (ATSDR, 2020).

Forms of Lead

Elemental lead, inorganic lead, and organic lead are the three primary kinds of lead that can be found in the environment. The type of lead

mentioned in the introduction is known as elemental lead, and the Earth's crust contains 0.002 percent of it. High lead content paint, dirt, dust, and a number of consumer goods are all common sources of inorganic lead. Depending on its chemical makeup, lead can have several colours, including white lead ($PbCO_3$), red lead (lead tetraoxide), and yellow lead (lead monoxide, lead chromate). Lead ethanoate tastes pleasant. Tetraethyl lead and tetramethyl lead are the two major types of organic lead used as fuel additives in gasoline to raise the octane number. Due to its high toxicity and ability to be absorbed through the skin, organic lead is especially hazardous (ATSDR, 2017). Leaded gasoline is thus no longer allowed to be used in motor vehicles in the US and other industrialized nations as of January 1, 1996 (ATSDR, 2007b).

Uses of Lead and Lead Compounds

Lead and lead alloys are frequently found in pipes, weights, shot and ammunition, cable coverings, lead sheets, and radiation protective aprons. They are also present in lead and lead alloys. Lead is mostly used in storage batteries for automobiles and other vehicles (ATSDR, 2007b). In addition to caulk, lead compounds are employed as pigments in paints, dyes, and ceramic glazes. To lessen its negative effects on humans and other animals, lead has, however, been used less frequently in these items in recent years. In the past, the United States added tetraethyl and tetramethyl lead to gasoline to raise the octane rating, but this practice was phased out between the 1980s and January 1, 1996. Off-road vehicles use tetraethyllead in their gasoline and of which Ghana may not be an exception (ATSDR, 2007b, 2020).

Occurrence and Sources of Lead

Lead is a substance that naturally occurs in the environment and is present in concentrated and accessible deposits all over the planet. Despite the fact that leaded gasoline is no longer used in the United States and other industrialised countries, burning leaded fuel still produces anthropogenic emissions that make up the bulk of the world's primary sources of lead today (ATSDR, 2007b). Other man-made sources of lead include ore mining and smelting, manufacturing and using lead-based paints, pigments, and glazes, electrical shielding, plumbing, storage batteries, solder, and welding fluxes, manufacturing and using lead-containing pesticides, burning coal and oil, and waste incineration (ATSDR, 2017, 2020). Currently, mined ores (primary source) and recycled scrap metal or batteries account for the majority of the lead utilized by industry (secondary source). It should be noted, though, that the majority of lead used now comes from secondary sources, particularly lead-acid batteries. Almost all of these batteries are recycled—roughly 97% (ATSDR, 2007b).

General Sources

Ash, battery manufacturing, car batteries, cigarette smoke, coal combustion, coloured inks, congenital intoxication, household dust, hair dyes, industrial emissions, lead pipes, lead-glazed earthenware pottery, mascara, metal polish, newsprint, paint, pencils, pesticides, rainwater, PVC containers, tin cans sealed with lead solder, tobacco, and toothpaste are all potential sources of lead exposure (ATSDR, 2017).

Food Sources

Foods containing lead include bone meal, canned fruit and juice, liver, milk, organ meats and liver.

Fate of Lead in the Environment

Although lead is a naturally occurring metal, human activity is mostly to blame for the high levels of lead found in the environment. Lead can be released into the environment during lead and other metal mining, as well as during the production or use of lead, lead alloys, and other lead compounds in factories. In addition, lead can be discharged into the air when trash, coal, or oil are burned (ATSDR, 2007b). In developing countries such as Ghana, where leaded gasoline is still used, a significant proportion of lead released into the environment comes from car exhaust fumes. Lead-contaminated particulate matter can travel through the air, water, and soil. The main source of lead found in soils is atmospheric deposition, especially when other nearby non-air sources, such as dust from deteriorating leaded paint, are unaffected. Lead is continuously transferred between the air, water, and soil through natural, chemical and physical processes such as weathering, runoff, precipitation, dry deposition of dust, and stream/river flow. Yet, it seems that sediments and soil are important lead sinks (ATSDR, 2020).

Lead can make its way into lakes, streams, and rivers through the movement of soil particles by rainwater. In addition, soft or acidic water can cause lead solder or pipes to leak a small quantity of lead into the water. For many years, lead can cling to soil fragments or water's bottom sediment. Lead contamination in the air, water, or soil can cause levels in plants and animals to rise over time. Most of the lead will pass through the bodies of animals that

consume polluted plants or animals. While sunlight, air, and water can convert some lead compounds into different forms of lead, elemental lead is not easily degraded (ATSDR, 2007b).

How People Are Exposed to Lead in the Environment

Humans can consume lead from the environment by drinking water, food, and dust. Those who live close to hazardous waste sites may be exposed to lead and chemicals that include lead through their food, water, air, food they eat, or dirt they ingest that contains lead. Consuming food or water that has been polluted with lead can potentially expose one to lead. Drinking water from sources with lead pipes can also expose people to lead, especially if the water is soft. Residents in older neighbourhoods whose homes were painted with lead paint may be more exposed to elevated amounts of lead in the soil and dust.

Similarly, residents of historic orchard land that had utilized lead arsenate pesticides or those who live close to busy highways may be exposed to increased lead levels. Through their line of work or recreational activities, such as manufacturing stained glass, people may also be exposed to lead. Consuming illegal whiskey produced using stills with lead-soldered parts, smoking cigarettes, consuming canned foods, and using consumer goods like storage batteries, toys, glazes for pottery, leaded crystal glassware, cosmetics, hair dye, jewellery, gun shots and ammunition, sinkers, among other things, are additional sources of exposure (ATSDR, 2007b, 2020). Lead exposure can also happen through the skin. Lead exposure prior to conception or ongoing exposure can cause pregnant women to pass lead into their blood and breast

milk, which can have long-term repercussions on the neurodevelopment of their unborn child (US CDC, 2024).

How Lead Enters and Leaves the Human Body

The bloodstream quickly spreads lead to other parts of the body after it enters the body through the lungs. After entering the lungs, large lead particles may be coughed up and ingested. Although swallowing is the primary method of lead absorption, relatively little of it actually reaches the blood and other organs. Just about 6% of the entire amount of lead absorbed by those who have recently eaten enters the blood from the stomach, according to study. After a day of fasting, 60–80% of the lead in the stomach might enter the blood. In general, if both adults and children ingest the same amount of lead, more lead will enter the blood of children than adults. Youngsters absorb lead from their diet at a rate of about 50% (ATSDR, 2007b).

Lead first enters the body through the soft tissues and organs, including the kidneys, liver, lungs, brain, spleen, muscles, and heart. The majority of the lead enters bones and teeth over the period of a few weeks. According to a study, the bones and teeth contain 94% of the lead that is in an adult's body. The majority of the lead that is in kids' bodies is kept in their bones, where it can remain for years. But in other circumstances, lead can leave the bones and re-enter the bloodstream and organs (e.g., during pregnancy and periods of breast feeding, after a bone is broken, and during advancing age) (ATSDR, 2007b). Lead that is not deposited in the bones leaves the body through faeces and urine after being ingested and distributed across the body's organs. Continuous lead exposure may result in a build-up of lead body burden in

tissues, particularly in the bone, as not all lead that enters the body is removed (ATSDR, 2007b).

How Lead Affects the Health of People

No matter how lead is exposed to humans, the effects are the same. The nervous system is the principal organ that lead toxicity primarily affects in both adults and children. According to research, prolonged workplace exposure to lead resulted in lower performance on various tests that gauge how well the neurological system is functioning. Lead exposure can also cause weakness in the wrists, ankles, or fingers (ATSDR, 2007b, 2017). Adults, especially those in their middle and later years, may additionally have anaemia and a moderate rise in blood pressure as a result. High levels of lead exposure can cause deadly brain and kidney damage in both adults and children (ATSDR, 2007b, 2017, 2020). High blood lead levels (BLL) during pregnancy increase the risk of preterm birth, miscarriage, neurological issues, intrauterine growth restriction (IUGR), spontaneous abortion, and/or low birth weight (ATSDR, 2007b, 2017, 2020).

Blood lead levels have also been related to a variety of brain impairments, including issues with cognition (thinking), trouble coordinating fine motions, such as picking up small items, and issues with organizing activities, decisions, and behaviors (executive functions) (Cecil et al., 2008). There is no concrete evidence that lead can cause cancer in people, at least not in humans. Based on substantial data from animal research and insufficient evidence from human studies, the United States Department of Health and Human Services (US DHHS) has established that lead and its related compounds are reasonably anticipated to be human carcinogens. While the

EPA has decided that lead is a potential human carcinogen, the International Agency for Cancer Research (IARC) has stated that inorganic lead is probably harmful to humans.

How Lead Affects the Health of Children

According to research, children are more vulnerable than adults. Furthermore, it is critical to understand that there is no "safe" blood lead level for children below which there is no danger of impaired intellectual or developmental function (ATSDR, 2007b). Lead prevents children's bodies from absorbing minerals and elements like zinc, calcium, and iron that are essential for growth and development. Lead toxicity rarely manifests in children until they are in school, frequently not until middle school, when the demands and expectations for academic performance rise. Although brain damage from lead exposure to children is the most immediate health concern, it is important to remember that lead poisoning during childhood can have long-term repercussions on the kidneys and IQ (ATSDR, 2017). Lead exposure during pregnancy, infancy, or the first few years of life may also hinder mental growth and lower IQ levels later in childhood. There is proof that these impacts might last past childhood (ATSDR, 2017).

How to Minimize Risks of Exposure to Lead in the Environment

To reduce exposure to lead, it is important to be aware of the different sources of lead in the environment and take steps to avoid contact with them. Implementing preventive measures can also help to minimize exposure to lead. Avoid:

- touch with old car batteries, open waste burning, and degrading lead-based paint on a home's walls, doors, and windows (ATSDR, 2007b);

- Smoking, smoke from automobile exhaust, smoke from open fires, old smelters, and lead-contaminated dust on a home's floor are all examples of smoke.

Recommendations and Regulations on Safe Levels of Lead in the Human Body to Protect Public Health

The following recommendations and regulations have been put up by some regulatory authorities to protect public health.

The US EPA sets a limit on the amount of lead that can be present in the air for public inhalation and that is 1.50 microgram per cubic meter ($\mu g/m^3$) averaged over three months. EPA also prohibits the use of leaded gasoline in compliance with the Clean Air Act Amendment of 1990 (CAAA). To lower the amount of lead in drinking water, the United States authorities developed the Lead Copper Rule (LCR) and the Lead Contamination Control Act of 1988 (ATSDR, 2007b; Illinois EPA, 2016).

The United States Consumer Protection Safety Commission (CPSC) has a regulation that restricts the quantity of lead to 0.06 percent in the majority of consumer paints. The Federal Hazardous Substances Act (FHSA) forbids the use of lead in items for kids that are judged dangerous. In order to reduce lead exposure, the Department of Housing and Urban Development (HUD) develops standards and rules. It also mandates that all federally sponsored housing, renovations, public housing, and Indian housing be evaluated for lead-based paint hazards and that any such hazards be addressed by caulking or removing the paint. The US EPA and HUD employ the standard that paint with a lead concentration of 1.0 mg/cm2 or above should be removed or treated when deciding whether lead-based paint in a dwelling

should be removed. Guidelines for lead paint risks, lead in dust, and lead in soil have been set by the US EPA. The US EPA has produced instructional brochures that can be downloaded through the National Lead Information Centre or located online in order to educate people about lead risks, lead poisoning prevention in the home, as well as the procedure for dealing with lead abatement process (ATSDR, 2007b).

OSHA guidelines state that during an 8-hour workday, the amount of lead in the air in a work location cannot exceed 50 g/m^3 (ATSDR, 2007b). OSHA mandates that a worker be removed from the work location where lead exposure is occurring if their blood lead level is 50 g/dL or above.

Meals packaged in cans with lead solders are regarded as unhealthy because lead is one of the toxic substances identified by the US FDA. Tin-coated lead foil, used to decorate wine bottles and keep bugs out, can taint the wine with lead, which is prohibited by the US FDA Food, Drug, and Cosmetic Act. Despite the fact that some of them contain lead amounts ranging from 0.1 to 10 ppm, the US FDA has also analysed a number of food ingredients and determined that, when utilised in line with accepted manufacturing practises, they are safe for consumption. Each state should create a plan to find children who may have been exposed to lead and test their blood for lead, according to the US CDC. As part of this strategy, the US CDC advises states to evaluate kids at particular ages:

1 and 2 years;

3–6 years if they have never been tested for lead;

to ascertain if they:

- obtain aid from public assistance schemes for the needy, such as Medicaid or the Supplemental Nutrition Program for Women, Infants, and Children;
- dwell in or usually visit a structure constructed before 1950;
- tour a property that was recently renovated and was constructed before 1978.; and/or
- having a playmate or sibling who has suffered from lead poisoning.

The US CDC defines a child as having a high blood lead level when it is at least 10 g/dL. Numerous states or local efforts help children with blood lead levels of 10 g/dL or above. If a child's blood lead level is 20 g/dL or greater, a medical evaluation and environmental investigation and remediation (MEEIR) should be carried out. Children with blood lead levels greater than 45 g/dL can need chelation therapy or other types of medical care (ATSDR, 2007b).

What is Mercury?

The chemical element mercury, with the chemical symbol "Hg," has the atomic number 80 and the atomic weight 200.592 (de Laeter et al., 2009; Prohaska et al., 2022). Mercury is the only metal that is liquid at room temperature and pressure. The 2019 Substance Priority List (SPL) of the Agency for Toxic Substances and Disease Registry puts mercury as the third most dangerous substance (ATSDR, 2019) based on the likelihood that humans could be exposed, its frequency, and toxicity. There are three categories into which mercury can be divided: elemental or metallic mercury, inorganic mercury, and organic mercury (Clarkson, 2002; WHO, 2017). The liquid form of elemental mercury is a gleaming, silvery-white metal. Devices like thermometers and switches contain it in its purest form. Some of the

metallic mercury will evaporate at normal temperature and turn into an odourless, invisible vapor. More vapor from metallic mercury is released as the temperature rises. A metallic aftertaste may result from inhaling this vapor (Clarkson, 2002; WHO, 2017).

Inorganic mercury are those compounds formed when mercury combines with other elements such as chlorine (e.g., $HgCl_2$), oxygen (e.g., HgO) or Sulphur (e.g., HgS). With exception of mercuric sulphide (also called cinnabar, HgS) which is red and changes into black on exposure to light, most inorganic mercury compounds exist as white crystalline substances or powders.

When mercury chemically reacts with carbon, organic mercury compounds, commonly referred to as organomercurials, are created. Methylmercury is one of these substances that is most prevalent. Dimethylmercury is used in small concentrations in various reference tests, and some organic mercury compounds, such phenylmercury, have been employed in commercial products in the past. The only organomercurial discovered at hazardous waste sites so far is dimethylmercury. Like inorganic mercury, methylmercury and phenylmercury are salts that can be found in nature. Methylmercury and phenylmercury are both white crystalline solids when they are pure, whereas dimethylmercury is a colourless liquid. Mercury can change its form due to a number of environmental microbes, including bacteria and fungi, as well as organic processes (ATSDR, 1999a). The most prevalent organic form of mercury that is formed naturally and by microbes from other forms of mercury is methylmercury. Environmentalists are concerned about it because, through a process called biomagnification, it can

build up to extremely high levels in the bodies of various freshwater, marine, and marine mammal species as well as some fish (ATSDR, 1999a, 2022b).

Occurrence and Sources of Mercury

A component of the earth's crust that is naturally occurring, mercury is released into the atmosphere as a result of volcanic eruptions, rock weathering, and human activity. The bulk of mercury releases are primarily the result of human activity, particularly the use of coal-fired power stations, domestic coal burning for heating and cooking, industrial activities, waste incineration, and the extraction of metals such as gold and mercury (WHO, 2017). Bacteria and fungus, among other microorganisms, are involved in the environmental transformation of mercury.

General Sources of Mercury

Congenital intoxication, cosmetics, dental amalgams, diuretics, fabric softeners, floor waxes, fungicides, insecticides, laxatives, lumber, paper manufacturing, algicides, antiseptics, barometers and thermometers, adhesives, air conditioner filters, algaecides, antiseptics, barometers and thermometers, battery production, body powders, broken thermometers, burning newspapers and construction materials, and calamine lotions (ATSDR, 1999a; WHO, 2017).

Food Sources of Mercury

Mercury can be found in certain food items such as cereal, grains, and seafood (particularly tuna and swordfish) and in contaminated water (ATSDR, 1999a).

Uses of Mercury and Its Compounds

Liquid metallic mercury has a variety of uses. It is used in a variety of industrial and domestic items, including thermostats, thermometers, barometers, some blood pressure monitors, and other scientific equipment, as well as in the production of caustic soda and chlorine gas (ATSDR, 1999a; WHO, 2017). Mercury is often used in streetlights, fluorescent lighting, and advertising signs. It also serves as a good electrical conductor and is a component of silent, position-sensitive switches. It is used to make chlorine gas and caustic soda. Moreover, metallic mercury is still utilized in rituals and spiritual practices for a number of Latin American and Caribbean religions, including Voodoo, Santeria, and Espiritismo, as well as in a variety of herbal and spiritual remedies throughout Asia and Latin America (ATSDR, 1999a). Exposure to mercury vapours in contaminated air offers a health risk to both users and non-users of these religions, even though not all followers of these faiths employ mercury in religious, ethnic, and ritualistic practices (ATSDR, 1999a). At places called Botanicas in Spanish and Haitian communities, metallic mercury is frequently sold under the traditional name of azogue (ATSDR, 1999a). It might be given as a herbal remedy or used in religious ceremonies. The elemental mercury is normally marketed in capsules or glass vials, although on rare occasions it may also be distributed throughout a building or vehicle or packaged in a sealed pouch to be worn as a necklace or carried in one's pocket. Some people put azogue in their bathwater, perfume, or love candles. The longer people breathe the harmful air, the greater their risk of becoming ill is. Everyone who lives in a house or apartment, including those who work there, is harmed by the usage of metallic mercury (ATSDR,

1999b). Creating amalgams, which are alloys consisting of metals like sodium, zinc, silver, gold, and cadmium, is a straightforward process that involves mercury and other metals. These amalgams extract gold from ores, increase the lifespan of dry cell batteries, and are utilized in dental fillings (silver) (e.g., zinc and cadmium)

Other inorganic mercury compounds, like mercuric iodide and ammoniated mercuric chloride, are employed in vaccines and skin-lightening creams. As fungicides, some inorganic mercury compounds are used (Clarkson, 2002). Mercuric chloride ($HgCl_2$), a common antiseptic and disinfectant, is a highly toxic salt that was previously used to clean wounds. Calomel, commonly known as mercury chloride (Hg_2Cl_2), is an antiseptic that kills microorganisms and is present in drugs like laxatives, worming remedies, and teething powders (ATSDR, 1999a). Mercuric sulfide (HgS), on the other hand, is used to make a red paint pigment called vermilion and also used in tattooing dyes while mercuric oxide (HgO) is used to make mercury batteries (https://education.jlab.org/faq/index.html). Mercurochrome, which contains 2% mercury, thimerosal, and phenylmercuric nitrate are three inorganic mercury compounds that are still used as antibacterial. They are used in small doses as preservatives in several prescription and over-the-counter drugs (ATSDR, 1999a). Only bacteria and fungi are capable of producing methylmercury in the environment. When it became clear that they had negative health effects, methylmercury and ethyl mercury were no longer allowed to be used as antifungal agents to protect seed grains. Phenylmercuric compounds were once used in interior and exterior paints as antifungal agents,

but they were banned when it was revealed that these paints leaked mercury vapours.

Fate of Mercury in the Environment

Due to exposure to wind, water, and volcanic activity, the minerals in rocks and soil naturally deteriorate, releasing mercury into the environment. Recent history has seen relatively consistent discharges of mercury from natural sources, despite an overall increase in ambient mercury levels. Anthropogenic activities like mining and the burning of fossil fuels have led to further mercury leaks into the environment. Methylmercury is the kind of mercury that can get into the food chain and accumulate there. When tiny fish consume foods containing methylmercury, the methylmercury absorbs into their tissues.

When larger fish eat smaller fish or other species that contain methylmercury, a sizeable portion of the methylmercury that was initially present in the prey gets bioaccumulated and biomagnified in the larger fish's body to many times the quantity present in the prey. Fish that live in contaminated waterways therefore have the highest concentrations of methylmercury in their bodies. These fish are typically older and larger. The highest levels of methylmercury are typically found in the systems of saltwater species like swordfish and sharks since they can live a long time and grow to enormous sizes. It is also possible for mushrooms to accumulate significant amounts when grown on methylmercury-contaminated soil. Yet, plants like corn, wheat, and peas only absorb a very small amount of methylmercury, even when grown in soils with far higher background levels.

How People Are Exposed to Mercury in the Environment

Because mercury is so prevalent in the environment, everyone is likely to be exposed to low levels of mercury through the air, water, and food. Mercury concentrations in metropolitan outdoor air have been shown to range between 10 and 20 nanograms per cubic metre (ng/m^3), despite the fact that these levels are significantly lower than those considered "safe" to breathe (ATSDR, 1999a). Much lower background levels can be observed outside of cities. Mercury concentrations in surface water are typically less than 5 parts per trillion (ppt) or 5 ng/L of water, which is almost 1,000 times lower than the allowed threshold for safe drinking water. On average, mercury concentrations in soil range from 20 to 625 ppm (ATSDR, 1999a). The total amount of mercury released from dental amalgams is influenced by several factors, including the total number of fillings, the surface area of each filling, the person's eating and chewing habits, and the chemical conditions in the mouth. It is estimated that dental amalgams release between 3 and 17 micrograms of mercury per day (3-17 g/day), accounting for up to 75% of a person's total daily mercury exposure (ATSDR, 1999a). Other factors such as the number of dental fillings, the number of fish consumed, and the mercury content of the fish, as well as exposure from less common sources such as taking mercury pills, using mercury-containing herbal remedies, and engaging in certain religious practises, can all affect this.

Religious practises also expose some people to mercury. Santeria (a Cuban-based religion whose adherents worship both African deities and Catholic Saints), Voodoo (a Haitian-based set of beliefs and rituals), Palo Mayombe (a hidden type of ancestral worship practised in the Caribbean), and

Espiritismo are examples of these faiths (a spiritual belief system native Puerto Rico). Although not all adherents of these religions utilise mercury, when mercury is used in religious, ethnic, or ceremonial behaviours, the broader community is exposed to mercury both during the practise and later through contaminated indoor air (ATSDR, 1999a). Accidental releases of mercury vapour into interior air, such as broken thermometers or damaged electrical switches, can be dangerous if prolonged exposure occurs. Humans can also be exposed to mercury vapour through contaminated air near hazardous waste sites, waste incinerators, or power stations that use mercury-containing fuels, but outside air is usually safe. Handling polluted soil, playing or ingesting contaminated surface soil, drinking well water, or eating fish from contaminated water near hazardous waste sites can all lead to mercury compound exposure.

Mercury can be ingested by fungicides, the topical use of expired drugs, antiseptics, disinfectants, and the ingestion of numerous pharmaceutical goods. Workers may be exposed to mercury vapour from breathing contaminated air, as well as other inorganic mercury compounds in the workplace. Jobs with a higher risk of exposure include manufacturing mercury-containing electrical equipment or vehicle parts, mercury-using chemical processing facilities, metal processing, and construction involving mercury-containing building components. Those who work in the medical field who utilize mercury-containing equipment run the risk of exposure. If a worker's clothing is polluted with mercury, family members could also be exposed to the material. Finally, a diet high in meat and fish can expose some people to methylmercury(ATSDR, 1999a; Clarkson, 2002). The U.S. Food

and Drug Authority (FDA) estimates that the average exposure to mercury in most people is around 50 nanograms per kilogram of body weight per day (i.e., 50 ng/kg/day) through food, though this level is not regarded to result in any harmful health effects (ATSDR, 1999a; Clarkson, 2002).

How Mercury Enters and Leaves the Human Body

Mercury can enter the body through inhaling contaminated air, swallowing contaminated water or food, or through skin contact. Although mercury enters the body by ingestion, inhaling mercury vapour is more dangerous since it enters the bloodstream straight from the lungs and can swiftly move to other organs of the body, including the brain and kidneys. Metallic mercury can persist in the body for weeks or months before converting to an inorganic form that can stay in the body indefinitely. Metallic mercury in the blood of pregnant women can also reach the growing baby. The majority of metallic mercury ingested into the body is expelled in the form of urine and faeces, but small amounts can also leave the body via exhaled air (ATSDR, 1999a; Clarkson, 2002).

Fewer than 10% of inorganic compounds taken are normally absorbed by the gastrointestinal tract, while up to 40% may occasionally enter the body through the stomach and intestines. A trace amount of inorganic mercury may enter the body through cutaneous contact. Inorganic mercury enters the bloodstream after entering the body and then moves to numerous tissues. In contrast to the brain, it accumulates in the kidneys. Additionally, it is difficult to transfer from mother blood to that of the growing foetus. Some of the inorganic mercury in nursing women' bodies is excreted in their breast milk. Over the course of several weeks or months, inorganic mercury also

eventually departs the body through urine or faeces (ATSDR, 1999a; Clarkson, 2002).

Mercury is most readily absorbed through the digestive system in the form of methylmercury. It enters the bloodstream and spreads to other regions of the body when absorbed by tainted food or fish. Dimethylmercury and other chemical compounds can easily enter the body through the skin. Most tissues, including the brain, may be easily reached by organic mercury after it has entered the bloodstream. Methylmercury can also enter the growing foetus's brain and tissues through the blood of a pregnant woman. Methylmercury, like metallic mercury, can change into inorganic mercury in the body and can linger for a very long time in the brain. Additionally, methylmercury can enter the breast milk of nursing women and is finally eliminated through the urine and faeces (ATSDR, 1999a; Clarkson, 2002).

How Mercury Affects the Health of People

Mercury is a neurotoxin and the extent to which its exposure affects the health of a person depends on a number of factors namely;

- the form of mercury – whether it is methylmercury or elemental (metallic mercury) or inorganic mercury;
- the amount or concentration of mercury involved in the exposure;
- the age of the person exposed (unborn infants are the most vulnerable);
- duration of the exposure;
- mode of exposure i.e., whether the exposure occurred through breathing, eating, skin contact, etc.; and
- the health of the person exposed (US EPA, 2019; WHO, 2017).

The effects of exposure to mercury can change based on the type, length, and intensity of the exposure. Metallic mercury and methylmercury are poisonous to the nervous system, and exposure to them can impair the immune, digestive, and neural systems as well as the lungs, kidneys, and metallic mercury. Severe cases of exposure can even result in death. The neurological, gastrointestinal, immunological, respiratory, and renal systems, as well as the lungs, can all be adversely affected by inhaling mercury vapor (WHO, 2017). Mercury toxicity affects the nervous system very strongly. Excessive levels of metallic mercury vapour in the air for a limited period of time can injure the mouth's lining and irritate the lungs and airways, resulting in chest tightness, a burning feeling in the lungs, and coughing (ATSDR, 1999a; WHO, 2017). Additional indications and symptoms include rashes on the skin, itchy eyes, nausea, vomiting, diarrhoea, and changes in heart rate or blood pressure. Injury to the lining of the mouth and lungs can also happen from lesser amounts of mercury vapour exposure.

Individuals who consume fish contaminated with high amounts of methylmercury or seed grains treated with methylmercury or other organic mercury compounds may suffer lifelong brain and kidney damage, according to cases of mercury poisoning reported in other nations (ATSDR, 1999a; WHO, 2017). Persistent brain damage can also develop from long-term exposure to excessive concentrations of metallic mercury. Loss of coordination, weak muscles, a loss of peripheral vision, problems speaking, and tingling or numbness in the hands, feet, and mouth region are some indicators of methylmercury poisoning (US EPA, 2019). Due to the fact that inorganic mercury has a difficult time crossing the blood-brain barrier, its

effects on the brain and nervous system are not well understood. However, it is well recognized that inorganic mercury is damaging to the skin, eyes, and digestive system and can harm the kidneys if consumed (WHO, 2017). Exposure to different forms of mercury through breathing it in, eating or drinking it, or having contact with it through the skin can result in neurological and behavioural changes

Symptoms that may occur with high exposure to inorganic mercury may include skin rashes and dermatitis, memory loss, tremors, mood changes, neuromuscular problems, insomnia, headaches, and difficulties with cognitive and motor functions (US EPA, 2019; WHO, 2017). Workers exposed to high levels of metallic mercury in the air (20 $\mu g/m^3$ or more) for several years may show subtle or mild, non-specific signs of central nervous system toxicity. Kidney effects have also been reported, including increased protein level in urine and kidney failure. Studies show that consuming water containing high levels of inorganic mercury over an extended period of time can lead to kidney damage (US EPA, 2019). Large doses of inorganic mercury can damage the stomach and intestines as well as the kidneys, resulting in symptoms including nausea, diarrhoea, and serious ulcers. Children who accidentally take mercuric chloride have also been related to heart issues like fast heartbeats and high blood pressure. However, there is a dearth of information on how long-term, low-level exposure to inorganic mercury affects people (ATSDR, 1999a).

Despite mercury's damaging effects on the neurological system, evidence from research in animals do not prove whether it can harm people. Mercury has not been linked to human cancer by the US Department of Health and Human Services (DHHS) or the International Agency for Research on

Cancer (IARC). However, the US Environmental Protection Agency (EPA) has classified mercury chloride and methylmercury as potential human carcinogens (ATSDR, 1999a).

How Mercury Affects the Health of Children

Children may be exposed to metallic mercury that has not been properly contained, mercury brought home on tools or clothing from work, or methylmercury-contaminated foods. Pregnant women who consume methylmercury or breathe contaminated air containing metallic mercury may convey the toxicity to their unborn child (ATSDR, 1999a; US EPA, 2019). Furthermore, new-borns may be exposed to methylmercury and inorganic mercury through breast milk when nursing. If their mothers consume contaminated fish and shellfish while pregnant, their infants may be exposed to methylmercury. This exposure may result in higher methylmercury levels in the baby's blood than in the mother's, which could harm the baby's growing brain and neurological system (ATSDR, 1999a; US EPA, 2019). These systems may be more vulnerable to methylmercury than adult systems. Children exposed to methylmercury in the womb may experience developmental delays in cognitive abilities, memory, attention, language, fine motor skills, and visual-spatial skills (U. S. EPA, 2019).

Mercury exposure can have the same negative health effects on children as it does on adults. Prolonged exposure to mercury vapour can result in death from respiratory failure and damage to the lungs, stomach, and intestines in severe cases. These symptoms are similar to those felt by those who worked in environments with metallic mercury vapours. High doses of mercurous chloride pills for worms or powders containing mercurous chloride

for teething pain caused elevated heart rates and blood pressure in children (ATSDR, 1999a; US EPA, 2019). Children who consumed grains contaminated with exceptionally high quantities of methylmercury had abnormal cardiac rhythms. Other signs of mercury poisoning in children who have taken mercurous chloride for constipation, worms, or teething discomfort include swollen red gums, excessive salivation, weight loss, diarrhoea, abdominal pain, and twitching or cramping in the arms and/or legs. Kidney damage is common following deadly levels of inorganic mercury exposure (ATSDR, 1999a; US EPA, 2019).

Research suggest that prolonged exposure to elemental mercury causes acrodynia in children who breathe elemental mercury vapour, consume foods or other items containing phenylmercury or inorganic mercury salts, or apply elemental mercury skin ointments (also known as "pink disease") (ATSDR, 1999a; US EPA, 2019). Acrodynia is characterised by agonising leg cramps, discomfort, and distinctive skin redness that develops to peeling of the hands, nose, and foot soles. Itching, swelling, fever, a quick heartbeat, high blood pressure, excessive sweating or salivation, rashes, anxiety, sleeplessness, and/or weakness are some of the other symptoms. Children and foetuses are more vulnerable to the neurological effects of metallic mercury and methylmercury during important developmental phases before birth and the first few months following birth. Exposure to metallic mercury during pregnancy may affect the foetus's ability to develop (ATSDR, 1999a; US EPA, 2019).

Breastfeeding mothers who have been exposed to methylmercury put their children at risk. The effects on the child may differ based on the amount

of exposure the foetus got. Some implications, such as minor IQ drops or brain impacts that require comprehensive neuropsychological testing, may not be readily apparent in cases where the exposure was low. Yet, when exposure levels are high, the consequences may be more severe. Mercury poisoning on a growing foetus is likely to take time to manifest, resulting in a newborn who appears healthy at birth but subsequently exhibits developmental delays, brain damage associated with mental retardation, coordination issues, and immobility (ATSDR, 1999a; US EPA, 2019). Excessive mercury exposure during pregnancy can cause blindness, uncontrollable muscle spasms and convulsions, muscle weakness, and verbal impairment in children (ATSDR, 1999b). The severity of the health repercussions is determined by the amount and length of exposure to mercury. When pregnant and nursing women were exposed to extremely high quantities of methylmercury, such as in cases of widespread poisoning from tainted grain used to make bread in Iraq or regularly consumed seafood in Japan, the severe effects were recognised (ATSDR, 1999a; US EPA, 2019).

How to Reduce Exposure to Mercury in the Environment

It's crucial to teach kids not to play with shiny, silvery liquids in order to safeguard them from the hazards of metallic mercury. Science instructors and school personnel should be aware of kids' curiosity with metallic mercury and educate them about the dangers of handling it. They should also make sure that metallic mercury is stored in a safe place that kids can't get without adult supervision. Children may breathe in harmful mercury vapor if metallic mercury is not stored in a closed container since it evaporates slowly. To avoid accidental poisoning, all mercury-containing medications should be kept out

of children's reach. Additionally, non-medical items like paints with mercuric sulphide or mercuric oxide and fungicides with mercury compounds should be preserved (ATSDR, 1999a).

When choosing between dental amalgam and a non-mercury material for fillings or whether to repair or replace an existing amalgam filling in a pregnant woman, it's necessary to see a dentist. It is key to pay attention to and abide by advisories on consumption of fish and animals in order to reduce exposure to mercury through the intake of fish, shellfish, and other types of food. The U.S. FDA also suggests that people limit their regular consumption of shark and swordfish, with the exception of pregnant women and women who are nursing (ATSDR, 1999b).

Recommendations and Regulations on Safe Mercury Levels in Air, Water and Food to Protect Public Health

Some regulations and recommendations for mercury include:

- A limit of 2 parts per billion (ppb) of inorganic mercury in drinking water has been established by the US EPA and US FDA.
- The US EPA is now reviewing mercury-related Water Quality Standards and advises that the level of inorganic mercury in rivers, lakes, and streams should not exceed 144 parts per trillion (ppt) of water in order to protect public health.
- According to the US EPA, daily exposure to inorganic mercury in drinking water at levels up to 2 ppb is unlikely to have any significant negative health repercussions for an adult of average weight.
- The FDA has set a maximum permissible quantity of methylmercury in seafood items sold in interstate commerce at 1 part per million (ppm).

It has the right to seize treated seed grain carrying more than one part per million of mercury, as well as shipments of fish and shellfish containing more than one part per million of methylmercury (ATSDR, 1999a).

The Occupational Safety and Health Administration (OSHA) of the United States regulates mercury quantities in the workplace. It has set a limit of 0.1 mg/m3 for organic mercury and 0.05 mg/m^3 for metallic mercury vapour in the workplace air to protect workers over an 8-hour shift and a 40-hour workweek. Furthermore, the National Institute for Occupational Safety and Health (NIOSH) recommends that the average level of metallic mercury vapour in the workplace air be kept to 0.05 mg/m^3 for a 10-hour work shift. (ATSDR, 1999b).

Essential Elements

Essential elements are mineral elements required by living organisms for normal growth, development and maintenance. The human body is made up of many elements. According to some studies, as many as 81 naturally occurring elements have been reported in some human tissues (Klein et al., 2017). It is worthy to note that essential elements in human breast milk play important role for the healthy growth and development of infants. Essential elements are macro/micro-mineral elements which are required by living organisms for normal growth, metabolism, development and maintenance (Zoroddu et al., 2019).

Based on the quantity required in the body, essential elements can be categorised into major or macronutrients and trace or micronutrients. Major or macronutrients are those elements needed in relatively large amounts for

normal growth and development whereas trace or micronutrients are needed in only small quantities (Callaban et al., 2020). Major or macronutrients include calcium, phosphorus, sodium, sulphur, chlorine, potassium and magnesium. The trace elements include iron, manganese, zinc, copper, selenium, cobalt, iodine, chromium, molybdenum and silicon. Each essential element may perform one or more of a variety of metabolic roles. Calcium, magnesium and phosphorus are all found in bones. Calcium is also important for nerve and muscle activity as well as cell signalling, while phosphorus is a key constituent in the energy transfer molecule ATP.

Potassium, sodium and chloride ions are the major electrolytic components of cells and body fluids and therefore, important determinants of electrical and osmotic potentials. The trace elements may function as cofactors or as constituents in complex molecules such as iron in haeme or cobalt in vitamin B_{12}. Studies have shown that deficiencies of essential elements are correlated with chronic diseases and increased rates of infection. On the other hand, excess amounts of these elements may also pose harm to life (Klein et al., 2017). Human breast milk contains over 20 different mineral elements, most of which are more prevalent in colostrum and become less abundant as lactation develops. These minerals include zinc, iron, and copper (Parr et al., 1991).

Calcium (Ca)

The second most abundant component in human breast milk is calcium, a vital mineral element. Calcium, in addition to being a vital component of bones, functions as a messenger in cell signalling pathways. It is essential for bone and tooth formation, blood clotting, and maintaining healthy

nerves and muscles. Growing infants between the ages of 0 and 6 months require 210 mg/day of calcium, while those between the ages of 7 and 12 months require 270 mg/day of calcium (Better Health Channel, 2023; US Institute of Medicine Standing Committee on the Scientific Evaluation on Dietary Reference Intakes, 1997).

Breast milk total calcium concentrations have been observed in certain studies to increase dramatically in the first 5 days of lactation (Kent et al., 1992), followed by a progressive drop during the length of lactation. In contrast, ionised calcium concentrations in breast milk remain consistent throughout lactation, implying a homeostasis similar to that found in blood (Kent et al., 1992).

Dietary intake alone is insufficient to explain differences in breast milk calcium concentrations between countries, according to studies (Dorea, 1999), which is consistent with the majority of studies that found no association between maternal dietary calcium intake and breast milk calcium concentrations (Feeley et al., 1983; Specker, 1984; Vaughan et al., 1979).Yet, in locations where calcium consumption is inadequate, dietary calcium may impact breast milk concentrations (Maru et al., 2013; Prentice & Barclay, 1991; Yoneyama et al., 1997).

Neither maternal status (Prentice & Barclay, 1991; Prentice et al., 1997; Ruz et al., 1982) nor dietary calcium or vitamin D interventions have been demonstrated to affect breast milk calcium concentrations (Jarjou et al., 2006; Nickkho-Amiry et al., 2008; Prentice et al., 1995; Prentice et al., 1998). Calcium contents in breast milk are reduced in nursing teenagers and women with iron deficiency anaemia (El-farrash et al., 2012; Lipsman et al., 1985;

Vitolo et al., 2004). Other factors, such as gestational age, sample methodologies (time of day, foremilk versus hindmilk, drip versus expression), parity, mother age, race, breastfeeding history, smoking, and oral contraceptive usage, are not related to breast milk calcium concentrations (Dorea, 1999; Feeley et al., 1983).

Sources of Calcium for Infants

While older infants acquire additional calcium from supplementary meals such cheese, yoghurt, some green leafy vegetables, fortified grain products, and tofu, younger new-borns primarily get calcium through breast milk or infant formula.

Calcium Deficiency

Lead poisoning and possibly increased susceptibility to the harmful effects of lead on the body are directly tied to calcium deficiency (Canfield & Jusko, 2008; Goyer & Cathryn, 1972).

Copper (Cu)

Copper as a trace mineral element, is an essential co-factor for enzymes involved in respiration at the cellular level, iron metabolism and connective tissue synthesis (Chen et al., 2022; Tapiero et al., 2003). Copper levels in breast milk have been shown in longitudinal studies to decline over time, at least for the first six months of breastfeeding (Kelleher & Lonnerdal, 2005). Longitudinal studies of copper concentrations in breast milk have found a decrease over time, at least for the first six month of lactation (Casey et al., 1989; Kelleher & Lonnerdal, 2005; Perrone et al., 1994; Rodriguez Rodriguez et al., 2000; Wasowicz et al., 2001).

Sources of Copper

Whereas majority of copper in serum (83 – 100%) is bound to ceruloplasmin (Salmenpera et al., 1986), ceruloplasmin carries only 20 – 25% of copper in breast milk (Lonnerdal et al., 1982). Research indicates that copper concentrations in breast milk are not associated with maternal status (Domellöf et al., 2004; Kelleher & Lonnerdal, 2005), dietary intake (Domellöf et al., 2004; Kelleher & Lonnerdal, 2005; Mahdavi et al., 2010; Rodriguez Rodriguez et al., 2000; Vuori, 1979) or supplémentation (Chierici et al., 1999). Furthermore, breast milk copper contents are unaffected by mother age, parity, smoking, iron supplementation, oral contraceptive usage, or infection (Orun et al., 2012; Silvestre et al., 2001; Silvestre et al., 2000), the concentrations of fore- and hindmilk are also the same (Dorea, 2000).

Breast milk copper concentrations are directly correlated with selenium concentrations (Perrone et al., 1994), and there is some evidence that an increase in soil selenium content may indirectly increase breast milk copper concentrations (Kantola & Vartiainen, 2001). Some of the sources of copper include shellfish, wheat-bran cereals, organ meats, seeds and nuts, chocolate and whole grain products (US NIH, 2022a).

Copper Deficiency

Copper is a component of many enzymes, including cytochrome oxidase, which play an important role in oxidative metabolism (Evans, 1973). Copper deficiency anaemia is caused in part by a shortage of copper-containing ferroxidases, such as caeruloplasmin, which are necessary for the oxidation of ferrous iron from the intestinal mucosa and storage in the reticulo-endothelial system (Doguer et al., 2018; Gulec & Collins, 2014). A

lack of copper-containing amine oxidases, which are essential for the cross-linking of elastin and collagen, can cause abnormalities in the walls of blood arteries, leading to mortality in animals from rupture of a major artery. The absence of these amine oxidases and ascorbic acid oxidase is most likely involved in the aetiology of the skeletal diseases that are common indications of copper deficiency. Tyrosinase is another copper-dependent enzyme; thus, copper deficit impairs melanin production (Hambidge, 1976)

Iron (Fe)

Iron is an essential trace mineral that plays a critical role in the formation of blood cells, including haemoglobin and myoglobin, as well as in the structure of various enzymes in the body. Infants require iron for growth and healthy blood cell development, as well as to prevent iron deficiency anaemia. The recommended daily intake of iron for infants between 0 and 6 months is 0.27 mg, while the recommended dietary allowance for infants between 7 and 12 months is 11 mg (US Institute of Medicine, 2001; US NIH, 2023).

For infants between the ages of 0 and 12 months, the safest consumption amount is 40 mg (US NIH, 2023). Breast milk, new-born formula, beef, liver, legumes, whole-grain foods, and dark green vegetables are just a few examples of the many foods that contain iron. The infant's iron status and the type of iron in the meal have an impact on how well the body can absorb iron from food. When iron storage are high, absorption is normally reduced, and it may rise when stocks are low (Dror & Allen, 2018).

Iron Deficiency (ID)

The efficient functioning of the muscle, brain, and red blood cells depends on iron. It can be lacking in the diet if people mostly eat staple foods with little meat or if they experience infections that result in blood loss. The most at risk for iron deficiency are children and mothers who are close to giving birth. Iron deficiency anaemia (IDA) in early childhood can lead to cognitive impairment in mid-childhood, as well as developmental delays and disability (WHO, 2009).

Iron deficiency can lead to various symptoms, such as anaemia, difficulty absorbing food, irritability, lack of appetite, pale skin, and fatigue. Research has also revealed that a lack of iron in infants and older children can be linked to behavioural issues that cannot be reversed, as well as abnormal brain functioning (Nokes et al., 1998). According to the WHO, iron deficiency affects almost 2 billion people worldwide and is the most prevalent dietary issue. Anaemia is thought to affect 600 million pre-schoolers and school-age children and approximately 469 million women of reproductive age. At least half of these instances are thought to be brought on by iron deficient anaemia (WHO, 2008).

Due to the strong demands for rapid growth and frequent iron deficiencies in diets, young children under the age of five are particularly vulnerable to iron deficiency anaemia (Dewey & Brown, 2003; US Institute of Medicine, 2001). Iron deficiency, whether or not it includes anaemia, can have significant negative impacts on the health of young children, such as higher risk of death during childbirth, slowed mental and physical growth, behavioural issues, decreased hearing and vision, and reduced physical

abilities (Algarín et al., 2003). Iron deficiency during early childhood can have permanent negative effects, such as poor academic achievement, reduced physical capabilities, and decreased productivity in adulthood (Haas & Brownlie, 2001; Iannotti et al., 2006; Lozoff et al., 1991, 2000). In low and middle-income countries, maternal short height and iron deficiency anaemia, which increases the likelihood of death during childbirth, are major factors in at least 18% of maternal fatalities (WHO, 2009).

The rates of anaemia have not significantly improved over the past 20 years, and maternal malnutrition increases the chance of infants being born with low weight, which raises the risk of death from infections or lack of oxygen in the new-borns (Ma et al., 2019; WHO/UNICEF, 2015). Additionally, anemia is linked to a higher risk of maternal death (Ramakrishnan & Yip, 2002). Worldwide, 56 million pregnant women (almost 50%) have anemia (WHO, 2013). Adolescent girls and women of reproductive age are especially at risk for iron insufficiency because they lose iron through monthly menstruation and because iron is frequently low in their diets (Pala & Dundar, 2008; Ramakrishnan & Yip, 2002).

Magnesium (Mg)

Magnesium is a type of mineral that contributes to over 300 metabolic processes and plays a structural role in bones (MedlinePlus, 2023a). Mobilization, the process of removing magnesium from the mother's bones and incorporating it into the milk supply, occurs during lactation. The average amount of magnesium found in breast milk is 31mg/L and most reported levels fall within a range of 20-40 mg/L (Dórea, 2000). Although there is variation among individuals, the magnesium levels in a woman's breast milk

tend to remain consistent throughout lactation. However, some studies have noted slight increases or decreases in magnesium levels during the first six months of lactation (Björklund et al., 2012; Dórea, 2000).

The magnesium levels in breast milk are not influenced by the amount of magnesium a mother consumes or takes as supplements, and they do not change due to the length of pregnancy, any metabolic disorders the mother may have, the number of times the mother has given birth, the mother's race, previous lactation history, smoking or oral contraceptive use (Dórea, 2000; Feeley et al., 1983).

Magnesium Deficiency

There are indications that lactating teenagers have lower levels of magnesium in their breast milk (Hunt & Schofield, 1969). This is due to the fact that a large amount of the magnesium in breast milk is bonded to proteins and low molecular weight components (Fransson & Lonnerdal, 1983) with minimal variations in the concentrations present in foremilk and hindmilk (Gillies & Neill, 1985; Neville et al., 1984).

Phosphorus (P)

An essential mineral called phosphorus is a part of nucleic acids and cell membranes and is involved in a number of biological functions including bone development, cell communication, energy production, and acid-base balance maintenance. Although the secretion of calcium and phosphorus in milk is regulated separately, both full-term and premature new-borns' breast milk has a median calcium to phosphorous ratio of 1.7 (Prentice et al., 1995). Similar to calcium, phosphorus levels are highest in early transitional milk and

steadily decline as breastfeeding progresses (Feeley et al., 1983; Harzer et al., 1986).

When compared to milk from other mammals, human milk contains much less phosphorus. This could be a way to prevent the growth of harmful bacteria in faeces, protect the kidneys of new-borns from disruptions in calcium metabolism, or to avoid metabolic acidosis (Manz, 1992). Phosphorus levels in breast milk are strictly controlled (Björklund et al., 2012) and does not seem to be affected by factors such as the mother's intake, age, number of pregnancies, race, breastfeeding history, methods of sampling, smoking, or use of oral contraceptives (Feeley et al., 1983; Harzer et al., 1986). When there is maternal familial hypophosphatemia, only (Jonas & Domiguez, 1989) or hyperparathyroidism (Hanukoglu et al., 1988) are breast milk phosphorus concentrations significantly decreased.

Sodium (Na)

Sodium is a sort of mineral that keeps the body's water balance in check, regulates blood flow, and keeps cells and other tissues functioning properly. The suggested minimum amounts of sodium that infants should be between 100 to 200 mg per day.

Sources of Sodium for Infants

It is mainly obtained from breast milk or regular, diluted new-born formula. Although the salt content of breast milk is relatively low, it is nevertheless adequate for growth.

Effects of Sodium Levels on Infants and Mothers

According to several researches, postpartum sadness and anxiety are strongly correlated with excessive salt intake through breast milk and a high

sodium to potassium ratio. High sodium levels in breast milk may be a sign of breastfeeding failure and are linked to higher anxiety and depressive symptoms in moms of infants without hypernatremic dehydration(Demirgoren et al., 2017).

Potassium (K)

Every cell, tissue, and organ in the human body needs potassium to function normally. It is necessary for appropriate digestion and muscular function and has a key role in the contraction of both skeletal and smooth muscles, which makes it essential for the health of the heart (Pohl et al., 2013). Magnesium and potassium are essential nutrients for growth and metabolism. For several enzymes involved in protein synthesis, fatty acid metabolism, glycogen production, glycolysis, and bone formation, it serves as a cofactor (Jong et al., 2022). Potassium plays a vital role in maintaining balance within human cells and is present in high concentrations. Human milk typically contains 50 mg of potassium per 100 mL. During breastfeeding, the body's need for potassium increases due to the secretion of the mineral in breast milk. Typically, breast milk from healthy women within the first four months postpartum contains between 12.8 and 15.0 mmol/l of potassium, with levels being highest in the first week and decreasing rapidly thereafter (Berry et al., 2013; Pohl et al., 2013).

Sulphur (S)

Sulphur is the third most common mineral in terms of percentage of total body weight and is a frequent macro-mineral identified in breast milk. Methionine, cysteine, cystine, homocysteine, homocystine, and taurine are

some of the amino acids that include sulphur (Parcell, 2002). Sulphur levels in human breast milk are a subject on which there is very little knowledge.

Selenium (Se)

The body uses selenium for a variety of crucial purposes, including as defending against free radicals, bolstering the immune system, maintaining thyroid and brain function, and improving cardiovascular health (Handy et al., 2021; Skroder et al., 2015). Selenium is a critical component of several selenium-containing proteins, such as peroxidases and deiodinases, which are powerful antioxidants and play a role in the metabolism of thyroid hormones. These proteins are essential for healthy development during early life (Skroder et al., 2015). As a part of the antioxidant glutathione peroxidase and in smaller concentrations as selenocystamine, selenocystine, and selenomethionine, selenium can be detected in breast milk (Michalke & Schramel, 1997). Infants are born with a certain amount of selenium stored in their bodies, but they also rely on the selenium present in their mother's milk. The concentration of selenium in colostrum is high, but it decreases as lactation continues. (Higashi et al., 1983; Tamarp & Tsuji, 1995; Wasowicz et al., 2001), parallel with the trend for the milk proteins into which selenium is incorporated.

The amount of selenium in our diet is determined by the selenium content of the soil where the food is grown, which can be referred to as organic selenium.

Terry et al. (2000), explains the considerable variation in breast milk selenium concentrations across geographical regions and is a significant factor in determining breast milk selenium concentrations (Dorea, 2002; Parr et al., 1991). There is a strong, albeit weak, association between serum or plasma

and breast milk selenium concentrations, according to several research (Carratu et al., 2003; Flax et al., 2015; Kumpulainen et al., 1985; Levander et al., 1987; Mannan & Picciano, 1987; Schramel et al., 1988). However, others have not found a significant correlation (Bianchi et al., 1999; Higashi et al., 1983; Micetic-Turk et al., 2000).

An approach to raise selenium levels in breast milk is by treating the soil or increasing the mother's diet with selenomethionine or sodium selenate, which is an effective preventative measure (Dylewski & Picciano, 2002; Flax et al., 2014). Breast milk selenium contents are unaffected by maternal age, BMI, iron supplementation, or smoking (Arnaud et al., 1993; Bianchi et al., 1999; Mandic et al., 1995). An adverse relationship was discovered between mother parity and breast milk selenium in late lactation in one study (Funk et al., 1990), but not in others (Arnaud et al., 1993; Mandic et al., 1995). Because milk fat includes 5% selenium, the variation in selenium concentrations found by some researchers but not others could be a statistical artefact due to milk selenium variability (Dorea, 2002).

Deficiency of Selenium

Conspicuous selenium deficiency is uncommon (Rayman, 2012). Individuals with extremely low levels of selenium in their blood may experience stunted growth, increased vulnerability to illnesses, and heart muscle disease (Combs, 2001). Individuals living with HIV often have low levels of Selenium, and these levels tend to decrease as the HIV infection becomes more severe. (FAO/WHO, 2004b).

The amount of selenium in people's bodies varies globally and is largely influenced by their diet, which is correlated with the selenium content

of the soil where the food is grown (Sillanpää & Jansson, 1992). Malawi is one of the places where the selenium content in the soil and the main food sources is lower than average (Chilimba et al., 2011). The amount of selenium consumed and found in the blood of adults in Malawi is often low due to limited intake (Eick et al., 2009; Gibson et al., 2011). HIV-positive women living in Malawi are likely to experience a dual difficulty in both having an inadequate intake of selenium and requiring more selenium than usual (WHO, 2004). Having an increased need for micronutrients during pregnancy and breastfeeding can make the burden of malnutrition more extreme (US Institute of Medicine, 2000). Mothers in countries like Finland and Poland, where the soil has low selenium concentrations, have been given supplements with both organic and inorganic selenium in order to boost the level of selenium in their blood and milk, while also increasing the amount of selenium their breastfed babies are receiving (Kumpulainen et al., 1985; Trafikowska et al., 1998). Adding inorganic selenium (in the form of sodium selenite) usually has a lesser effect than consuming organic selenium (selenomethionine) (Xia et al., 2005)

Zinc (Zn)

Zinc has been acknowledged for its role as a trace mineral for over a century, but it was not until the 1960s that people started to understand the effects of zinc deficiencies. It is very important for proper development, both pre- and post-natal, and it is involved in many metabolic processes. It aids in taste perception, immunity, tissue growth and maintenance, protein synthesis, wound healing, and tissue repair. Twenty enzymes that require zinc to

function have been identified, and they are associated with nucleic acid metabolism and protein synthesis (Hambidge, 1976).

Infants between 0 – 6 months should consume an average of 2 milligrams of zinc per day, and for those between 7 – 12 months, the recommended amount is 3 milligrams per day. The maximum safe amount for infants of both age group is 4 and 5 milligrams respectively (US Institute of Medicine, 2001; US NIH, 2022b).

Sources of Zinc

Breastfeeding, infant formula, and a variety of animal and plant-based meals, such as chicken, egg yolks, cheese, liver, yogurt, meat, legumes, cereals, whole-grain breads, and other enriched goods, can all provide zinc to infants. Breast milk is a suitable source of zinc for the first half a year, but not enough for babies who are older (US Institute of Medicine, 2001). Starting at 6 months, it is important to ensure that infants receive zinc from food sources such as meats and fortified infant cereal, alongside breast milk or infant formula, to meet their nutrient needs (US Institute of Medicine, 2001).

Zinc Deficiency in Infants

Babies who lack zinc suffer from inhibited physical growth, a weakened immune system, an increased risk of death from diarrhoea and respiratory illnesses, and greater general susceptibility to disease (Domellöf et al., 2004). The amount of zinc in breast milk significantly decreases from the first milk to the milk in between colostrum and mature milk (Silvestre et al., 2001), followed by a gradual decline throughout lactation (Casey et al., 1989). According to estimates, an infant receives an average of 4 mg of zinc per day from breast milk in colostrum, 1.75 mg at one month, and 0.7 mg at six

months(Brown et al., 2009). Breast milk zinc concentrations are refractory to maternal status (Dewey et al., 1984), intake (Akamori et al., 2009; Hannan et al., 2009; Kelleher & Lonnerdal, 2005; Mahdavi et al., 2010; Vuori, 1979), and supplémentation (Chierici et al., 1999; Silvestre et al., 2000). Older infants' breast milk zinc levels have been found to be lower (Rodriguez Rodriguez et al., 2000; Silvestre et al., 2000), multiparous (Kelleher & Lonnerdal, 2005), and iron-deficient (El-farrash et al., 2012) women. No link has been established between the zinc levels of breast milk and whether the mother smoked, took iron or multivitamins or minerals, or the length of the pregnancy (Orun et al., 2012; Silvestre et al., 2000).

Having too little zinc in the body can lead to acrodermatitis enteropathica, poor immune system performance, diarrhoea, and stunted growth. To measure the amount of zinc in a person, doctors often look at their serum zinc levels; however, this can be impacted by illnesses, stress, and overall growth. Therefore, while serum zinc levels are useful in determining zinc levels in healthy infants in general, they are not always the best way to measure it in individuals (Butte et al., 2002).

Persistent Organic Pollutants (POPs)

Besides heavy metals, another class of chemicals of great environmental and health concern to mankind are Persistent Organic Pollutants (POPs). Unlike other conventional pollutants like toxic heavy metals which are confined to their sources, POPs can travel over a long-range distance in the atmosphere, a characteristic that enhances their widespread distribution throughout the globe, even finding their way into places where they had never been manufactured nor used (El-Shahawi et al., 2010; US EPA,

2009). Studies have shown that traces of POPs (persistent organic pollutants) can be found in breast milk, fats of fish and animals worldwide, with particularly high rates in the Arctic regions of the north and south (WFPHA, 2000). POPs are chemically stable and are resistant to virtually all forms of natural degradation in the environment and are linked with adverse effects on health and the environment (WHO, 2003a).

A rising body of knowledge suggests that POPs have spread throughout the global environment, endangering both terrestrial and aquatic ecosystems and posing a hazard to human health. That practically all living things on Earth today have detectable levels of POPs in their tissues is a shocking realization. It appears that trace amounts of toxic chemicals from a group called Persistent Organic Pollutants (POPs) can be found in a variety of food sources - from baked goods to produce, meat, poultry and dairy (El-Shahawi et al., 2010; US EPA, 2009). A single food item can have five or more POPs present, the most common being the pesticides DDT and Dieldrin (Schafer & Kegley, 2002).

Dieldrin is administered in amounts that are significantly higher than those suggested by the US Environmental Protection Agency and US Agency for Toxic Substances and Disease Control. Moreover, research suggests that hazardous pollutants known as POPs are present in human blood and breast milk around the globe (Solomon & Weiss, 2002)

What Are Persistent Organic Pollutants?

POPs are synthetic organic chemicals that were commonly produced during the 20th century, primarily used as pesticides and industrial chemicals. While they initially brought benefits such as increased crop yields and pest

control, it was later discovered that they caused harm to wildlife and human health through contamination. There are worries about the effects of long-term low-level exposure in humans, particularly in foetuses, infants, and children who may be more vulnerable to harm due to the rapid growth and development of their organ systems. Studies in laboratory animals have demonstrated the impact of POPs on various organs (WHO, 2010b).

The effects of POPs on health can be difficult to detect, and exposure during key stages of children's development may not have obvious symptoms until later in life. Measuring POPs exposure through analysing mother's milk is a non-invasive way to obtain information about exposure to these chemicals.

The 12 POPs (also known as the "Dirty Dozen") initially earmarked for early global action are all incidentally, organo-halogen compounds. They include aldrin and dieldrin, endrin, chlordane, DDT, heptachlor, mirex, polychlorinated dibenzodioxins (PCDD) polychlorinated dibenzofurans (PCDF), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), and toxaphene. The POPs are made up of 8 pesticides, two industrial chemicals and two inadvertently produced/released industrial by-products. Aldrin, endrin, dieldrin, mirex, heptachlor, DDT, Chlordane and toxaphene are pesticides, PCBs and HCB are industrial chemicals while PCDD and PCDF are inadvertently produced/released by-products (US EPA, 2009).

General Characteristics of POPs

The physical characteristics that make POPs desirable for a number purposes also make them agents of environmental health problems. All POPs:

- Can persist in the environment for many years;

- Have the tendency to concentrate in fatty tissues and bioaccumulate in ecosystems as they move up the food chain;
- Have long range transport, traveling long distances in global air and water currents, generally moving from tropical and temperate regions to concentrate in the northern latitudes; and (a phenomenon known as grasshopper effect) and
- Have been linked with serious health effects in humans and other living organisms, even at very low exposures (Schafer & Kegley, 2002; US EPA, 2009).

According to studies, POPs have recently spread worldwide and are now found in the environment on a global scale, endangering land and water ecosystems and posing a threat to human health. All living things on the world have POPs in their tissues (Schafer & Kegley, 2002). The levels of POPs found in sea mammals are so high that their bodies would be considered hazardous waste according to US regulations (Schafer & Kegley, 2002) , POPs have been well-documented to be present in breast milk and human blood (Bhuiyan et al., 2009). According to research, even minute quantities of POP exposure during crucial developmental stages, notably in the womb, can result in long-term harm. The consequences of these exposures might not become apparent for a very long time, but they might be noticeable in the exposed parents' offspring.

POPs are still made, used, and stored in many nations despite their negative effects. Even when there are laws or rules in place to regulate them, their enforcement is sometimes insufficient, and as a result, these controls cannot shield individuals from exposure to POPs that have migrated from

other regions where they are still in use. These chemicals, which are still present in the environment and are still being produced in certain nations despite being outlawed in many others, contaminate the world's food supply (Schafer & Kegley, 2002). Additionally, their long-range transport means that they can be found in places where they have never been produced or used before

Modes of Exposure to POPs

POPs are extensively present, and many people are exposed to them. Humans are exposed to these chemicals through a variety of channels, mostly through food, air, and everyday items, according to a WHO report (WHO, 2010b). To enhance the properties of products like flame retardants and surfactants, these compounds are added. POPs are therefore present in practically every detectable concentration on Earth. Organochlorine pesticides like DDT, industrial chemicals like PCBs, and by-products of industrial processes, notably polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), often known as dioxins, are the most frequently encountered POPs. POPs bioaccumulate in organisms and undergo biomagnification along the food chain, with the largest concentrations being found in creatures at the top of the food chain. As a result, the human body contains background amounts of POPs (UNEP, 2023; WHO, 2020).

Health Effects of Exposure to POPs

POP exposure can have a wide range of negative health impacts on the body, including cancer, endocrine disruption, immune system, central nervous system, and reproductive system damage, as well as developmental problems, chronic diseases, and cancer. It may even cause death in some circumstances

(WHO, 2010b). Many POPs have been discovered to disrupt the proper functioning of the endocrine system in both humans and animals.

According to research, more and more POPs (lipophilic persistent organic pollutants) have been discovered in human breast milk over time. POP remnants are still present in significant concentrations in human breast milk even though they have been banned for many years (Tanabe & Kunisue, 2007). It's intriguing that a mother's exposure to environmental toxins is partially mitigated by human milk. Given the high fat content of breast milk, prolonged maternal exposure to persistent lipophilic pollutants (POPs) may result in a slow accumulation of these chemicals in the mother's adipose tissue (Barrett, 2013; US EPA, 2009). After being released from maternal adipose reserves, these chemicals are then excreted into breast milk during milk production. In comparison to maternal blood or cord blood samples, it is expected that breast milk will include higher organohalogen lipophilic pollutants. The existence of POPs in human breast milk has raised concerns, according to a study of the literature, because these lipophilic compounds are easily absorbed by new-borns (Massart et al., 2008).

Persistent Organic Pollutants in Human Breast Milk

Numerous studies have linked the organochlorine cyclodiene family to the pesticides and insects detected in human breast milk (Massart et al., 2008).

Aldrin and Dieldrin

In a review Massart et al. (2008) indicated that pesticides like Aldrin and Dieldrin were frequently utilized in the 1970s. They are a class of chlorinated cyclodiene insecticides used as preventative measures and treatments for wood against ant, termite, and anobiid borer infestation.

Dieldrin is produced when aldrin breaks down in the body and environment. Dieldrin does not readily evaporate or attach to soil particles and is quite persistent in the environment. Aldrin and Dieldrin leftovers are directly absorbed by plants from the soil. Animals, including humans, store dieldrin in their fat, and it is expelled very slowly (ATSDR, 1993, 2002).

Aldrin and Dieldrin were strongly restricted or outright prohibited in more than 70 countries as of 1995 (WHO, 1989). At least 28 nations have had studies done on the presence of Aldrin and Dieldrin in breast milk, and most of those nations identified the compounds in more than 99 percent of the samples (WHO, 1998). The level of dieldrin in breast milk is typically reported to be 6 times higher than the level in blood because of its lipophilic nature and the high lipid concentration of human milk (WHO, 1998).

The prevalence of detection remained high as countries restricted and outlawed the use of Dieldrin, although the amounts in breast milk considerably fell by up to 10 times in the years after the restriction (Calabrese, 1982; Furst et al., 1994; Khanjani & Sim, 2006; Noren & Meironyt, 2000). On the other hand, in countries such as Kenya or the southern United States where Dieldrin was heavily used, the levels of Dieldrin in breast milk were often significantly higher (Massart et al., 2008; Rogan et al., 1980).

Chlordane

Chlordane is a common organochlorine cyclodiene pesticide composed of over 26 distinct compounds. It was used as an agricultural pesticide on lawns, gardens, and to control termites, according to the literature (US EPA, 1976), but it is now illegal in at least 47 countries and severely restricted in another 14 (National Center for Biotechnology Information, 2024; US EPA,

1976) Similar to many persistent organic pollutants, chlordane and its breakdown products have been found in soil, fish, bird, and mammal tissues up to 20 years after initial treatment (Savage et al., 1981). A total of 16 nations have studied the presence of chlordane metabolites in breast milk (Isobe et al., 2009; Khanjani & Sim, 2006; Noren & Meironyt, 2000; Savage et al., 1981; Taguchi & Yakushiji, 1988; Wickstrom, Pyysalo, & Siimes, 1983).

Data from places where chlordane was used revealed a significant change in breast milk levels, according to usage trends. For instance, in the 1970s in the United States, nursing mothers in the southern states had an average of 113 ng/g lipid (range from 108-118 ng/g) of chlordane in their milk as opposed to 79 ng/g (ranging from 76-82 ng/g) in women residing in other regions (US EPA, 1976). This disparity was most likely caused by agricultural use and more frequent termite control at home (Savage et al., 1981).

In Japan in the 1980s, women who lived in homes that utilised chlordane for termite control had 4.4 times more chlordane in their breast milk than women who did not live in chlordane-treated homes (Taguchi & Yakushiji, 1988). Slight amounts of chlordane have been detected in breast milk from Indonesia and Australia, but the finding of slightly growing levels of chlordane compounds in Japanese primiparae indicates that levels in Japanese breast milk have not decreased since 1998 (Isobe et al., 2009; Khanjani & Sim, 2006; Savage et al., 1981; Taguchi & Yakushiji, 1988; Wickstrom et al., 1983). The occurrence of chlordane residues, however, is not limited to the locations where the chemical was used. For example, despite the fact that the chemical was never used in Finland and was strictly restricted in neighbouring countries, chlordane was discovered in the breast milk of

Finnish women in the mid-1980s (Wickstrom et al., 1983). Bioaccumulation has been linked to exposure in Baltic fish (Wickstrom et al., 1983). The average level of chlordane metabolites identified in breast milk in the decades following the majority of Europe's ban on chemical use has been reducing, despite the fact that chlordane is still present in the environment (Noren, 1993; Noren & Meironyt, 2000). This evidence comes from Sweden.

Dichlorodiphenyltrichloroethane (DDT)

DDT is an organochlorine insecticide that was widely employed in agricultural settings and public health programmes to eradicate malaria (Lpez-carrillo et al., 1996). DDE, the major DDT metabolite, has a half-life of 6 years, whereas DDT has a half-life of about 4 years (Noren & Meironyt, 2000).

As a result, DDT and its by-products can persist in soil and sediments for more than 15 years and have been reported to accumulate in animal tissue via the bioaccumulation process (Massart et al., 2005). Ulrich and colleagues (Ulrich et al., 2000) revealed that organochlorine pesticide concentrations in diverse human populations that had not been exposed to these chemicals were often only 2.7 to 120 times lower than the quantity of o,p'-DDT known to produce hormonal abnormalities in mice. o,p'-DDT concentrations in blood were found to be as high as 32 ng/mL in Israel (Toppari et al., 1996), approximately double the threshold estrogenic blood level of 18 ng/mL recorded in live mouse investigations. Yet, because DDE and DDT bind to fat, their concentrations in breast milk are sometimes six to seven times higher than in the mother's blood (Massart et al., 2005) .

DDT and DDE are mostly absorbed by humans through the food chain, where they are retained in the fat of fish, dairy, and meat products (Hovinga, Sowers, & Humphrey, 1993; Lpez-carrillo et al., 1996). DDT exposure is generally associated with PBC pollution in the environment. Organochlorines are obtained by fish from fresh water body sediments; for example, high amounts of DDT and DDE have been repeatedly identified in fish captured in the Great Lakes. (Hovinga et al., 1993). Moreover, DDT, DDE, and PCBs have been found in cattle, poultry, eggs, and dairy products as a result of environmental exposure (Solomon & Weiss, 2002). A population-based study in Germany discovered that serum DDT and PCB levels were positively linked with beef, lamb, and saltwater fish diet (Moysich et al., 2002). Organochlorine residues have also been discovered in fruits, vegetables, and cereals; however, the quantities of organochlorine in food vary greatly depending on the source. Food grown at houses with high soil content of DDT and PCBs could have higher levels of these compounds than fruits and vegetables purchased at a shop (Laden et al., 1999). DDT was banned for all usage in 49 countries as of 1995, with the exception of vector control in another 23 (ATSDR, 2022a).

The average level of DDT in breast milk varies greatly between countries. Following DDT restrictions and bans in some countries (in the mid-1970s), average breast milk levels decreased significantly in many countries (Chao et al., 2006; Furst et al., 1994; Jaraczewska et al., 2006; Noren & Meironyt, 2000; Schade & Heinzow, 1998; Wickstrom et al., 1983), but not all (Subramanian et al., 2007; Tanabe & Kunisue, 2007). Similarly, other Asian researchers (Sudaryanto et al., 2005; Yu et al., 2003) found accumulated

DDT/DDE in breast milk that was close to or even greater than the Health Canada acceptable daily consumption standards (Oostdam et al., 2005).

Endrin

A common application for the insecticide endrin is on field crops like cotton and cereals. Additionally, it has been used as a rodenticide to get rid of mice and voles. Animals quickly break it down, and unlike other compounds of a similar kind, it does not build up as much fat. Through volatilization and soil runoff, it can contaminate surface water and be released into the atmosphere. Endrin's half-life in soil can range from 2 to 12 years, depending on the environment (Honeycutt & Jones, 2014). It can bioconcentrate in organisms due to its persistence and high partition coefficient. Endrin's chemical characteristics, such as its poor solubility in water, high environmental stability, and semi-volatility, facilitate its long-distance transit, and it has been found in freshwater from the Arctic (ATSDR, 2021a). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) indicated a tolerable daily intake of 0.0002 mg/kg body weight (WHO, 2021), however current intake is typically below this level (JMPR - 1997).

Heptachlor

Heptachlor is an organochlorine cyclodiene pesticide that has been used on seed grains and food crops to control termites and as an insecticide. The major metabolite of heptachlor, heptachlor epoxide, is extremely persistent and can be identified 14-16 years after administration (ATSDR, 2007d; WHO, 1984). Heptachlor epoxide can be taken up directly by plants from the soil and bioaccumulates in animals. Heptachlor has been prohibited or restricted in over 60 countries; nevertheless, some of these countries

continue to allow its usage for termite and other pest control, while many poor countries continue to use it for agricultural purposes (IARC, 1991; WHO, 1984). Despite being outlawed in the United States in 1988, US customs data revealed that considerable amounts of heptachlor were exported until 1994 (ATSDR, 2007d). As governments imposed restrictions and prohibitions on heptachlor, quantities detected in breast milk fell, typically by more than tenfold (Chao et al., 2006).

Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are synthetic chemicals that have been widely used since 1930 for various industrial purposes, such as in electrical equipment, as heat transfer fluids, as additives in paint and plastics, and in carbonless copy paper (ATSDR, 2011a). A class of synthetic compounds known as PCBs have been employed in several industrial applications, including electrical equipment and as product additives. They are present in the environment and can build up in organic materials like biological tissues, sediments, and soils. Exposure to PCBs has been associated to a number of negative health impacts, such as altered liver function, skin rashes and acne, as well as more serious conditions like hyperkeratosis and skin darkening (Guo et al., 1999; WHO, 2003b). Large populations of individuals have occasionally been exposed to PCBs as a result of instances involving contaminated rice oil, which can cause symptoms like eye and skin irritation, exhaustion, and nausea (ATSDR, 2011a).

Children exposed to PCBs in the womb, specifically in the Taiwan incident, have been found to have various health issues such as hyperpigmentation, deformities, and cognitive and behavioural problems up

until 7 years of age Chen et al., 1994; Guo et al., 2004). However, by age 12, these children's development seemed to catch up with their peers. Children born 7-12 years after exposure also had mild delays in development. These effects are believed to be caused by the persistence of PCBs in the body and prenatal exposure, which is consistent with studies on children exposed to PCBs through their mothers' consumption of contaminated fish (Guo et al., 1999, 2004). Additionally, those exposed in the Yucheng incident had weakened immunity and various infections (Guo et al., 1999). Examining their blood also revealed decreased immunity markers and an abnormal response to certain stimuli. After 3 years, some of these effects had subsided, but there were also reports of increased cancer deaths among workers in the electrical capacitor manufacturing industry, specifically in males and a higher incidence of blood and gastrointestinal cancers (ATSDR, 2011a ; Bertazzi et al., 1987; Guo et al., 2004).

The fact that PCBs persist in the environment and have a high tendency to stick to fats in organisms, allows them to accumulate in organisms over time. Studies have shown that fish exposed to PCBs through their diet tend to have lower concentrations of PCBs in their bodies than those exposed to PCBs in the water, indicating that fish absorb PCBs directly from the water rather than through their food (ATSDR, 2011a). The primary way that people are exposed to PCBs is through their food, particularly through the consumption of fish (ATSDR, 2011a).

PCBs are a type of persistent organic pollutant that belong to a larger group of chemicals known as organohalogens, which also includes insecticides, dioxins, furans, brominated biphenyls, and ethers. These

chemicals have similar toxic effects and can be found in various forms, with PCBs having 209 different congeners. The main source of exposure to these chemicals is through food, particularly fish (Needham & Wang, 2002). All of the congeners of these chemical families, including PCBs, dioxins, and related compounds, have the potential to be widespread and persistent environmental pollutants. Due to their lipophilic and hydrophobic nature, these chemicals tend to accumulate in soil and sediment, increase in concentration as they move up the food chain in aquatic animals, and increase in concentration even more as they move up higher levels of the food chain (Massart et al., 2006).

Humans consume meat, dairy, fish, and plants, all of which are sources of food for other animals, which can lead to the accumulation of certain persistent environmental pollutants, such as PCBs, dioxins and related compounds. These compounds tend to concentrate in soil and sediment, and can also bioaccumulate in aquatic animals, and biomagnify up the food chain.

However, not all congeners of these compounds have the ability to bioaccumulate in the food chain and not all will be found in human fatty tissue. For example, only a small number of PCDDs and PCDFs can be stored in human fatty tissue (Needham & Wang, 2002). The presence of dioxin-like chemicals, including 12 specific PCBs, in human tissues is closely linked to the levels found in the mother's body. The concentration of these chemicals in breast milk or cord blood can vary based on the amount of exposure the mother has had (Massart et al., 2005). The accumulation of PCBs and dioxin-like compounds in the human body over an extended period, primarily in fat tissue, in addition to the slow rate of metabolism and excretion, results in a body burden of these chemicals (Massart et al., 2005). The persistence of

PCBs, PCDDs and PCDFs in the body is high, with a half-life of up to 15 years, due to the accumulation in fat tissue and slow metabolic degradation and excretion rate (Flesch-Janys, 1996; Wolff et al., 1992). Most of PCB monitoring studies were performed in Europe (Cerná et al., 2012; Dewailly et al., 1993; Noren & Meironyt, 2000; Polder et al., 2008; Wilhelm et al., 2007). Due to the lack of standardized measurement protocols, it is challenging to determine the changes in PCB contamination levels in breast milk over time.

Yet, other research imply that pollution levels may have dropped modestly during the last 25 years (Longnecker et al., 1997). In other countries, such as Sweden, the Czech Republic, and Germany, where data collection procedures have remained consistent over time, there has been a noticeable decline in PCB levels over the last 25 years (Cerná et al., 2012; Noren & Meironyt, 2000; Wilhelm et al., 2007). According to research, the amounts of PCBs discovered in breast milk are much greater than those found in blood, with some studies indicating a 4-10 times difference (Longnecker et al., 1997). In one study (Patandin et al., 1999), for example, children aged 1 to 5 received 6.3-6.5 pg TEQ/kg body weight and breast-fed babies received 112-118 pg TEQ/kg body weight. Maternal PCB and dioxin-like concentrations are positively associated to maternal age and adversely related to prior breastfeeding duration (Albers et al., 1996; Massart et al., 2005). The majority of human exposure to PCBs and related chemicals comes from food, with oral intake accounting for more than 90% of daily exposure, while other sources such as water, air, and soil contribute less than 10% (Theelen et al., 1993).

The role of diet in breast milk pollution has been studied. Fish intake in the Great Lakes region of the United States has been linked to a higher body

burden of PCBs (Falk et al., 1999). Inuit and fishery groups in Canada have higher PCB levels in breast milk than urban ones (Massart et al., 2008). Consumption of fish and marine mammals is not the sole source of worry in the diet. Breast milk levels in the Czech Republic were higher than in nearby areas and countries due to the use of PCB-containing paint in grain silos (Cerná et al., 2012). Furthermore, throughout the lactation phase, short-term dietary regimens with low dioxin doses exhibited no reduction in breast milk dioxin concentrations (Pluim et al., 1994).

In a study conducted in Accra, Ghana Asamoah et al. (2018), researchers examined the levels of PCBs in the breast milk of some Ghanaian women in suspected hotspot (Agbogbloshie) and relatively non-hotspot (Kwabenya) areas to determine whether the levels of PCBs in mothers milk pose any health risk to breastfed infants. A total of 128 individual human breast milk samples were collected from primiparae and multiparae (Agbogbloshie - 105 and Kwabenya - 23) and analysed using GC - MS/MS. A total of 3.64 ng/g lipid weight and LOD-29.20 ng/g lipid weight were the mean total values and range of 7PCBs, respectively. Agbogbloshie (a hotspot location) and Kwabenya (a non-hotspot location) had mean levels of 4.43 ng/g lipid wt. and 0.03 ng/g lipid wt., respectively (Asamoah et al., 2018). They discovered that PCB-28 contributed the most PCBs in the milk samples (29.5%), whereas PCB-101 contributed the least (1.74%). The estimated daily consumption of PCBs and total PCB concentrations in this study were lower than in prior studies conducted around the world. Based on the estimated hazard quotient and the Health Canada suggested threshold level of 1 g/kg bw/day, baby health was not projected to be jeopardised. Taking the Agency for Toxic Substances and

Disease Registry's minimum allowable quantity of 0.03 g/kg bw/day into account.

Polybrominated diphenyl ethers (PBDEs) are common flame retardants. They are utilised in computer and television plastics, as well as building materials, furniture, and textiles (Meironyte et al., 1999). As with many POPs, the principal route of human exposure is through food. Tens of thousands of different food samples have been tested for contaminants, including PCBs, in several countries throughout the years. The vast majority of samples are derived from milk, meat, and seafood. Food becomes contaminated with PCBs in three ways uptake from the environment by fish, birds, livestock (via food-chain), and also into crops;

- migration from packaging materials into food (around 1 mg/kg, but in some cases up to 10 mg/kg);
- direct contamination of foodstuff or animal feed as the result of an industrial accident or incident.

The levels of PCBs found in different foodstuff are:

- animal fat: 20 to 240 µg/kg
- cow's milk: 5 to 200 µg/kg
- butter: 30 to 80 µg/kg
- fish: 10 to 500 µg/kg, on a fat basis. Certain fish species (eel) and fish products (fish liver and fish oils) may contain much higher levels, up to 10 mg PCBs/kg •vegetables, cereals, fruits, and a number of other products: <10 µg/kg

Large fish, shellfish, marine mammals, meat, milk, and other dairy products are the main PCB-related food sources of concern. The median values

observed in fish across a number of nations are in the range of 100 g/kg (on a fat basis). However, it seems that PCB levels in fish are gradually declining (Visha et al., 2018; Webster & Fryer, 2022).

Per-and Polyfluoroalkyl Substances (PFAS)

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) represent a category of synthetic organic chemical contaminants that have garnered increasing attention due to their potential adverse impacts. Existing research underscores the contamination of human breast milk by PFAS, raising concerns regarding potential deleterious health effects in children.

Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)

Perfluorooctanoic acid perfluorocaprylic acid; PFOA (IUPAC name: 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoroactanoic acid; molecular: $C_8HF_{15}O_2$; molar mass 414.1 g/mol) and perfluorooctanesulfonic acid / heptadecafluorooctane sulfonic acid; PFOS (IUPAC name: 1,1,2,2,3,3,4,4,5,5, 6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonic acid; molecular formula: $C_8F_{17}SO_3$; molar mass 500.1 g/mol) are chemical contaminants of emerging concern. They are synthetic fluorinated organic compounds that are part of a larger group of chemicals referred to as perfluoroalkyl substances, PFAS (US EPA, 2017). The PFAS group of chemical compounds is made up of two subgroups namely, perfluoroalkyl substances and polyfluoroalkyl substances.

PFOS and PFOA are perfluoroalkyl substances i.e., chemical compounds where all the hydrogen atoms on all the carbon atoms (apart from the carbon atoms connected with the functional groups) have been switched out for fluorine atoms. Polyfluoroalkyl substances, on the other hand, are compounds

for which hydrogens on the carbon atoms have been partially replaced by fluorine atoms.

Though PFOS and PFOA are essentially synthetic organic chemicals, nevertheless, they could also be formed by environmental degradation or by metabolism in larger organisms from a large group of related PFAS or precursor compounds (ATSDR, 2018). For instance, rainbow trout transform perfluorinated acids to PFOS (CRC CARE, 2013).Through PFAS-containing product use and disposal as well as industrial processing, these substances have been discharged into the environment (Liu & Mejia, 2013). PFOS and PFOA are the two perfluoroalkyls made in the largest amount in the United States of America. To decrease releases and the amount of toxic compounds in their products, businesses have either stopped production or started modifying manufacturing procedures. Many perfluoroalkyls are being replaced with alternative chemicals in some facilities (ATSDR, 2015).

Physical and Chemical Properties of PFAS

PFOS is a white powdered potassium salt at room temperature and atmospheric pressure with a boiling point range of 258 – 260 ℃, organic carbon partition coefficient, Koc = 2.57 and water solubility of 680 mg/L at 25 ℃ (ATSDR, 2015). PFOA is also a white powder or waxy white solid with a boiling and melting points of 192 ℃ and 54 ℃ respectively, organic carbon partition coefficient, Koc = 2.06 and water solubility of 9.5 x 10^3 mg/L at 25 ℃ (ATSDR, 2015). Chemically, PFOS and PFOA are relatively inert substances. They can withstand high temperatures, lower surface tension, and are resistant to grease and water. These compounds are frequently used to treat the surfaces of carpets, textiles, leather, paper, and cardboard due to their

properties. Additionally, they serve as a surfactant in firefighting foams, as a mist suppressor during chrome plating, and in the mining and oil industries (ATSDR, 2015; US EPA, 2017). These compounds' industrial success-making qualities also cause persistency, bio-accumulation, and, in certain circumstances, their toxicity in the environment.

According to the existing available research, PFOS and PFOA are difficult to partition between soils, water, and sediments. Partitioning may be impacted by a variety of variables, including solution ionic composition, solubility (PFOS solubility declines with increasing seawater organic content), binding strength, pH, and the presence of other pollutants such hydrocarbons (Lasier et al., 2011). It has been established that PFOS and PFOA are metabolically and chemically inert, defying biotic and chemical breakdown (Boudreau et al., 2003). PFOS finding in people and wildlife is more proof that PFAS are known to bioaccumulate and biomagnify in food webs (Konwick et al., 2008). Additionally, it has been discovered that edible aquatic animals like mollusks, crabs, and fish bioaccumulate PFOS and PFOA (Yang et al., 2014). We know a fair amount about the toxicological effects of PFOS and PFOA exposure on aquatic creatures. Numerous studies have shown that aquatic creatures exposed to PFOS and PFOA can experience negative impacts on their ability to reproduce, develop their immune systems, and grow. Multiple pathways involved in embryonic and larval development, reproduction, and stress response are impacted by waterborne PFOS exposure, and these pathways can cause developmental toxicity in oviparous and viviparous freshwater fish (Oakes et al., 2005; Wang et al., 2011). Adult zebrafish exposed to chronic PFOS have been shown to have decreased body

length and weight, changed sex ratios, and impaired gamete function (Wang et al., 2011). An intertidal copepod treated to PFOS at doses up to 1 mg/L showed decreased individual development and reproduction (Han et al., 2015).

Sources and Occurrence of PFOA and PFOS

PFOS and PFOA are the two best known PFAS compounds manufactured in large volumes and sold in the United States by 3M. In 1947, 3M, an American Multinational Conglomerate, started producing PFOS and PFOA by electrochemical fluorination. Dupont started purchasing PFOA in 1951 from the then Minnesota Mining and Manufacturing Company for the manufacture of Teflon, a product that brought Dupont a billion-dollar-a-year profit by the 90s. Dupont referred to PFOA as C8 (ATSDR, 2015). The original formula for Scotchgard, a water repellent applied to fabrics, was discovered accidentally in 1952 by two 3M chemists, Patsy Sherman and Samuel Smith (Sherman & Smith, 1971; SPS, 2021).

Since the 1950s, perfluorinated compounds (PFCs) have been produced for a variety of commercial and industrial uses. In the United States, perfluoroalkyls were produced in huge quantities. To decrease releases and the amount of toxic compounds in their products, businesses have either stopped production or started modifying manufacturing procedures. Many perfluoroalkyls are being replaced by other chemicals in some facilities. In addition to surface water and groundwater, soil and sediment also contain perfluoroalkyls (ATSDR, 2015). The highest concentrations of perfluoroalkyls in the environment are often found close to production or use sites for these chemicals. However, they have also been discovered in far-off places like the Arctic and the ocean. They might be transported over great distances.

Perfluoroalkyls are extremely stable substances that do not easily degrade in the environment (ATSDR, 2015). Within days to weeks, perfluoroalkyls in the air should drop to the ground. Groundwater and flooding have the potential to transport them through the soil, and when the wind blows, they may become airborne.

PFOS and PFOA are resistant to common environmental degradation processes and very persistent in the environment. They are consequently widely dispersed across the upper trophic levels and can be found in soil, air, and groundwater at various locations around the US. Because of their potential for bioaccumulation and toxicity, PFOS and PFOA should raise concerns for both the environment and people's health (ATSDR, 2021b).

Uses of PFOS and PFOA

PFOS and PFOA are chemical compounds that are known to be extremely stable. They have been employed in a variety of applications due to their remarkable ability to repel oil and water. As a result, PFOS and PFOS-related substances have been used in a wide range of products around the world, including aqueous film forming foams (AFFF), semiconductors, hydraulic fluids, photolithography, Scotchgard tape, grease repellents for packaging, surface treatments for rugs and carpets, paper and packaging, coatings and coating additives, industrial and household cleaning products, pesticides and insecticides, and pesticides and insecticides (CRC CARE, 2013, 2017; Seow, 2013).

PFAS compounds may also be produced from household goods and possibly from meals. PFOA salts are used to create fluoropolymers that are fire resistant as well as oil, stain, grease, and water repellent (US EPA, 2014).

PFOA is found in trace concentrations in Teflon™ (polytetrafluoroethylene), which is used in nonstick cookware, waterproof and breathable garment membranes, and the aerospace, automotive, building and construction, chemical processing, electronics, semiconductors, and textile sectors (CRCCARE, 2014; Seow, 2013). PFOA is also produced by the breakdown of some fluorotelomers, such as 8:2FtS, which can be used in stain, grease, and water resistant surface treatment products, paints, coatings, cleaning products, fire-fighting foams and engineering coatings, and AFFF fire-fighting foams (Backe et al., 2013; Seow, 2013). Others include emulsifiers, wetting agents, processing aids in the manufacture of fluor (ATSDR, 2015; US EPA, 2017). PFOS and other PFAS compounds were (and continue to be) utilized in the production of aqueous film forming foam (AFFF), which is used to extinguish liquid hydrocarbon fires (ASTSWMO, 2015; DoD - SERD, 2014).

Humans Exposure to PFAS

Chemicals containing perfluoroalkyl are often present. According to studies, perfluoroalkyls have been discovered in all environmental media, including air, surface water, groundwater (including drinking water), soil, and food. Humans are most exposed to PFOS and PFOA through inhalation and oral ingestion of contaminated water, food, and soil (including biomagnification in the food chain) (ATSDR, 2015; US EPA, 2014).

The risk associated with cutaneous exposure uptake appears to be lower in general, notwithstanding the paucity of information (ATSDR, 2015). Hand-to-mouth activities on treated carpets could expose young children, while breast milk could expose infants (ATSDR 2015). Human blood serum frequently contains PFOS, though the levels have been dropping since 2002.

People could be exposed by any of these media. Due to contaminated drinking water, some American communities that lived close to fluoropolymer production sites were exposed to high levels of PFOA, PFOS, and other perfluoroalkyls (ATSDR, 2008a). Potential exposure paths include consuming food and water, using consumer goods, or inhaling particulate matter (such dust and dirt) that contains PFAS (ATSDR 2015; EPA 2009b). PFOA and PFOS, which are commonly connected to manufacturing facilities, industrial use, or dumping, have been found in drinking water supplies (ATSDR, 2015; Guerranti et al., 2013).

Fate and Transport of PFAS in the Environment

PFAS are widely diffused in the environment and have been detected in soil, sediment, surface waterways, and groundwater, both near and far from point sources. Its intermediate solubility and environmental persistence allow them transportable across great distances and through different mediums. PFOS and PFOA can be carried into groundwater, as well as surface and subterranean fluids, by runoff and leaching. The detection of PFOS in isolated parts of the Arctic caps shows that precursor compounds can be transferred through the atmosphere and across long distances by ocean currents (US EPA, 2014). PFOS and PFOA have also been shown to be persistent in aquatic settings, having a proclivity to adsorb to particulate materials and bioaccumulate in aquatic organisms (Hazelton et al., 2012). Because of its extremely low Due to the lower solubility of PFOS and PFOA in seawater, aquatic ecosystems, particularly marine systems, may be a substantial potential sink for PFOS. Volatilization from water to air is uncommon, according to Henry's Law Constant (Ahrens et al., 2009).

The detection of PFOS in groundwater and surface water systems around PFOS manufacturing facilities and firefighting training centres in the United States lends credence to this (Boudreau et al., 2003; Konwick et al., 2008). Furthermore, particles will eventually settle in sediments, generating a PFOS sink. While sediments are a sink for PFOS, they are unlikely to be a sink for PFOA since PFOA has greater aqueous concentrations than PFOS and is less strongly adsorbed than PFOS, says Environment Canada.

The degree of sorption to sediments or soils during transport influences PFAS mobility in water. By removing some of the PFAS from an aqueous solution, sorption can reduce the total contaminant mass migration velocity relative to water velocity (CONCAWE, 2016). Furthermore, wastewater treatment plants discharge PFAS into the environment, damaging both aquatic and terrestrial environments (Bossi et al., 2008; Hu et al., 2011). Higher quantities of PFOS and PFOA in wastewater treatment plant effluent may emerge from the wastewater treatment process, possibly due to the transition of precursor chemicals (Becker et al., 2008;Chen et al., 2012). Since biosolids have a high organic content, PFOS and PFOA easily adsorb to sludge in wastewater treatment plants and concentrate there (Zareitalabad et al., 2013). PFOS is thought to sorb to biosolids faster than PFOA (Becker et al., 2008; Chen et al., 2012). The precursor products of biosolids can act as long-term environmental sources of PFOS and PFOA (Venkatesan & Halden, 2014).

Health Effects of PFAS on Humans

The harmful health consequences of PFAS on people and the environment have been extensively documented in the literature. According to a case Minnesota brought against 3M in 2010, the business was aware that

these chemicals were building up in people's blood over forty years earlier. Similar to Michigan scientists, researchers from 3M also found PFOS and PFOA in fish, but they did so in the 1970s (Lerner, 2018). In the same decade, 3M researchers discovered that the substances they were creating were dangerous. According to reports, the corporation may have even possessed evidence of the substances' effects on the immune system at that time. Studies are only now motivating the ATSDR, as well as many States and the European Union, to propose lower levels (Lerner, 2018).

In a lawsuit brought in 2010 by the Minnesota attorney general, 3M was accused of contaminating groundwater with PFAS compounds while "knowing or should have known" that doing so would harm the environment and human health, as well as "result in injury, destruction, and loss of natural resources of the state." The “3M" acted with a purposeful disregard for the considerable danger of injury to the citizens and wildlife of Minnesota, according to the complaint. On February 20, 2018, 3M, however, reached a settlement with the plaintiffs for $850.

According to recent investigations, detectable levels have been found in the environment as well as in human blood, tissues, and breast milk (Du et al., 2013). PFOS and PFOA are easily absorbed after oral intake, accumulate largely in the blood serum, kidney, and liver, and have a half-life of roughly two to nine years in humans, according to toxicology studies (US EPA, 2014). In addition to deregulate lipid and lipoprotein metabolism, PFOS and PFOA bind to proteins (-lipoproteins and liver fatty acid binding protein), preferring partitioning to liver, blood, and kidney tissue. Due to their dual lipo- and hydrophobic characteristics, they do not build up in fatty tissues.

Human blood serum levels for PFOS and PFOA have been measured in several research conducted on regions of Australia. Measurements from several rural areas in the years 2002–2003 revealed PFOS levels ranging from 19.1 to 36.1 ng/mL and PFOA values ranging from 7 to 14.5 ng/mL (Kärrman et al., 2007). PFOS values in 2010 – 2011 ranged from 4.4 to 17.4 ng/mL, and PFOA levels ranged from 3.1 to 6.5 ng/mL, according to pooled data from deidentified surplus pathology samples (Toms et al., 2014). The decline in concentrations is linked to the cessation of PFOS and related compound industrial production in 2002. Due to a lack of data, neither the hazardous effect of acute exposure on people nor the long-term negative consequences on humans are known.

Due to the extended half-lives of PFOS and PFOA, there is worry that repeated exposure could raise body concentrations to levels that could have negative effects (Seow, 2013).

Studies on animals have shown a mild acute oral toxicity that may have negative effects on the liver, thyroid, and gastrointestinal systems (CRC CARE, 2013). Through short- and medium-term oral research, potential developmental, reproductive, and other systemic effects in rodents were discovered. Rats exposed to PFOS developed liver tumors and had impacts on their neuroendocrine system. There is conflicting information available about PFOS and PFOA's carcinogenicity. PFOS and PFOA are not yet classified as carcinogenic by the US EPA (US EPA 2016a, b). However, the Danish EPA (DEPA, 2015) believes that the designation of PFOS and four of its derivatives as acutely hazardous, carcinogenic, and harmful to reproduction is harmonized. DEPA (2015) has also suggested that PFOA be categorized for

eye irritation, acute toxicity, target organ toxicity, and carcinogenicity. According to the IARC Monographs, PFOA may cause human cancer (Group 2B). PFOS and PFOA have not been discovered to have mutagenic characteristics, however excessive dosages may cause animal carcinogenesis (US EPA, 2016c, 2016b).

PFOA exposure has been linked to high cholesterol, elevated liver enzymes, a weakened immune system, thyroid abnormalities, pregnancy-induced hypertension and preeclampsia, and cancer, according to epidemiological research on humans (testicular and kidney) (US EPA, 2016b). Human epidemiological studies established associations between PFOS exposure and high cholesterol and adverse reproductive and developmental effects (US EPA, 2016c).

PFOS and PFOA are toxic to laboratory animals, producing reproductive, developmental and systemic effects in laboratory tests (Inoue et al., 2004; Post et al., 2012; US EPA, 2016b, 2016c). The US EPA has established that there is suggestive evidence that PFOS and PFOA may cause cancer (US EPA, 2016b, 2016c). The American Conference of Governmental Industrial Hygienists (ACGIH) has classified PFOA as a Group A3 carcinogen – confirmed animal carcinogen with unknown relevance to humans (ATSDR, 2015). The World Health Organization's International Agency for Research on Cancer has found that PFOA is possibly carcinogenic to humans, Group 2B (IARC, 2016).

Using this weight-of-evidence approach, the available epidemiology data identify several potential health hazards of PFOA, PFOS in humans as listed below.

PFOA

- Pre-eclampsia and hypertension brought on by pregnancy
- Increases in serum enzymes and drops in serum bilirubin levels are signs of liver injury.
- Increasing levels of serum lipids, especially of total and LDL cholesterol
- greater likelihood of thyroid illness
- reduced vaccination antibody response
- increased chance of developing asthma
- heightened danger of declining fertility
- Small birth weight losses (<20 g or 0.7 ounces for 1 ng/mL increase in blood perfluoroalkyl level) were seen.(US EPA, 2017).

PFOS

- Pre-eclampsia and hypertension brought on by pregnancy
- Increases in serum enzymes and drops in serum bilirubin levels are signs of liver injury.
- Increasing levels of serum lipids, especially of total and LDL cholesterol
- greater likelihood of thyroid illness
- reduced vaccination antibody response
- heightened danger of declining fertility
- Small birth weight losses (20 g or 0.7 ounces for 1 ng/mL increase in blood perfluoroalkyl level) were seen (US EPA, 2017).

Effects of PFOS and PFOA on Children

According to studies that are currently available, there is no connection between blood PFOA levels and birth abnormalities in offspring of mothers who reside in areas with high PFOA water contamination. Higher levels of serum PFOA or PFOS have been linked to lower child birth weights in investigations of the general population and residents living close to PFOA industrial facilities. Although slight, the birth weight loss might not have an impact on the baby's wellbeing. Increases in blood cholesterol were discovered in a study of kids who drank water with high amounts of PFOA, which was consistent with the results in adults. Mice born to females who swallowed relatively high quantities of PFOS during pregnancy showed birth abnormalities. The highest PFOS levels found in workers were at least 10 times lower than the blood levels linked with these effects. Early death has been linked to PFOA and PFOS oral intake (US EPA, 2017).

Follow-up studies of the C8 cohort would be helpful in identifying whether the observed effects are caused by perfluoroalkyl exposure and would allow for a longitudinal assessment of health impacts in children. Information on children's toxicokinetic is required. Studies on half-life have been done on adults; it is important to know whether they apply to kids. No research has looked at whether young animals are more or less vulnerable to perfluoroalkyl toxicity than adults. It would be beneficial to get more information on this subject.

Regulations and Guidelines on Safe Levels of PFOS and PFOA

The current health warnings for PFOA and PFOS levels are based on exposure via drinking water consumption rather than from skin contact or air

inhalation (ATSDR, 2015). The EPA took into account sources of exposure to PFOA and PFOS other than drinking water, such as air, food, dust, and consumer products, when developing the recommendations. The US EPA suggested provisional drinking water health recommendations for PFOA and PFOS in 2009 of 0.4 µg/L and 0.2 µg/L, respectively (ATSDR, 2015) while in soil, the readings for PFOA and PFOS were 6 µg/L and 16 µg/L, respectively. The revised drinking water advisory for the US EPA is 0.07 µg/L for both PFOA and PFOS (US EPA, 2016a). Perfluoroalkyl chemicals in air are currently not subject to any regulatory restrictions set by OSHA or NIOSH.

Previous Studies on PFOS and PFOA

The first food that is universally advised to be given to new-borns soon after birth is the mother's breast milk, whose components are crucial for the neonate's nutritional needs. Public health professionals typically advise breastfeeding from birth to at least 6 months because it contains cellular, biochemical, and immunological components that can offer protection for the new-born against infections, diarrhoea, and malnutrition. Despite this fundamental fact, lactation is widely known to be a significant channel for contaminant exposure for neonates through breast milk, and is frequently employed as a bio-indicator to assess the body burden of certain PFOS and PFOA in babies (Gu et al., 2010; Lankova et al., 2013; Rodriguez et al., 2009). Additionally, infants are known to be more susceptible to pollutants because to their smaller size and are exposed to much higher levels of chemicals than adults (Liu et al., 2011). The majority of the details on PFOS and PFOA in this segment were taken from a review written by Macheka-Tendenguwo et al. (2018).

Evidence of PFAS in Human Milk

PFAS have been created for more than 60 years, but only recently have they been extensively examined in Asia, Europe, and North America in relation to their presence in human breast milk (Apelberg et al., 2007; Hoffman et al., 2010; Liu et al., 2010). The discovery of PFAS in human breast milk was originally reported in 2004 (Kuklenyik et al., 2004), PFAS values from frozen breast milk samples obtained as far back as 1996 and 1999 were later published by additional researchers (Tao et al., 2008a; Volkel et al., 2008). Since then, a number of further studies have been conducted, raising concerns about exposure during lactation (Lankova et al., 2013; Liu et al., 2010). The most often found PFAS in people, according to research, are perfluorohexanesulphonic acid (PFHxS), PFOA, and PFOS (Kärrman et al., 2007; Sundström et al., 2011).

There is currently no proof that PFAS are present in breast milk from Africa, Antarctica, Australia, or South America, despite the fact that they have been detected in significant quantities in the environment and in blood. Published studies predict that these compounds are spread across a wide range of geographic regions based on their structural properties and concentration levels. In North America, measurements of PFAS in breast milk have been made in Canada and the USA (Kuklenyik et al., 2004; Tao, et al., 2008a)

The greatest levels of PFAS have been found in breast milk samples taken from regions with a lot of industrial activity and high levels of economic development (Liu et al., 2010; OECD, 2005; Tao et al., 2008a). For instance, in 12 provinces of China, both rural and urban areas had their PFAS amounts measured. According to the findings, there are high levels of industrialization

and higher quantities of PFOA (814 pg/mL), PFUnDA (196 pg/mL), and PFOS (100 pg/mL) in metropolitan Shanghai. The lowest amounts, on the other hand, came from rural Ningxia, one of China's least developed regions, with values for PFOA, PFUnDA, and PFOS concentrations that ranged from below the limit of detection (<LOD) to 6 pg/ml (Liu et al., 2010). There may be a connection between PFAS concentrations and industrial expansion, according to literature. In a study (Tao et al., 2008a) showed that people from nations with higher gross domestic products (GDP) had higher concentrations of PFOS than people from nations with lower GDP. For example, PFAS concentrations in samples from Cambodia, India, and Vietnam were discovered to be 40–50% lower than those reported in more industrialized nations like China and Germany (Tao et al., 2008a; 2008b). With an estimated 85% presence in samples from India and 100% in the other studied nations, the levels of PFAS observed in samples from Asia revealed that PFOS was the predominant component in human breast milk (Tao et al., 2008a).

With a mean of 232 pg/mL, the highest PFOS concentrations ever observed came from Ehime in Japan. The excessive manufacturing and utilization of consumer items containing these pollutants was blamed for these levels (Tao et al., 2008a). In more recent studies, PFAS levels from other parts of Japan were substantially lower than those from Ehime, but they were still on the high end (Fujii et al., 2012). The peak PFAS concentrations for PFOA were found to be as high as 89 pg/ml and 93.5 pg/mL, respectively, in these samples, which were obtained from Hokkaido and Kyoto. There have been reports of other high PFOA levels in breast milk.

Levels of PFAS (i.e., PFOS and PFOA) in Breast Milk and Nursing History

A study by (Tao et al., 2008a) uncovered the possibility that PFOA levels in breast milk may be lowered by prior breastfeeding. Additionally, numerous research have supported the notion that moms who were breastfeeding for the first time had a higher load of PFAS than mothers who had previously breastfed (Barbarossa et al., 2013; Guerranti et al., 2013; Kadar et al., 2011; Thomsen et al., 2010). Thomsen and his colleagues reported that non-occupationally exposed multiparous women who breastfed had relatively lower levels of PFAS in their milk than women who were nursing for the first time (Thomsen et al., 2010). When compared to women who were nursing for the first time, the mean amounts of PFOS and PFOA were reduced by 44 and 59 percent, respectively.

PFOS concentrations were found to be over the limit of detection (LOD) in 90% of primiparas women's breast milk samples and 62% of multiparous mothers' samples in a comparable Italian investigation (Barbarossa et al., 2013). In primiparas mothers, PFAS concentrations ranged from 15 to 288 pg/mL (mean 57 pg/mL) and from 15 to 116 pg/mL (mean 36 pg/mL) in multiparous women. In Spain, average PFAS concentrations ranged from 13 to 397 pg/mL (mean 96 + 101 pg/mL) in women who were nursing for the first time, and from 13 to 167 pg/mL (mean 40 pg/mL + 31 pg/mL) in women who had previously breastfed. Also, all primiparous participants' samples included PFOA at levels higher than the LOD, although only 42% of multiparous donors' samples did. The preceding research data, which includes further investigations (Hamm et al., 2010; Kishi et al., 2017). Breastfed infants

may be exposed to PFAS from the environment mostly through breast milk, which also serves as a channel for their delayed elimination from the mother's body. Given the apparent relationship between PFAS concentrations and the number of previous pregnancies, it is logical to predict that first-born infants will be exposed to more PFAS than siblings born later. This would only be true if the mother's exposure remained constant during the time range. Following the first birth, the likelihood of a subsequent birth increases in direct proportion to the mother's level of exposure (body burden).

According to one study (Whitworth et al., 2012), the amount of time between pregnancies had a substantial effect on PFAS levels in the body. Breast milk contamination may become as high as it was during the first lactation if there is a long period between births. The scientific community has yet to question or support this hypothesis. Among non-occupationally exposed individuals, PFAS concentrations are frequently gender specific, with females typically having lower levels than males (Kärrman et al., 2007; Olsen et al., 2003). This outcome could be explained by the depuration of these compounds after prolonged breastfeeding.

According to a study (Haug et al., 2011), total PFOA and PFOS in babies would provide > 83% and > 94% of exposure within 6 months through nursing, respectively. Such high levels of exposure can contribute to a variety of problems later in life, including attention deficit hyperactivity disorder, excessive cholesterol, and cardiovascular disease (Ode et al., 2014; Steenland et al., 2009). According to certain recorded accounts, the presence of PFOS in the human body is linked to liver and bladder cancer, as well as contributing to reproductive and developmental damage during growth (Apelberg et al., 2007;

Haddow et al., 1999; Inoue et al., 2004; Thibodeaux et al., 2003). PFOA toxicity has been related with buildup in the blood, pancreas and liver (ATSDR, 2015; Olsen et al., 2007) as well as harm to the thyroid and immunological system, which may raise chances of allergies (Haddow et al., 1999). Nevertheless, contrasting results were published by Okada et al. (2014), who determined that there were no correlations between exposure to both PFOS and PFOA and allergies and infections in infants aged 18 months. Excessive PFOA exposure in mice has also been linked to poor developmental effects, which may also be seen in people, according to research (Rodriguez et al., 2009).

Blood, pancreas, and liver buildup of PFOA have all been linked to harmful effects (ATSDR, 2015; Olsen et al., 2007) In addition, thyroid and immune system dysfunction may increase the likelihood of allergies (Haddow et al., 1999). However, contradictory results were documented by (Okada et al., 2014), who found no correlation between exposure to PFOS and PFOA and allergies or illnesses in 18-month-old infants. Additionally, studies on rats has shown that exposure to high amounts of PFOA has detrimental effects on development, which may also be seen in humans (Rodriguez et al., 2009).

Factors Affecting Effective Evaluation of Contaminants in Human Breast Milk

The finding of toxic chemicals in human breast milk brings up several crucial issues for pediatric care, public health policy, and the study of environmental health. In a review Landrigan et al. (2002) listed the top five obstacles to an accurate assessment of pollutants in human breast milk. These are a few of them:

Lack of Comprehensive Data on Contaminants

The types of substances that are most likely to be found in breast milk have given rise to a wealth of information, yet this database is incomplete and deficient. From tiny samples of women in a relatively small number of geographic locations, data have only been gathered on a small number of substances (NRDC, 2001). There is a critical need for more information on exposure trends, contamination levels, and trends.

Lack of Consistent Protocols

For the collection and analysis of pollutants in samples of human breast milk, there is no established protocol. This makes it challenging to compare data among studies. Even if there are more statistics available in other countries, there are no established standards. A few examples of methodological flaws in published studies include inconsistent sampling and analysis protocols, incomplete details of sampling techniques, nonrepresentative sampling (in terms of geography, parity, and age), brief sampling times, few study participants, and a limited number and variety of chemicals analyzed (Lakind et al., 2001).

Lack of Toxicokinetic Data

Women may be exposed to lipophilic substances through a variety of pathways, including air, food, water, the workplace, and the home. During lactation, lipophilic substances can be mobilized from body fat after being stored and accumulated over time. Chemicals typically passively move into breast milk from plasma, and the solubility and lipophilicity of the chemical determine its content in the milk (Anderson & Wolff, 2000). Six months of lactation can result in a transfer of 20% or more of the mother body burdens of

some persistent contaminants, such as PCBs. Chemicals' toxicokinetic in breast milk are not fully understood.

Lack of Data on Health Outcomes

Data on the potential health effects of newborn exposure to toxins during breastfeeding are scarce. The majority of high-dose poisonings where the mother was clinically unwell have so far been associated with consequences on the nursing infants (Landrigan et al., 2002). Only a small number of chemical contaminants, most notably PCBs, have undergone the prospective epidemiologic studies necessary to assess chronic effects that might manifest at lower exposure levels. There is not much data on long-term effects or chemical interactions.

Lack of Evidence-Based Health Standards

Despite the fact that the majority of breast-feeding mothers have measurable levels of several environmental pollutants in their milk (Landrigan et al., 2002), there are no accepted normal or abnormal values for clinical interpretation that are determined from toxicologic or epidemiologic investigations. As a result, evidence-based recommendations cannot be made.

Risk Assessments

Traditionally, adult body weights and food consumption data have been used to estimate risk (Sonawane, 1995). How harmful exposure to chemical residues in human milk is for newborns and children depends on a mother's eating habits, the types and amounts of chemical residues in her milk, and the toxicological potency of those chemicals. To perform a full examination of the potential health risks to infants and children exposed to toxins from breast milk, all of these factors, as well as the unique

vulnerabilities of newborns and children, must be taken into consideration. Children and infants may show heightened sensitivity to the harmful effects of substances since their tissues are developing and growing quickly in these age groups (Landrigan, 1999). Infants and kids consume more milk fat and particular foods per unit of body weight than do adults, which could expose them to proportionally larger levels of chemical exposure. These early-life exposures may increase the risk of persistent toxic effects in neonates and young children compared to exposures that take place later in life. In order to take these factors into consideration and properly safeguard infants and young children, it is required to expand present techniques of health risk assessment.

Additionally, it must be remembered that there is only a limited quantity of data on baby and child food intake patterns and chemical residual levels in milk that are appropriate for use in risk assessment. Two additional sources of chemical exposure that should be considered when evaluating the risk are drinking water and the water used to make infant formula. The current risk assessment process takes into consideration water intake, however neither nondietary exposures nor exposures in drinking water are taken into account when estimating the risk for the total chemical exposure in breastmilk. These restrictions may lead to an underestimation of the burden of all exposures to young children and infants.

Chapter Summary

Exposure of infants to environmental chemicals such as toxic heavy metals and POPs through breast milk is a major public health challenge to breastfeeding. Heavy metals toxicity can cause neurological, brain impairment and diverse problems in children. Furthermore, the disturbance of the

endocrine system brought on by low-level exposure of youngsters to POPs including OCPs, PCBs, and PFAS can have a negative impact on their health. This chapter includes rules and recommendations on acceptable levels of pollutants in breast milk as well as factors that can hinder an accurate assessment of those contaminants.

CHAPTER THREE

METHODOLOGY

Introduction

This chapter describes the method employed to carry out the study, type of samples, sample and storage, sample preparation and the analytical methodology. The Ho Teaching Hospital (HTH) was the site of the study.

Study design

The participants' information and samples of breast milk were collected using a cross-sectional design in this study. The Ho Teaching Hospital (HTH) was the site of the study because it is a Teaching Hospital and attracts a wider public patronage.

Study Settings and sampling Site

The Ho Teaching Hospital (HTH), (previously known as the Volta Regional Hospital) obtained its current status as a Teaching Hospital in 2019. It is situated at a popular location known as Trafalgar, along the Ho-Aflao Highway about 800 meters from the Medical Village junction. It occupies an area of approximately 650 x 500 square meters. The present staff strength of the hospital is over 316 with a bed capacity of 240 and also serves as a referral hospital for other hospitals and clinics within the Volta Region. Among the many services it offers are Outpatient Department (OPD), In-patient Department (IPD), Surgical Services, including Orthopaedics, Obstetrics and Gynaecology, Internal Medicine, Dental Care, Ear, Nose, and Throat, Public Health Services, Accident and Emergency, Child Health, Mental Health, Eye Care, Pharmacy, Radiology and Imaging, Physiotherapy Services, Laboratory Services, Blood Bank and Transfusion Services, Administration, Catering, and

Cafeteria. The main catchments population is basically Ho and its environs. However, referrals are received from all over the districts and beyond the region. Foreigners such as Togolese, Beninnoise and Nigerians also considerably patronize their services (Ghana's Ministry of Health, 2019).

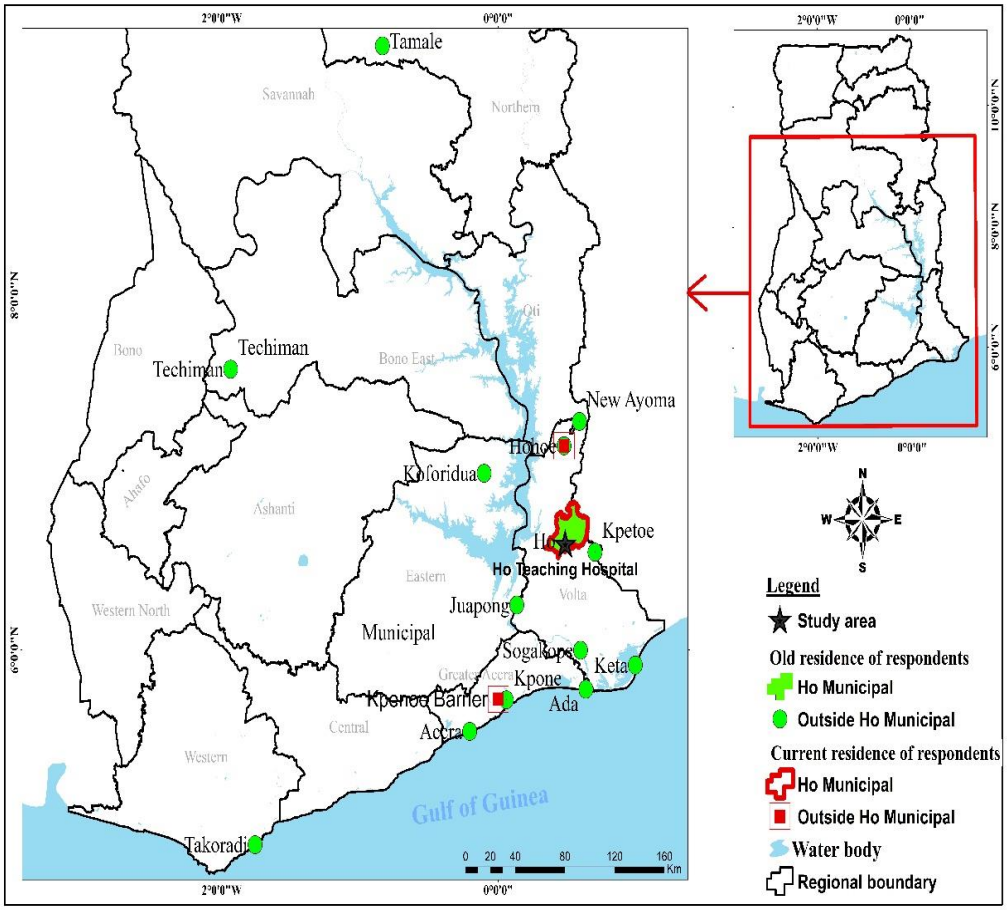

Figure 7: A map of Study/Sampling Site Showing Residences of Study Participants. Source: Author's Construct (2023)

Despite the fact that samples were taken at the Ho Teaching Hospital in the Ho Municipality, some of the study subjects had previously resided elsewhere.

Ethical Considerations

The University of Health and Allied Sciences Research Ethics Committee reviewed and approved the study protocol, and Ho Teaching Hospital provided institutional consent for the investigation (UHAS – REC).

During pre-natal and post-natal care at the hospital, our research staff notified all possible respondents about the study and offered them to participate (see the informed consent form at appendix B).

Inclusion/Exclusion Criteria

Inclusion Criteria

All voluntary and healthy mothers between the ages of 18 and 44 who gave birth at the HTH Maternity Ward during the study's time period were included.

Exclusion Criteria

Mothers who satisfy the above criteria but gave birth to twins were excluded. Also, mothers who qualify to be enrolled in the study but could not be followed to their homes for the collection of mature milk were not selected to take part in the study.

Samples and Sampling Method

The main sample investigated in the study was human breast milk (HBM). To choose study participants, a purposive sampling technique was used. During the study, a total of 47 participants were recruited. Healthy mothers between the ages of 18 and 44 who gave birth at the HTH's maternity department throughout the study's time period were eligible to participate. Each participant had 40 milliliters of breast milk taken from them in three separate amounts at three different lactational stages: colostrum from birth to day four, transitional milk from day five to two weeks, and after two weeks (mature milk).

Arrangements were made with the midwife in charge of the maternity ward to facilitate collection of the samples. Collection of colostrum started

few hours after birth while the mother was in the hospital. Likewise, transitional milk was collected during the one-week postnatal discharge at the hospital. Thereafter, in cases where it was not possible to get a participant in the facility to collect the mature milk, she was followed to her residence to do so. The process continued until we enrolled a total of forty-seven (47) participants.

For the purpose of analysing the breast milk of mothers, a comprehensive questionnaire was administered to each participant to elicit information on biodata, body mass, height, parity, place of residence, socio-economic status, educational level, lifestyle and dietary pattern.

Sample Size Determination and Sampling

The estimated sample size for the study was 50 participants. The population for the study was all healthy mothers who gave birth at the Ho Teaching Hospital Maternity Ward during the period of the study. Since the population was infinite, we used the Cochran formula

N = $\frac{Z^2pq}{e^2}$ **……(3.1)** where N is the population size, e = margin of error/desired level of precision, p = the estimated proportion of the population who have the desired attributes, q = 1 – p and values z = 1.96. Assuming that half of the mothers giving birth at the HTH have xenobiotics or pollutants in their breast milk, this gave a variability and p = 0.5. Now, at 95% confidence interval and at 10% margin of error, we had N = $\frac{1.96\ (0.5).(0.5)}{0.1\ x\ 0.1}$ = 49

We wanted to work with a round figure, so we added one more to obtain a population size of 50 participants. However, our success rate of getting participants to take part in the study was 94% (i.e. 47/50).

Since purposive sampling method was used, any mother who delivered at the maternity ward during the time of the study and fell within the inclusion criteria was selected. The process continued until we enrolled 47 participants for the study. All COVID-19 preventive measures like social distancing, washing of hands with soap and water and proper wearing of masks were strictly observed during the sampling process.

Sample Collection, Treatment and Storage

Breast milk samples were collected either manually or using a breast milk pump. 20 ml each of the three different types of breast milk samples was obtained from each participant at the different periods of breastfeeding viz; from birth to day four (colostrum), from day five onwards up to 2 weeks (transitional milk) and from 2 weeks onwards (mature milk). The samples were then maintained in a refrigerator at -20 °C (Figure 8) until extraction and analysis.

Figure 8: Frozen Human Breast Milk Samples in a Freezer. Source: Field Data (2021)

Materials and Reagents

Reagent grade chemicals – Conc. HNO_3 (AR, 70% w/v, 1.42 sp.gr. from BDH), 1:1 hydrochloric acid, HCl (36% w/v, Merck), Reagent water

(AFS-Water-Purification-Systems, Merck KGaA), Thermofisher Scientific iCAP 7400 Duo ICP-OES Spectrometer equipped with a Teledyne ASX-560 autosampler, hot plate (Karizma KZ – HP 2000 Double Hot Plate –2000 W), 100 ml Griffin beakers, watch glasses, 50.0 ml sterile plastic sampling containers, Ultraspec multi-element standards with Lot Numbers CSG02-08-21US, CSG02.1-08-21US (from De Bruyn Spectroscopic Solutions), Chromatographic grade ethyl acetate (Merck), analytical grade acetonitrile, ACN, (99.8% from Sigma-Aldrich) sodium chloride (99.99%; BDH), anhydrous magnesium sulphate ACS (RG, 98.00%), trisodium citrate dehydrate (99.00%, Thermo Scientific^{TM}), disodium hydrogen citrate sesquihydrate 99.00%, Thermo Scientific^{TM}), primary secondary amine, PSA, (Thermo Scientific^{TM}), formic acid, mixed PCBs and pesticides standards were all obtained from Dr. Ehrenstorfer.

Sample Preparation and Extraction of PFAS from Breast Milk Samples

In this investigation, PFAS from human breast milk were sampled and analysed using the method outlined by (Kadar et al., 2011). The process involved a liquid-liquid extraction (LLE) followed by a two successive Solid Phase Extraction (SPE) [first on Oasis® HLB (Hydrophilic-Lipophilic Balance) and next on carbon graphitized (Envicarb) cartridges] purification systems. For the purpose of performing protein precipitation, about 5 mL of each breast milk sample was placed in a polypropylene (PP) tube, spiked with 0.5 ng ^{13}C-PFOA/^{13}C-PFOS internal standards, followed by the addition of 9 mL of acetone.

The PP tube was sealed, fully mixed using a vortex chemical mixer for 30 seconds, and then let to extract for 10 minutes at a room-temperature of

30 ℃ ultrasonic bath. Afterwards, the sample was centrifuged and the supernatant transferred into another PP tube and evaporated to approximately 3 mL under gentle stream of nitrogen at 45 °C. After that, 8 mL of 0.1 M formic acid solution was added in order to adjust the pH for the first purification step on the Oasis® HLB cartridge. After conditioning the cartridge with 10 mL of methanol and 10 mL of 0.1 M formic acid, the sample was loaded onto the cartridge.

After all the solution had passed through the column, two washings were performed, first, with 5 mL of 0.1 M formic acid and then with 5 mL of 0.1 M formic acid/methanol (50:50. v/v) solution. At this point, the analytes were eluted with 6 mL of a mixture of methanol/ammonia solution ($MeOH/NH_4OH$) 33% (99:1, v/v) and afterwards concentrated to approximately 2 mL under nitrogen. A second purification was performed using a Supelclean™ ENVI-Carb™ cartridge, previously activated with 10 mL of methanol. After placing a new tube under the column, the sample was loaded and then eluted with 6 mL of a methanol/glacial acetic acid (80:1, v/v) solution. The eluate was evaporated to dryness under nitrogen and reconstituted with 50 µL of methanol/water (50:50, v/v) solution for instrumental analysis. Procedural blanks and laboratory reagent blanks were treated in the same manner.

Instrumental Analysis of PFAS

The final cleaned up extracts was analyzed using ultraperformance liquid chromatography-tandem mass spectrometer (UHPLC–MS/MS). Analysis of the PFAS from human breast milk was performed in an accredited laboratory in Europe.

Quality Assurance and Quality Control for PFAS

In order to ensure the validity and integrity of the analytical procedure, all the solvents and reagents employed were treated exactly the same way as the samples, including exposure to all glassware and equipment that were used in the analytical procedures. With each batch of ten samples, about 10.0 µ of methanol (CH_3OH) was injected into the HPLC- MS/MS instrument to check and prevent potential background and carry-over contamination. Analysis of procedural blanks was carried out for each batch of samples using Milli – Q water. Moreover, the internal standard method was employed in the quantification of target PFAS in the human breast milk samples.

Six-points calibration curve with R^2 regression model or linearity greater or equal to 0.99 were used to quantify target analytes. Since the method or procedural blanks did not show any detectable levels of PFAS, consequently, the LODs were defined as the signal-to-noise ratio and ranged from 1.30 – 24 ng/L. Due to nonavailability of suitable standard reference material for human breast milk and in line with earlier studies (Liu et al., 2010), the efficiency of extraction and recovery were determined by analysing four (4) samples of cow's milk spiked with 50 ng/L ^{13}C-PFOA internal standard. The efficiency of recovery of the target analytes ranged from 100% - 112% for all the PFAS as summarised in Table 1.

For the purposes of identification of each PFAS, retention times of PFAS in the standards were compared with those in the samples at a tolerance range of ± 2.5%. Furthermore, in line with the 2002/657/EC Decision, comparisons of the relative ion intensities of the spiked samples were made with the relative ion intensities of the PFAS standard solutions at the same

levels of concentration as employed in the drawing of the calibration graph. The limits of detection (LODs), limits of quantitation (LOQs), linearity, retention times and the percentage recoveries are summarized in Table 1.

Table 1: Summary of Quality Control and Recovery Studies on PFAS in Breast Milk

Type of PFAS	LOD/ (ng/L)	LOQ/ (ng/L)	Retention time (s)	Linearity (R^2)	Spiked sample (ng/L)	Mean Recovery(%)
PFHxA	1.30	4.30	4.81	0.9971	50.00	100.00
PFHpA	8.10	27.00	5.28	0.9967	50.00	110.00
PFOA	20.00	65.00	5.57	0.9981	50.00	112.00
PFOS	24.00	79.00	5.85	0.9911	50.00	107.00

Source: Laboratory Data (2023)

The percentage recovery of 100% was produced by perfluorohexanoic acid (PFHxA) while the recovery of 112% was produced by perfluorooctanoic acid (PFOA).

Health Risk Assessment of PFAS in Human Breast Milk

For the purpose of evaluating and assessing any possible health risks of PFAS (PFHxA, PFHpA, PFOA & PFOS) to infants through consumption of breast milk, the tolerable daily intake (TDI) method was employed. Using this method, the daily amount of PFAS ingested via breastfeeding per kilogram body weight for each infant was computed using the formula:

$$\text{TDI (ng/kg.Bw/Day)} = \frac{C_{\text{PFAS (ng per litre x VBM}}}{\text{BW (kg)}}, \text{ where}$$

C_{PFAS} = Mean concentrations of PFAS in breast milk;

VBM = Mean daily volume of breast milk consumed by infant; and

BW = average body weight of infant in kilogram

In this study, the mean volume of consumption of breast milk by an infant between 0 – 6 months old of 0.742 L and an average infant body weight of 6.0

kg suggested by the Environmental Protection Agency (US EPA, 2008) and (EFSA, 2020) guidelines were used in calculating the TDI of PFAS. The injection rate of breast milk volume of 0.742 L/kg/day employed was in line with those reported in other countries (Fujii et al., 2012; Taoet al., 2008a). Based on this relationship, the TDI of the PFAS calculated are shown in Table 11.

Sample Preparation for the Determination of POPs (Chlordane, DDT, Aldrin, Dieldrin, Endrin, PCBs, and Heptachlor) in Breast Milk

For the purpose of extracting and clean-up of the human breast milk samples for analysis, a modified form of QuEChERS was employed. About 10.00 g (± 0.05 g) of breast milk sample was weighed into a 50.0 mL centrifuge tube, then 10.0 mL acetonitrile (ACN) was added and vortexed for one minute. Thereafter, a mixture of:

- 4.00 g ± 0.20 g anhydrous magnesium sulphate,
- 1.00 g ± 0.05 g sodium chloride,
- 1.00 g ± 0.05 g trisodium citrate dihydrate and
- 0.50 g ± 0.03 g disodium hydrogen citrate sesquihydrate were added, vortexed for 1 minute, and centrifuged for 5 minutes at 4000 rpm.

This was followed by dispersive solid phase extraction (DSPE). 6.0 mL aliquots of the ACN layer was then transferred into a 15.0 mL centrifuge which contained 150.0 mg primary secondary amine (PSA), 150.0 mg C18 and 900.0 mg $MgSO_4$. The tube was closed and shaken vigorously for 30 seconds and centrifuged at 4000 rpm for 5 minutes. The cleaned extract was then concentrated below 40 °C on a rotary evaporator just to dryness, and 4.0 mL of it was transferred into a round bottomed flask where it was quickly

adjusted to a pH of about 5, by adding 40.0 mL of a solution of 5 percent formic acid in acetonitrile (v/v), and the filtrate.

It was then re-dissolved in ethyl acetate by adding 1.0 mL using a pipette. The sample was put in a 2.0 mL container and analysed using a gas chromatograph machine (Agilent 7890 B GC combined with Agilent Technologies GC Sampler 80/MS (Agilent 7000 C Triple Quadruple). The extract was kept frozen until it was ready to be measured. Gas chromatography and electron capture detection (GC-ECD) were used to evaluate the compounds Aldrin, endrin, heptachlor, chlordane, dieldrin, and DDT, whereas gas chromatography and mass spectrometry (GC-MS) were used to analyse PCBs.

Quality Assurance Protocols for Determination of PCBs

For the purpose of validating the method of extraction to determine the recovery of analytes and discover matrix influences or selectivity of the method, some samples were spiked with known concentrations of PCB standards and analysed to determine their recovery. The recovery studies of the PCB congeners ranged from 16% - 112% as shown in Table 2.

Table 2: Performance of the GC-MS/MS Method for PCBs Analysis in Breast Milk

PCB Congener	Retention Time/min	LOD/ ppb	LOQ/ ppb	50 ppb Spiked	Recovery (%) of QCs 20 ppb	50 ppb	100 ppb	200 ppb
18	11.853	0.0033	0.01	118.2	90	108	93	101.5
28	14.007	0.0033	0.01	105.3	95	100	106	94.5
31	19.885	0.0033	0.01	106	100	100	96	101.0
44	16.400	0.0033	0.01	120	75	100	96	109.0
52	15.495	0.0033	0.01	112.6	85	100	98	106.0
101	23.329	0.0033	0.01	106.4	85	102	104	99.0
118	23.329	0.0033	0.01	105.8	110	98	95	101.5
138	25.165	0.0033	0.01	75	95	102	94	102.0
149	23.937	0.0033	0.01	81	95	98	101	100.0
153	24.144	0.0033	0.01	76	90	102	96	101.0
170	27.420	0.0033	0.01	79	100	102	94	100.0
180	28.248	0.0033	0.01	47	80	100	95	106.5
194	29.979	0.0033	0.01	38.	100	102	93	109.5
209	31.490	0.0033	0.01	30.9	75	102	94	101.5

Source: Laboratory Data (2021)

Generally, higher PCB congeners gave poor yields (PCB 209, PCB 194, PCB 180, PCB 170 and PCB 138 respectively) while the lower congeners like PCB 18, PCB 28, PCB 31, PCB 44 and PCB 153 gave high yields for the recovery studies. Table 3 gives the PCB congeners and their systematic names.

Table 3: The 14 PCB Congeners of Interest and their IUPAC Names

PCB Congener	IUPAC Systematic Name
PCB 18	2,2',5 - trichlorobiphenyl
PCB 28	2,4,4'- trichlorobiphenyl
PCB 31	2,4',5 – trichlorobiphenyl
PCB 44	2,2',4,4' - tetrachlorobiphenyl
PCB 52	2,2',5,5' - tetrachlorobiphenyl
PCB	2,2',4,5,5' - pentachlorobiphenyl
PCB 118	2,3',4,4',5 – pentachlorobiphenyl
PCB 138	2,2',3,4,4',5' - hexachlorobiphenyl
PCB 149	2,2',3,4',5',6 – hexachlorobiphenyl
PCB 153	2,2'4,4',5,5' - hexachlorobiphenyl
PCB 170	2,2',3,3',4,4',5 – heptachlorobiphenyl
PCB 180	2,2',3.4,4',5,5' - heptachlorobiphenyl
PCB 194	2,2',3,3',4,4',5,5' - octachlorobiphenyl
PCB 209	2,2',3,3',4,4'.5,5',6,6' - decachlorobiphenyl

Source: Author's Construct (2023)

Formula for Calculation of Recoveries

The percentage of recovered samples from the spiked samples was calculated using the formula below:

Recovery (%R) = $\frac{C_s - C_u}{C_n}$**……(3.2)**, where

C_s = Measured concentration (peak area) of spiked sample aliquot

C_u = Measured concentration (peak area) of unspiked sample aliquot

C_n = Nominal concentration (peak area) of the standard or spike.

Laboratory Reagent Blank

For each batch of extracts, one laboratory reagent blank (LRB) (made up of all chemicals except the sample) was likewise run. The reagents were processed in the same manner as the samples, including exposure to all glassware and equipment. The LRB were used to determine whether there were any method analytes or other interferences in the laboratory environment, reagents, or apparatus.

Calibration Standard for PCB Congeners

Before analysing, the device was calibrated with a standard solution containing a combination of PCB congeners. The calibration standard solutions were purchased pre-packaged. Calibration was performed at seven (7) sites. Calibration standard solutions containing 5 ppb, 10 ppb, 20 ppb, 50 ppb, 100 ppb, 200 ppb, and 500 ppb mixed PCBs standards were used to calibrate the instrument in order to determine the detector's linear response. The appendix contains calibration curves for all of the PCB congeners studied. Table 4 summarises the GC - MS/MS operating settings for the study of PCB congeners.

Table 4: Summary of GC Operating Conditions for the Determination of PCBs

Apparatus:	
Instrument	Description
Gas Chromatograph	Agilent 7890 B GC with Agilent Technologies GC sampler 80
Analytical column	30m +10m EZ Guard x 0.25mm internal diameter fused silica capillary coated with VF-5ms (0.25µm film) from Agilent or equivalent.
Liner	Agilent 5190 -3167:900UL Single Tapered
Mass Spectrometer	Agilent 7000C Triple Quadrupole
Temperatures:	
Item	Conditions
Injector	Splitless mode, temperature 280°C
Oven	70°C / 2min $\frac{25°C/min}{}$ 150°C $\frac{3°C/min}{}$ 200°C $\frac{8°C/min}{}$ 280°C/13.133min
MSD Transfer Line	325°C
Gases:	
Gas	Flow rate
Helium (carrier)	2.25 ml/min constant flow
Collision gas (Nitrogen)	1.5 ml/min constant flow
Septum purge	30mL/min at 0.75min
Pressure	27.5psi
Mass Spectrometry	
Segment start time	4.0 min
Segment end time	45.0 min
Source Temperature	300°C
Scan Mode	MRM
Ionization mode	EI

Source: Laboratory Data (2021)

After establishing the GC operating parameters, the same settings were utilised to analyse the standards, quality control check samples, laboratory reagent blanks, and breast milk sample extracts.

Retention Time Windows

Retention time windows are critical for identifying target molecules. Absolute retention periods were used in addition to relative retention times to

determine PCBs as congeners using the internal standard technique. To compensate for modest variations in absolute retention periods caused by sample loadings and typical chromatographic variability, retention time frames were created. The width of the retention time window was carefully chosen to reduce the possibility of both false positive and false negative outcomes.

Qualitative Identification

The identification of PCBs as congeners using the GC - MS/MS method is based on agreement between peak retention times in the sample chromatogram and retention time windows defined by the examination of standards of target analytes. When a peak from a sample extract falls exactly within the retention time range established by a certain analyte of interest, this is referred to as tentative identification.

Analytical Procedures for the Quantification of POPs (Organochlorine Pesticides) from Human Breast Milk Samples

POPs (organochlorine pesticides) were detected in human breast milk samples using a Varian CP 3800 gas chromatograph equipped with an Electron Capture Detector and a CombiPAL Autosampler. The samples were processed through a 30m +10m EZ Guard column with a 0.25mm internal diameter and coated with Varian Inc.'s VF-5ms (0.25m film) or a comparable product.

Instrumental Analysis of Target Organochlorine Pesticides

Aldrin, Dieldrin, Endrin, Chlordane, Heptachlor, and pop-DDT were the POPs (Organochlorine pesticides) targeted for detection in this investigation. A gas chromatograph equipped with an electron capture detector was used to analyse each cleaned up extract (GC – ECD). The recovery

requirements for POPs at known concentrations were examined. The target analytes in the breast milk samples were identified and semi-quantified. The samples were identified by comparing their retention periods, peak forms, and peak patterns to the recovery standards. The sample peak areas or heights were quantified in relation to the standard peak areas or heights. The LOD and LOQ for POPs determination were set at 0.00167 g/kg and 0.005 g/kg, respectively. Table 5 summarises the GC - ECD operating conditions for determining organochlorine pesticides.

Table 5: Summary of the GC Operating Conditions for the Determination of the Organochlorine Pesticides

Apparatus:	
Instrument	Description
Gas Chromatograph	Varian CP-3800 GC-ECD with a CombiPAL Autosampler
Analytical column	30m +10m EZ Guard x 0.25mm internal diameter fused silica capillary coated with VF-5ms (0.25µm film) from Varian Inc or equivalent.
Temperatures:	
Item	Conditions
Injector	Splitless mode, temperature 270°C
Oven	70°C / 2min $\xrightarrow{25°C/min}$ 180°C/1min $\xrightarrow{5°C/min}$ 300°C
Detector-ECD	300°C
Gases:	
Gas	Flow rate
Nitrogen (carrier)	1 ml/min constant flow
Make-up	29 ml/min

Source: Laboratory Data (2021)

After establishing the proper operating conditions for the gas chromatograph, the same settings were used to analyse laboratory reagent blanks, standards, quality control samples, and extracts from breast milk samples.

Quality Control /Assurance Protocol for the Organochlorine Pesticides

Laboratory Reagent Blank

Each set of ten extracted samples contained extraction blanks, spiked blanks, and quality control (QC) samples. QC samples were used to check shifts in retention times (RT), variations in mass accuracy, and peak intensity to monitor the instrument's performance. For each batch of extracts, one laboratory reagent blank (made up of all chemicals except the sample) was likewise run. The reagents were processed in the same manner as the samples, including exposure to all glassware and equipment. The LRB were used to determine whether there were any method analytes or other interferences in the laboratory environment, reagents, or apparatus.

In order to ensure that the GC- ECD operating conditions were optimum for the determination, quality control procedures and validation steps were undertaken. Figure 9, 10 & 11 respectively shows a blank sample chromatogram, a chromatogram containing quality control samples and a chromatogram of a human breast milk sample spiked with 0.05 ppb mixed POPs standards.

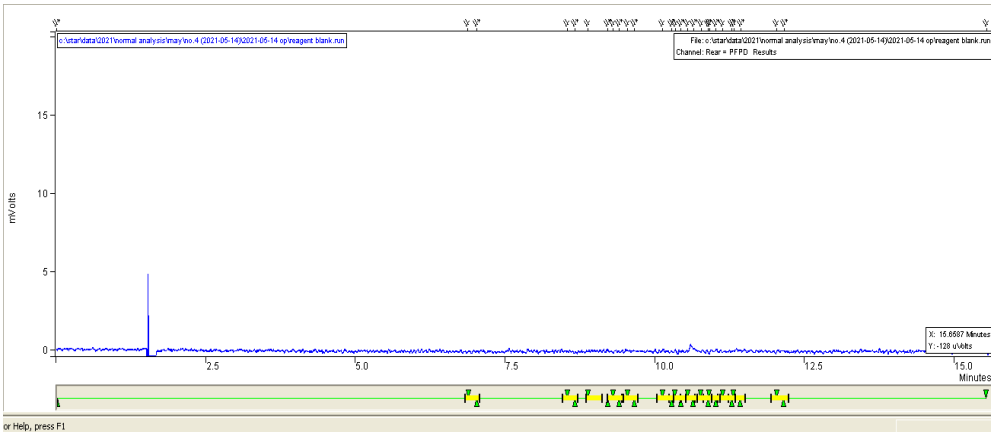

Figure 9: A Blank Milk Sample Chromatogram Using GC-ECD.
Source: Laboratory Data (2021)

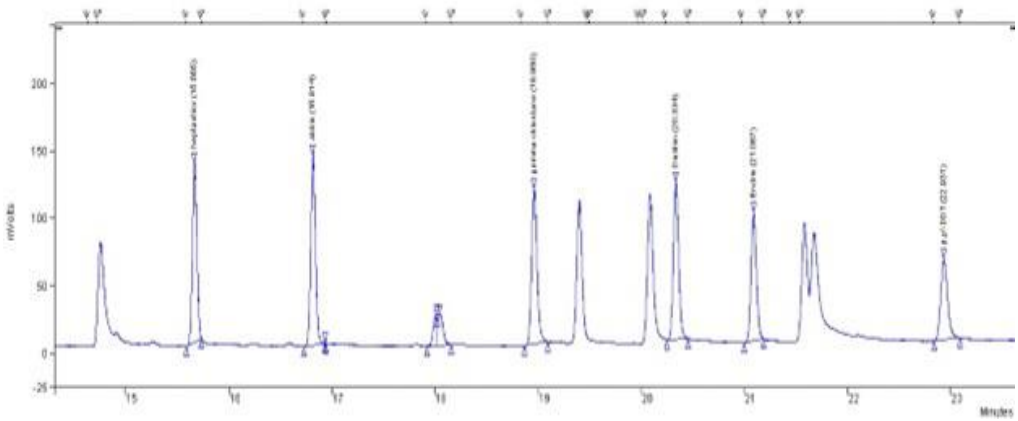

Figure 10: A Chromatogram of Quality Control Samples Using GC-ECD
Source: Laboratory Data (2021)

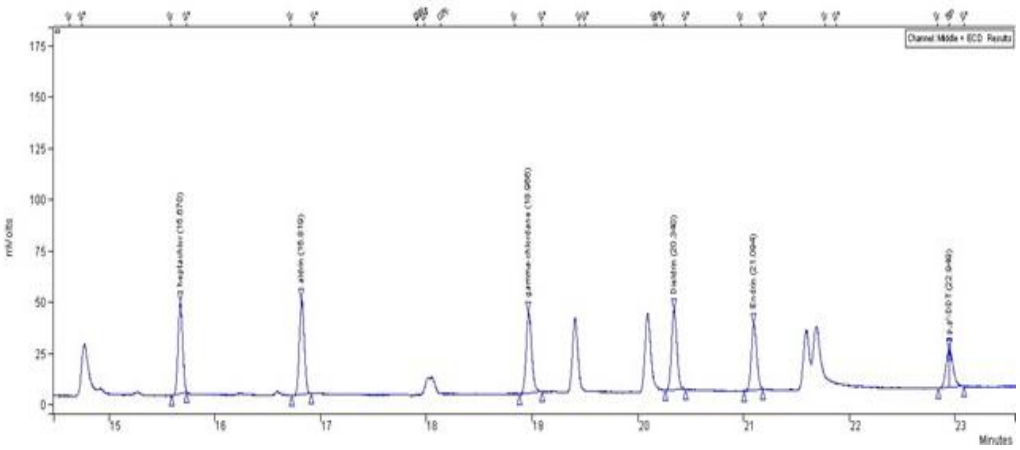

Figure 11: A Chromatogram of the Results of Breast Milk Sample Spiked with 0.05 ppb mixed POPs Standard Using GC-ECD. Source: Laboratory Data (2021)

Calibration of the GC–ECD Machine for Analysis of Breast Milk Samples

Known concentrations of mixed standards of POPs (organochlorine pesticides) were run through the instrument to calibrate the GC – ECD. Thereafter, 0.1000 mg/kg of the mixed POPs quality control standard was examined to deduce the recovery of the target organochlorine pesticides. The quality control standard's target organochlorine pesticide recoveries ranged from 66.9 percent to 104.5 percent. Results of the quality control showed that Dieldrin gave the least percentage recovery whereas Aldrin and DDT gave the highest recoveries of 104.3% and 104.5% respectively as shown in Table 6.

Table 6: Performance Validation of the GC-ECD Method for POPs (Organochlorine Pesticides) QC Samples Analysis

Name of POP (Pesticide)	Retention Time (min)	LOD/ (ppb)	LOQ/ (ppb)	Run of 0.1000 kg Organochlorine QC Sample		
				Expected Results	Observed Results	Recovery (%)
Aldrin	14.06	0.00167	0.005	0.1000	0.1043	104.3
Dieldrin	16.719	0.00167	0.005	0.1000	0.0669	66.9
Endrin	17.226	0.00167	0.005	0.1000	0.0755	75.5
γ-Chlordane	15.945	0.00167	0.005	0.1000	0.075	75.0
Heptachlor	13.036	0.00167	0.005	0.1000	0.0772	77.2
p,p'-DDT	18.152	0.00167	0.005	0.1000	0.1045	104.5

Source: Laboratory Data (2021)

Performance Validation and Reproducibility

Some samples were spiked with known concentrations of the mixed organochlorine pesticides standard and examined to assess their recovery in order to validate the extraction process to discover matrix effects and ascertain the recovery of analytes and selectivity of the procedure. The percentage recoveries of the spiked samples for the target organochlorine pesticides ranged from 71.0% - 91.6%, except for DDT which gave zero percent recovery as shown in Table 7.

Table 7: Performance of the GC-ECD Method for Human Breast Milk Sample Spiked with Mixed POPs (Organochlorine Pesticides)

Name of POP (Pesticide)	Retention Time (min)	LOD/ (ppb)	LOQ/ (ppb)	Breast Milk Sample Spiked with 0.05 ppb Standards		
				Expected Results	Observed Results	Recovery (%)
Aldrin	16.277	0.00167	0.005	0.05	0.0407	81.4
Dieldrin	19.76	0.00167	0.005	0.05	0.0355	71.0
Endrin	20.51	0.00167	0.005	0.05	0.0458	91.6
γ-Chlordane	18.41	0.00167	0.005	0.05	0.0414	82.8
Heptachlor	15.154	0.00167	0.005	0.05	0.0354	70.8
p,p'-DDT	22.346	0.00167	0.005	0.05	0.0465	93.0

Source: Laboratory Data (2021)

Retention Time Windows for the Organochlorine Pesticides Determination

Retention time windows are critical for identifying target molecules. Absolute retention times were used in addition to relative retention periods to identify the target POPs using the internal standard technique. To compensate for modest variations in absolute retention periods caused by sample loadings and typical chromatographic variability, retention time frames were created. The width of the retention time window was carefully chosen to reduce the possibility of both false positive and false negative outcomes.

Quantitative Identification of POPs (Organochlorine Pesticides)

The target POPs (organochlorine pesticides) were tentatively identified by comparing sample peak patterns and retention times to POPs standard peak patterns and retention times. The external standard approach was mostly used to quantify POPs. The peak height of a representative peak in the sample was compared to the peak height of the identical peak in the POPs standard examined closest in time to the sample. The injected concentration is calculated using a direct ratio to the standard, and the final concentration is adjusted for sample size.

Experimental Procedures

Analytical Procedures for the Determination of Heavy Metals and Essential Elements

The procedure of breaking down the breast milk samples to analyse metallic components follows EPA Method 3010A, with minor adjustments. A ten millilitre (10.0 mL) aliquot of each breast milk sample was measured and placed in a 100.0 mL Griffin beaker, followed by the addition of 1.0 mL pure

HNO_3 (analytical grade from Sigma Aldrich), and the beaker was covered with a watch glass and set on a hot plate (Karizma KZ – HP 2000 Double Hot Plate – 2000 W). The sample was carefully evaporated to roughly 5.0 mL, ensuring that it did not boil and that no part of the beaker went dry. The beaker was chilled, and 1.0 mL of concentrated HNO_3 was added to the sample. The beaker was returned to the hot plate, and the temperature was raised to allow for a gentle reflux. The sample was constantly cooked on a hot plate, with extra 1.0 mL of acid added as needed until the digestion was complete (generally indicated when the digestate was light in colour or does not change in appearance with continued refluxing). The beaker was chilled, and 1.0 mL of 1:1 HCl was added. The beaker was then covered and refluxed for 15 minutes. The beaker walls and watch glass were rinsed with distilled water, filtered, and topped up to 50.0 mL with distilled water before being transferred to the ICP - OES equipment for analysis. Laboratory reagent blank samples were likewise processed in the same way. Table 8 summarises the instrumental parameters and the operating conditions of the ICP – OES.

Table 8: Instrument Operating Parameters for the ICP – OES Machine

Instrument Parameters	Operating Conditions
Parameter description	Rf power 1.15 kW
Nebulizer gas flow rate	0.70 L/min
Auxiliary gas flow rate	1.50 L/min
Plasma gas flow rate	15.0 L/min
Argon gas (high purity)	99.99%
Sample uptake rate	1.8 mL/min
Nebulizer type	Concentric glass
Torch type	Duo Torch (Axial and Radial)
Nebulizer pressure	200 kPa
Pump rate	50 rpm
Sample uptake delay	30 s
Replicates	3

Source: Laboratory Data (2021)

Principles of Inductively Coupled Plasma Optical Emission Spectrometry Application

Optical Emission from Inductively Coupled Plasma ICP-OES Spectroscopy is a spectrometric technique used to identify trace elements in aqueous solutions. The ICP-OES continually aspirates or nebulizes a sample solution into an inductively coupled argon plasma discharge, where analytes of interest are transformed to excited-state gas-phase atoms or ions. When excited-state atoms or ions return to their ground state, they radiate energy in the form of light at wavelengths unique to each element. The amount (concentration) of energy emitted at the selected wavelength determines the intensity of that energy element in the tested sample. Hence, by detecting which wavelengths and intensities are emitted by a sample, the elemental composition of the given sample relative to a reference standard may be determined.

Quality Assurance Protocols for Determination Heavy Metals and Essential Elements

All relevant quality assurance measures were observed. For each batch of processed analytical samples, blanks (calibration blank and method blank), replicate samples and calibration verification standard (CVS) were employed to determine the efficiency and accuracy of the analytical procedure. The reagents went through the same procedures as the samples, including being in contact with all used glassware and tools. The laboratory reagent blanks were used to ascertain the presence of any method analytes or interferences in the environment, reagents, or equipment of the lab. The reporting limit of the analytical method were Al = 0.0400 mg/L; As = 0.0010 mg/L; Cd = 0.0020

mg/L; Pb = 0.0070 mg/L; and Hg = 0.0050 mg/L. The calibration standards were analysed initially and periodically. Known concentrations of the multielement standard solution containing aluminium, arsenic, cadmium, lead and mercury were run through the instrument to calibrate the ICP – OES for linearity.

Regarding quality assurance procedures, percentage recoveries of the heavy metals analysed were Al(103.76% - 104.08%); As(95 – 101.42%); Cd(94.69% - 106.30%); Pb(99.10% - 110.82% and Hg(89.80 - 90.92%) while the essential elements were Ca(110.89 – 111.42%; Cu(101.85 – 105.10%); Fe(100.00 –102.62%); Mg(104.08 –106.73%); K(97.71–98.79%); P(109.17–110.11%); Se(99.86 – 103.30%); S(103.18 – 112.51%); Na(94.44 – 96.51%) and Zn(94.24– 104.24%) which were all in line with US EPA Method 200.7-30 and within the acceptable limits prescribed by the scientific community.

Dietary Risk Assessment for Heavy Metals in Human Breast Milk

Estimation of dietary exposure of infants to Al, As, Cd, Pb and Hg through breastfeeding was carried out using the US EPA guidelines (US EPA, 2002a, 2008). Based on these guidelines, exclusively breastfed infants would have an average weight of 4.6 kg within the first month of birth and would consume approximately 510 mL per day of breast milk, while the upper limit of consumption was set at 950 mL per day. The daily estimated intake of heavy metals was then calculated using the formula:

$$\text{EDI (ngkg}^{-1}\text{ BW day}^{-1}) = \frac{CTrace\ Metals\ x\ DVBreast\ Milk}{BW} \quad \textbf{.....(3.3)}$$

where

EDI (ngkg^{-1} BW day^{-1}) = Estimated daily intake of trace heavy metal through breast feeding; CTrace Metals = Mean concentration (µg/L) of each toxic

heavy metal in breast milk; BW = Average body weight of infant based on US EPA guidelines.

Hazard quotients (HQ) of the toxic heavy metals were also computed to determine noncarcinogenic risks posed to infants through the consumption of these metals in breast milk according to US EPA guidelines (US EPA, 2002b, 2008). The noncarcinogenic risk was determined using the formula:

$$HQ = \frac{EDI\ (\frac{\mu g}{L}/DW/day}{RfD} \text{ ..... (3.4);}$$

Where:

HQ = Hazard quotient;

EDI = Estimated daily intake of toxic heavy metals through breastfeeding;

RfD = Reference dose, according to the World Health Organization (Cotruvo, 2017).

Using the hazard quotient assessment, if HQ < 1, it implies negligible noncarcinogenic health risk on infants. However, if HQ > 1, it implies an acceptable level of noncarcinogenic risk threat to the health of infant (US EPA, 2002b, 2008). In this study, the standard updated RfD values employed for the calculation of the HQ for the various toxic heavy metals were Al = 0.0004 mg/kg/day bw (US EPA, 2012; WHO 1993); As = 0.0003, Cd = 0.0010 mg/kg/day, Pb = 0.0036 mg/kg/day and Hg = 0.00016 mg/kg/day.

Additionally, the carcinogenic risks (CRs) of the toxic heavy metals were estimated using the equation:

$CR_{ing} = ADD_{Ing}. X\ SF_{Ing}$ ……..(3.5), where

CR_{Ing} = Carcinogenic risk due to the ingestion of toxic heavy metals in breast milk,

ADD_{Ing} = Average daily dose of toxic heavy metals through ingestion of breast milk. The SF = Cancer slope factor (mg/kg/day) for the toxic heavy metals

The Average Daily Dose via ingestion of breast milk, ADD_{Ing}, is mathematically defined as:

$$ADD_{Ing} = \frac{C \; x \; IR \; x \; ED}{BW \; x \; AT} \quad \text{.....(3.6), where}$$

C = concentration of toxic heavy metal in breast milk

IR = ingestion rate

ED = exposure duration

BW = body weight (kg) and

AT = Averaging time

The carcinogenic equation was used to assess the probability of an individual developing a cancer over a lifetime due to exposure to potential toxic heavy metals. The slope factor (SF) is toxicity value which defines in quantitative terms the relationship between dose and response. According to the US EPA, the acceptable range for carcinogenic risk assessment is $1.0x\ 10^{-6}$ to 1.0×10^{-4}.

In order to demonstrate the lifetime carcinogenic risk that the breastfeeding infants in the study might experience, the CR values were computed for As, Cd and Pb. In this study, oral reference dose (RfD_{oral}) was used for the respective metals. The slope factor values used for As, Cd and Pb were 1.5 mg/kg/day, 5.0×10^{2} mg/kg/day and 8.5 mg/kg/day respectively.

All the parameters were calculated using IBM SPSS Statistics Version 24 and Excel Analysis Tool Pak.

Carcinogenic risk assessment could not be made for Al and Hg because their slope factor values could not be found.

Data Analysis for POPs and Toxic Heavy Metals and Essential Elements

POPs – PFAS, PCBs & OCPs

For the purpose of analysing human breast milk for PFAS (forever chemicals), 29 breast milk samples were collected from mothers who had just delivered at the Ho Teaching Hospital. Data analyses were performed using IBM SPSS Statistics version 24, Excel 2016 and XLSTAT 2022. Descriptive statistics were used to summarize the study variables and to check out for range of values. Data normality were tested for using Shapiro-Wilk, Anderson Darling, Lilliefors and Jarque-Bera. Correlation test and non-parametric tests were performed on the qualitative variables using Kruskal-Wallis test and the differences compared at 95% significant level.

Though 141 breast milk samples were analysed for PCBs and OCPs, yet no results were obtained for the PCBs and OCPs because they were all below detection limits.

Toxic Heavy Metals and Essential Elements

A total of 141 breast milk samples made up of colostrum, transitional milk and mature milk were analysed for toxic heavy metals and essential elements. Statistical analyses were performed using IBM SPSS (Version 24), Excel Tool Pak and XLSTAT 2022.4.1.1377. Descriptive statistics were used to summarize the study variables and to check out a range of values. Correlation tests and non-parametric tests were performed on the qualitative variables using Kruskal-Wallis test and the differences compared at 95% significant level.

Chapter Summary

Similar to women in other locations, the women at the Ho Teaching Hospital who were the subject of this study have spent their entire lives breathing in, eating, and drinking environmental pollutants like heavy metals and POPs. This chapter has described the study design, study site, ethical clearance, samples size determination, sampling procedures, sample collection and storage to determine environmental contaminants in breast milk. The chapter also discussed sample preparation and extraction of POPs and toxic heavy metals from human breast milk samples, clean-ups and instrumental analysis. Other procedures such as quality assurance protocols, statistical analysis and risk assessment have been captured in this chapter.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

This chapter presents and discusses the results of the study. The study was conducted at the Ho Teaching Hospital to determine the concentrations of persistent organic pollutants (POPs), toxic heavy metals and essential elements in human breast milk. The target POPs were PFAS, OCPs - aldrin, dieldrin, DDT, endrin, heptachlor, hexachlorobenzene and 14 congeners of polychlorinated biphenyls (PCBs). The toxic heavy metals were aluminium (Al), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) while the essential elements were calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), sulphur (S), selenium (Se) and zinc (Zn). The objectives of the study were to:

- determine the levels of POPs such as PFAS (perfluoroalkyl substances/forever chemicals) in human breast milk; OCPs such as Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, and PCBs in the colostrum, transitional milk and mature breast milk of lactating mothers;
- determine the levels of aluminium, arsenic, cadmium, lead and mercury in the colostrum, transitional milk, and mature breast milk of lactating mothers;
- determine levels of essential elements, namely; calcium, copper, iron, magnesium, phosphorus, sodium, potassium, sulphur, selenium and zinc in the breast milk of lactating mothers;

- compare the levels of toxic heavy metals, essential elements and POPs in the various portions of human breast milk samples;
- compare the levels of these contaminants in human breast milk with allowable safety levels set out by international regulatory bodies such as the World Health Organization;
- evaluate and assess the extent of contaminants exposure to infants and levels of risk associated with breast milk as a principal source of infants' nutrition.

Determination of PFAS in Human Breast Milk

Table 9 presents a summary of the sociodemographic characteristics of the study participants for PFAS analysis.

Table 9: Sociodemographic Profiles of Study Participants and their Babies

Characteristic	N(%)	Mean ± SD	Range
Maternal age (yrs.)			
<32 years	18(62.07)	29.83 ± 5.67	20.0 - 31.0
32 yrs. and above	11(37.93)		32.0 - 44.0
Maternal education level			
None	1(3.45)		
JHS	10(34.48)		
Secondary	6(20.69)		
Tertiary	12(41.38)		
Maternal employment status			
Teacher	4(13.79)		
Civil servant	7(24.14)		
Student	1(3.45)		
Trader	7(24.14)		
Other	10(34.48)		
Maternal weight (kg)	29(100)	77.93 ± 12.82	56.0 -110.8
Maternal height (cm)	29(100)	159.20 ± 8.43	132.0 - 168.0
Maternal BMI (kg/m^{-2})	29(100)	30.99 ± 5.93	22.1 - 42.5
Parity			
Primiparous	3(10.34)		
Multiparous	26(89.66)		
Gestational age	29(100)	39.17 ± 1.7	33.00- 42.0
Baby sex			
Male	16(55.17)		
Female	13(44.83)		

Source: Field Data (2023)

All the women recruited into the study were mothers who had just given birth at the Maternity Ward of the Ho Teaching Hospital. Their mean age was 29.83 ± 5.67 years, with 18 (62.07%) having their age ranging from 20 - 31 years while 11 (37.93%) were 32 years and above. The weights and heights of the study participants ranged from 56.00 – 110.00 kg and 132.00 – 168.00 cm respectively with a mean BMI of 30.99 kgm^{-2}. With regard to educational levels of participants, 12(41.38%) had tertiary level education, 10(34.48%) were JHS graduates, 6(20.69%) had secondary level education while 1(3.45) had no formal education. In terms of parity, majority of the mothers 26(89.66%) were multiparous with only 3(10.40%) being primiparous or first-time mothers. The results of normality test performed on the data indicated that the data in general were not normally distributed as shown in Table 10.

Table 10: Summary of Normality Test Results of PFAS Data

Variable\Test	Shapiro-Wilk	Anderson-Darling	Lilliefors	Jarque-Bera
PFHxA	<0.0001	<0.0001	<0.0001	0.139
PFHpA	0.000	<0.0001	<0.0001	0.292
PFOA	<0.0001	<0.0001	<0.0001	0.016
PFOS	<0.0001	<0.0001	<0.0001	0.012
Age (Yrs.)	0.693	0.738	0.672	0.915
Mass (kg)	0.438	0.390	0.140	0.428
Height (cm)	0.001	0.002	0.008	0.001
BMI (kg/m^2)	0.177	0.308	0.347	0.488

Source: Field Data (2023)

This conclusion was based on the fact that the computed p-value was greater than the significance level alpha = 0.05. Hence the variable from which the sample was taken did not follow normal distribution.

In the analysis of each human breast milk sample, the individual levels of four PFAS namely- perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA) and perfluorooctanesulphonate (PFOS) were determine and the sum of the mean concentrations calculated. In line with EFSA recommendations (Schrenk et al., 2020) concerning lower bound PFAS, instrumental readings which were below detection limits (BDL) were treated as zero.

Levels of PFAS in Human Breast Milk

Levels of four PFAS were quantified in the human breast milk samples collected in this study. The complete results of the PFAS analysis together with quality control and retention times are presented in the appendix D, E, F and F. All the 4 types of PFAS were detected in the human breast milk samples in varying concentrations and the summary results are shown in Table 11.

Table 11: Descriptive Statistics of PFAS in the Breast Milk of Mothers (ng/L)

Type of PFAS	Detection Frequency (%)	Min ıg/L)	Max (ng/L)	Mean ± SD (ng/L)	Covariance (% CV)	Range (ng/L)	Total ∑PFAS
PFHxA	28.45%	LOD	8.90	2.65 ± 3.31	124.91	LOD - 8.90	76.80
PFHpA	55.17%	LOD	9.70	3.14 ± 3.14	100	LOD - 9.70	91.00
PFOA	86.21%	LOD	93.00	61.23 ± 29.57	48.29	LOD - 93.00	1775.60
PFOS	86.21%	LOD	190.00	83.14 ± 38.61	46.44	LOD - 190.00	2411.00

Source: Laboratory Data (2021)

The mean concentrations of the individual PFAS analysed in all the breast milk samples were respectively PFHxA (2.65 ± 3.31 ng/L), PFHpA (3.14 ± 3.14 ng/L), PFOA (61.23 ± 29.57 ng/L) and PFOS (83.14 ± 38.61 ng/L). Figure 12 illustrates the pattern of distribution of the PFAS measured in breast milk.

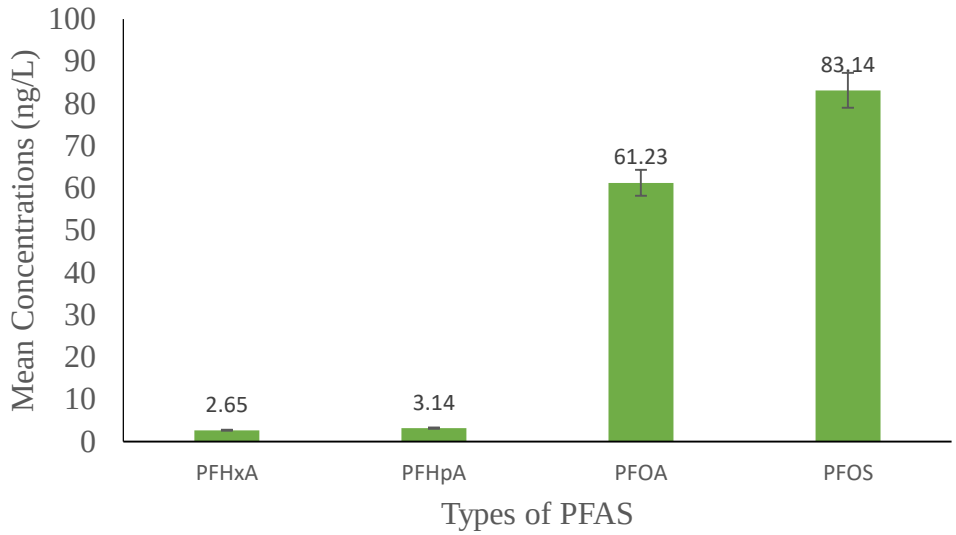

Figure 12: A Graph of the Mean Levels of PFAS in Human Breast Milk (With 5% Error Bars) Source: Laboratory Data (2023)

Of the four PFAS measured in human breast milk, PFHxA had the lowest detection frequency of 28.45% in all the samples, followed by PFHpA with a relatively high detection frequency of 55.10% whereas PFOA and PFOS had the highest detection frequency of 86.21% each. However, in a study in the United States Zheng et al. (2021) reported of 64% detection frequency for PFHxA with a median concentration of 9.69 pg/mL (1 pg/mL = 1 ng/L). However, the mean concentration of PFHpA which was 6.10 pg/mL in that was higher than the mean concentration observed in the present study. Zheng et al. (2021) expressed worry about the alarming increase in the concentrations of PFHxA comparable to PFOA, a PFAS of increasing toxicity of concern. An earlier study in Hangzhou, China (Jin et al., 2020) observed a relatively high mean concentration of 41.00 pg/mL for PFHxA and another in the United States (Kuklenyik et al., 2004) observed even a far higher concentration of 820.00 pg/L PFHxA in breast milk.

In this present study, PFOS was the predominant compound found in the breast milk, followed by PFOA. This observation is consistent with those reported (Barbarossa et al., 2013; Rawn et al., 2022; Volkel et al., 2008; Zheng et al., 2021). In Asia (Tao et al., 2008b) also reported PFOS to be predominant in human breast milk from the Philippines. They reported of mean concentrations of PFOS from breast milk ranging from 46.1pg/mL in India to 232.00 pg/ml in Japan. The results of the present study is supported by a study in the Czech Republic where (Lankova et al., 2013) found PFOA and PFOS to be the most abundant PFAS detected in breast milk, with concentrations in the range of 12.00 – 128.00 pg/mL (mean = 50.00 pg/mL; median = 44.00 pg/mL) and 7.00 – 114 pg/mL (mean = 33.00 pg/mL; median = 30.00 pg/mL) for PFOA and PFOS respectively.

It is worthy to note in the current study that the highest concentrations of PFAS found in individual samples obtained for PFOS (190.00 ng/L) and PFOA (93.00 ng/L) were lower compared with the highest concentrations of 288.00 ng/L and 241.00 ng/L respectively reported for these compounds in samples provided by primiparous mothers in a study in Italy (Barbarossa et al., 2013). Maximum concentrations of PFOS and PFOA 865.00 ng/L and 907.00 ng/L respectively have been reported in human breast milk from Spain (Llorca et al., 2010). High concentrations of PFOS, 639.00 and 309.00 ng/L respectively have been observed in breast milk samples from Hungarian and German women (Volkel et al., 2008).

Correlation Coefficient Matrix of PFAS in Human Breast Milk

The data was subjected to Pearson's correlation analysis and the results obtained is given in Table 12.

Table 12: Pearson Correlation Matrix for PFAS in Breast Milk

Variables	Age (Yrs)	PFOS	PFOA	Mass (kg)	BMI (kg/m^2)	PFHxA	PFHpA	Height (cm)
Age (Yrs)	1							
PFOS	0.203	1						
PFOA	0.179	**0.784**	1					
Mass (kg)	-0.143	-0.235	-0.253	1				
BMI (kg/m^2)	-0.055	-0.016	-0.222	**0.796**	1			
PFHxA	0.010	0.179	0.266	-0.524	-0.516	1		
PFHpA	-0.176	0.155	0.157	-0.219	-0.032	0.336	1	
Height (cm)	-0.138	-0.280	-0.005	0.069	-0.542*	0.125	-0.217	1

Values in bold are different from 0 with a significance level alpha = 0.05. Source:

Laboratory Data (2023)

Results of the tests revealed a strong positive and significant correlation between PFOS and PFOA concentrations in human breast milk (r = 0.784; p<0.0001). This result was in agreement with the one reported in a Germany study where (Volkel et al., 2008) found a strong positive correlation between PFOS and PFOA concentrations in breast milk samples (r = 0.75; p = 0.008). The strong positive correlation between PFOS and PFOA in breast milk suggests they originate from the same anthropogenic source. In a related Asian study in Japan (Tao et al., 2008a) observed a significant positive correlation among PFOS, PFOA and PFHxS in human breast milk from Japan (Spearman's coefficient, p<0.01). They also found a significant correlation (Spearman's p<0.05) between PFOS and PFHxS in breast milk from the Philippines, Vietnam and Malaysia.

Moreover, the current study also found a strong positive correlation between BMI and mother's weight (r = 0.796; *p*<0.0001). There were also negative correlations between PFHxA and mother's weight (r = -0.525; *p* = 0.004), PFHxA and BMI (r = -0.516; *p* = 0.004) and then maternal height and BMI (r = -0.542; *p* = 0.002). The negative correlations found between maternal weight, BMI and PFHxA suggest that as mother's weight and BMI increase, levels of PFHxA decreases and vice versa.

Relationship Between PFAS Levels in Breast Milk and Maternal Characteristics and Infants' Sex

Non parametric tests were performed on the data using Kruskal-Wallis Test at 0.05 significant level to determine whether there existed any significant difference between levels of PFAS in mother's breast milk and sex of their infants (male or female) and some selected maternal characteristics such as

age, weight, height, BMI, parity, gestational age, educational level, employment status. However, the study results did not find any significant difference between infant's sex and PFAS levels in mother's milk. Similarly, no significant relationship was found between any of these maternal characteristics with levels of PFAS determined in human breast milk. Likewise, the study did not find any significant relationship between maternal diet, drinking water source and total PFAS concentrations in breast milk. These findings suggest that levels of PFAS in mothers' breast milk are not affected by infant's sex, maternal characteristics or lifestyle.

The study findings are consistent with a study conducted in Barcelona, Spain where (Llorca et al., 2010) did not find any significant relationship between PFAS concentrations in human breast milk and maternal age and infant's sex. Other researchers (Tao et al., 2008a) did not find any significant relationship between concentrations of PFOS, PFOA and PFHxS with mother's age when samples were analysed for individual countries. However, when they considered all the samples together, concentrations of PFOS in breast milk significantly and positively correlated with mother's age (Spearman's $p<0.001$), a comparison which suggested a possible increase in PFOS burden with mother's age.

The current study also did not find any significant relation between PFAS levels in breast milk and maternal parity which is consistent with a study in the United States (Zheng et al., 2021) which did not find any statistically significant relationship between concentrations of any of the PFAS in human breast milk and maternal parity. However, in Canada (Rawn et al., 2022) found a statistically significant relationship between levels of

PFAS in human breast milk and maternal parity. Rawn et al. (2022). observed that primiparous women had higher total PFAS concentrations in breast milk than multiparous women (ANOVA $p < 0.01$). This observation suggests mother-to-child transfer of PFAS burden either during the course of pregnancy, lactation or both. They also observed a weak inverse relationship between maternal age and total PFAS concentrations in breast milk (r = -0.184; ANOVA $p = 0.018$). Nevertheless, the pattern of relationship was not visually apparent.

Comparison of Levels of Total PFAS in the Study with those Reported in Other Studies

The levels of the individual total PFAS, ∑PFAS; ∑PFHxA, ∑PFHpA, ∑PFOA and ∑PFOS and their corresponding means, measured in the breast milk samples in the present study ranged from below limits of detection (LOD) to 76.80 ng/L (2.65 ± 3.31 ng/L), 91.00 ng/L (3.14 ± 3.14 ng/L), 1775.60 ng/L (61.23 ± 29.57 ng/L) and 2411.00 ng/L (83.14 ± 38.61 ng/L) respectively. It was evident from the data that PFOS and PFOA contributed greatly to the total PFAS burden in breast milk, followed by PFHpA, with PFHxA contributing the lowest amount. In an American study Zheng et al. (2021) reported of total concentrations of 16 PFAS (∑PFAS, the sum of 16 PFAS concentrations) ranging from 52.00 to 1850.00 pg/mL (median = 121.00 pg/L). The total concentrations of 4 PFAS calculated in the current study were far greater than those reported by Zheng et al. (2021). These results suggest a higher contamination of breast milk by PFAS in this present study. Moreover, Zheng et al. (2021) also found PFOS and PFOA as the predominant PFAS (median 30.40 and 13.90 pg/L respectively) among the 16 PFAS quantified in

human breast contributing averagely 32% and 15% respectively to ∑PFAS concentrations. This report was consistent with findings in this current study where the contributions of PFOS and PFOA (mean = 83.14 and 61.23 ng/L) were respectively 55.37% and 40.78% to ∑PFAS (the sum of four PFAS) concentrations in breast milk, though the figures in this current study were higher compared to those reported in the American study. Similarly, in a Canadian breast milk study Rawn et al. (2022) reported of the predominance of PFOA and PFOS in human breast milk samples analysed, with PFOA contributing the most (≈ 30%) to total PFAS concentrations. A study in Spain (Guzman et al., 2016) also observed the overall concentrations of PFAS ranging from 13.00 – 397.00 pg/mL (mean = 96.00 ± 101.00 pg/mL) in primiparous women while in those of multiparous women the values ranged from 13.00 – 167.00 pg/mL (mean = 40.00 ± 31.00 pg/mL).

It is of importance to mention that using arithmetic means and ranges of individual PFAS in different studies offer a better and more comprehensive basis for comparison of PFAS levels in breast milk. In Italy (Barbarossa et al., 2013) observed PFOS concentrations in breast milk ranging from 15.00 – 288.00 ng/L (mean = 57.00 ng/L) in primiparous mothers and 15.00 – 116.00 ng/L (mean = 36.00 ng/L) in their multiparous counterparts. Although the range of concentrations for PFOS was higher in their study than the current study, yet, the means were lower. With respect to PFOA, they observed concentrations in primiparous women ranging from 24.00 – 241.00 ng/L (mean = 76.00 ng/L) and 24.00 – 100.00 ng/L (mean = 43.00 ng/L) in multiparous women. It is obvious that the range of concentrations and the

mean for PFOA for primiparous were relatively higher than those obtained in the current study.

Similarly, mean concentrations of PFOS and PFOA of 232.00 ng/L and 77.70 ng/L reported in human breast milk from Japan in 1999 (Tao et al., 2008b) were all higher than the mean concentrations observed in this study. However, the mean levels of these compounds in the current study were higher than the concentrations reported in a Canadian study in 2008 – 2011 (PFOS - 35.50 ng/L, PFOA – 41.30 ng/L respectively) (Rawn et al., 2022). Similarly, the mean PFOS concentration of 46.10 ng/L in human breast milk reported in an Indian study (Tao et al., 2008b) was also lower than the one found in the current study.

Furthermore, a number of studies have reported the mean concentrations of PFOA in breast milk some of which were higher than those determined in this current study whereas others were lower than the concentration found in this study. The mean concentrations of PFOA found in previous studies that were higher than the mean concentration of 61.23 ng/L measured in the current study include 438.00 pg/mL reported in Massachusetts, USA, (Tao et al., 2008a), 106.00 pg/mL in Shoushan, China (So et al., 2006), 87.00 pg/mL in Hangzhou breast milk, China (Jin et al., 2020) and 78.00 pg/mL in Ehime, Japan (Tao et al., 2008b). Conversely, the mean concentrations of PFOA reported in previous studies which were lower than the concentrations in the current study were 50.00 pg/mL in the Olomouc region, Czech Republic (Lankova et al., 2013) and 41.00 pg/mL in Toulouse, France (Cariou et al., 2015).

In the case of PFOS, much higher mean concentrations have been reported in several studies across the world. In Ehime, Japan (Tao et al., 2008a) reported a mean concentration of 232.00 pg/mL; in Uppsala, Sweden, (Kärrman et al., 2007) reported a mean concentration of 201.00 pg/mL; in Hangzhou, China (So et al., 2006) reported a mean concentration of 121.00 pg/mL while in Munich, Germany (Volkel et al., 2008) observed a mean concentration of 116 pg/mL. All these mean concentrations of PFOS were far higher than the mean concentration of 83.14 ng/L found in this study.

Varying concentrations of PFOS and PFOA have been reported in many studies, and in most instances, these two PFAS tend to predominate in human breast milk samples. In another study in China (Liu et al., 2010) reported of the mean and median concentrations (46.00 pg/mL and 49.00 pg/mL; 46.00 pg/mL and 34.50 pg/mL respectively) for PFOS and PFOA. All these values were lower than those found in the present study. Liu et al. (2010) observed a large variation in the geographical distribution of PFAS in human breast milk. They reported of high concentrations of PFOA (814.00 pg/mL) for the rural sample and 616.00 pg/mL for the urban samples in human milk from Shanghai. Other researchers Kadar et al. (2011) reported of PFOS and PFOA as the most dominant PFAS in human breast milk with respective median values of 74.00 pg/mL (range 24.00 – 171.00 pg/mL) and 57.00 pg/mL (range 18.00 – 102.00 pg/mL). Although there exists ample evidence from literature which suggest a positive relationship between concentrations of PFAS in breast milk and industrial development, the results of PFAS concentrations in the current study was not consistent with that evidence or observation because Ghana is not a developed country.

Belgium, however, is reported to have one of the highest levels of PFAS recorded in literature with elevated concentrations of PFOS and PFOA of 28,200.00 pg/mL and 3.500.00 pg/mL respectively (Roosens et al., 2010). The total concentrations of PFAS recently measured in South Africa (Macheka et al., 2022) were very low compared to those discussed above.

This study was the first conducted in Ghana to determine levels of perfluoroalkyl substance in human breast milk. The results obtained are in consistent with those reported in similar studies carried out in developed countries such as the United States (Tao et al., 2008a; Zheng et al., 2021), Canada (Rawn et al., 2022), China (Lignell et al., 2013; Liu et al., 2010; So et al., 2006; Tao et al., 2008b), France (Kadar et al., 2011), Hungary (Volkel et al., 2008), Sweden (Kärrman et al., 2007), Spain (Kärrman et al., 2010; Llorca et al., 2010) Japan (Tao et al., 2008b) and Germany (Fromme et al., 2009; Volkel et al., 2008). Though other PFAS were quantified in these studies, almost invariably, PFOAS and PFOA often emerged as most predominant PFAS.

Results of Health Risk Assessment of PFAS in Human Breast Milk

The results of health risk assessment of PFAS in human breast milk are presented in Table 13.

Table 13: Calculated TDI (ng/kg.bw/day) Values of PFAS in Breast Milk

Type of PFAS	Mean conc. (ng/L)	TDI Value (ng/kg.bw/day)	TWI (ng/kg.bw/wk)*
PFHxA	2.65	0.33	2.29
PFHpA	3.14	0.39	2.72
PFOA	61.23	7.57	53.00
PFOS	83.14	10.28	71.97

**Tolerable Weekly Intake (TWIs) of PFAS were calculated by multiplying the Tolerable Daily Intake (TDI) values by 7.* Source: Laboratory Data (2023)

Dietary intake serves as a vital route for the exposure of human adults to PFAS (Fromme et al., 2009). The TDI values calculated in this study were not high and compared favourably well with those reported from other studies (Aceti et al., 2021; Fujii et al., 2012; Tao et al., 2008a; 2008b). For the mean concentrations of each of the PFAS detected in human breast milk, the corresponding TDI's and TWI values ranged from 0.33 – 10.28 ng/kg.bw/day and 2.29 – 71.97 ng/kg.bw/week respectively. The TDI values of PFOS and PFOA were the highest. All the TDI values calculated in the study were lower than those reported in some studies (Aceti et al., 2021; Guzman et al., 2016; Liu et al., 2010; Llorca et al., 2010; Zheng et al., 2021) for breastfeeding infants. They were also lower than those proposed by the European Food Safety Authority (EFSA, 2020) for average median PFAS concentrations in breast milk for PFOS (38.00 – 75.00 ng/kgbw/week) and PFOA (40.00 ng/kgbw/week). It can therefore, be concluded from the dietary intake information that the concentrations of PFAS detected in the study were not high enough to cause any significant health risks to breastfeeding infants.

Sociodemographic Profile of Participants for the Analysis of Heavy Metals, POPs and Essential Elements at Lactational Stages

Table 14 summarizes the sociodemographic characteristics of participants for the study.

Table 14: Sociodemographic Profile of Participants and their Babies

Maternal Characteristics	Variables	Frequency (n = 47)	Mean + SD	Percentage (%)
Education	None	2		4.3
	JHS	11		23.4
	SHS/Secondary	11		23.4
	Vocational	1		2.1
	Tertiary	22		46.8
Age (Years)	11 Up to 20	1		2.1
	21 – 30	19		40.4
	31 – 40	24	31.19 ± 5.48	51.1
	41 – 50	3		6.4
Body Mass (kg)	40 – 59	4		8.5
	60 – 79	24	77.97 ± 12.42	51.1
	80 – 99	17		36.2
	100 - 119	2		4.3
Height (cm)	131 - 140	1		2.1
	141 – 150	4		8.5
	151 – 160	12	160.19 ± 8.05	25.5
	161 – 170	27		57.4
	171 - 180	3		6.4
BMI (kg)	18.0 – 24.9	5		10.6
	25.0 – 29.9	18		38.3
	30.0 – 34.9	16	30.63 ± 5.03	34.0
	35.0 – 39.9	5		10.6
	40.0 – 44.9	3		6.4
Gestational Age (wks.)	33 – 35	5		10.6
	36 – 38	13	38.47 ± 1.98	27.7
	39 – 41	29		61.7
Parity	Primiparous	8		17
	Multiparous	39		83
Occupation	Civil servant	15		31.9
	Teacher	5		10.9
	Trader	15		31.9
	Student	2		4.3
	Other	10		21.3
Working Tools	Metals	2		4.3
	Electrical equipment	30		63.8

	Printers	11	23.4
	Others	4	8.5
Current Residence	Ho Municipality	44	93.6
	Hohoe	2	4.3
	Have	1	2.1
Common Diet	Sea food	5	10.6
	Fresh water	4	8.5
	Both	38	80.9
Water Sources	Well	2	4.3
	Tap water	39	83.0
	Other	6	12.8
Infant Sex	Male	26	55.3
	Female	21	44.7

Source: Field Data (2022)

The mean age of the mothers recruited for the study was 31.19 ± 5.48 years, with the minimum and maximum ages being 21 and 44 years respectively. Their average BMI was 30.63 ± 5.03 kgm^{-2}. Of the forty-seven mothers recruited, 10.6% (5/47) had normal weight, 38.3% (18/47) were overweight while 51.0% (24/47) were obese. Most of the mothers were multiparous 39(83%) while the rest, 8 (17%) were primiparous or first-time mothers.

The gender distribution of their babies was 26 males (55.3%) and 21 females (44.7%). The birth weights of the babies ranged from 1.5 – 4.0 kg.

Levels of Other POPs in the Colostrum, Transitional Milk and Mature Breast Milk of Mothers

Besides the PFAS, seven different persistent organic pollutants (POPs) namely, Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor and PCBs were investigated in the colostrum, transitional milk and mature breast milk of lactating mothers at the Ho Teaching Hospital. The POPs were divided into

two different categories – organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) which are industrial chemicals. Result of the analysis carried out on all the 141 breast milk samples showed that all the six OCPs and fourteen PCB congeners were below limits of detection. The LODs and LOQs for the OCPs and PCBs were 0.00167 mg/kg, 0.005 mg/kg and0.01 ppb and 0.03 ppb respectively. Even though PFAS, OCPs and PCBs are all members of POPs which are lipophilic and easily mobilised into breast milk, yet, appreciable amounts of PFAS were detected in the breast milk samples whereas none of the OCPs and PCBs were found. Besides the different extent to which mothers may be exposed to these chemicals in the environment, it may also be due to the different physicochemical properties of PFAS which enhance their relative mobilisation from fat tissues into breast milk compared to OCPs and PCBs.

The non-detection of POPs in the breast milk samples analysed could be considered a positive development. The results of the study were probably an indication of the fact that the campaign initiated by the Stockholm Convention on May 17, 2004, on the elimination of POPs in the environment is yielding positive results. However, the non- detection should not be taken as a total absence of the OCPs and PCBs in the mothers' breast milk, and for that matter, in the environment. It may probably mean that some of the POPs were present in the breast milk but their concentrations were not high enough to trigger or cause significant health risks in the nursing infants. The ingestion of POPs (i.e., OCPs and PCBs) is of high toxicological relevance due to their long-term health effects. There is, therefore, the need for environmental samples to be analysed to understand the general pattern of distribution of

POPs in the environment. While not dwelling much on their acute toxicity, research indicates that low levels of exposure to some POPs may lead to possible endocrine disruption in humans (El-saeid et al., 2021), a situation which led to a ban on use of many OCPs for agriculture purposes and also for the control of mosquitoes.

The results of this study is similar to the one conducted in Assiut University Hospital, Egypt where the researchers did not find OCPs like Aldrin, Endrin, Heptachlor and chlordane from the breast milk samples within the detection limit (Salem & Ahmed, 2002). The study results were also in line with a study conducted in Brazil (Kowalski et al., 2010) where they detected no contamination of human breast milk with PCBs in Rio Blanco/AC.

The study results were, however, in sharp contrast to those reported in Accra, Ghana by Asamoah et al. (2018) who assessed the levels of PCBs in the breast milk of some Ghanaian women at suspected hotspot (Agbogbloshie) and relatively non-hotspot (Kwabenya) areas to ascertain if the levels of PCBs in mothers milk posed any health risk to the breastfed infants. A total of 128 human breast milk were sampled from both primiparae and multiparae (Agbogbloshie – 105 and Kwabenya – 23) and the samples were analyzed using GC – MS/MS. The total mean levels and range of Σ7PCBs were 3.64 ng/g lipid wt. and <LOD–29.20 ng/g lipid wt. respectively. Mean concentrations from Agbogbloshie (hot-spot area) and Kwabenya (non-hotspot areas) were 4.43 ng/g lipid wt. and 0.03 ng/g lipid wt. respectively. The present study site was, however, not regarded as a ‘hot-spot’ or contaminated site area for PCBs nor OCPs.

It is important to note that the public health significance of PCBs contamination in human populations and the concomitant effects on breast-fed infants cannot be overemphasised. Some researchers have reported a decline in the levels of POPs in human milk in recent times (Guerranti et al., 2011) which is a positive development in view of the negative effects of POPs on human population. Per the requirements of the Stockholm Convention on POPs, levels of certain POPs in human breast milk would serve as an indicator of the effectiveness of the treaty in eliminating or reducing emissions of selected POPs.

Even though the study results and those of other researchers in other parts of the world showed non detection of POPs in human breast milk, however, monitoring of POPs in human breast milk is still important and should be encouraged. As indicated by Pirsaheb et al. (2015) OCPs are still applied in some developing countries, (of which Ghana is no exception), especially, in the control of pests and weeds. It is therefore, appropriate and relevant to inform and educate the general public about the harmful effects of such chemicals, and more importantly, the need for the government to ban or place restrictions on their use. This study will contribute to efforts toward providing baseline data on current levels of POPs in Ghana.

Levels of Toxic Heavy Metals and Essential Elements in Breast Milk

Forty-seven (47/50) fresh breastfeeding mothers were recruited for the study, representing 94% participation. This current study is probably the first in Ghana and perhaps, among a few in the world to simultaneously analyse toxic heavy metals and essential elements in the three portions of human breast during the lactation period. The results of the concentrations of heavy

metals and essential elements determined in the colostrum, transitional milk and mature milk are now presented and discussed. The link between the levels of some toxic heavy metals and essential elements in mothers' breast milk and some sociodemographic characteristics of mothers such as parity, age, educational levels, maternal employment and infants sex are also discussed.

Levels of Toxic Heavy Metals in the Colostrum, Transitional Milk and Mature Milk of Mothers

Levels of Toxic Heavy Metals in the Colostrum of Mothers

The results of the determination of toxic heavy metals in the colostrum of mothers are given in Table15 while the overall is in the Appendix I. The results indicate that close to 30% of the samples did not detect the presence of any of the heavy metals analysed in the colostrum of mothers. However, the detection frequency of each of the toxic heavy metals is shown in Table 15.

Table 15: Descriptive Statistics of Toxic Heavy Metals in the Colostrum of Mothers

Heavy Metal	Detection Frequency	Min (µg/L)	Max (µg/L)	Mean (µg/L)	SD (µg/L)	Coefficient of Variation(%CV)
Al	87.23	0.050	0.823	0.104	0.128	123.07
As	87.23	0.011	0.217	0.038	0.034	89.47
Cd	38.29	0.002	0.028	0.006	0.006	100.00
Pb	89.36	0.007	0.032	0.009	0.004	44.44
Hg	51.06	0.005	0.008	0.006	0.001	16.67

Source: Laboratory Data (2022)

Levels of heavy metals detected in the colostrum milk ranged from 0.006 ± 0.001 µg/L (Cd, Hg) to 0.104 ± 0.128 µg/L (Al). Aluminium had the highest mean concentration (0.104 ± 0.128 µg/L), followed by arsenic (0.038 ± 0.034 µg/L), lead (0.009 ± 0.004 µg/L), mercury (0.006 ± 0.001 µg/L) and cadmium (0.0.006 ± 0.006 µg/L) in that order. Figure 13 illustrates the levels of heavy metals detected in the colostrum milk of mothers. The highest

concentration of aluminium in colostrum may be due to the widespread sources of aluminium in the natural environment and also the fact that most people use aluminium for varied purposes, ranging from cooking utensils to baking sheets and many others (Juhaiman, 2010; Kawahara & Kato-negishi, 2011; Marta et al., 2006; Mohammad et al., 2011; Semwal et al., 2006).

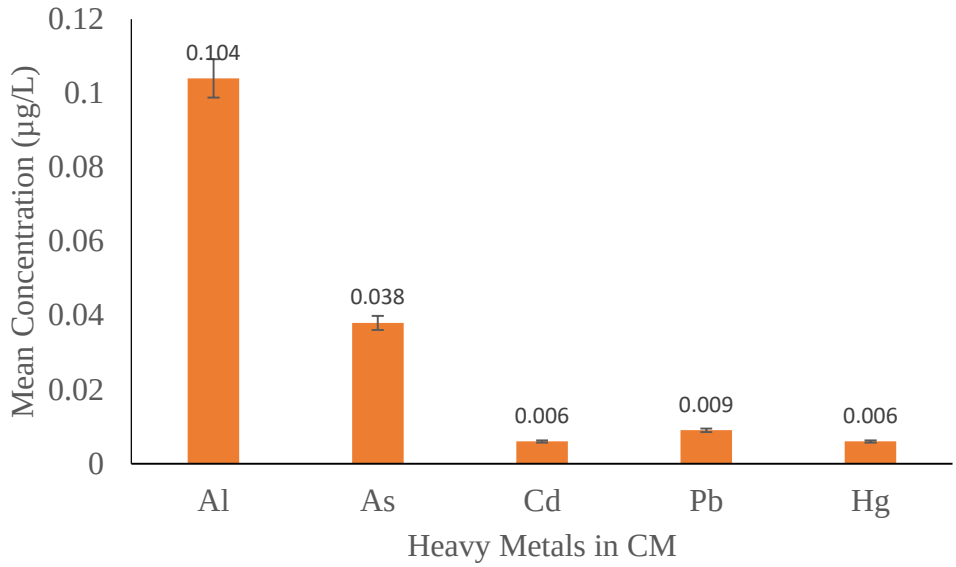

Figure 13: A Graph of the Concentrations of Toxic Heavy Metals in the Colostrum of Mothers (With 5% Standard Error Bars).
Source: Laboratory Data (2022)

Moreover, there was a great deal of variability in the concentrations of the different toxic heavy metals in the colostrum milk. The heavy metal with the highest variation was Al, with coefficient of variation of 123.07%, followed by Cd (100%), As (89.47%) and Pb (44.44%). Of the five toxic heavy metals analysed in the colostrum, only Hg gave the smallest and best distribution in the data set, with coefficient of variation of 16.67%.

Concentrations of toxic heavy metals detected in the colostrum of mothers were low compared to those reported in other studies. Levels of Al, As, Cd and Pb detected in this study were much lower than those reported in Taiwan (Chao et al., 2014). which were 56.45 ± 22.77 ng/mL(Al), 1.60 ±1.50

ng/mL(As), 1.37 ± 0.94 ng/mL(Cd) and 13.22 ± 3.58 ng/mL(Pb) respectively. Unlike their study where they recorded the highest concentration for Al followed by Pb, As and Cd, this study recorded the highest level for Al, followed by As, Pb; Hg and Cd recording the lowest level of 0.006 ± 0.006 µg/L. The levels of Pb and Cd in colostrum milk were lower than those reported in Ankara, Turkey (Turan et al., 2001) where the concentrations of lead and Cd were 14.6 ± 5.5 µg/L and 2.8 ± 1.7 µg/L respectively. Moreover, in Portugal Almeida et al. (2008) reported higher concentrations of As (7.80 ± 2.20 µg/L) and Pb (1.55 ± 1.38 µg/L) in colostrum than the levels detected in the present study. Furthermore, in another study in Austria Rossipal & Krachler (1998) reported higher concentrations of Cd (1.30 ± 1.20 µg/L), Pb (2.30 ± 2.90 µg/L) and Hg (7.70 ±11.00 µg/L) respectively in colostrum than the concentrations of these toxic heavy metals found in this study.

Correlation Matrix of Toxic Heavy Metals in Colostrum of Mothers

With the exception of Al which had quite a weak but significant positive correlation with Pb (r = 0.442, p = 0.002) in CM, all the other heavy metals showed non-significant correlation as shown in Table 16.

Table 16: Correlation Matrix for Toxic Heavy Metals in Colostrum

Variables	BMI	Cd	Pb	Al	GES AGE	As	AGE	Hg
BMI	1							
Cd	0.163	1						
Pb	0.035	0.158	1					
Al	-0.027	-0.144	0.442	1				
GES AGE	-0.224	-0.011	-0.207	-0.429	1			
As	-0.061	0.015	0.003	-0.155	0.111	1		
AGE	0.011	-0.168	-0.103	0.073	-0.131	-0.154	1	
Hg	-0.053	0.066	-0.003	0.109	0.135	0.137	-0.217	1

Values in bold are different from 0 with a significance level = 0.05.
Source: Laboratory Data (2022)

The weak but significant positive correlation observed between Al and Pb suggest they have similar physiological pathway. The study also found a significant negative correlation between Al and gestational age of the mothers. This observation may be due to the fact that Al in breast milk may have an inverse relation with gestational age.

Relationship between Levels of Toxic Heavy Metals in Colostrum Milk and Sociodemographic Characteristics of the Mothers

Table 14 summarizes the sociodemographic characteristics of the study participants and babies. Using nonparametric analysis on the data with Kruskal-Wallis Test at 95% significance level, the study attempted to find out the relationship between toxic heavy metals in colostrum milk and sociodemographic characteristics such as maternal age, BMI, gestational age, parity, education level, infants sex, drinking water sources (DWS), diet and employment status of mothers. The results showed that there was no significant relationship between heavy metals levels in CM and maternal age, maternal BMI, infants sex, gestational age, DWS, diet and employment status of mothers.

The findings are in line with those reported by other researchers (Björklund et al., 2012; Jeong et al., 2017; Khanjani et al., 2018; Kunter et al., 2017; Lee et al., 2016; Olowoyo et al., 2021) who did not find any significant relationship between trace elements in breast milk and maternal age, maternal weight, maternal education, infants sex and DWS. Some researchers (Chao et al., 2006; Gundacker et al., 2002) however, found relationships with these factors.

In a study in Taiwan (Chao et al., 2014) observed a significant correlation between levels of Pb in colostrum milk and maternal age, educational level and parity. They found that the age of a lactating mother had a significant relationship with Pb levels in CM ($r = 0.396$, $p = 0.007$). They reported that lactating mothers who were 30 years and above had higher levels of Pb in their CM (14.48 ng/mL) than those who were less than 30 years (12.11 ng/mL). Besides, they found a significant correlation between Pb levels in CM and maternal educational level ($r = 0.311$, $p = 0.038$). They observed that lactating mothers with College level education and above had higher mean levels of Pb in their CM (14.48 ng/mL) than those with High School education and below (11.64 ng/mL). Furthermore, (Chao et al., 2014) also found a significant correlations between Pb levels in the CM and parity. They observed that multiparous mothers had higher Pb levels in their colostrum milk than primiparous mothers.

The latter observation was consistent with findings in this present study which also found a significant difference between Pb levels in the CM and parity. However, primiparous mothers were found to have higher levels of Pb in their CM than their multiparous counterparts. The box plot in Figure 14 compares means levels of Pb in both primiparous and multiparous mothers while Figure 15 summarizes the mean values of the various toxic heavy metals and their corresponding Kruskal-Wallis p-values.

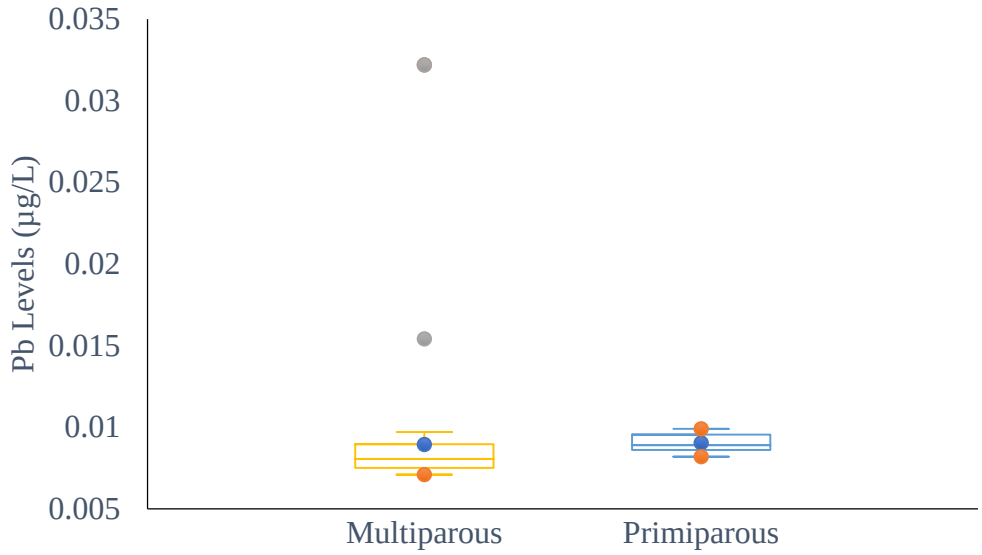

Figure 14: A Gragh of the Mean Levels of Pb (µg/L) in Primiparous and Multiparous Mothers. Source: Laboratory Data (2022)

From the box plot, the mean concentrations of Pb in primiparous mothers were slightly higher than those of multiparous mothers. This was confirmed from the mean of ranks (primiparous = 34.36; multiparous = 22.19) from multiple pairwise comparison. The slightly higher levels of Pb observed in primiparous mothers may be explained by the fact that first time mothers (primiparous mothers) have higher Pb body burden than multiparous mothers. Consequently, the rate of mobilisation of Pb into breast milk was also higher. Figure 15 below illustrates toxic heavy metals and Kruskal-Wallis p-values indicating significant relationship.

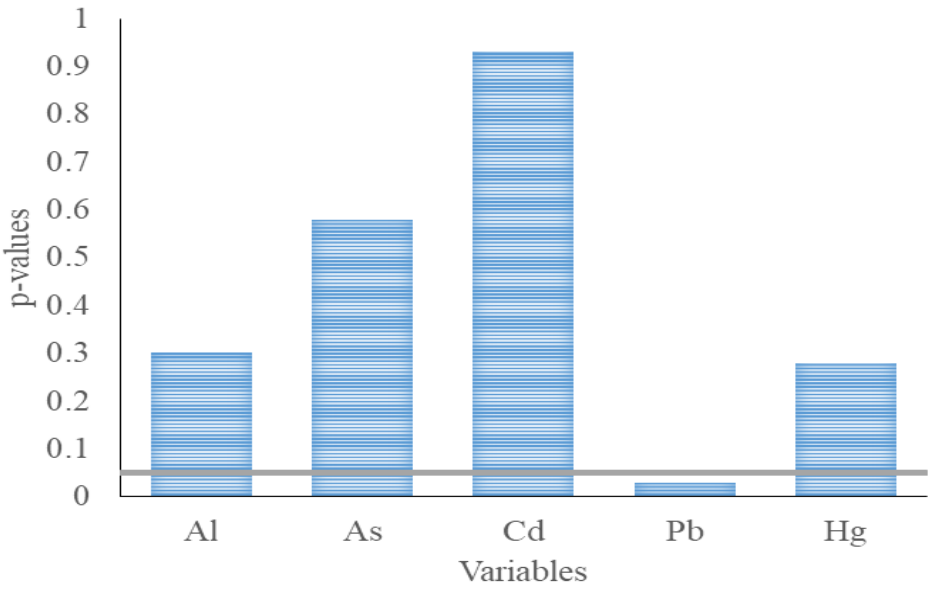

Figure 15: A Graph of Toxic Heavy Metals and Kruskal-Wallis P-Values
Source: Laboratory Data (2022)

The Kruskal-Wallis p-value (two-tailed) for Pb in Fig.15 (p = 0.030) indicates a significant relationship between Pb levels in mothers and parity.

Levels of Heavy Metals in the Transitional Milk of Mothers

The results of the determination of heavy metals in the transitional milk of mothers are also shown in Table 17 while the general data is in the Appendix J.

Table 17: Descriptive Statistics of Toxic Heavy Metals in the Transitional Milk of Mothers

Heavy Metal	Detection Frequency(%)	Min (µg/L)	Max (µg/L)	Mean (µg/L)	SD (µg/L)	Coefficient of Variation (%CV)
Al	82.98	0.050	0.789	0.123	0.153	124.39
As	82.98	0.012	0.290	0.036	0.043	119.44
Cd	36.17	0.002	0.003	0.003	0.000	0.00
Pb	74.47	0.007	0.997	0.037	0.167	451.35
Hg	51.06	0.005	0.008	0.006	0.001	16.67

Source: Laboratory Data (2022)

The mean concentrations of heavy metals detected ranged from 0.003 ± 0.000 µg/L (Cd) to 0.123 ± 0.153 µg/L (Al). Here again, aluminium indicated the highest concentration with cadmium having the lowest concentration. Lead, however, gave a high concentration in the transitional milk than arsenic. Figure 16 illustrates the variation of concentrations of toxic heavy metals in the transitional milk of mothers.

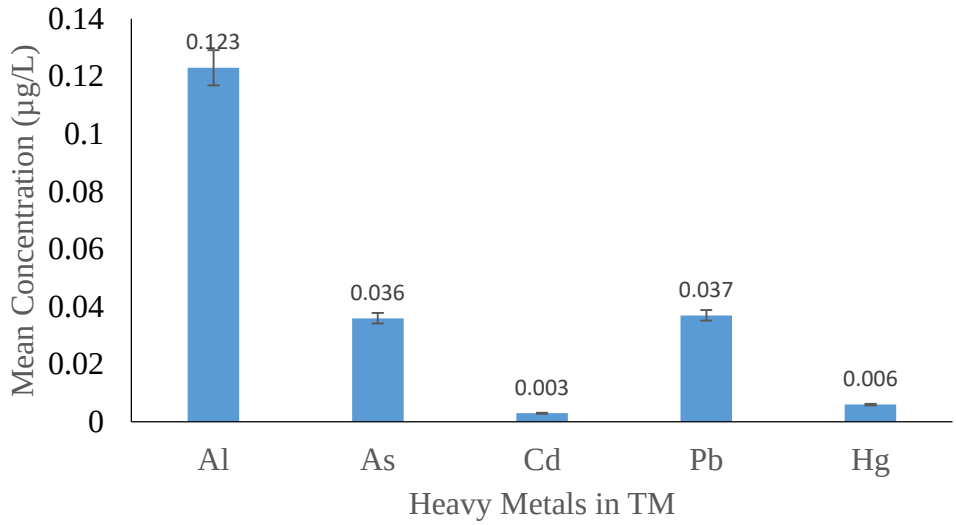

Figure 16: A Graph of Concentrations of Heavy Metals in the Transitional Milk of Mothers (With 5% Standard Error Bars). Source: Laboratory Data (2022)

There was a wide variation in the concentration of toxic heavy metals in the TM of mothers. The heavy metal which exhibited the highest variation was Pb with a coefficient of variation of 451.35%, followed by Al (124.39%), As (119.44%) and Hg (16.67%) respectively. Cd was the only toxic heavy metal which did not show any variation in it concentrations in the data for TM. Essentially, only Cd and Hg offered the best distribution in the data.

The levels of heavy metals detected in transitional milk were again lower in this study than those reported in Taiwan (Chao et al., 2014) and Austria (Rossipal & Krachler, 1998). The current study, however, found a

slightly increasing levels of Al, As and Pb from colostrum to transitional milk except Cd and Hg whereas Chao et al. (2014) observed a decreasing trend for all the heavy metals investigated (i.e. Al, As, Cd & Pb).

Correlation Matrix for Heavy Metals in the Transitional Milk of Mothers

The correlation matrix of toxic heavy metals in transitional milk (TM) of mothers is shown in Table 18.

Table 18: Correlation Matrix of Toxic Heavy Metals in TM

Variables	Hg	GES AGE	Pb	Al	AGE	Cd	As	BMI
Hg	**1**							
GES AGE	0.209	**1**						
Pb	-0.128	-0.205	**1**					
Al	0.022	-0.145	**0.369**	**1**				
AGE	-0.025	-0.131	0.110	0.285	**1**			
Cd	-0.023	0.026	0.017	0.142	0.070	**1**		
As	-0.104	-0.069	0.009	-0.063	0.037	-0.239	**1**	
BMI	-0.184	-0.224	-0.082	-0.069	0.011	-0.043	-0.195	**1**

Values in bold are different from 0 with a significance level = 0.05
Source: Laboratory Data (2022)

From Table 18, correlation analysis on the data from the transitional milk found only one weak positive significant correlation between Pb and Al ($r = 0.369$; $p = 0.011$). The significant but weak positive correlation between Al and Pb suggests that they come from the same anthropogenic source. This implies that Al and Pb can coexist in the same environment. Some studies have established positive correlations between Al and Pb in the knee joint structures (Li et al., 2021). It has also been reported that generally, the concentrations of Al in the soil are positively correlated under most natural conditions (Xu et al., 2022) due to Al and Pb enrichment in clay minerals.

Sociodemographic Factors Influencing Levels of Toxic Heavy Metals in the TM of Mothers

The analysis of questionnaires from all the 47 lactating mothers and the concentrations of Al, As, Cd, Pb and Hg during the transitional milk stage of lactation revealed no significant relationship between heavy metals levels in TM and maternal age, parity, maternal BMI, infant sex, diet, drinking water sources and employment status. However, a significant relationship was found between maternal education and Cd levels in TM of mothers. Figure 17 illustrates a significant relationship ($p = 0.038$) between Cd levels in TM and maternal education.

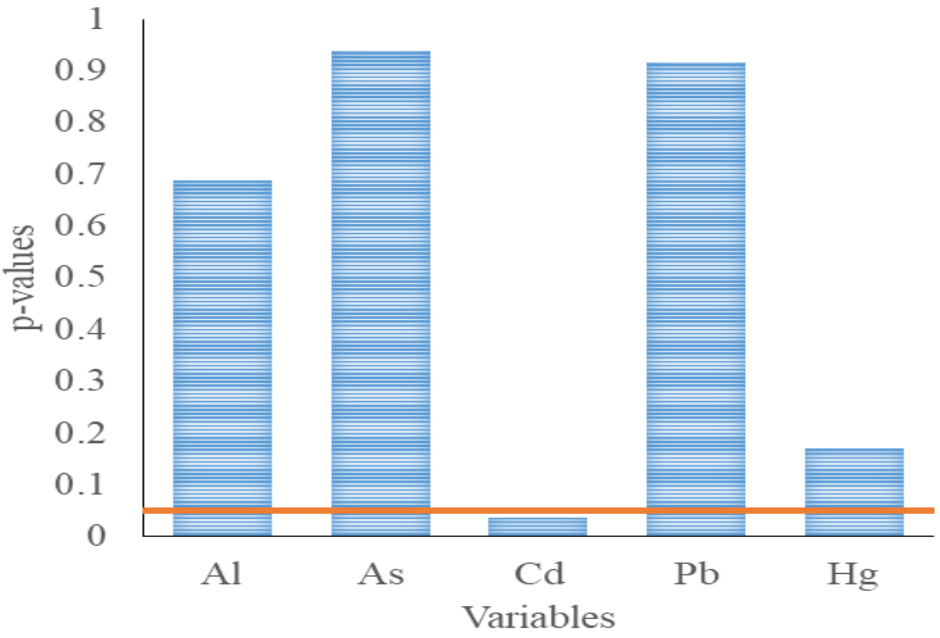

Figure 17: A Graph of Toxic Heavy Metals Against Kruskal-Wallis P-Values
Source: Laboratory Data (2022)

The study found that mothers without any formal education had higher Cd concentrations in their TM than those with formal education (Figure 18).

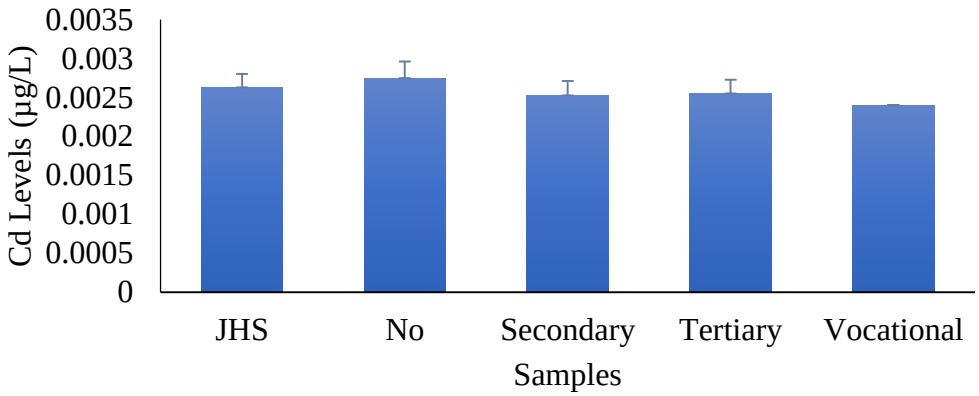

Figure 18: A Graph of Cd Levels in TM Against Maternal Educational Levels
Source: Laboratory Data (2022)

This finding may be a bit misleading because if level of education were the sole predictive factor in determining concentrations of Cd in mothers' milk, then those with tertiary education would have had less but that was not the case. On the contrary, mothers with vocational education rather had the least levels of Cd in their transitional milk (Fig. 18).

Levels of Toxic Heavy Metals in the Mature Breast Milk of Mothers

In the mature breast milk, about 33.62% of the samples analysed revealed that the heavy metals were below limits of detection. The descriptive statistics of toxic heavy metals detected in the mature milk of mothers is shown in Table 19 while the general data is presented in Appendix K.

Table 19: Descriptive Statistics of Toxic Heavy Metals in the Mature Breast Milk of mothers

Heavy Metal	Detection Frequency (%)	Min (µg/L)	Max (µg/L)	Mean (µg/L)	SD (µg/L)	Coefficient of Variation(%CV)
Al	80.85	0.049	0.564	0.105	0.088	83.81
As	78.72	0.012	0.293	0.038	0.045	118.42
Cd	40.43	0.002	0.004	0.003	0.000	0.00
Pb	74.47	0.007	0.021	0.009	0.003	33.33
Hg	57.45	0.005	0.007	0.006	0.000	0.00

Source: Laboratory Data (2022)

Levels of toxic heavy metals detected in mature milk of mothers ranged from 0.003 ± 0.000 µg/L (Cd) to 0.105 ± 0.088 µg/L (Al). Cadmium recorded the least concentration whereas aluminium recorded the highest concentration. Overall analysis of the levels of toxic heavy metals in the mature breast milk showed that cadmium recorded the least concentration followed by mercury, lead and arsenic in that order. Figure 19 illustrates the distribution of toxic heavy metals in the mature breast milk of mothers.

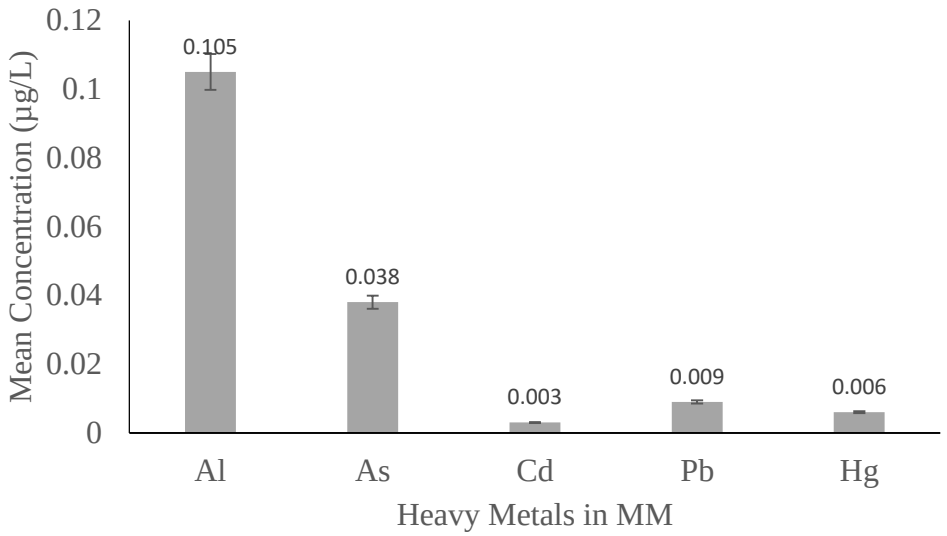

Figure 19: Variation in the Concentrations of Toxic Heavy Metals in the Mature Milk of Mothers (With 5% Error Bars). Source: Laboratory Data (2022)

The coefficient of variation calculated showed quite a wide range of variability in the concentrations of some toxic heavy metals in the data set for mature breast milk. The highest variation was exhibited by As (118.42%), followed by Al (83.81%) and Pb (33.33%) respectively. There were no variations in the concentrations of Cd (0.00%) and Hg (0.00%). Thus Cd and Hg gave the best distribution in the data set for mature human breast milk.

Generally, the concentrations of the toxic heavy metals detected were not very high. They were lower than those reported in Taiwan (Chao et al.,

2014), levels of As (5.80 ±1.10 µg/L) and Pb (0.94 ± 1.05 µg/L) reported in Portugal (Almeida et al., 2008), and levels of Cd (0.26 ± 0.19 µg/L), Hg (< 0.52 µg/L) and Pb (0.90 ± 1.70 µg/L) reported in Austria (Rossipal & Krachler, 1998).

Correlation Matrix for Toxic Heavy Metals in the Mature Milk of Mothers

The correlation matrix of toxic heavy metals in the MM of mothers is presented Table 20.

Table 20: Correlation Matrix of Toxic Heavy Metals in the Mature Breast Milk of Mothers

Variables	BMI	GES AGE	Al	As	Pb	Hg	Cd	AGE
BMI	**1**							
GES AGE	-0.224	**1**						
Al	-0.031	**-0.341**	**1**					
As	-0.085	-0.022	-0.234					
Pb	-0.082	-0.093	0.115	-0.279	**1**			
Hg	-0.085	0.108	-0.001	-0.063	0.259	**1**		
Cd	0.040	-0.036	-0.004	-0.122	0.120	0.096	**1**	
AGE	0.011	-0.131	0.072	0.161	-0.044	-0.056	-0.124	**1**

Values in bold are different from 0 with a significance level alpha=0.05
Source: Laboratory Data (2022)

There was only one negative significant correlation found between Al in mature milk and gestational age of mothers (r = -0.341; p = 0.020).

The weak negative significant correlation observed between Al levels in mature breast milk and gestational age of mothers suggested an inverse relationship. It could be explained that as Al levels in the MM increases, gestational ages of mothers decreases or vice versa.

Maternal Factors Influencing Levels of Toxic Heavy Metals in the Mature Milk of Mothers

The study attempted to find out whether there existed any significant difference between levels of toxic heavy metals in mature breast milk and maternal characteristics and infants' sex by subjecting the data to nonparametric test using Kruskal-Wallis test at 95% significant interval.

The results did not find any significant relationship between levels of heavy metals in the MM breast milk and maternal age, maternal BMI, infant sex, drinking water sources and maternal employment status. It, however, found significant relationships between:

1) Pb levels in the MM and maternal parity;
2) Pb levels in the MM and maternal diet; and also
3) Al levels in the MM and maternal education.

With regards to parity, the study found a significant difference between Pb levels in MM and maternal parity. It observed that, primiparous mothers had higher Pb levels in their mature breast milk than multiparous mothers. Figure 20 illustrates a significant relationship between Pb levels in MM and maternal parity ($p = 0.039$). The results of the study were in contrast to those reported in Taiwan (Chao et al., 2014) in which multiparous women were found to have higher levels of Pb in their breast milk than primiparous women. Though trace metal levels have been known to be associated with maternal parity, however, the reasons for these observation are not fully understood and cannot be explained satisfactorily.

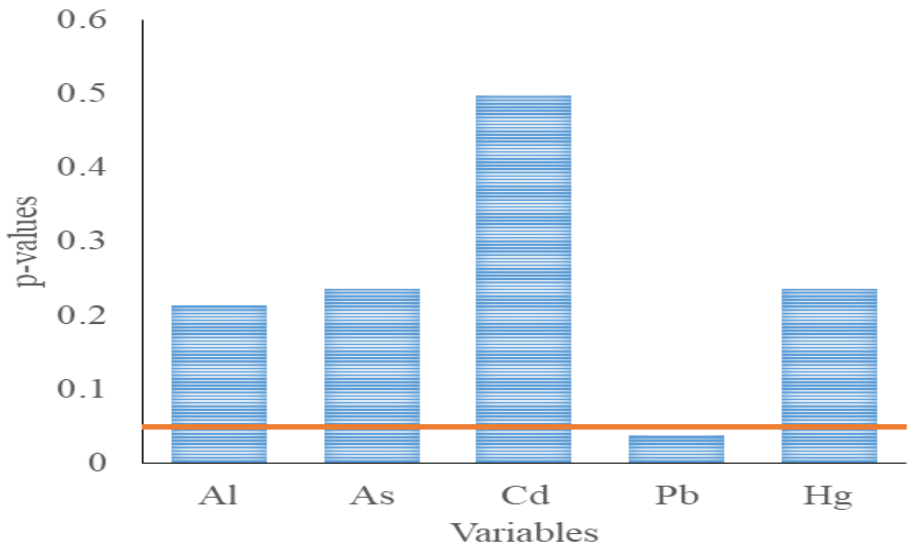

Figure 20: Relationships of Toxic Heavy Metals in MM and Kruskal-Wallis P-Values. Source: Laboratory Data (2022)

The higher levels of Pb observed in the mature breast milk of primiparous mothers in this study may possibly be explained by the fact that, being first time mothers, the degree or extent of mobilisation of stored Pb in their bones tissues into their breast milk progresses steadily during the period of lactation. Thus, Pb levels in their mature milk would be higher than of multiparous mothers. The box plot in figure 21 also gives a comparison of the levels of Pb in primiparous and multiparous mothers.

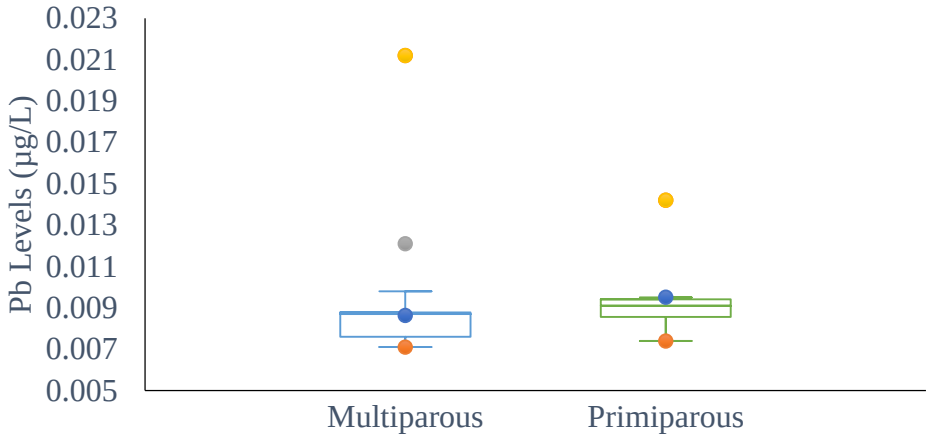

Figure 21: A Box Plot Comparing Pb Levels in Primiparous and Multiparous Mothers. Source: Laboratory Data (2022)

Figure 21 indicates a slightly higer levels of Pb in primipaous mothers than in multiparous mothers.

Additionally, the study found a significant relationship between Pb levels in mature breast milk and maternal diet (p = 0.026) as shown in Figure 22.

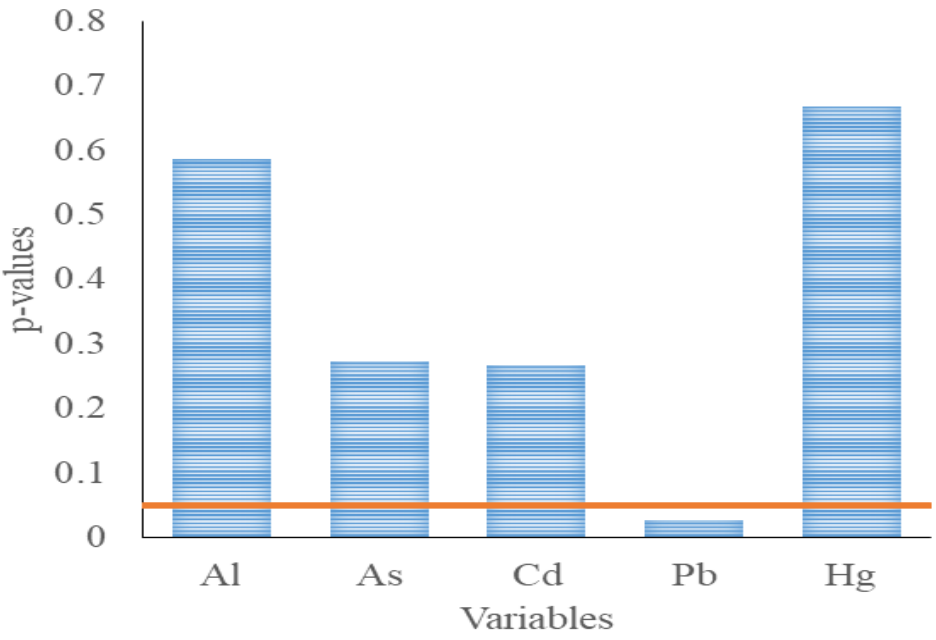

Figure 22: Relationship of Toxic Heavy Metals in Mature Breast Milk and Kruskal-Wallis P-Values. Source: Laboratory Data (2022)

The Kruskal-Wallis p-value (p = 0.026) in Figure 22 indicates a significant difference between Pb levels in the MM and maternal diet. It was observed that mothers whose common diets was predominantly seafood had higher Pb levels in their mature breast milk than those whose common diet were from freshwater or mixed sources. The box plot in Figure 23 shows that the highest levels of Pb was found in the mature breast milk of mothers whose main diet was seafood.

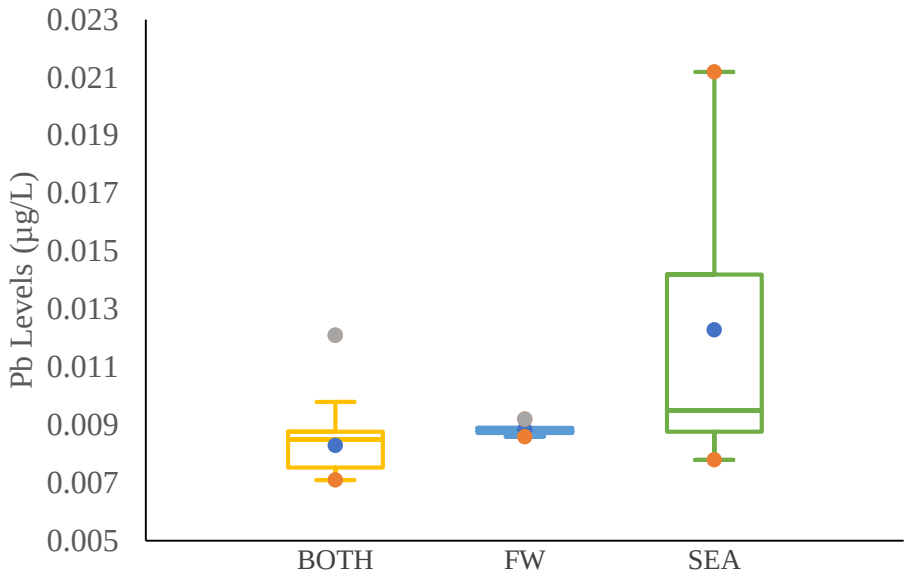

Figure 23: A Box Plot Comparing Pb Levels in Mature Human Breast Milk and Maternal Diet. Source: Laboratory Data (2022)

Diet has been reported to be one of the major exposure pathways to trace metal elements (El-Kady & Abdel-Wahhab, 2018). Though seafood has been known to be the major route of exposure to As and Hg (Dursun et al., 2016; Gundacker & Zodl, 2005), however, in the present study, Pb exposure has been found to be associated with it as well.

Furthermore, the study also found a significant difference between Al levels in the mature human breast milk and maternal education. It was observed that mothers who had secondary level education had a slightly higher Al concentration in their MM than mothers with other levels of education. The box plot in Figure 24 illustrates this observation.

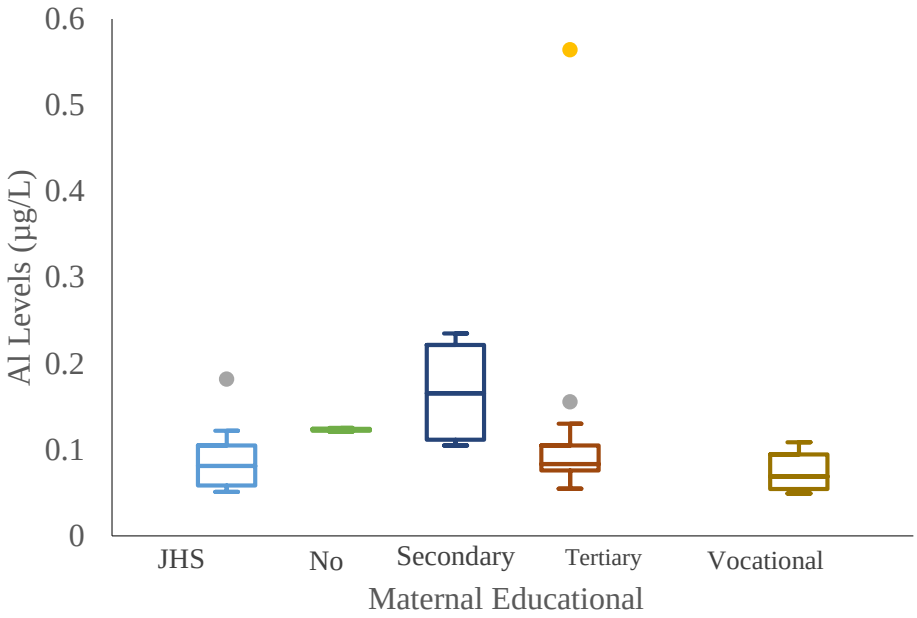

Figure 24: A Box Plot Comparing Toxic Heavy Metals Levels in MM and Maternal Education. Source: Laboratory Data (2022)

Figure 25 on the other hand, illustrates the significant difference between Al in MM and maternal education (p = 0.037).

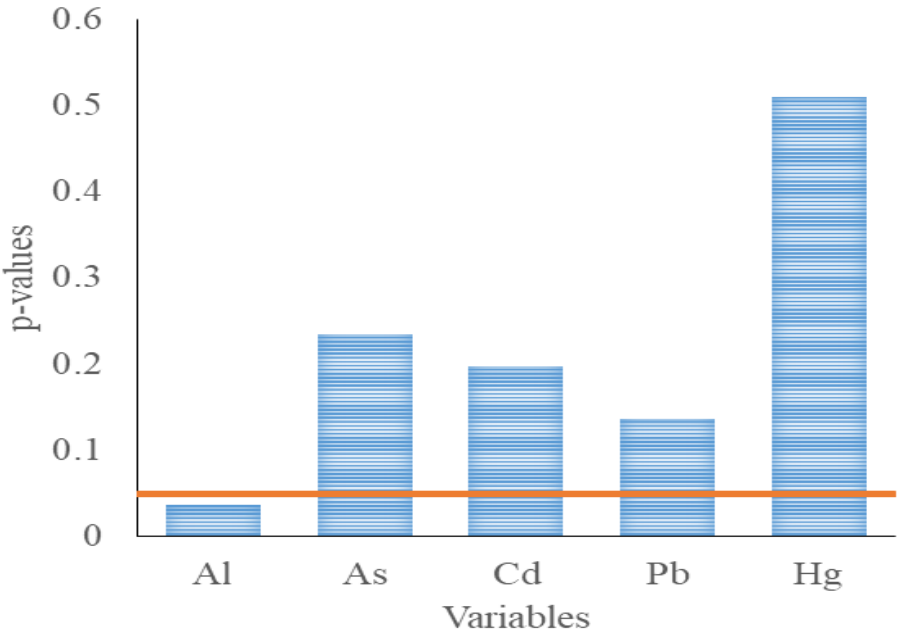

Figure 25: Levels of Toxic Heavy Metals and Kruskal-Wallis P-Values Indicating Significant Relationship. Source: Laboratory Data (2022)

The study findings were similar to those reported in Taiwan where (Chao et al., 2014) observed a significant correlation between Pb levels in colostrum milk and maternal education (r = 0.311; p = 0.038). They found that mothers who had attained College level education and above had higher levels of Pb in their colostrum milk (14.48 ± 3.43 ng/ml) than mothers who had High School education and below (11.64 ± 3.17). Though these observations have been made concerning the relationship between trace metals levels in breast milk and maternal education, the chemistry and mechanism behind these observations are not yet understood and cannot be satisfactorily explained.

Total Mean Concentrations of Heavy Metals in the Total Breast Milk of Mothers

The total mean levels of toxic heavy metals in mothers' breast milk are shown in Table 21 while the general data is presented in Appendix L.

Table 21: Descriptive Statistics of Toxic Heavy Metals in Total Breast Milk of Mothers with WHO Acceptable Limits

Heavy metal	Detection frequency (%)	Min (µg/L)	Max (µg/L)	Mean ± SD (µg/L)	Coefficient of variation (%CV)	WHO Acceptable limits (µg/L)
Al	87.23	0.051	0.351	0.108 ± 0.076	70.37	4.000*
As	87.23	0.012	0.267	0,037 ± 0.038	102.70	0.20 – 0.600
Cd	36.17	0.002	0.003	0.003 ± 0.000	0.00	<1.00
Pb	89.36	0.007	0.337	0.018 ± 0.051	283.33	2.00 – 5.00
Hg	61.70	0.005	0.007	0.006 ± 0.001	16.67	1.40 – 1.70

**The limit of Al can be more than the stated value.* Source: Laboratory Data (2022)

It represents the total mean concentrations of the toxic heavy metals analysed in the colostrum, transitional milk and mature breast milk. The levels were from 0.003 ± 0.000 µg/L (Cd) to 0.108 ± 0.076 µg/L (Al). It is evident that among the heavy metals analysed, aluminium recorded the highest concentration, followed by arsenic (0.037 ± 0.038 µg/L), lead (0.018 ± 0.051 µg/L), mercury (0.006 ± 0.001 µg/L) and cadmium (0.003 ± 0.000 µg/L)

receptively. Figure 26 illustrates the pattern of distribution of toxic heavy metals in total breast milk.

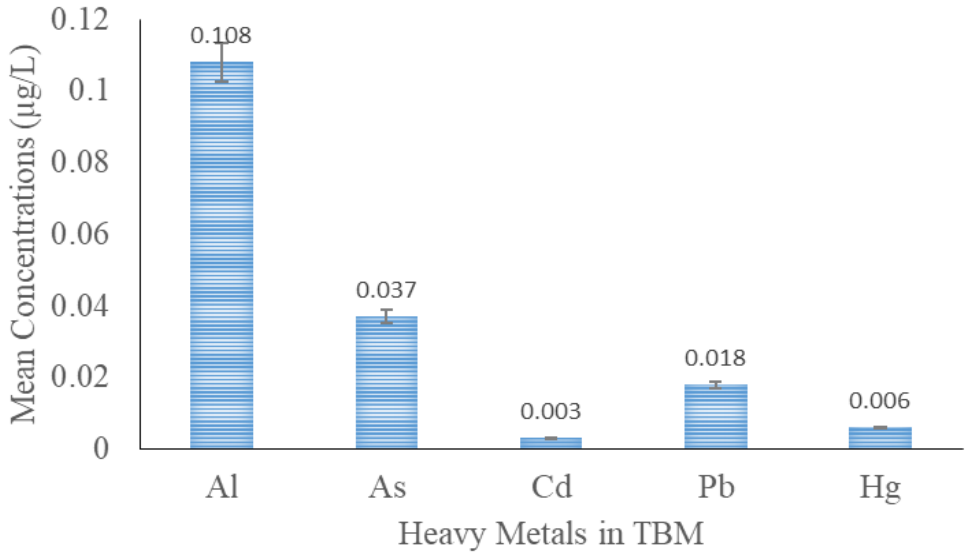

Figure 26: A Graph of the Mean Levels of Toxic Heavy Metals in TBM
Source: Laboratory Data (2022)

There was quite a wide range of variation in the concentrations of different toxic heavy metals in the data set for total breast milk. The highest variation was exhibited by Pb with a coefficient of variation of 283.33%, followed by As (102.70%), Al (70.37%) and Hg (16.67%). There were no variations in the concentrations of Cd in the data set for total breast (%CV = 0.00). Essentially, the toxic heavy metals in total breast milk which presented the best distribution were Cd and Hg.

Distribution of Levels of Toxic Heavy Metals in Total Breast Milk of Mothers

The results of toxic heavy metals determination from total breast milk of mothers showed the general pattern of distribution in the descending order Al > As > Pb > Hg > Cd (Figure 25). Generally, levels of all the heavy metals detected were very low and just a little above the limit of detection, except

aluminium. The concentrations ranged from 0.022 – 0.003 µg/L (Cd, the lowest) to 0.051 – 0.351 µg/L (Al, the highest). The detection frequencies of all the samples ranged from 36.17% (for Cd) to 89.36% (for Pb). The highest mean levels of 0.108 ± 0.076 (Al) and 0.037 ± 0.038 (As) were all below the acceptable values and below the published guideline values of these toxic heavy metals set by the WHO (FAO/WHO, 2011; World Health Organization, 1996).

Al in Breast Milk

With regard to aluminium, few research has been conducted to determine its levels in breast milk. However, almost in all studies across the world where levels of aluminium have been quantified, the concentrations were more often than not, high. This may be as a results of it being used in manufacturing processes and packaging of materials (Sipahi et al., 2014). There is also the possibility of Al leaching through cooking utensils and food containers into food (Juhaiman, 2010; Marta et al., 2006; Mohammad et al., 2011). Some study indicates that about 60% of dietary intake of aluminium typically comes from milk, milk products and cereal products (Pennington, 1988), while in another study (Hardisson et al., 2017) reported that diet (such as vegetables, fish and seafood as well as roots and tubers) was the most common exposure routes of aluminium into the human body. In recent times, aluminium has been implicated in a number of chronic medical conditions like dementia and Alzheimer's disease (Kawahara & Kato-negishi, 2011). Some studies indicate that brain functions of children exposed to aluminium could be seriously affected (Dorea, 2014). According to some studies Al levels in human milk decrease significantly over the nursing period, ranging from 0.056

mg/L in colostrum to 0.013 mg/L in mature breast milk (Chao et al., 2014). It has also been reported that women aged more than 25 years have high levels of Al in their breast milk (Mandic et al., 1995). However, the present study did not find that. The detection frequency of Al in present study was 87.23%.

Unlike aluminium, a large volume of studies has been carried out on levels of As, Cd, Pb and Hg in human breast milk from different parts of the world using different analytical methods. The mean levels of the toxic heavy metals in the study with their corresponding ranges were As(0.037 ± 0.038 µg/L; range: 0.012 – 0.267 µg/L), Cd(0.003 ± 0.000 µg/L; range: 0.002 – 0.003 µg/L), Pb(0.018 ± 0.051 µg/L; range: 0.007 – 0.337 µg/L) and Hg(0.006 ± 0.001 µg/L; range: 0.005 – 0.007 µg/L). All these concentrations were lower than those previously reported in Ghana (Bansa et al., 2017; Bentum et al., 2010)and also those of Cd and Pb (Koka et al., 2011).

As in Breast Milk

The detection frequency of As in all the samples analysed was 87.23%. This detection was close to the one reported in a recent study Olowoyo et al. (2021) which was 89%. However, the mean levels detected in this study were comparatively lower than in the study just cited and those reported in the reviewed literature by (Kunter et al., 2017; Oliveira et al., 2020; Parr et al., 1991; Samiee et al., 2019)

The levels were also far lower than those reported in the United States, Namibia, Poland and Argentina (Klein et al., 2017). All samples with arsenic did not exceed the maximum tolerance limit of 0.20 – 0.60 µg/L. The main sources of inorganic arsenic are seafood, soils, sediments and groundwater, cereals (e.g. rice and rice products), poultry, mushrooms and some fruit juices

(American Cancer Society, 2020; CDC, 2017; Chung et al., 2014; WHO, 2018b).

Cd in Breast Milk

The detection frequency of Cd in all the samples analysed in the study was 36.17%. This was far lower than those reported in Pretoria (Olowoyo et al., 2021). It was however, higher than those reported in a study in Southern Spain (Motas et al., 2021) where the detection frequency was 6%, though the levels of Cd detected were higher than those found in this study. The mean levels of 0.003 ± 0.000 µg/L with a range of 0.002 – 0.003 µg/L of Cd in this study were also lower than those reported in Pretoria (Olowoyo et al., 2021), Southern Spain (Motas et al., 2021), Taiwan (Chao et al., 2014), United Arab Emirates (Abdulrazzaq et al., 2008), Lebanon (Bassil et al., 2017), Ankara, Turkey (Turan et al., 2001), Saudi Arabia (Al-Saleh et al., 2003), Kota, Turkey (Elmastas et al., 2005), Iran (Rahimi et al., 2009), Greece (Leotsinidis et al., 2005), Sweden (Björklund et al., 2012), Italy (Abballe et al., 2008; Kosanovic et al., 2008)and in many other studies across the world. The mean levels of Cd detected in all the mothers investigated were very low and for that matter may not pose any significant health risks to their infants. The low levels of Cd recorded may be partly due to the fact that all the mothers who took part in the study have had little exposure to Cd. Also, they were non-smokers since smoking is known to be the principal source of Cd in the human body. Another route of Cd exposure is through diet from crops grown in polluted soils and contaminated water.

Pb in Breast Milk

With regard to inorganic lead, the detection percentage of all the samples analysed in the study was 89.36%. This was far higher than those reported on human breast milk studies elsewhere in Southern Spain (Motas et al., 2021) but still lower than the one reported in Pretoria (Olowoyo et al., 2021); though the mean levels of Pb recorded in this study were lower compared to theirs. The mean levels of Pb detected in total breast milk from the current study were 0.018 ± 0.051 µg/L, with a range of 0.007 – 0.337 µg/L. Also, the mean concentrations of Pb recorded were lower than those reported in other breast milk studies elsewhere in Pretoria (Olowoyo et al., 2021), Southern Spain (Motas et al., 2021), Poland (Winiarska-Mieczan, 2014), Taiwan (Chao et al., 2014), Egypt (Hasballah & Beheary, 2017), United States, Namibia, Poland and Argentina (Klein et al., 2017). Common sources of lead exposure in the environment are the air, the soil, water, gasoline, and consumer products such as paint, pipes and plumbing materials, ceramics, solders, batteries, ammunition and cosmetics (US EPA, 2012b). Some studies have reported that higher levels of Pb in human milk have been associated with women who live closer to industrial sites (Chao et al., 2014; Goudarzi et al., 2013), urban areas (Leotsinidis et al., 2005) or use of lead-containing cosmetics (Shawahna et al., 2016). Lead found in human breast milk does not come from the mother's exposure during lactation. Rather, it emanates from lead stored in the mother's bone during her lifetime which is mobilized into blood and breast milk during lactation (Gomaa et al., 2002; Rothenberg et al., 2000; Silbergeld, 1991).

Hg in Breast Milk

The detection frequency of Hg in all the samples analysed was 61.70%. The detection frequency for Hg in this study was a little higher than the one reported in a study conducted in Southern Spain (Motas et al., 2021) which was 58%. However, the mean concentrations of Hg recorded in this study were lower. The mean levels of Hg recorded for total breast milk in the study were 0.006 ± 0.001 µg/L, with a range of 0.005 – 0.007 µg/L (Table 19). Generally, the levels of Hg recorded in the study were lower than those in other breast milk studies in some part of the world such as Southern Spain (Motas et al., 2021), United Arab Emirates (Kosanovic et al., 2008) and Italy (Abballe et al., 2008). Although diet is considered to be the main source of Hg exposure in the general population, yet, since the mean levels of Hg detected in all the samples were generally low, there was no need to investigate the dietary pattern of the participants. In recent times, there is a growing concern about Hg exposure through high fish diet and its adverse effects on the foetus or neurological development of children (Grandjean et al., 1999; Murata et al., 1999).

The low levels of Hg detected in this study may not have any significant risks on the health of infants. Another source of Hg exposure is through dental amalgam which results in the accumulation of Hg levels in breast milk. However, the study did not investigate the effects of Hg levels in mothers with dental amalgam fillings and those without.

Correlation Matrix of Heavy Metals in Total Breast Milk of Mothers

The correlation matrix of toxic heavy metals in total breast milk of mothers is presented in Table 20.

Table 20: Correlation Matrix of Heavy Metals in Total Breast Milk of Mothers

Variables	AGE	Hg	Cd	GES AGE	Al	Pb	As	BMI
AGE	**1**							
Hg	-0.149	**1**						
Cd	0.057	0.184	**1**					
GES AGE	-0.131	0.197	-0.219	**1**				
Al	0.126	0.031	0.125	**-0.365**	**1**			
Pb	0.047	-0.125	0.142	**-0.305**	**0.414**	**1**		
As	-0.026	0.098	-0.102	0.133	-0.151	-0.197	**1**	
BMI	0.011	-0.096	0.056	-0.224	0.011	0.070	-0.065	**1**

Values in bold are different from 0 with a significance level alpha=0.05
Source: Laboratory Data (2022)

The study found only one significant positive correlation between Pb with Al (r = 0.414; p = 0.004) in total breast milk. The significant positive correlation between Al and Pb suggests they come from the same anthropogenic source or other words, they exist together. The study also found significant weak negative correlations between Al and gestational age (r = -0.365; p = 0.012) and also between Pb and gestational age (r = -0.305; p = 0.038). These negative correlations suggest inverse relationship between Al and Pb in total breast milk.

Maternal Factors Influencing Levels of Toxic Heavy Metals in Total Breast Milk (TBM)

The study explored to find out possible maternal characteristics that influenced levels of toxic heavy metal levels in TBM by subjecting the TBM data to nonparametric analysis using Kruskal-Wallis test at 95% significant interval. The results found no significant difference between heavy metals levels in total breast milk and maternal age, maternal education, BMI, maternal diet, drinking water sources, maternal employment status and

infants' sex. However, the study found a significant difference between Cd levels in total breast milk and parity (p = 0.037) as shown in Figure 27.

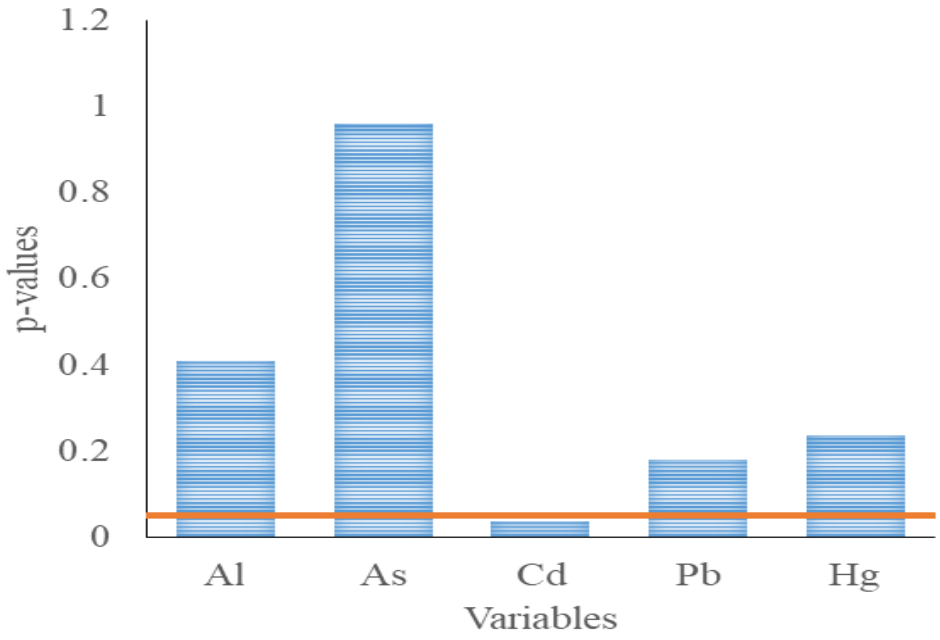

Figure 27: A Graph of Toxic Heavy Metals in TBM and Kruskal-Wallis P-Values. Source: Laboratory Data (2022)

The study found that primiparous mothers had higher Cd levels in their total breast milk than multiparous mothers as shown in the box plot in Figure 28.

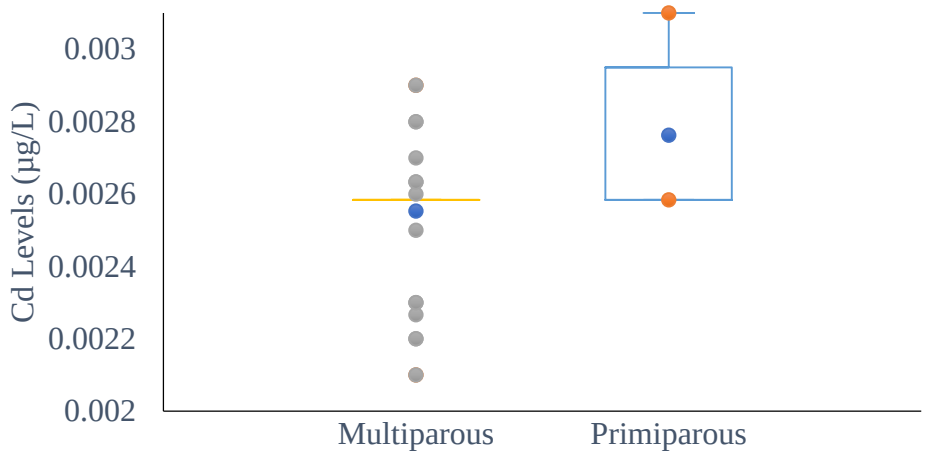

Figure 28: Relationship Between Cd Levels in Total Breast Milk and Parity Source: Laboratory Data (2022)

Generally, for mothers who are not cigarette smokers, the reasons behind this observation is difficult to explain. It may, however, be due to exposure to partial smoking or consumptions of food substances contaminated with Cd. Even though some studies have reported of the relationship between Cd levels in breast milk and infants' sex (Jeong et al., 2017) and also between Cd levels and maternal employment status (Olowoyo et al., 2021), no such relationship has been reported between Cd levels in human milk and parity.

Levels of Essential Elements in the Various Portions of Human Breast Milk

Besides the toxic heavy metals, ten (10) essential mineral elements were also analysed in the study to determine their levels in the three portions of human breast milk namely, colostrum (CM), transitional milk (TM) and mature milk (MM). The results obtained are hereby presented and discussed.

Levels of Essential Elements Detected in the Colostrum of Mothers

The essential elements analysed in this study were calcium, copper, iron, magnesium, phosphorus, sodium, potassium, sulphur, selenium and zinc. Of these, calcium, magnesium, phosphorus, sodium, potassium and sulphur are considered macronutrients (macro-elements) whereas copper, iron, selenium and zinc are regarded as micronutrients (micro- or trace elements). The descriptive statistics of the essential elements in the CM of mothers are presented in Table 21 while the full results are presented in Appendix M.

Table 23: Descriptive Statistics of Essential Elements in the Colostrum Milk of Mothers

Essential Elements	Detection Frequency (%)	Min (mg/L)	Max (mg/L)	Mean (mg/L)	SD (mg/L)	Coefficient of Variation (%CV)
Ca	100.00	121.00	590.00	244.45	80.93	33.10
Cu	100.00	0.11	0.92	0.58	0.17	29.31
Fe	100.00	0.62	2.81	1.40	0.54	38.57
Mg	100.00	18.40	36.30	23.96	3.72	15.53
P	100.00	86.00	282.00	193.23	46.76	24.19
Na	100.00	196.00	626.56	403.91	143.03	35.41
K	100.00	442.00	815.00	602.63	77.62	12.88
S	100.00	138.00	317.00	202.57	46.03	22.72
Se	100.00	0.07	0.21	0.11	0.03	27.27
Zn	100.00	1.36	7.37	3.79	1.38	36.41

Source: Laboratory Data (2022)

There was a great deal of variability in the concentrations of the essential mineral elements in the data set, with coefficient of variation ranging from 12.88% (K) to 38.57% (Fe). The essential elements which exhibited the smallest and better variation in their concentrations were K, with a coefficient of variation of 12.88%, followed by Mg (15.33%). Those with coefficient of variation greater 20% were not good enough.

The mean concentrations of the essential elements detected in the colostrum ranged from 0.11 ± 0.03 mg/L to 602.63 ± 77.62 mg/L. Among the trace mineral elements, selenium recorded the lowest concentration (0.11 ± 0.03 mg/L), followed by copper (0.58 ± 0.17 mg/L), iron (1.40 ± 0.54 mg/L) and zinc (3.79 ± 1.38 mg/L) respectively in that order. The macronutrient which recorded the highest concentration in the colostrum milk was potassium (602.63 ± 77.62 mg/L), followed by sodium (403.91 ± 143.03 mg/L), calcium (244.45 ± 80.93 mg/L), sulphur (202.57 ± 46.03 mg/L), phosphorus (193.23 ±

46.76 mg/L) and magnesium (23.96 ± 3.72 mg/L) respectively as shown in Figure 29.

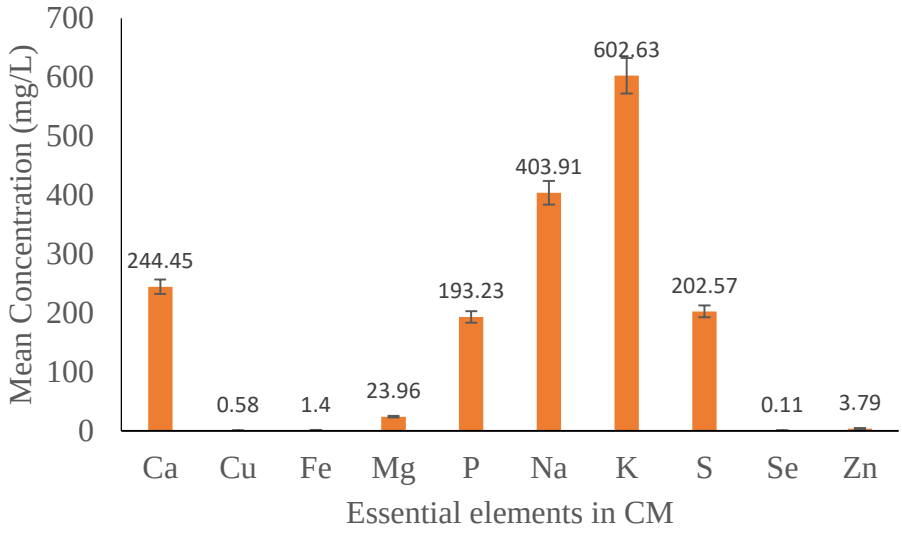

Figure 29: A Graph of the Mean Concentrations of Essential Elements in the Colostrum Milk of Mothers (With 5% Error Bars). Source: Laboratory Data (2022)

Correlation Matrix of Essential Mineral Elements in Colostrum Milk of Mothers

Spearman's correlation analysis performed on the data from colostrum milk of mothers gave the results in Table 24. The study found significant correlations among quite a number of the essential elements.

Table 24: Correlation Matrix for Essential Elements in Colostrum Milk of Mothers

Variables	P	Mg	AGE	Zn	K	Ca	Fe	Na	S	Cu	Se	BMI	GES AGE
P	**1**												
Mg	-0.258	**1**											
AGE	-0.053	**0.331**	**1**										
Zn	-0.202	**0.319**	**0.386**	**1**									
K	**-0.308**	**0.388**	0.211	**0.464**	**1**								
Ca	-0.029	0.162	0.117	**0.299**	**0.557**	**1**							
Fe	-0.004	0.090	0.182	0.255	**0.427**	**0.333**	**1**						
Na	0.098	0.144	0.190	0.237	**0.297**	**0.309**	**0.500**	**1**					
S	**0.336**	0.198	0.239	0.236	0.257	0.094	**0.300**	**0.510**	**1**				
Cu	0.199	0.166	**0.288**	0.129	0.161	0.206	0.209	0.218	0.564	**1**			
Se	-0.099	0.195	0.268	0.266	0.255	-0.090	-0.013	0.008	0.058	-0.203	**1**		
BMI	0.086	0.037	0.011	0.118	-0.119	-0.025	0.044	0.151	0.202	0.050	-0.135	**1**	
GES AGE	0.057	-0.082	-0.131	-0.140	-0.054	-0.081	-0.154	0.078	-0.074	0.060	-0.089	-0.224	**1**

Values in bold are different from 0 with a significance level = 0.05. Source: Laboratory Data (2022)

For instance, there were significant moderately strong positive correlations between K and Zn ($r = 0.464$; $p = 0.001$), K and Ca ($r = 0.557$; $p<0.0001$), Fe and K ($r = 0.427$; $p = 0.003$), Na and Fe ($r = 0.500$; $p = 0.000$), S and Na ($r = 0.510$; $p = 0.000$), Cu and S ($r = 0.564$; $p<0.0001$) (Table 24). In Latvia Aumeistere et al. (2019) found that Ca strongly correlated with K ang Mg. The current study also found a strong positive correlation between Ca and K but a weak positive correlation between Ca and Mg.

The moderately strong positive correlations among the essential elements suggest that those elements are related to one another or they come from the same anthropogenic source. Those with negative correlations suggest they have inverse relationship with one another in CM. This implies that as the concentration of one essential element increases, the concentration of the other would decrease and vice versa.

Possible Maternal Factors Influencing Levels Essential Mineral Elements in Colostrum of Mothers

The study attempted to determine possible maternal characteristics that influenced the levels of essential elements in CM by subjecting the CM data to nonparametric analysis using Kruskal-Wallis test at 95% confidence interval. The analysis found no significant relationship between the essential elements in CM and maternal age, education, diet, BMI, infant sex and drinking water source. In similar manner, some studies such as those of Olowoyo et al. (2021) did not find any significant relationship between levels of trace elements with maternal age, maternal education, parity, infants' sex and sources of drinking water. In a study, Aumeistere et al. (2019) reported that maternal age, parity and infant's sex did not have any significant influence on the levels of

essential elements in breast milk. This study, however, found a significant relationship between K levels in the CM and employment status of mothers as shown in Figure 30 (p = 0.057). Figure 29 illustrates concentrations of K levels in the CM of mothers against employment. The results revealed that mothers who were self-employed had the highest concentrations of K in their colostrum milk than other categories of mothers.

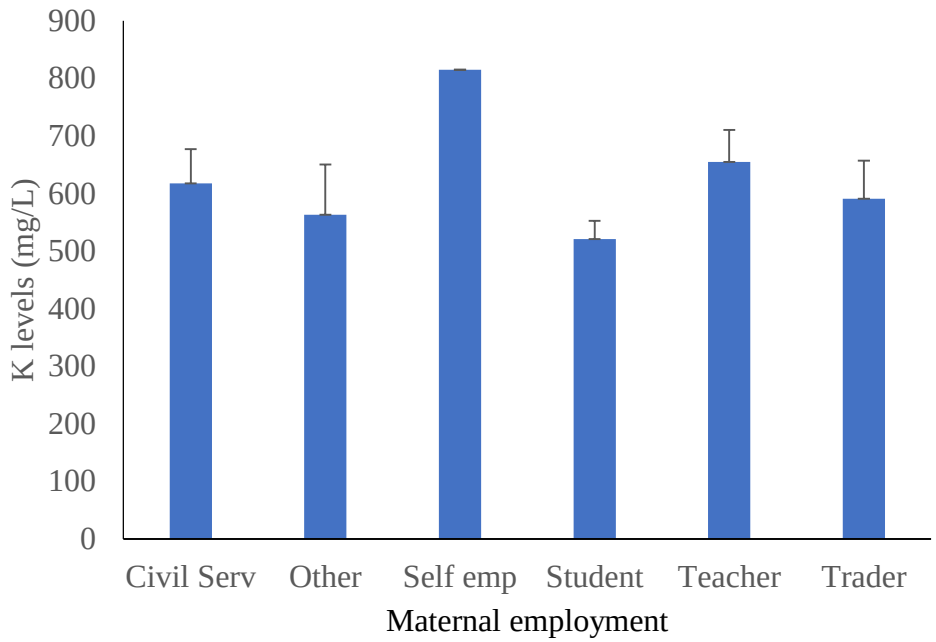

Figure 30: A Graph of K levels in CM Against Maternal Employment Status
Source: Laboratory Data (2022)

The findings are a bit difficult to explain but one may probably suggest that the self-employed mothers have better socio-economic status and therefore, they afford balanced diet containing enough. Some studies have found significantly higher levels of some trace elements in the breast milk of unemployed women (Cherkani-hassani et al., 2016; Orun et al., 2012) which rule out the possibility of occupational exposure of mothers to these trace metal elements.

The study also found a significant relationship between levels of Zn in CM and maternal parity as shown in Figure 31.

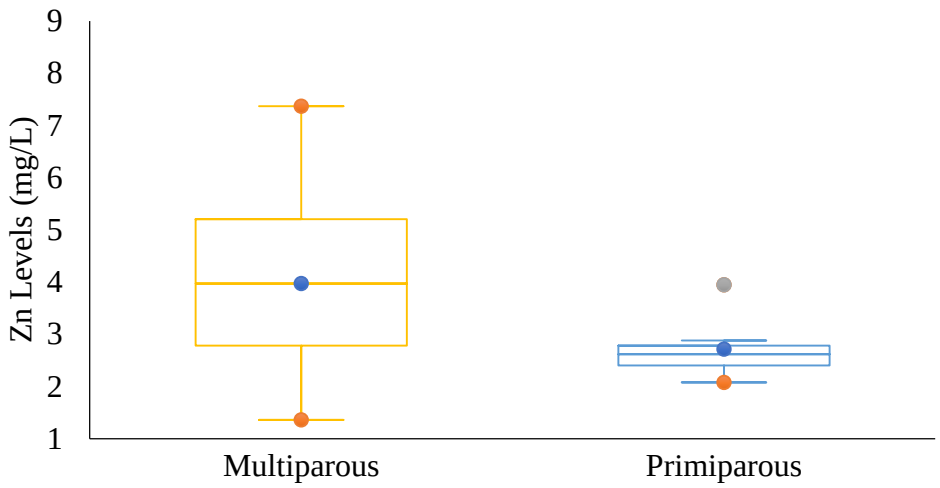

Figure 31: A box Plot Comparing Zn Levels in Primiparous and Multiparous Mothers. Source: Laboratory Data (2022)

It shows that there was a significant difference between levels of Zn in primiparous and multiparous mothers ($p = 0.028$). Essentially, multiparous mothers were found to have a higher concentrations of Zn in their colostrum milk than their primiparous counterparts. Though it is difficult to explain these observations satisfactorily, it may be suggested that physiologically, the system of a multiparous mother had already initiated the production of Zn during previous birth(s). Therefore, it is easier for them to produced more of it in the colostrum milk right after birth. However, in the case of primiparous (first time) mothers, their system will transit from the original state before being eventually switched into that state where these essential elements production will be initiated for the nourishment of the neonate.

Levels of Essential Elements in the Transitional Milk of Mothers

The summary of the descriptive statistics of essential elements detected in the transitional milk in the study are shown in Table 25 while the full results are presented in the appendix N.

Table 25: Descriptive Statistics of Essential Elements in the Transitional Milk of Mothers

Essential Elements	Detection Frequency (%)	Min (mg/L)	Max (mg/L)	Mean (mg/L)	SD (mg/L)	Coefficient of Variation (%CV)
Ca	100.00	148.00	308.00	218.02	42.12	19.32
Cu	100.00	0.30	0.91	0.55	0.14	25.45
Fe	100.00	0.58	1.87	1.16	0.40	34.48
Mg	100.00	16.90	36.30	23.99	3.85	16.05
P	100.00	130.00	240.00	175.60	25.46	14.49
Na	100.00	138.00	1008.00	349.63	151.34	43.29
K	100.00	321.00	816.00	530.15	92.45	17.44
S	100.00	101.00	284.00	165.47	40.83	24.68
Se	100.00	0.07	0.21	0.09	0.03	33.33
Zn	100.00	0.91	7.37	3.16	1.17	37.03

Source: Laboratory Data (2022)

The mean concentrations of the essential elements detected in the transitional milk ranged from 0.09 ± 0.03 mg/L to 530.15 ± 92.45 mg/L. Among the trace mineral elements, selenium recorded the lowest concentration of 0.09 ± 0.03 mg/L, followed by copper (0.55 ± 0.14mg/L), iron (1.16 ± 0.40 mg/L) and zinc (3.16 ± 1.17 mg/L) respectively. Figure 32 illustrates the results.

The study observed a wide range of variability in the concentrations of the essential elements in the data set for TM. From Table 25, the coefficient of variation of the different essential elements analysed ranged from 14.49% to 43.29%. Out of the ten essential elements, only P, Mg, K and Ca exhibited good distribution in the data set with coefficient of variations of 14.49%,

16.05%, 17.44% and 19.32% respectively. The rest of the element gave coefficient of distributions greater than 20% which do not represent good data distribution.

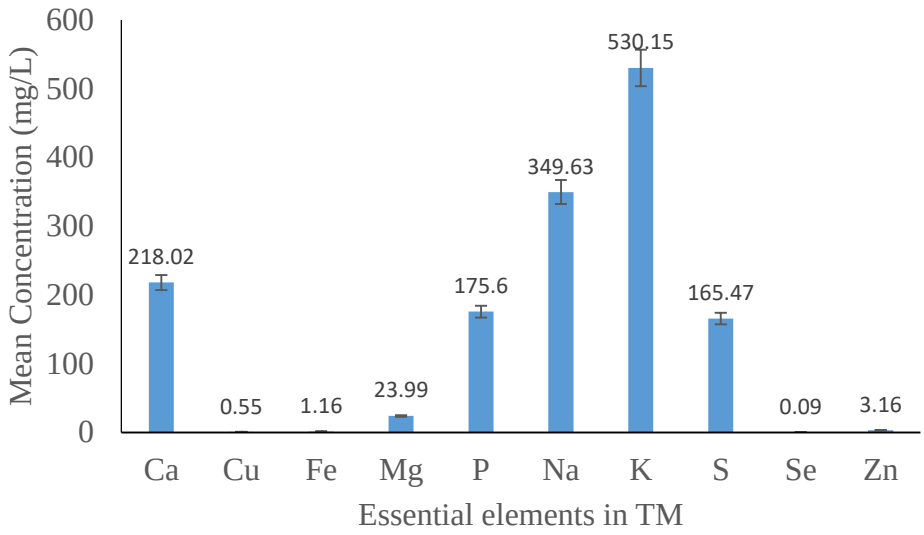

Figure 32: A Graph of the Mean Concentrations of Essential Mineral Elements in the TM of Mothers (With 5% Error Bars). Source: Laboratory Data (2022)

Among the macronutrients, potassium recorded the highest concentration of 530.15 ± 92.45, followed by sodium (349.63 ± 151.34 mg/L), calcium (218.02 ± 42.12 mg/L), phosphorus (175.59 ± 25.46 mg/L), sulphur (165.47 ± 40.83 mg/L) and magnesium (23.99 ± 3.85 mg/L) respectively.

Correlation Matrix of Essential Mineral Elements in the TM of Mothers

Data from the transitional milk were subjected to correlation analysis and the results obtained shown in Table 26.

Table 26: Correlation Coefficient of Essential Mineral Elements in TM of Mothers

Variables	BMI	Mg	P	Zn	Ca	K	S	Na	Fe	Cu	AGE	Se	GES AGE
BMI	**1**												
Mg	0.112	**1**											
P	0.216	**-0.299**	**1**										
Zn	0.046	0.139	-0.253	**1**									
Ca	0.113	0.146	0.229	0.279	**1**								
K	-0.069	0.230	-0.133	**0.309**	**0.457**	**1**							
S	0.097	0.161	0.105	**0.352**	**0.347**	**0.363**	**1**						
Na	-0.030	0.281	-0.094	0.211	**0.325**	**0.318**	**0.514**	**1**					
Fe	0.090	0.157	-0.014	0.151	0.252	**0.351**	0.270	**0.409**	**1**				
Cu	-0.002	0.068	0.088	0.076	0.208	0.058	**0.433**	0.204	**0.292**	**1**			
AGE	0.011	0.275	-0.134	**0.321**	0.020	0.086	0.118	0.278	0.133	0.269	**1**		
Se	-0.031	-0.027	-0.061	**0.303**	0.067	0.239	0.249	0.159	-0.065	-0.120	0.164	**1**	
GES AGE	-0.224	-0.015	0.024	-0.248	-0.149	-0.130	-0.153	0.142	-0.251	-0.022	-0.131	-0.256	**1**

Values in bold are different from 0 with a significance level = 0.05. Source: Laboratory Data (2022)

The study found a number of significant correlations among the essential elements in TM. The essential elements which showed significant correlations in the TM were indicated in bold figures. Some significant positive correlations among the essential elements included Na and S ($r = 0.514$; $p = 0.000$), Cu and S ($r = 0.433$; $p = 0.003$), S and Zn ($r = 0.352$; $p = 0.016$), Fe and K ($r = 0.351$; $p = 0.016$), K and Zn ($r = 0.309$; $p = 0.035$), Fe and Na ($r = 0.409$; $p = 0.005$), K and Ca ($r = 0.457$; $p = 0.001$), S and Ca ($r = 0.347$; $p = 0.017$) and many others in Table 23. It is instructive to know that P was the only essential element which showed a significant but weak negative correlation with Mg ($r = - 0.299$; $p = 0.042$),

This observation suggests an inverse relationship between P and Mg in TM. This inverse relationship meant that P and Mg could not exist together simultaneously in breast milk. Thus as the concentration of P increased, that of Mg would decrease and vice versa.

The positive and significant correlations among the essential elements in TM may probably reflects similar mechanisms in their pathway from plasma to milk in the mammary glands as suggested by some authors (Kelleher & Lonnerdal, 2005; Lonnerdal, 2000). However, there is the need for more information and empirical evidence to rationalize these assumptions.

Possible Maternal Factors Influencing Levels of Essential Mineral Elements in the TM of Mothers

A number of factors have been suggested to account for variations in the levels of essential elements in human breast milk. In order to determine the relationship between essential mineral elements levels in TM and maternal characteristics as well as infants' sex, the data was subjected to nonparametric

analysis using Kruskal-Wallis test at 95% significant interval. The results found no significant relationship between the levels of essential elements in TM and maternal BMI, parity, maternal education, infants' sex, drinking water sources, diet and maternal employment status. It, however, found a significant difference between the concentrations of Cu in TM and maternal age (p = 0.030) as shown in Figure 33.

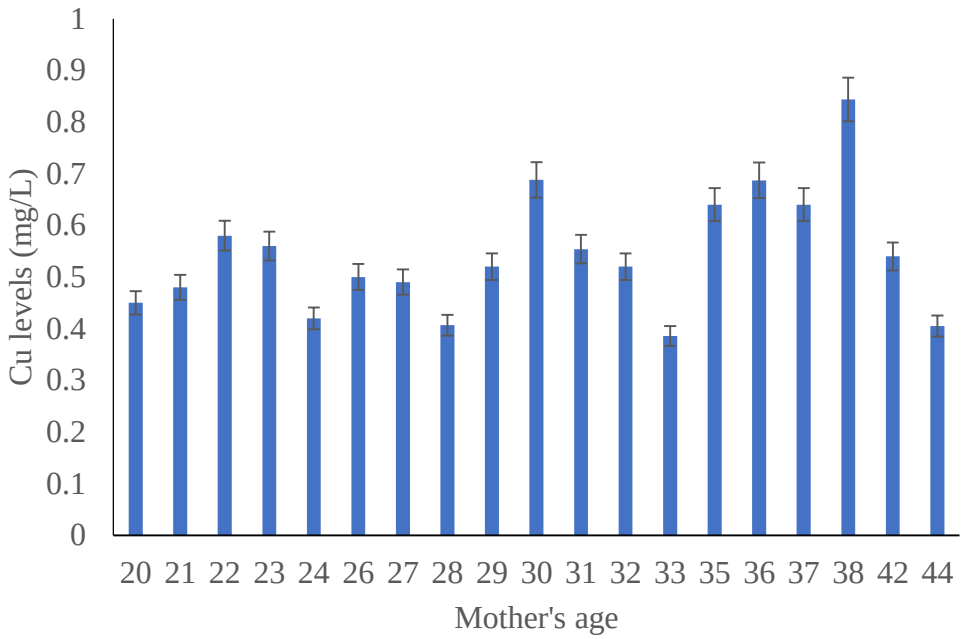

Figure 33: A Graph of Cu Levels in TM and the Age of Mothers. Source: Laboratory Data (2022)

The Kruskal-Wallis p-value of Cu (p = 0.030) indicates a significant relationship between Cu levels in TM and maternal age. The results revealed that mothers who were 38 years old had the highest levels of Cu in their TM than mothers who were either younger or older as shown in Figure 33. The findings are in line with those reported in study in Southern Spain where mothers with the highest level of copper in their breast milk 35 years old and above (Motas et al., 2021).

The observation is similar to those made in a study in Poland (Goc et al., 2012) but in a different direction. They reported that the lowest concentration of Ca and Mg were observed among younger women (20 – 25 years). The researchers revealed that whereas the highest level of Ca was detected from the oldest group of women (32 – 35 years), the highest levels of Mg was observed in women from 25 – 31 years old. In the current study, mothers with highest levels of Cu in their TM were older (38 years).

These findings are in line with those reported by (Chao et al., 2014; Gundacker et al., 2002) that maternal age had effects on the levels of trace inorganic metals in human milk. The current study results were, however, contrary to those reported by (Lin et al., 1998) who found no influence of maternal age on the levels of essential elements in transitional milk. Other researchers like (Björklund et al., 2012; Hsueh et al., 2017; Jeong et al., 2017; Khanjani et al., 2018; Kunter et al., 2017; Olowoyo et al., 2021) found no influence of maternal age on trace metal levels in breast milk. It is, however, important to mention that even among the researchers who found a relationship between maternal age and trace metal elements concentration in breast milk, the evidence was often contradictory.

Whereas some studies reported significantly higher levels of trace metal elements in older women such as in Taiwan (Chao et al., 2014), Japan (Nishijo et al., 2002), Slovakia (Ursinyova & Masanova, 2005), Saudi Arabia (Younes et al., 1995) and India (Sharma & Pervez, 2005), other studies from Zanzibar (Norouzi et al., 2010), Japan (Honda et al., 2003) and Iran (Vahidinia et al., 2019) reported significantly higher concentration in younger women, though the différences were not signifiant. The reasons for these variations are

not known but may be due to physiological mechanisms involved in the regulation of mammary gland mineral transport during lactation.

Levels of Essential Elements Detected in the Mature Breast Milk of Mothers

The summary of the descriptive statistics of the mean concentrations of the essential elements detected in the mature breast milk of mothers are presented in Table 27 while the entire results are presented in Appendix O.

Table 27: Descriptive Statistics of Essential Elements in Mature Breast Milk of Mothers

Essential Elements	Detection Frequency (%)	Min (mg/L)	Max (mg/L)	Mean (mg/L)	SD (mg/L)	Coefficient of Variation (%CV)
Ca	100.00	132.00	457.00	202.68	65.79	32.46
Cu	100.00	-26.00	0.92	0.45	0.14	31.11
Fe	100.00	0.54	2.21	1.00	0.39	39.00
Mg	100.00	17.10	32.90	24.67	3.55	14.39
P	100.00	112.00	240.00	151.17	28.18	18.64
Na	100.00	86.00	527.00	269.07	117.01	43.48
K	100.00	324.00	596.00	456.34	76.45	16.75
S	100.00	62.00	198.00	130.45	37.56	28.79
Se	100.00	0.07	0.35	0.09	0.05	55.56
Zn	100.00	0.15	4.65	2.20	1.13	51.36

Source: Laboratory Data (2022)

The mean concentrations of the essential elements in MM ranged from 0.09 ± 0.05 mg/L(Se) to 456.34 ± 76.45 mg/L(K). Selenium recorded the lowest mean concentration among the micronutrients in the mature breast milk. The pattern of concentration of the micronutrients followed the increasing order: selenium < copper < iron < zinc. However, with regard to the macronutrients, potassium recorded the highest concentration, followed by sodium, calcium, phosphorus, sulphur and magnesium in that order. Figure 34

illustrates the concentrations of the essential elements detected in the mature breast milk.

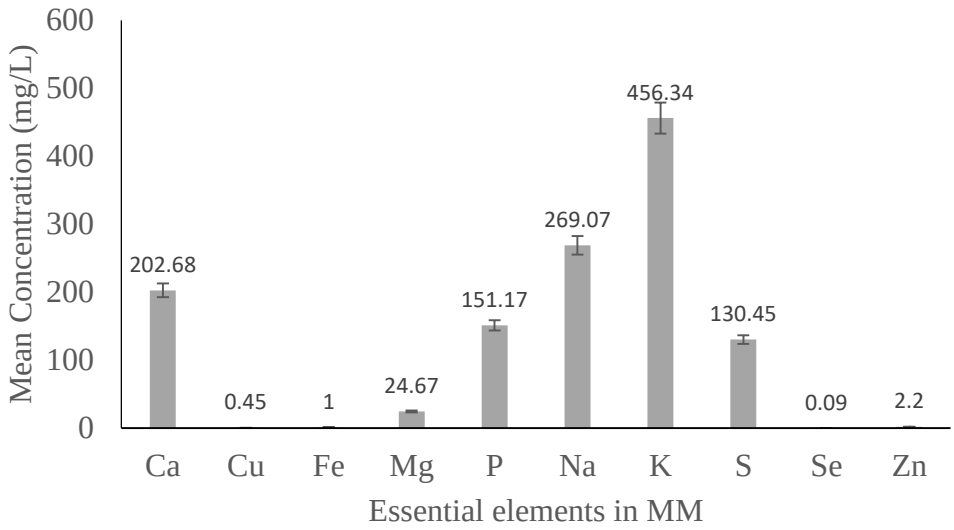

Figure 34: A Graph of the Mean Concentrations of Essential Elements in the MM of Mothers (With 5% Error Bars). Source: Laboratory Data (2022)

There were large variations in the concentrations of the essential elements in the data set for MM. The coefficients of variation of the essential elements ranged from 14.39% to 55.60%. However, out of the ten (10) essential elements, only Mg (CV = 14.39%), K (CV = 16.75%) and P (CV = 18.64%) presented good distributions for the data set.

Correlation Matrix of Essential Mineral Elements in the Mature Milk of Mothers

Data from the mature breast milk were subjected to correlation analysis and the results obtained presented in Table 28.

Table 28: Spearman's Correlation Matrix for Essential Elements in the Mature Milk of Mothers

Variables	P	Mg	Se	K	Ca	Zn	AGE	Fe	Na	S	Cu	BMI	GES AGE
P	**1**												
Mg	**-0.306**	**1**											
Se	-0.100	0.222	**1**										
K	-0.104	**0.303**	**0.421**	**1**									
Ca	-0.104	0.222	0.036	**0.604**	**1**								
Zn	-0.225	**0.360**	0.186	0.255	**0.462**	**1**							
AGE	-0.125	**0.297**	0.270	0.184	0.139	**0.342**	**1**						
Fe	-0.022	0.116	0.194	**0.340**	**0.314**	0.183	0.214	**1**					
Na	-0.103	**0.304**	0.110	0.265	**0.321**	0.043	0.138	**0.469**	**1**				
S	0.218	0.148	0.019	0.245	0.061	-0.022	0.193	0.265	**0.483**	**1**			
Cu	0.019	0.107	-0.224	0.069	0.065	-0.081	0.247	0.153	0.261	**0.576**	**1**		
BMI	-0.072	-0.015	-0.057	-0.080	-0.232	0.027	0.011	0.074	0.110	0.223	0.021	**1**	
GES AGE	-0.001	-0.086	-0.061	-0.067	0.033	-0.140	-0.131	-0.086	0.095	-0.119	0.043	-0.224	**1**

Values in bold are different from 0 with a significance level = 0.05

Source: Laboratory Data (2022)

The study also found a number of significant correlations between the essential elements in MM. It is important to mention that Mg showed a significant negative correlation with P ($r = 0.306$; $p = 0.037$). Some essential elements showed moderately strong positive and significant correlations e.g. Ca and K ($r = 0.604$; $p<0.0001$), Cu and S ($r = 0.576$; $p<0.0001$), S and Na ($r = 0.483$; $p = 0.001$), Na and Fe ($r = 0.469$; $p = 0.001$), Zn and Ca ($r = 0.462$; $p = 0.001$), Zn, and Mg ($r = 0.360$; $p = 0.013$), K and Se ($r = 0.421$; $p = 0.003$), K and Mg ($r = 0.303$; $p = 0.039$). The occurrence of these strong positive correlations is an indication of the fact that they were all coming from the same source.

A few weak negative correlations were observed between some of the essential mineral elements in MM but only the one between Mg and P was significant. The significant negative correlation between Mg and P in mature breast milk suggest inverse relation between Mg and P. this observation implied that Mg and P cannot occur together simultaneously in the same environment.

Maternal Factors Influencing Levels of Essential Mineral Elements in the MM of Mothers

The study explored to determine possible maternal characteristics that influence the levels of essential elements in the MM by subjecting the MM data to nonparametric analysis using Kruskal-Wallis test at 95% confidence interval. The results did not find any significant difference between the levels of essential elements in mature milk and maternal BMI, maternal education, infants' sex, drinking water source, diet and employment status. These observations were in line with those reported by other researchers like (Jeong

et al., 2017; Khanjani et al., 2018; Kunter et al., 2017; Lee et al., 2016; Lin et al., 1998; Olowoyo et al., 2021) who also made similar observations.

The current study, however, observed a significant relationship between levels of Zn in the mature milk and maternal parity ($p = 0.006$) as shown in Figure 35.

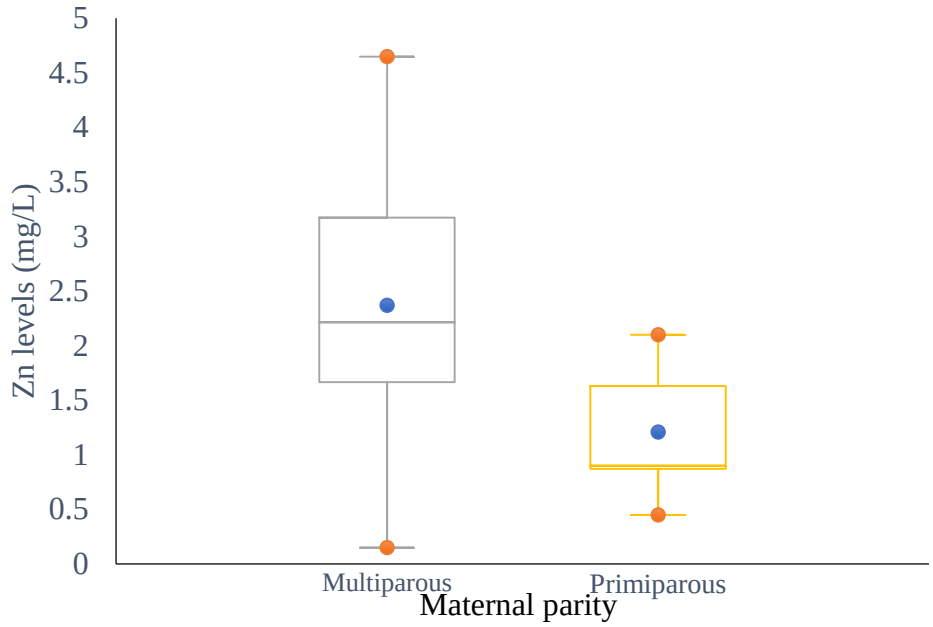

Figure 35: A Box Plot Comparing the Levels of Zn in Primiparous and Multiparous Mothers. Source: Laboratory Data (2022)

The study results showed that higher levels of Zn were found in multiparous mothers than in primiparous mothers. The results of the study were contrary to those reported by (Goc et al., 2012) who observed that concentration of essential element in the transitional milk of multiparous mothers were lower than those of primiparous mothers. However, when comparing parity in women, they found a significant difference between levels of Ca in primiparous and multiparous mothers. In the case of Mg levels in primiparous and multiparous women, they did not observe any significant

difference. In a similar study, Arnaud and Favier (1995) discussed the effects of the concentrations of Cu in multiparous and primiparous mothers.

Likewise, Honda et al. (2003) also discussed the effect of parity on Zn levels in human breast milk. Other studies like (Björklund et al., 2012; Lin et al., 1998; Olowoyo et al., 2021) did not observe any significant effects of parity on levels of trace metal elements in human milk. Moreover, this study also found that multiparous mothers had higher Zn concentrations in their mature milk than primiparous mothers.

Mean Concentrations of Essential Elements in the Total Breast Milk of Mothers

The mean concentrations of all the essential elements detected in colostrum, transitional milk and mature milk were summed up and the total mean levels computed to obtain the total mean concentrations shown in Table 29. The entire results are presented in the appendix P.

Table 29: Descriptive Statistics of Essential Elements in Total Breast Milk of Mothers

Essential Elements	Detection Frequency (%)	Min (mg/L)	Max (mg/L)	Mean (mg/L)	SD (mg/L)	Coefficient of Variation (%CV)
Ca	100.00	149.33	373.67	220.22	50.17	22.78
Cu	100.00	0.24	0.77	0.52	0.13	25.00
Fe	100.00	0.58	1.94	1.19	0.39	32.77
Mg	100.00	17.99	32.43	24.20	3.18	13.14
P	100.00	113.67	225.00	174.05	24.19	13.89
Na	100.00	148.67	561.33	340.87	115.11	33.77
K	100.00	375.67	659.67	529.71	71.88	13.57
S	100.00	105.00	257.00	165.45	35.74	21.60
Se	100.00	0.07	0.19	0.10	0.03	33.00
Zn	100.00	0.81	5.64	3.01	1.13	37.54

Source: Laboratory Data (2022)

The total mean concentrations of the essential elements in the overall mothers' milk ranged from 0.10 ± 0.03 mg/L (for Se) to 529.71 ± 71.88 mg/L (for K). The concentrations of the trace mineral elements or micronutrients were in the increasing order:

Selenium < Copper < Iron < Zinc whereas those of the macronutrients were also in the increasing order: Magnesium < Sulphur < Phosphorus < Calcium < Sodium < Potassium. Figure 36 illustrates the total mean concentrations of the essential elements in the overall human breast milk.

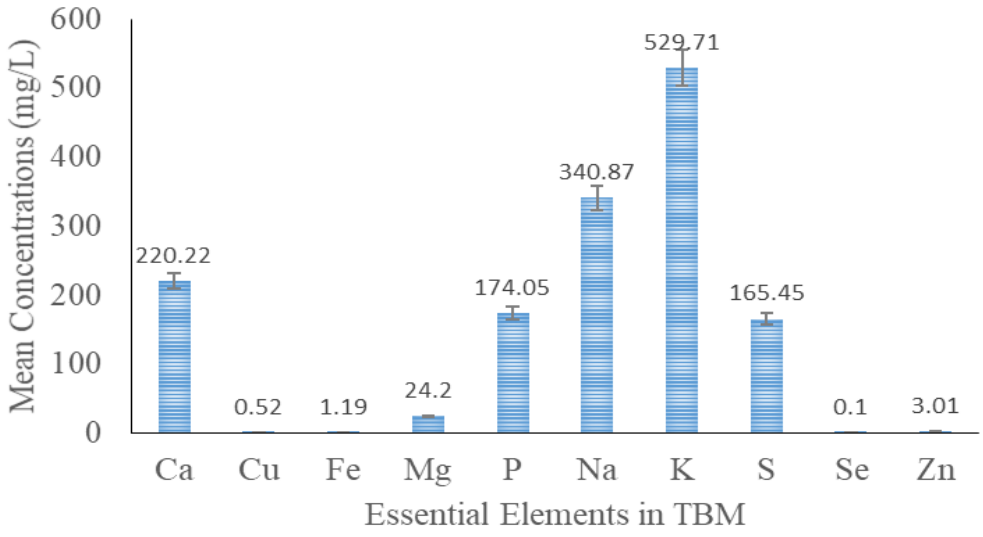

Figure 36: A Graph of the Mean Levels of Essential Elements in Total Breast Milk of mothers (With 5% Error Bars). Source: Laboratory Data (2022)

There were quite large variabilities in the concentrations of essential mineral elements in the data set but better than the one observed for the mature milk data. The coefficients of variation of the essential elements in the total breast milk data ranged from 13.14% to 37.54% (Table 27). The essential elements which gave good distributions in the data set were Mg (CV = 13.14%), K (CV = 14.57%) and P (CV = 13.89%). These indicate low relative

variability or better distribution in the dataset. They imply the data points are relatively close to the mean, which suggest stability and consistency.

Correlation Matrix of Essential Mineral Elements in the TBM of Mothers

The results of correlation analysis on the data obtained from total breast milk (TBM) are shown in Table 30.

Table 30: Correlation Matrix of Essential Elements in the TBM of Mothers

Variables	P	Mg	AGE	Zn	K	Ca	Fe	Na	S	Cu	Se	BMI	GES AGE
P	**1**												
Mg	-0.258	**1**											
AGE	-0.053	**0.331**	**1**										
Zn	-0.202	**0.319**	**0.386**	**1**									
K	**-0.308**	**0.388**	0.211	**0.464**	**1**								
Ca	-0.029	0.162	0.117	**0.299**	**0.557**	**1**							
Fe	-0.004	0.090	0.182	0.255	**0.427**	**0.333**	**1**						
Na	0.098	0.144	0.190	0.237	**0.297**	**0.309**	**0.500**	**1**					
S	**0.336**	0.198	0.239	0.236	0.257	0.094	**0.300**	**0.510**	**1**				
Cu	0.199	0.166	**0.288**	0.129	0.161	0.206	0.209	0.218	**0.564**	**1**			
Se	-0.099	0.195	0.268	0.266	0.255	-0.090	-0.013	0.008	0.058	-0.203	**1**		
BMI	0.086	0.037	0.011	0.118	-0.119	-0.025	0.044	0.151	0.202	0.050	-0.135	**1**	
GES AGE	0.057	-0.082	-0.131	-0.140	-0.054	-0.081	-0.154	0.078	-0.074	0.060	-0.089	-0.224	**1**

Values in bold are different from 0 with a significance level = 0.05

Source: Laboratory Data (2022)

The study found moderately strong significant correlations among some of the essential elements in TBM. Significant correlations observed were those in which the correlation coefficient figures have been boldened in Table 28. Some of the essential elements which showed moderately strong positive and significant correlations in total breast milk were Cu and S ($r = 0.564$; $p<0.0001$), Ca and K ($r = 0.557$; $p<0.0001$), S and Na (0.510; $p = 0.000$;), Na and Fe (0.500; $p = 0.000$), Fe and K (0.427; $p = 0.003$), K and Mg (0.388; $p = 0.007$), S and P (0.336; $p = 0.021$), Fe and Ca (0.333; $p = 0.023$) and many others. The occurrence of these moderately strong and significant correlations among the essential elements may probably reflect indication of the fact that they are coming from the same anthropogenic source or they can exist together simultaneously.

The only negative significant correlation observed among the essential elements in total breast milk was between K and P (-0.308; $p = 0.036$). The significant negative correlation between K and P suggested an inverse relationship between the two elements in breasts. It implied that K and P cannot exist together in breast milk simultaneously. In other words, as the concentration of K increases, that of P would decrease or vice versa.

Possible Maternal Factors Influencing Levels of Essential Mineral Elements in the TBM of Mothers

The study did not find any significant relationship between essential elements levels in total breast milk and maternal age, maternal BMI, maternal education, drinking water sources, diet and maternal employment status. It, however, found some significant relationships between:

1) concentrations of essential elements in TBM and infants' sex; and

2) concentrations of essential elements in TBM and parity.

In the first place, the study observed a significant relationship between Ca levels in TBM and infants' sex (p = 0.032; Cl = 0.05) as shown in Figure 37.

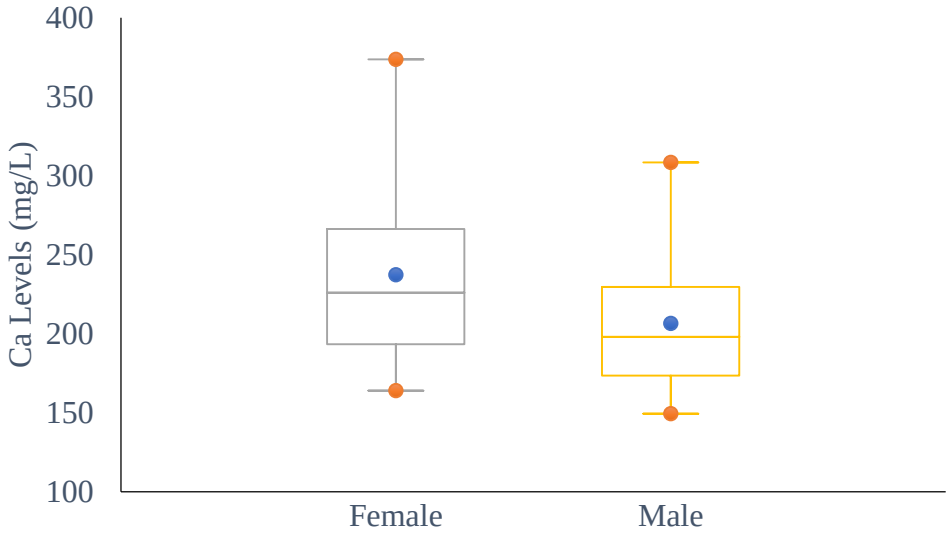

Figure 37: A Graph of Significant Relationship Between Ca Levels in TBM and Infants' Sex. Source: Laboratory Data (2022)

Mothers of female infants in the study had higher Ca levels in their TBM than those who gave birth to males.

The study results were corroborated by those of Sanchez et al. (2020), who reported significant differences in Na and P levels in breast milk of mothers. They observed that mothers of female infants had higher levels of Na in their breast milk than those of male infants while mothers of male infants had higher levels of P in their breast milk than mothers of female infants.

Moreover, in a related study, (Powe et al., 2010) reported that mothers of male infants produced milk that had 25% greater energy content than mothers of females (P = 0.001). Even though the current study and a few others have observed a significant difference between infants' sex and levels of mineral elements content in breast milk, the available literature does not

establish such relationships. Meanwhile, it has been established that Pb levels usually occur at significantly elevated levels in male infants than females (Lee et al., 2016)

Furthermore, the study found significant relationships between Se (p = 0.031) and Zn (p = 0.018) levels in total breast milk and maternal parity. It was observed that Se and Zn levels in total breast milk had significant relationships with parity as shown in Figure 38.

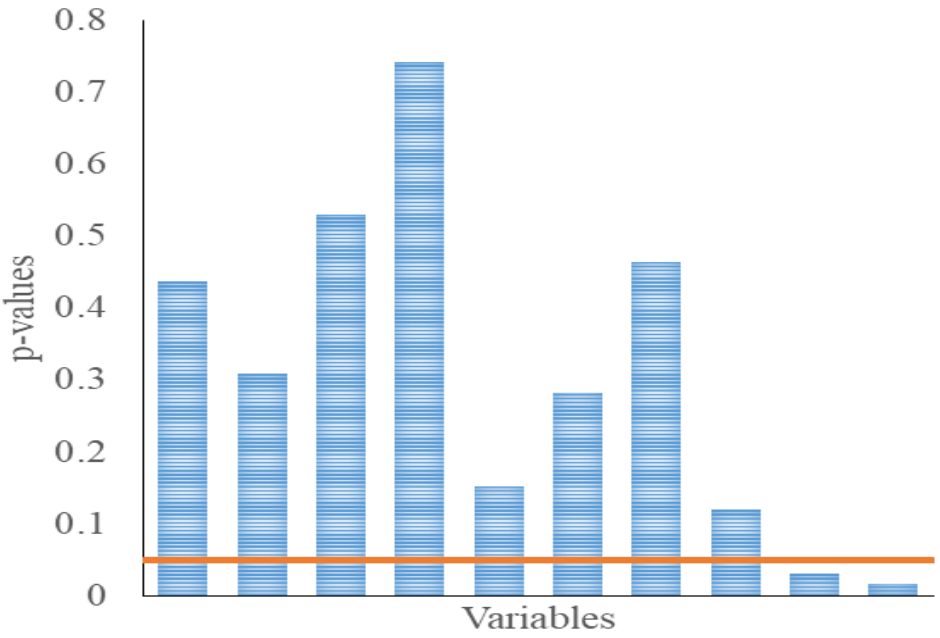

Figure 38: A Graph of P-Values Indicating Significant Relationship Between Se and Zn Levels in TBM and Parity. Source: Laboratory Data (2022)

The results established that multiparous mothers had higher levels of Se and Zn contents in their total breast milk than their primiparous counterparts as illustrated by the box plots in Figures 39 & 40.

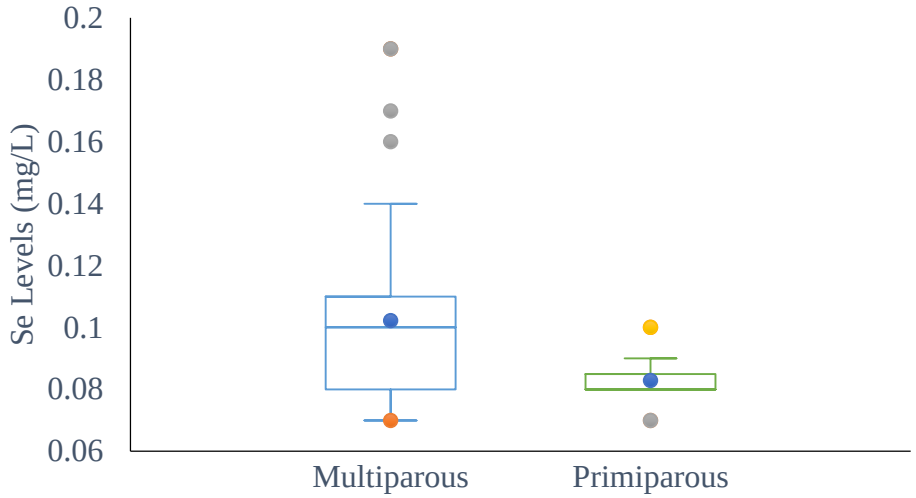

Figure 39: A Box Plot Comparing Se Levels in Primiparous and Multiparous Mothers. Source: Laboratory Data (2022)

The box plot in Figure 38 indicates higher levels of Se in the total breast milk of multiparous mothers than those primiparous mothers. Not much is known about the variation of Se levels with maternal parity but it may be ascribed to biological and physiological mechanisms responsible for the regulation of the mammary gland essential mineral elements transport during lactation to meet nutritional needs of the neonate.

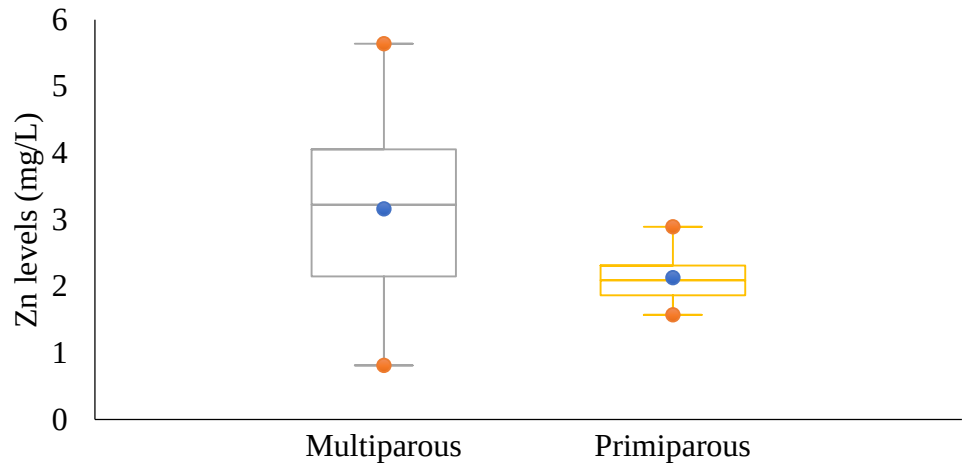

Figure 40: A Box Plot Comparing Zn Levels in Primiparous and Multiparous Mothers. Source: Laboratory Data (2022)

Likewise, Figure 39 indicates higher levels of Zn associated with the total breast milk of multiparous mothers than their primiparous counterparts. The reasons behind the variation of maternal parity with levels in mothers' breast milk are not completely understood and cannot be satisfactorily explained. However, some studies have suggested that the variations could be related to the changes in milk-binding ligands of these essential mineral elements (Suzuki et al., 1991).

The results were in line with those of Arnaud and Favier (1995) who reported that parity affected Cu concentrations in breast milk and also (Honda et al., 2003) also observed the effects of parity on Zn concentrations in breast milk as well as (Goc et al., 2012) who reported of the effects of parity on Ca and Mg concentrations. However, some studies like those of (Aumeistere et al., 2019; Björklund et al., 2012; Jeong et al., 2017; Khanjani et al., 2018; Kunter et al., 2017; Lee et al., 2016; Lin et al., 1998; Olowoyo et al., 2021) did not observe any significant effects of parity on levels of trace metal elements.

Table 31 gives the combined correlation data of both heavy metals and essential elements analysed in the study.

Table 31: Correlation Coefficients of both Heavy Metals and Essential Elements in the breast milk of mothers

	Al	As	Cd	Pb	Hg	Ca	Cu	Fe	Mg	P	Na	K	S	Se	Zn
Al	1.00														
As	-0.08	1.00													
Cd	-0.25	0.02	1.00												
Pb	0.30	-0.02	-0.14	1.00											
Hg	-0.17	0.31	0.02	-0.14	1.00										
Ca	.419**	0.01	-0.04	.367*	0.01	1.00									
Cu	0.13	0.09	-0.09	0.26	0.13	0.13	1.00								
Fe	0.19	0.17	0.00	-0.12	-0.19	0.25	0.16	1.00							
Mg	0.08	0.00	0.06	-0.19	-0.03	0.02	0.24	0.03	1.00						
P	-0.08	-0.28	-0.21	0.28	-0.09	0.01	0.14	-0.01	-0.17	1.00					
Na	0.05	0.13	-0.41	-0.17	0.01	0.23	0.20	.483**	0.15	0.06	1.00				
K	.351*	0.27	-0.34	-0.04	0.01	.473**	0.18	.417**	.302*	-0.25	.358*	1.00			
S	0.06	0.29	-0.20	-0.05	0.05	0.08	.507**	.311*	0.18	0.28	.495**	.307*	1.00		
Se	0.12	0.11	-0.42	-0.18	-0.21	-0.10	-0.11	-0.05	0.22	0.02	-0.07	0.08	0.09	1.00	
Zn	0.17	0.19	-0.23	-0.29	0.08	0.20	0.03	0.25	0.26	-.303*	0.24	.419**	0.15	0.20	1.00

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

Source: Laboratory Data (2022)

Some of the essential elements and toxic heavy metals were moderately positively or negatively correlated with each other in breast milk as observed in CM, TM & MM. For example, Na positively correlated with Fe ($r = 0.483$), S ($r = 0.495$) and negatively correlated with Cd ($r = -0.411$). Calcium also positively correlated with Al ($r = 0.419$), Pb ($r = 0.367$), and K (0.473). Also, K and Zn positively correlated ($r = 0.419$), and S and Cu ($r = 0.507$). There was a negative correlation between the toxic element Cd and Se ($r = -0.420$). The positive correlations suggest that these elements might originate from the same anthropogenic source or they could exist together simultaneously in breast. In other words, they imply similar and identical relationships among these elements breast milk. However, the negative correlations implied inverse relationships among those elements, and therefore, they could not exist together in the same environment i.e. when the concentration one element increases, the concentration of the other decreases and vice versa.

Variations in the Levels of Essential Elements in Total Breast Milk of Mothers

This present study is probably the first one in Ghana determining levels of essential elements in human breast milk. The descriptive statistics of the essential elements detected in total human breast milk are presented in Table 27. A total of ten (10) essential elements were analysed from the breast milk of mothers. The essential elements were classified into two categories: macro-elements or major mineral elements (Ca, Mg, P, Na, K, & S) and trace mineral elements (Cu, Fe, Se and Zn). All the ten essential mineral elements were detected in all the samples analysed in varying concentrations.

Calcium in Breast Milk

The Mean levels of Ca detected in total breast milk were 220.22 ± 50.17 mg/L with a range of 149.33 t0 373.67 mg/L. Research indicates that calcium is the second most abundant mineral element in breast milk and its levels are regulated by homeostasis mechanisms in maternal serum. The levels of Ca detected in this study were lower than those reported in breast milk studies from , Switzerland (Sabatier et al., 2019), Indonesia (Daniels et al., 2019), North Western Spain (Sanchez et al., 2020), Republic of Latvia (Aumeistere et al., 2019), Spain (Mandia et al., 2021), Ethiopia (Maru et al., 2013), United States, Poland and Argentina (Klein et al., 2017).

Again the study, however, recorded higher mean concentrations of Ca than those reported in Nigeria (Thacher et al., 2006), Brazil (Andrade et al., 2014), Namibia (Klein et al., 2017), USA (Perrin et al., 2016) and Korea (Noh & Lee, 2021). The high concentration of Ca recorded in the study may be due to adequate intake of Ca among study population. Some studies have shown that breast milk total calcium concentrations increase sharply in the first 5 days of lactation (Kent et al., 1992), followed by a gradual decline for the duration of lactation. This observation is consistent with the study's findings. Studies have shown that calcium levels are invariably tightly associated with citrate or casein in human milk (Jarjou et al., 2006). A review of a number of studies conducted between 1940 and 1990 showed that the median levels of Ca measured in breast milk were 252 mg/L, with most of the samples collected between one and six months of lactation, having concentrations ranging from 100 to 300 mg/L (Dorea, 1999). The mean levels of Ca recorded

in the current study were comparable to those reported from other parts of the world.

Copper in Breast Milk

Copper is a trace mineral element which acts as an essential cofactor for enzymes involved in respiration at the cellular level, iron metabolism and connective tissue synthesis. The mean levels of copper detected in the total breast milk were 0.52 ± 0.13 mg/L, with a range of 0.240 mg/L to 0.77 mg/L. The results compared favourably with those reported in other studies across the world like Portugal (Almeida et al., 2008), Korea (Kim et al., 2012). The values were however, far less than those reported in Spain (Mandia et al., 2021; Motas et al., 2021) but higher than those reported in Sweden (Björklund et al., 2012), Ethiopia (Maru et al., 2013), China (Lin et al., 1998), Japan (Yamawaki et al., 2005) and Korea (Kim & Yi, 2020) . Some studies indicate that there is a decrease in copper concentration in human breast milk during lactation, reaching a minimum of 0.08 – 0.10 mg/L in mature milk between 6 months and 1 year (Lin et al., 1998).

This pattern of variation is consistent with majority of published results by other authors during the same period of lactation (Abdulrazzaq et al., 2008; Almeida et al., 2008; Yamawaki et al., 2005). One study reported a slight increase in copper concentration in breast milk during the first month in colostrum from 0.25 – 0.29 mg/L to 0.37–0.41 mg/L in mature milk (Lin et al., 1998). This trend was well observed in the present study. Some studies have also reported a decline in breast milk copper during iron supplementation (Bloxam et al., 1989; Hambidge et al., 1987).

Iron in Breast Milk

The mean levels of Fe detected were 1.19 ± 0.39 mg/L, with a range of 0.58 to 1.94 mg/L. The mean concentrations of Fe recorded in this study were not high and comparable to those reported in other breast milk studies in the world such as Japan (Yamawaki et al., 2005), Namibia and the United States (Klein et al., 2017), The present study, however, recorded relatively higher concentrations than those reported in Ethiopia (Maru et al., 2013), USA (Hannan et al., 2009; Perrin et al., 2016), Latvia (Aumeistere et al., 2019), Switzerland (Sabatier et al., 2019), Indonesia (Daniels et al., 2019), Brazil (Peixoto et al., 2019), North Western Spain (Sanchez et al., 2020), Spain (Mandia et al., 2021), Uppsala in Sweden (Björklund et al., 2012), Southern Spain (Motas et al., 2021), as well as those reported in Poland and Argentina (Klein et al., 2017). Iron is a vital component of haemoglobin, myoglobin and serves as a structural component for many enzymes in the body. Iron is needed by infants for growth and formation of health blood cells as well as prevention of iron deficiency anaemia.

Adequate intake (AI) of iron for infants between 0 – 6 months is 0.27 mg/day of iron, recommended dietary allowable (RDA) for infants 7 – 12 months is 11 mg/day of iron while the Tolerable Upper Intake Level (UL) for infants 0 – 12 months is 40 mg/day of iron. Research indicates that in breast milk, Fe is bound mainly to low molecular weight peptides, fat globules and lactoferrin, having a mean saturation of lactoferrin between 2.2% to 12% (Dorea, 2000). Human milk Fe concentrations reach a maximum in colostrum and declines afterwards through the first year of lactation (Hannan et al., 2009;

Kelleher & Lonnerdal, 2005; Perrone et al., 1994; Wasowicz et al., 2001) with a reported mean values of 0.04 – 1.92 mg/L (Dorea, 2000).

Magnesium in Breast Milk

The mean levels of magnesium detected in the study were 24.196 ± 3.182 mg/L; with the minimum and maximum levels being 17.99 mg/L and 32.43 mg/L respectively. Magnesium is regarded as the second most abundant extracellular cation after sodium and its concentrations in breast milk are regulated by homeostasis mechanisms which ensures its stability irrespective of metabolic, constitutional or dietary variables. Hence levels of Mg reported in the literature are very similar in all populations, ranging from 10.0 mg/L to 50.0 mg/L.

The mean levels of Mg detected in the present study was comparable to those reported in other parts of the world such as United States of America (Friel et al., 1999), China (Shi et al., 2011), Brazil (Andrade et al., 2014), New Zealand (Butts et al., 2018), Switzerland (Sabatier et al., 2019), Indonesia (Daniels et al., 2019), Ethiopia (Maru et al., 2013), Spain (Mandia et al., 2021), Japan (Yamawaki et al., 2005) and North Western Spain (Sanchez et al., 2020). However, the mean concentration values of Mg in this study were less than those reported in Latvia (Aumeistere et al., 2019) and Sweden (Björklund et al., 2012).

Magnesium is a major mineral element which plays a structural role in bone and is also involved in more than 300 metabolic reactions. (Fiorentini et al., 2021; MedlinePlus, 2023b) The median magnesium concentrations in breast milk is 31 mg/L, with most reported means within the range of 20–40 mg/L (Dórea, 2000). This indicates that the mean lean levels of Mg detected in

this study were within the limits reported worldwide. In spite of interindividual variation, breast milk magnesium concentrations in the same woman are fairly stable during the course of lactation, although various researchers have reported slight increasing or decreasing trends during the first 6 months (Björklund et al., 2012; Dórea, 2000).

Phosphorus in Breast Milk

The mean breast milk levels of phosphorus measured in this study were 174.05 ± 24.19 mg/L, with the minimum and maximum levels being 113.67 and 225 mg/L respectively. These mean breast milk phosphorus concentrations observed were comparable to those reported in Sweden (Björklund et al., 2012) but higher than those reported in Japan (Yamawaki et al., 2005). Others include Nigeria (Thacher et al., 2006), Switzerland (Sabatier et al., 2019), Indonesia (Daniels et al., 2019) and North Western Spain (Sanchez et al., 2020). All these studies reported slightly lower concentrations of P than in the current study.

In a review, Dorea (1999b) discussed a comprehensive survey of studies dealing with Ca and P in breast milk conducted over the last 50 years which shows a wide variability in the concentrations of these elements in human milk. The researcher indicated that the mean concentrations of P ranged from 84 to 278 mg/L with a median of 143 mg/L. These findings are consistent with several studies that compare Ca and P concentrations of human breast milk both within and between countries and have reported that maternal nutrition may not affect Ca and P concentrations of human milk (Dorea, 1999). Phosphorus concentrations of human milk are low compared with milk of other mammals. Low breast milk concentrations of 84 to 106 mg/L have

been reported (Jonas & Domiguez, 1989). Moreover, very low levels of breast milk phosphorus of 17 to 58 mg/L (i.e. 1.7 g/dl to 5.8 g/dl) have also been documented (Hanukoglu et al., 1988). Low levels of P in breast milk have been associated with improved Ca absorption and quite a number of benefits for neonates (Manz, 1992).

Sodium in Breast Milk

The mean levels of sodium detected in total breast milk in this study were 340.87 ± 115.11 mg/L, with a range of 148.67 to 561.33 mg/L. Sodium is a mineral required to maintain water balance in the body, regulate blood volume and to ensure the proper functioning of cell membranes and other tissues. The estimated minimum requirements of sodium for infants range from 100 to 200 mg/day. The mean levels of sodium detected from total breast milk in the study were higher than those reported in the United States of America (Perrin et al., 2016), Japan (Yamawaki et al., 2005), Sweden (Björklund et al., 2012), India (Roy et al., 2014), Latvia (Aumeistere et al., 2019) and Indonesia (Daniels et al., 2019).

However, values far greater than those observed in this study have also been reported by some authors. In Indian concentration of 527 mg/L of Na has been reported in breast milk (Roy et al., 2014) while mean levels of 176.94 mg/L and a range of 44.75 to 942.67 mg/L have also been reported in North Western Spain (Sanchez et al., 2020). Moreover, very low mean breast milk sodium levels have been reported among nursing mothers in Guatemala Li et al., 2016). Elevated breast milk sodium concentration is regarded as responsible for elevated sodium intake (Manganaro et al., 2007). Some studies have shown that compared to normal breast milk Na levels and Na/K ratio,

high breast milk sodium and high Na/K ratio, with serious consequences on infants, are associated with depression and anxiety in the postpartum period (Demirgoren et al., 2017). High sodium consumption is a precursor for elevated blood pressure, and high blood pressure is a major risk factor for heart disease and stroke. The United States Department for Agriculture Dietary Guidelines for 2020-2025 advises individuals 14 years and older to limit their consumption of Na to 2,300 mg/day (USDA, 2020).

Potassium in Breast Milk

The mean levels of potassium (K) detected in total breast milk in the present study were 529.71 ± 71.88 mg/L with the minimum and maximum levels being 375.67 and 659.67 respectively. The mean levels of K detected in total breast milk and the range observed were not too high but fell within the limits and range reported worldwide in breast milk studies such as i Latvia (Aumeistere et al., 2019), United States of America (Perrin et al., 2016), Switzerland (Sabatier et al., 2019), Indonesia (Daniels et al., 2019) and North Western Spain (Sanchez et al., 2020). Potassium is vital for the proper function of all cells, tissues, and organs in the human body. It is also crucial to heart function and plays a key role in skeletal and smooth muscle contraction, making it important for normal digestive and muscular function (Pohl et al., 2013).

The concentration of potassium is human milk is approximately 50 mg per 100 mL i.e. 500 mg/L (EFSA NDA Panel, 2016). During lactation, potassium requirement is increased because of secretion of potassium in the breast milk. The mean potassium content of breast milk in healthy women 0 to 4 months postpartum is reported to be 12.8 to 15.0 (range 7.9–23.2) mmol/L

or 2304 – 2700 mg/L (range 1422 to 4176 mg/L) with higher values in the first week and decreasing quickly thereafter (Berry et al., 2013; Pohl et al., 2013).

Sulphur in Breast Milk

The mean levels of Sulphur (S) detected in total breast milk in this study were 165.454 ± 35.738 mg/L, with a range of 105.00 to 257.00 mg/L. To the best of the knowledge of the researcher, no study has been carried out on sulphur in breast milk from the beginning of the twentieth century to date. This makes the comparison of the mean levels of sulphur detected in this study with other studies a bit challenging. However, one study conducted on breast milk sulphur around the middle of the 19th century and another getting to the close of that century has been chanced upon. In a study in Canada (McNally et al., 1991) determined total sulphur in human colostrum and mature milk. The mean concentrations of total sulphur reported in their study were 10.4 ± 4.0 mmol/L and 4.5 ± 0.8 mmol in colostrum and mature human milk respectively. These mean levels of sulphur are equivalent to 1890 ± 72 mg/L and 810 ± 14.4 mg/L respectively. It is very clear that the levels of sulphur detected in this study were far less than those reported by McNally et al. (1991) In another study in Michigan, USA (Macy, Icie, 1949), determined levels of both electropositive and electronegative elements including sulphur in human colostrum, transitional milk and mature milk. The mean concentrations of sulphur reported on human colostrum, transitional milk and mature milk were 230, 200 and 140 mg/L respectively. The mean levels of sulphur observed in this study were quite consistent with those reported by Macy (1949). Though, it is quite obvious that the levels of S decline as

lactation progresses, it is important to mention that the factors that control the variation of S in human breast milk are not yet known.

Selenium in Breast Milk

Selenium (Se) was another trace mineral element analysed in this study. The mean levels of Se detected in the study were 0.10 ± 0.03 mg/L, with the minimum and maximum levels being 0.07 and 0.19 mg/L respectively. The mean levels of Se detected in this study were not consistent with most studies reported across the world, though there exist a wide range of variability in the values both within and between countries. The levels of Se in total breast milk recorded in the study were comparable to those reported in Latvia (Aumeistere et al., 2019) and Guatemala (Li et al., 2016). They were, however, far lower than those reported in New Zealand (Butts et al., 2018), Switzerland (Sabatier et al., 2019), Indonesia (Daniels et al., 2019), Brazil (Peixoto et al., 2019) and South Western Spain (Sanchez et al., 2020).

Though human infants are born with selenium reserves but they also depend on the selenium supplied by their mothers' milk (Dorea, 2002). Some studies have shown that selenium concentrations are high in colostrum and decrease as lactation progresses (Higashi et al., 1983; Tamarp & Tsuji, 1995; Wasowicz et al., 2001), parallel with the trend for the milk proteins into which selenium is incorporated (Dorea, 2002).

Zinc in Breast Milk

The mean levels of zinc (Zn) detected in this study participants were 3.01 ± 1.13 mg/L, with a range of 0.81 to 5.64 mg/L. According to the relevant literature, Zn may be 15 times more concentrated in breast milk than in the mothers' plasma. This may be indicative of an evidence of active

transport mechanism and its vital role in children's development (Almeida et al., 2008; Bratter et al., 1997). A rapid and significant decrease in the concentration of Zn in breast milk throughout the lactation period has also been reported, especially during the first month (Almeida et al., 2008).

Comparison of Levels of Heavy Metals in Colostrum, Transitional Milk and Mature Milk

The five heavy metals analysed namely, Al, As, Cd, Pb and Hg were present in the colostrum, transitional milk and mature milk in varying concentrations, though the levels were not all that remarkable. Figure 41 gives comparison of the trends in the concentrations of the five toxic heavy metals in the colostrum, transitional milk and mature milk of the mothers investigated.

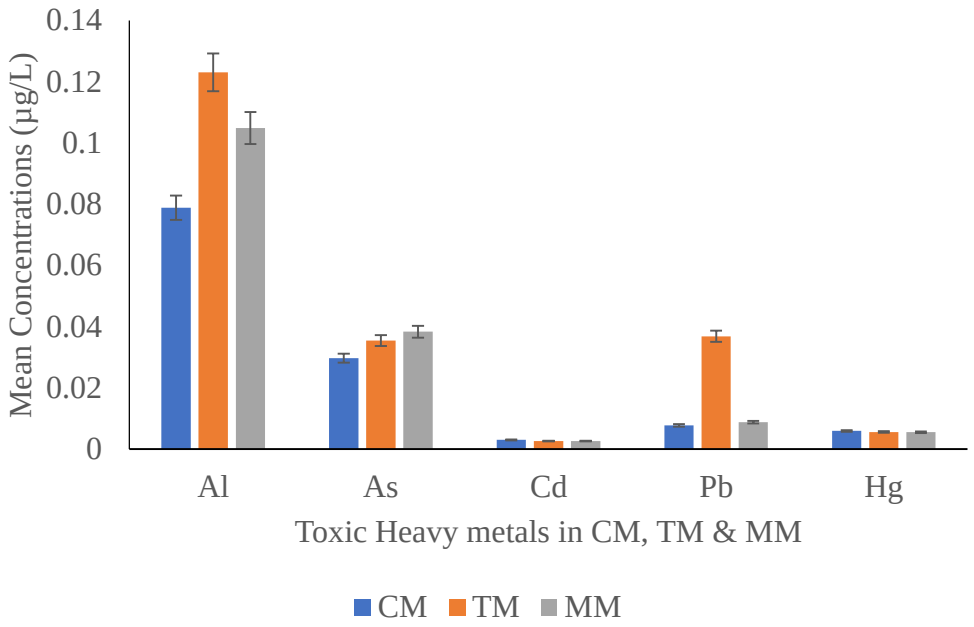

Figure 41: Comparisons of the Levels of Toxic Heavy Metals in Colostrum, Transitional Milk and Mature Milk (With 5% Error Bars). Source: Laboratory Data (2022)

The results showed that aluminium had the highest mean concentrations in all the three portions of breast milk, followed by arsenic,

lead, mercury and cadmium having the lowest. Comparing the mean concentrations of each of the metals analysed in the colostrum, transitional milk and mature milk, the following deductions can be made. The concentration of aluminium was highest in the transitional milk, with the general pattern of concentrations in the three portions of breast milk in the increasing order: Colostrum < Mature Milk < Transitional Milk (Fig. 41).

With arsenic, the mean concentration was highest in the mature milk, followed by the transitional milk while the colostrum had the lowest concentration. Lead, on the other hand, had the highest mean concentration in the transitional milk, followed by the mature milk and then the colostrum in that order.

In the case of mercury, the mean concentrations detected in the colostrum, transitional milk and mature milk were almost the same but with just a slightly higher concentration in the colostrum and transitional milk (0.006 ± 0.001 µg/L) as compared to 0.005 ± 0.000 µg/L in the mature breast milk.

Cadmium showed the least mean concentrations among all the heavy metals analysed, with a pattern of variation similar to that of mercury. The highest mean concentration of 0.004 ± 0.006 µg/L occurred in the colostrum milk while the mean concentrations in the transitional milk and mature milk were the same (0.003 ± 0.000 µg/L).

The comparison of the levels of the five heavy metals in the different portions of breast milk can be summarised as follow in ascending orders:

Colostrum: Cd < Hg < Pb < As < Al

Transitional milk: Cd < Hg < As < Pb < Al

Mature milk: Cd < Hg < Pb < As < Al

The pattern of variation observed in the concentrations of the different toxic heavy metals in the study during the three stages of lactation was irregular but quite similar to the pattern reported by other researchers (Almeida et al., 2008; Chao et al., 2014; Rossipal & Krachler, 1998).

ANOVA of Heavy Metals Analysed in Colostrum, Transitional Milk and Mature Milk

The study employed ANOVA to analyse the means obtained from the data with respect to colostrum milk, transitional milk and mature to determine whether there were any significant differences in the levels of Al, As, Cd, Pb and Hg during the three stages of lactation. The results obtained have been summarized under Table 32 – 36. By way of summary, the study did not observe any significant differences in the levels of the five heavy metals analysed during the three stages of lactation. This was clearly illustrated by figure 38.

Table 32: ANOVA of Al in CM, TM, and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	0.009203	2	0.004601	0.287656	0.751	3.075144
Within Groups	1.839601	115	0.015997			
Total	1.848804	117				

Source: Laboratory Data (2022)

Since the *P*-value of $p = 0.751$ was greater than the Cl = 0.05, it implies that the levels of Al in breast milk during the three stages of lactation were not significant, though some variations were observed during the lactation periods.

Table: 33: ANOVA of As in CM, TM, and MM of Mothers

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.000195	2	9.73E-05	0.058435	0.943	3.075853
Within Groups	0.189842	114	0.001665			
Total	0.190037	116				

Source: Laboratory Data (2022)

Based on the ANOVA results of $p = 0.943$, the levels of As in breast milk during the three stages of lactation were not significant.

Table 34: ANOVA of Cd in CM, TM, and MM of Mothers' Milk

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.82E-05	2	1.91E-05	1.595846	0.213	3.18261
Within Groups	0.000599	50	1.2E-05			
Total	0.000637	52				

Source: Laboratory Data (2022)

From Table 34 and based on the ANOVA results of $p = 0.213$ and Cl = 0.05, it was concluded that the levels of Cd detected during the three stages of lactation were not significant.

Table 35: ANOVA of Pb in CM, TM, and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	0.01892	2	0.00946	1.094453	0.338	3.078819
Within Groups	0.950819	110	0.008644			
Total	0.96974	112				

Source: Laboratory Data (2022)

From Table 35 and based on the ANOVA results of p = 0.338 and Cl = 0.05, it was concluded that the levels of Pb in breast milk during the three stages of lactation were not significant.

Table 36: ANOVA of Hg in CM, TM, and MM of Mothers

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.85E-06	2	9.23E-07	2.199132	0.118	3.123907
Within Groups	3.02E-05	72	4.2E-07			
Total	3.21E-05	74				

Source: Laboratory Data (2022)

From Table 36 and based on the ANOVA results of p = 0.118 and Cl = 0.05, it was concluded that the levels of Hg in breast milk during the three stages of lactation were not significant.

Exploratory Factor Analysis of Toxic Heavy Metals in Breast Milk

The use of EFA was helpful in determining whether or not the measurement items were loaded onto their associated latent variables in the correct manner. IBM SPSS Statistics (v.26) was used to run the EFA. There was a total of five (5) latent variables, namely; Aluminium (Al), Arsenic (As), Cadmium (Cd), Lead (Pb) and Mercury (Hg) concentration levels at the various stages of breast milk. During the EFA, measurement items that loaded on different or numerous constructs, as well as those that loaded on factors with weak factor loadings (less than 0.1), were removed from the analysis. After removing items that had factor loadings of less than 5 or cross-loading on multiple constructs, the items that remained after this process are presented in Tables 37 and 39.

The findings indicated that the total variance extracted (TVE) was 74.1 percent Colostrum Milk (CM) and 98.95 percent in Transition Milk (TM), which is much higher than the required minimum of 50 percent. However, factor extraction could not load for variables in Matured Milk because at least one variable has variance of zero.

In the total mean concentrations of heavy metals in breast milk of mothers, the total variance extracted was 69.89 percent. The Kaiser-Meyer-Olkin (KMO) measure of sampling Adequacy should be at least 0.6 (Kaiser & Rice, 1974; Shrestha, 2021), however the current study reached 0.876, which suggests that sample adequacy was high. In order to establish the degree to which the variables are associated with one another and meet the requirements of the EFA, it is necessary to obtain a result for the Bartlett's Test of Sphericity that is statistically significant. For this data, Bartlett's Test was significant ($p < 0.001$), and therefore, factor analysis was appropriate. Because this would be a sign of positive definiteness in the data that was utilised for the estimation, the Determinant of Correlation should likewise not be equal to zero (0).

Exploratory Factor Analysis of Mean Concentration of Heavy Metals in Colostrum Milk

A crucial proportion of the variation was explained by factor 1 which accounted for 48.04% of the total variation. Essentially, factor 1 was concerned with the association between Al and Pb. Factor 2 which was also concerned with Hg, As, and Cd also contributed 26.06% of the total variation. It is essential to mention that these elements (Hg, As, and Cd) were minor contributors in factor 1. Results of the factor analysis are presented in Table 37 while the scree plot is illustrated by Figure 42.

Table 37: Rotated Component Matrix of Heavy Metals in CM

	Components	
Measurement items	1	2
Al	0.980	
Pb	0.970	
Hg		0.935
As		0.819
Cd		-0.301
Variance (%)	48.04	26.06
Cumulative Variance (%)	48.04	74.09
Extraction Method: Principal Component Analysis.		
Rotation Method: Varimax with Kaiser Normalization.		
a. Rotation converged in 3 iterations.		

Source: Laboratory Data (2022)

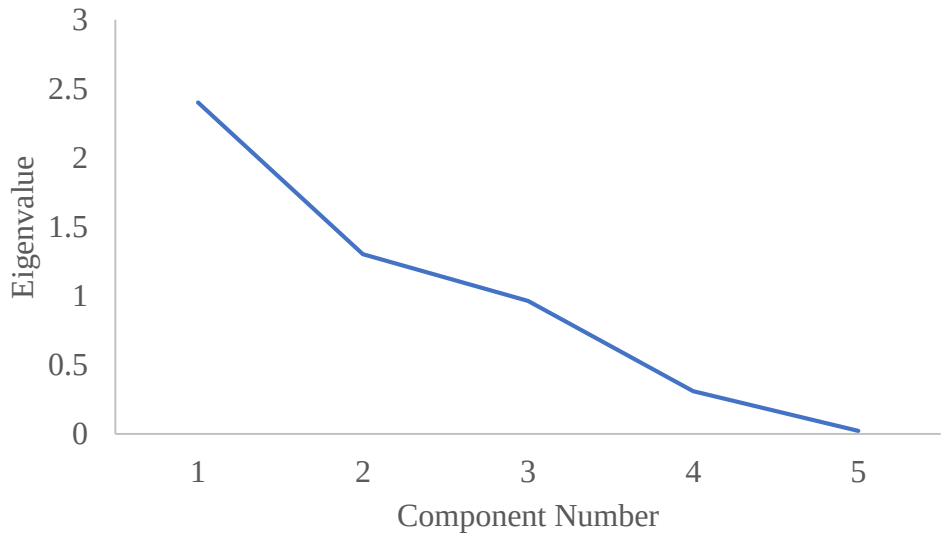

Figure 42: A Scree Plot of the Extracted Components in the CM of Mothers
Source: Laboratory Data (2022)

Exploratory Factor Analysis (EFA) of Mean Concentrations of Toxic Heavy Metals in TM

The results of the EFA of heavy metals in transitional breast milk (TM) are presented in Table 38 while the scree plot is shown on Figure 43.

Table 38: Rotated Component Matrix of Heavy Metals in TM

	Components	
Measurement items	1	2
As	-0.999	
Cd	0.979	
Al	0.934	
Hg	-0.802	
Pb		0.994
Variance (%)	73.87	25.08
Cumulative Variance (%)	73.87	98.95
Extraction Method: Principal Component Analysis.		
Rotation Method: Varimax with Kaiser Normalization.		
a. Rotation converged in 3 iterations.		

Source: Laboratory Data (2022)

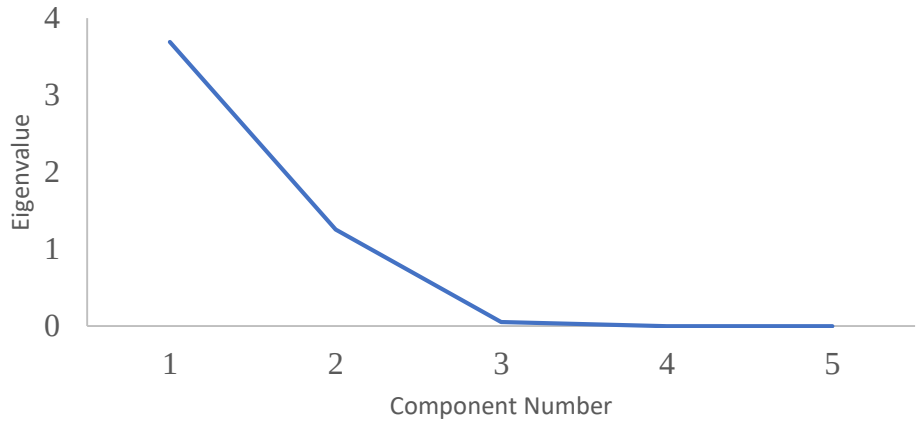

Figure 43: A Scree Plot of the Extracted Components in the TM of Mothers
Source: Laboratory Data (2022)

Exploratory Factor Analysis of Mean Concentration of Toxic Heavy Metals in Total Breast Milk (TBM)

In the mean concentration of heavy metals in the TBM of mothers, there was a total of three components extracted as shown in Table 39 while the scree plot is illustrated on Figure 44. Factor 1 which accounted for 37.81% of the variation was attributed to Pb and Al. Hg and As also caused a variation of

32.08% in factor 2 while Cd contributes to factor 3. Nearly 22.03% of the variance is assigned to this factor.

Table 39: Rotated Component Matrix of Heavy Metals in TBM

	Components		
Measurement items	1	2	3
Pb	0.913		
Al	0.878		
Hg		0.944	
As		0.742	
Cd			0.984
Variance (%)	37.81	32.09	22.03
Cumulative variance (%)	37.81	69.89	91.92

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 6 iterations.

Source: Laboratory Data (2022)

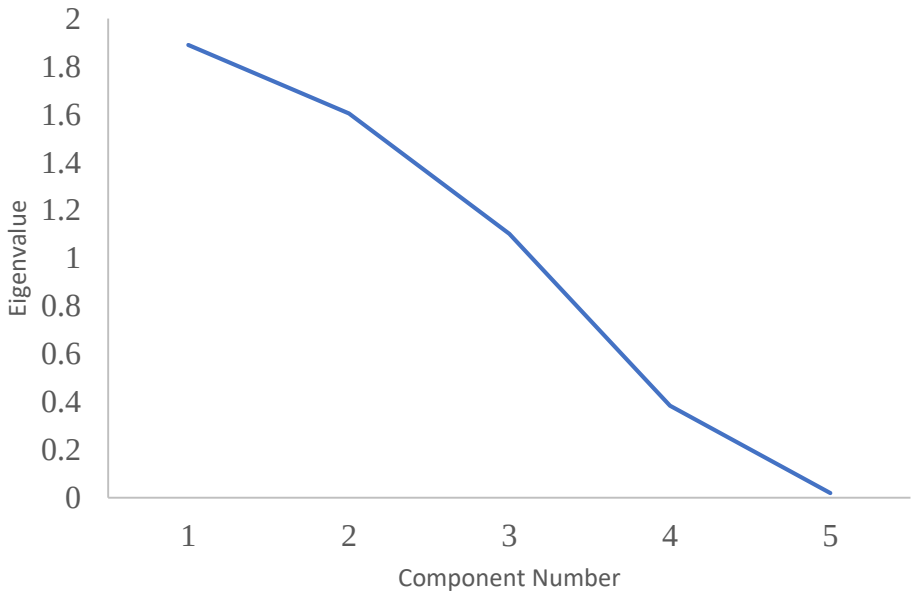

Figure 44: A Scree Plot of the Extracted Components in Total Breast Milk
Source: Laboratory Data (2022)

Comparison of Levels of Essential Elements in Colostrum, Transitional Milk and Mature Milk

The study also quantified the concentrations of essential elements in the three portion of human breast milk and the results are illustrated by Figure 45. The results show that the macro-elements – potassium, sodium, calcium, phosphorus, sulphur and magnesium respectively recorded relatively high concentrations in all the three portions of breast milk while the micro-elements or trace elements namely; copper, iron, selenium and zinc also recording relatively low concentrations in line with what is known in literature about their quantities in the human body.

There was also something interesting about the levels of essential elements detected in the breast milk. Among all the essential elements (both macro-elements and micro-elements), with exception of magnesium which recorded a relatively higher concentration in the mature milk, all the rest (Ca, Cu, Fe, P, Na, K, S, Se & Zn) recorded lower concentrations in the mature milk. The general pattern of the levels of the essential elements detected can be summarised in an ascending order as: Mature < Transitional milk < Colostrum.

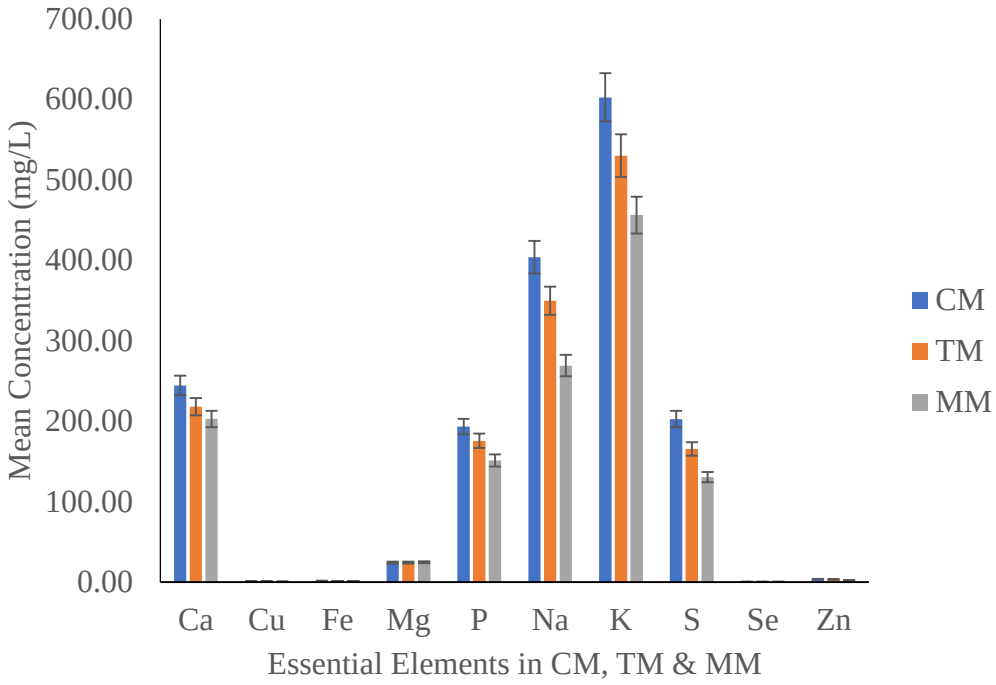

Figure 45: Comparisons of the Levels of Essential Elements in Colostrum, Transitional Milk and Mature Milk. Source: Laboratory Data (2022)

Thus the highest concentrations of these elements were observed in the colostrum milk, followed by the transitional milk; with the least concentration in the mature breast milk. So there is a general trend of decline in the concentrations of these elements as lactation progresses. High levels of K and Na were observed across all stages of the breast milk. Ca, S and P recorded approximately the same levels in the three stages while Cu, Fe, Zn, and Se recorded little to trace concentrations at all stages.

The pattern of variation of concentrations of essential mineral elements analysed in our study (illustrated in Figure 44) is consistent with those reported by other authors around the world with some slight variations with respect some elements. It is instructive to know that there is a general gradual decline in the levels of essential elements as the period of lactation progresses. The results is confirmed a study reported in Nigeria (Akanle et al., 2001)

where the mean concentrations of the elements namely Na, Mg, Ca, K, Fe, Zn and Cu determined decreased progressively from colostrum to mature milk respectively as shown below: Na : 3500 ± 1490, 2850 ± 1150 and 2000 ± 900 µg/g; Mg: 370 ±210, 360 ± 150 and 240 ± 130 µg/g; Ca: 2700 ± 690, 2400 ± 1240 and 2100 µg/g; K: 4800 ± 2100, 4300 ± 1760 and 4100 ± 1600 ±g/g; Fe: 15.7 ± 2.6, 11.4 ± 3.2 and 7.4 ± 2.3 µg/g; Zn: 36 ± 11, 25 ± 7 and 18 ± 6 µg/g; Cu: 7.3 ± 1.5, 5.3 ± 1.7 and 4.5 ± 1.8 µg/g. Other researchers like (Macy, 1949) also reported a similar pattern involving Ca, Mg, K, Na, P and S where the concentrations of all the elements decreased progressively from colostrum to transitional milk through to mature milk respectively. In a study in Portugal involving levels of trace metal elements in human milk, (Almeida et al., 2008) reported that the concentrations of the essential mineral elements Cu, Zn and Se declined from colostrum to mature milk. Another researcher who investigated levels of total sulphur in human breast milk also reported this pattern of variation (McNally et al., 1991).

Moreover, in England, Barltrop & Hillier (1974) also investigated the levels of major essential elements Ca and P in transitional milk and mature human milk during the period of lactation. They found that the concentrations of these elements decrease progressively from transitional milk to mature milk Even though the P content of transitional milk (P = 29.7) was higher than that of Ca (Ca = 23.9) in the transitional milk but the Ca content in the mature milk (Ca = 19.9) was higher than that of P (P = 18.5). Nevertheless, the overall pattern of concentration decline from transitional milk to mature was maintained. All these findings were in line with the present study. In another development, a study conducted in Poland, (Wasowicz et al., 2001)

investigated the variation of the levels of the essential trace elements Se, Cu and Zn in human breast milk at the three stages of lactation. They found that the mean levels of the trace elements Cu decreased progressively from colostrum to mature milk during the lactation period which was in perfect agreement with our findings.

Even though there exist a large volume of literature documenting the variation of concentrations of essential mineral elements during the period of lactation from colostrum to mature milk, it is still not clearly known the factors responsible for the observed pattern. With regard to decrease in the concentrations of trace mineral elements Cu, Fe, Se and Zn during lactation, Rossipal & Krachler (1998) offered some possible explanation to rationalize it. They explained that the observed pattern might probably be due to an alteration in the binding capacity of the trace elements since essential trace elements are mainly bound to protein in human milk and the concentration of this major protein decreases during the course of lactation.

However, it may be speculated that the observed pattern of variations may be mainly due to a natural mechanism of supplying nutritional needs of the infant with regard to mineral elements during the various lactation stages to foster healthy growth and development of infants.

ANOVA of Essential Elements in Colostrum Milk, Transitional Milk and Mature Milk

The study employed ANOVA to analyse the means obtained from the three datasets with respect to colostrum milk, transitional milk and mature to ascertain whether there was any significant difference in the levels of Ca, Cu, Fe, Mg, P, Na, K, S, Se and Zn during the three stages of lactation. The results

obtained have been summarized in Tables 40 – 49. With exception of Mg (P = 0.578), and Se (p = 0.068) there were significant differences in the levels of all the other essential elements with respect to the three stages of lactation. This significant pattern of distribution of essential elements in breast milk as lactation progresses is clearly illustrated by Figure 45.

Table 40: ANOVA of Ca in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	41955.84	2	20977.92	4.974037	0.008	3.061716
Within Groups	582012.8	138	4217.484			
Total	623968.7	140				

Source: Laboratory Data (2022)

From Table 40 and based on the ANOVA results of p = 0.008 and Cl = 0.05, it was concluded that the levels of Ca detected in breast milk during the three stages of lactation were very significant.

Table 41: ANOVA of Cu in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	0.407871	2	0.203936	9.345996	0.000	3.061716
Within Groups	3.011248	138	0.021821			
Total	3.419119	140				

Source: Laboratory Data (2022)

From Table 41 and based on the ANOVA results of p = 0.000 and Cl = 0.05, it was concluded that the levels of Cu detected in breast milk during the

three stages of lactation were very significant. It implies that there was a gradual decline in the concentration of Cu from CM to TM and then to MM.

Table 42: ANOVA of Fe in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	3.766644	2	1.883322	9.340459	0.000	3.061716
Within Groups	27.82502	138	0.201631			
Total	31.59166	140				

Source: Laboratory Data (2022)

From Table 42 and based on the ANOVA results of p = 0.000 and Cl = 0.05, it was concluded that the levels of Fe detected in breast milk during the three stages of lactation were very significant.

Table 43: ANOVA of Mg in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	15.11926	2	7.559628	0.550134	0.578	3.061716
Within Groups	1896.317	138	13.74143			
Total	1911.436	140				

Source: Laboratory Data (2022)

From Table 43 and based on the ANOVA results of p = 0.578 and Cl = 0.05, it was concluded that the levels of Mg detected in breast milk during the three stages of lactation were not significant.

Table 44: ANOVA of P in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	41940.95	2	20970.48	17.33965	0.000	3.061716
Within Groups	166896.4	138	1209.394			
Total	208837.3	140				

Source: Laboratory Data (2022)

From Table 44 and based on the ANOVA results of $p = 0.000$ and Cl = 0.05, it was concluded that the levels of P detected in breast milk during the three stages of lactation were very significant.

Table 45: ANOVA of Na in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	432644	2	216322	11.37501	0.000	3.061716
Within Groups	2624388	138	19017.31			
Total	3057032	140				

Source: Laboratory Data (2022)

From Table 45 and based on the ANOVA results of $p = 0.000$ and Cl = 0.05, it was concluded that the levels of Na detected in breast milk during the three stages of lactation were very significant.

Table 46: ANOVA of K in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	502912.8	2	251456.4	36.95198	0.000	3.061716
Within Groups	939083.2	138	6804.951			
Total	1441996	140				

Source: Laboratory Data (2022)

From Table 46 and based on the ANOVA results of $p = 0.000$ and Cl = 0.05, it was concluded that the levels of K detected in breast milk during the three stages of lactation were very significant.

Table 47: ANOVA of S in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	122290.4	2	61145.22	35.2981	0.000	3.061716
Within Groups	239050.8	138	1732.252			
Total	361341.2	140				

Source: Laboratory Data (2022)

From Table 47 and based on the ANOVA results of p = 0.000 and Cl = 0.05, it was concluded that the levels of S detected in breast milk during the three stages of lactation were very significant.

Table 48: ANOVA of Se in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	0.007124	2	0.003562	2.741628	0.068	3.061716
Within Groups	0.179301	138	0.001299			
Total	0.186425	140				

Source: Laboratory Data (2022)

From Table 48 and based on the ANOVA results of p = 0.068 and Cl = 0.05, it was concluded that the levels of Se detected in breast milk during the three stages of lactation were not significant.

Table 49: ANOVA of Zn in CM, TM and MM of Mothers

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	60.28561	2	30.14281	19.83197	0.000	3.061716
Within Groups	209.7475	138	1.51991			
Total	270.0331	140				

Source: Laboratory Data (2022)

From Table 49 and based on the ANOVA results of $p = 0.000$ and Cl = 0.05, it was concluded that the levels of Zn detected in breast milk during the three stages of lactation were very significant.

With the exception of sulphur, the significant differences observed in this current study in the varying and decreasing concentrations of the essential mineral elements during lactation periods are well known and have been reported by many researchers (Akanle et al., 2001; Almeida et al., 2008; Arnaud et al., 1993; Arnaud & Favier, 1995; Lin et al., 1998; Rossipal & Krachler, 1998; Wasowicz et al., 2001). Of the ten essential mineral elements analysed in this current study except Mg, their concentrations were highest in the CM, followed by that in the TM, with the MM recording the least concentrations. The concentrations of both the macro-elements (Ca, Mg, Na, K, P & S) as well as the micro-mineral elements (Cu, Fe & Zn) are known to be tightly regulated by maternal homeostasis processes (Bates & Prentice, 1994) because the mammary gland seems to have developed mechanisms to adjust the levels of these elements in breast milk to sufficiently furnish the breast-fed neonates with the appropriate levels of these minerals irrespective of maternal status (Lonnerdal, 2000). An exception to this rule is Se which its levels in breast milk are dependent on maternal status. Some researchers like (Rossipal & Krachler, 1998) put forward some reasons to explain these observation. These include the alteration in the binding capacity for trace elements in human milk which may be caused by a change in the concentration of a binding media such as proteins or ligands, and an increase of bioavailability of some trace elements for the mammary gland leading to increase in their actual concentration in breast milk. However, the entire

mechanism of the gradual decline of levels of mineral elements from colostrum to mature milk is not yet properly understood. Infants will therefore, have access to lower amounts of mineral elements as lactation progresses.

Magnesium was the only mineral element which did not show any meaningful variability in its concentrations during the three stages of lactation. There was virtually no change in its concentration from the CM (23.96 ± 3.72 mg/L) to TM (23.99 ± 3.85 mg/L); though there was a slight increase in its concentration from TM to MM (24.67 ± 3.55 mg/L). Other researchers (Aumeistere et al., 2019; Björklund et al., 2012; Daniels et al., 2019; Sanchez et al., 2020; Shi et al., 2011) reported higher mean concentrations of Mg than observed in this study.

An essential macro-mineral element of interest in this study was sulphur. To the best of the knowledge the researcher, S was the least studied among the elements quantified in breast milk. Apart from few researchers (Macy, Icie, 1949) and (McNally et al., 1991) who determined the concentrations of S in colostrum and mature human milk, very little is known about it in the available literature. In a study, Parcell (2002) indicated that the role of elemental S in human nutrition had not been studied to any meaningful extent, even though S is the sixth most abundant macro-mineral element in breast milk and the third most abundant mineral in terms of percentage total body weight. Moreover, S forms part of some important amino acids such as methionine, cysteine, cystine, homocysteine, homocystine, and taurine. According to Parcell (2002), dietary sulphur-containing amino acids (SAAs) analysis and protein supplementation could be indicated for vegan athletes, children or patients with HIV due to an increased risk for SAA deficiency in

these groups. However, as to why the study of S in breast milk and S in human nutrition have virtually escaped the lenses of researchers worldwide is hard to explain.

The present study observed a very significant difference ($p = 0.000$) in the levels of S during the three stages of lactation, a trend which was similar to and consistent with the other mineral elements. Other researchers (Macy, Icie, 1949; McNally et al., 1991) also observed a similar pattern of decline in the levels of S during lactation periods.

Mean Levels of Essential Elements in the CM, TM, MM and TBM of Mothers

Table 50 gives a summary of the mean levels of essential elements determined in the breast milk of mothers at the Ho Teaching Hospital during the three stages of lactation – Colostrum, CM (Day 1 – Day 5); Transitional milk, TM (Day 6 – 2 Weeks) and Mature milk, MM (after 2 Weeks and beyond). It also includes the recommended levels of the essential elements in breast milk (Kim & Yi, 2020)

Table 50: Mean Levels of Essential Elements (EEs) (mg/L) in CM, TM, MM and TBM and their Respective Ranges and the Recommended Levels in Breast Milk

Observe Levels of Essential Elements at Lactational Stages (mg/L)				Recommended levels in breast milk (mg/L)**	
Essential element	Colostrum milk	Transitional milk	Mature milk	Colostrum	Mature milk
Ca	244.45 ± 80.93	218.02 ± 42.12	202.68 ± 65.79	250.00	200.00 – 250.00
Cu	O,58 ± 0.17	0.55 ± 0.14	0.45 ± 0.14	0.50– 0.80*	0.10 – 0.30*
Fe	1.40 ± 0.54	1.16 ± 0.40	1.00 ± 0.39	0.50 – 1.00	0.30 – 0.70
Mg	23.96 ± 3.72	23.99 ± 3.85	24.67 ± 3.55	30.00 – 35.00	30.00 – 35.00
P	193.23 ± 46.76	175.60 ± 25.46	151.17 ± 28.18	120.00 – 160.00	120.00 -140.00
Na	403.91 ± 143.03	349.63 ±151.34	269.07 ± 117.01	300.00 – 400.00	150.00 – 250.00
K	602.63 ± 77.62	530.15 ± 92.45	456.34 ± 76,45	600.00 – 700.00	400.00 – 550.00
S	202.57 ± 46.03	165.47 ± 40.83	130.45 ± 37.56	NA	NA
Se	0.11 ± 0.03	0.09 ± 0.03	0.09 ± 0,05	25.0 – 32.0*	10.00 – 25.00*
Zn	3.79 ± 1.38	3.16 ±1.17	2.20 ± 1.13	5.00 – 12.00*	1.30*

**Kim & Yi (2020); *Levels in micrograms per litre (µg/L).

Source: Laboratory Data (2022)

Exploratory Factor Analysis of the Mean Concentrations of Essential Elements in the Colostrum Milk (CM)

In the Colostrum milk of mothers, factor one accounted for 27.77% of the variation of essential elements. This is evident in Table 52 that factor one is concerned with association between Na, Fe, Cu, and K, Se, Zn, and S contributed about 14.52% in factor two. Factor three contributed 13.52% of the variation in essential elements in breast milk of mothers with focus on elements such as P, Ca, and Mg. The results of the factor analysis are presented in Table 51 while Figure 46 illustrates the scree plot of the extracted components.

Table 51: Rotated Component Matrix of Essential Elements in CM

	Components		
Measurement items	1	2	3
Na	0.700		
Fe	0.655		
Cu	0.631		
K	0.570		
Se		0.844	
Zn		0.623	
S		0.600	
P			0.822
Ca			0.553
Mg			-0.523
Variance (%)	27.77	14.52	13.52
Cumulative variance (%)	27.77	42.29	65.73

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 6 iterations.

Source: Laboratory Data (2022)

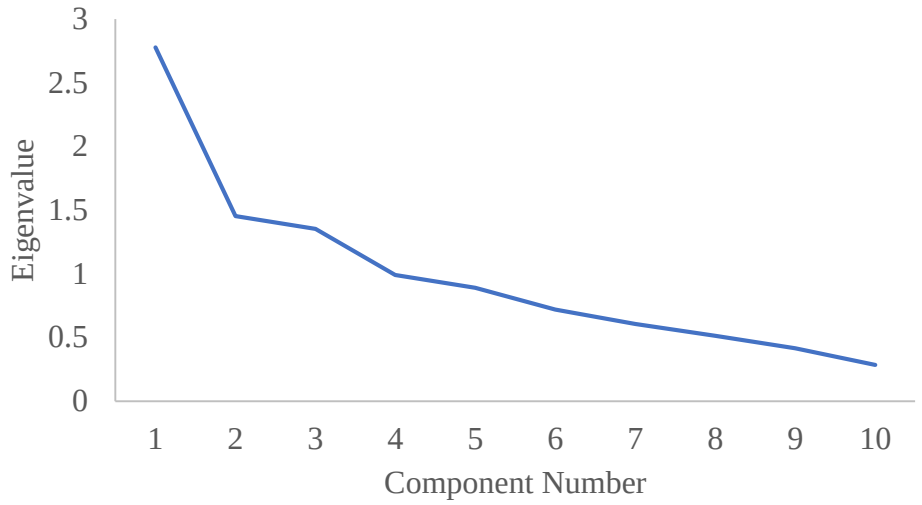

Figure 46: A Scree Plot of the Extracted Components of Essential Elements in the CM. Source: Laboratory Data (2022)

Exploratory Factor Analysis of Mean Concentrations of Essential Elements in the TM

In the transition milk of mother's data, a total of four components/factors were extracted. Factor one which was explained by variables such as Ca, Fe, and K accounted for 26.15% of the variation in the data. Variables such as Cu, S, and Na were responsible for the 18.53% variation accounted for factor two. Zn, P, and Mg also accounted for 12.75% of the variation reported in factor three while Se caused a variation of 11.49% in factor four. Results of the factor analysis is summarized in Table 52 while Figure 47 illustrates the extracted components.

Table 52: Rotated Component Matrix of Essential Elements in the TM

	Components			
Measurement items	1	2	3	4
Ca	0.855			
Fe	0.696			
K	0.612			
Cu		0.799		
S		0.787		
Na		0.538		
Zn			0.414	
P			-0.805	
Mg			0.640	
Se				0.855
Variance (%)	26.15	18.53	12.75	11.49
Cumulative variance (%)	26.15	44.67	57.42	68.91

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 6 iterations.

Source: Laboratory Data (2022)

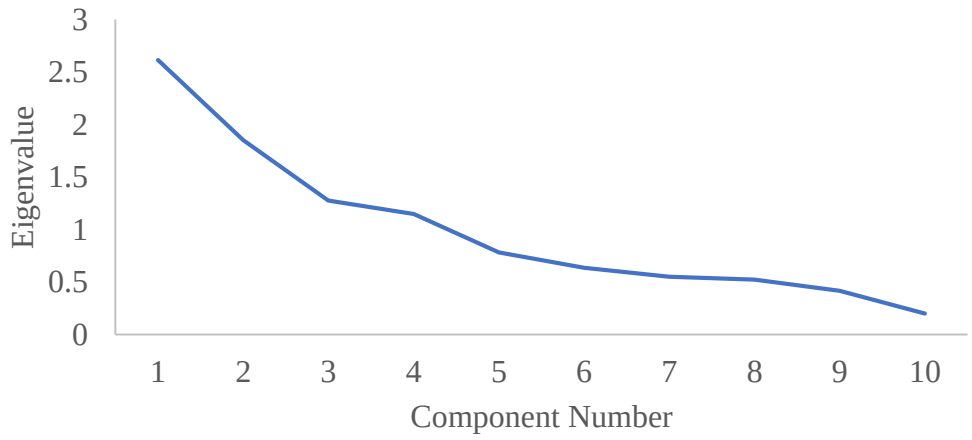

Figure 47: A Scree Plot of the Extracted Components of Essential Elements in the TM. Source: Laboratory Data (2022)

Exploratory Factor Analysis of the Mean the Concentration of Essential Elements in MM

The exploratory factor analysis revealed that factor one accounted for 30.39% of the total variation in the mature milk of mothers. This was concerned with three elements; Cu, P, and S. Factor two which was concerned with Na and Fe accounted for 16.19% of the total variation. Significant elements such as K, Ca, Zn, and Mg caused a variation of 14.56% explained by factor 3. Factor 4 which accounted for 10.66% of the total variation was concerned with Se. Results of the factor analysis is summarised in Table 53 while Figure 48 shows the extracted components.

Table 53: Rotated Component Matrix of Essential Element in the MM

	Components			
Measurement item	1	2	3	4
Cu	0.820			
P	0.796			
S	0.775			
Na		0.821		
Fe		0.709		
K			0.759	
Ca			0.758	
Zn			0.616	
Mg			0.515	
Se				0.955
Variance (%)	30.39	16.19	14.56	10.66
Cumulative variance (%)	30.39	46.58	61.14	71.79

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 7 iterations.

Source: Laboratory Data (2022)

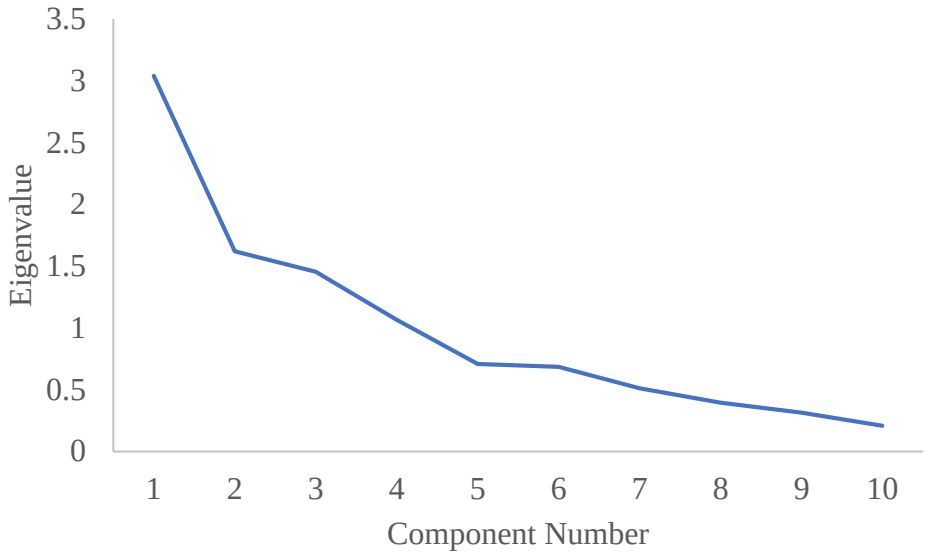

Figure 48: A Scree Plot of the Extracted Components of Essential Elements in MM. Source: Laboratory Data (2022)

Exploratory Factor Analysis of the Mean Concentrations of Essential Elements TBM

A significant proportion (28.56%) of the variance is explained by factor one. It is evident fromTable 54 that this factor is concerned with an association (in decreasing order) between K, Fe, Ca, and Na. Of these, the loadings of the others six elements may be considered minor as they contribute to one or two other factors. The second factor relates S, Cu, and P. It accounts for 16.55% of the variance. Factor three has major contributions from Mg, Se, and Zn. Nearly 13.11% of the variance is assigned to this factor. Table 54 gives the extracted components while Figure 49 illustrates the scree plot.

Table 54: Rotated Component Matrix of Essential Elements in TBM

	Components		
Measurement item	1	2	3
K	0.769		
Fe	0.677		
Ca	0.672		
Na	0.590		
S		0.813	
Cu		0.681	
P		0.636	
Mg			0.733
Se			0.700
Zn			0.527
Variance (%)	28.56	16.55	13.11
Cumulative variance (%)	28.56	45.10	58.21

Extraction Method: Principal Component Analysis.

Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 7 iterations.

Source: Laboratory Data (2022)

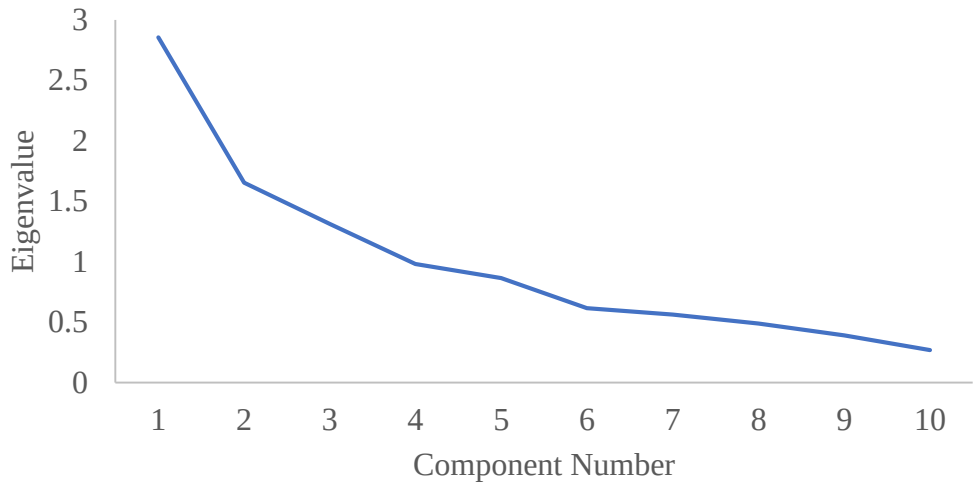

Figure 49: A Scree Plot of the Extracted Components of Essential Elements in Total Breast Milk. Source: Laboratory Data (2022)

Comparison of the Levels of POPs in the CM, TM and MM of Mothers

All the POPs analysed (6 organochlorine pesticides and 14 PCB congeners) in the colostrum, transitional milk and mature milk were below detection limits of 0.00167 mg/kg for the OCPs and 0.001 ppb for the PCBs, and therefore, comparisons of their levels in the three portions of breast milk could not be made.

Comparisons of Levels of Heavy Metals in human Breast Milk with Allowable Safety Levels Set by the World Health Organization

Table 55 compares the levels of toxic heavy metals in colostrum, transitional milk, mature milk and total breast milk with WHO acceptable limits of heavy metals in human breast milk.

Table 55: Comparison of Levels of Heavy Metals in CM, TM, MM and TBM with WHO Recommended Safe Limits (µg/L)

Heavy Metals	CM	TM	MM	TBM	Permissible levels
Al	0.104 ± 0.128	0.123 ± 0.153	0.105 ± 0.088	0.108 ± 0.076	4.00*
As	0.038 ± 0.034	0.035 ± 0.043	0.038 ± 0.045	0.037 ± 0.038	0.20 – 0.60*
Cd	0.004 ± 0.006	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	<1.00*
Pb	0.009 ± 0.004	0.037 ± 0.167	0.009 ± 0.003	0.018 ± 0.051	2.00 – 5.00*
Hg	0.006 ± 0.001	0.006 ± 0.001	0.005 ± 0.000	0.006 ± 0.001	1.40 – 1.70*

*WHO permissible levels of heavy metals in human breast milk

Source: Laboratory Data (2022)

The mean concentrations of all the heavy metals determined in the study were generally very low. With the exception of Al which does not have a defined permissible limit set by the WHO, the concentrations of all the other heavy metals were below the limit set by the WHO. The results for As, Cd, Pb and Hg were comparable to those reported on breast a milk study in Spain (Martínez et al., 2019) except that of Al which was a little higher. The concentrations of Al, As, Cd and Pb in present study were also lower compared with 56.45 ± 22.77 ng/mL, 1.50 ±1.50 ng/mL, 1.37 ± 0.94 ng/mL and 13.22 ± 3.58 ng/mL respectively reported in Taiwan (Chao et al., 2014). Al concentrations determined in this study were far lower than the 584 µg/L reported in Uppsala by (Björklund et al., 2012). In the case of As, Cd and Pb, the present study values were also lower than those reported in Sweden (Björklund et al., 2012) and Pretoria (Olowoyo et al., 2021). With respect to Cd and Pb, the study values were also lower than those reported in Austria (Rossipal & Krachler, 1998), Turkey (Turan et al., 2001), Portugal (Almeida et al., 2008) and Poland (Winiarska-Mieczan, 2014). It is important to note that biochemistry of the interactions of these toxic metals and proteins in breast milk may vary to a considerable extent and therefore, results in inherent variations in the levels of these toxic metals in breast milk. Furthermore, with regards to As and Pb, the study's values were far less than those determined in Namibia (Poland, United States of America and Argentina (Klein et al., 2017). Moreover, the concentrations of As, Cd, Pb and Hg determined in the current study were lower than those previously determined in Ghana (Bansa et al., 2017; Bentum et al., 2010; Koka et al., 2011).

Finally, the concentrations of Hg determined in the present study were lower than those reported by (Rossipal & Krachler, 1998). Al, As, Cd, Pb and Hg levels in our study were similar to those reported among Hungarian women (Ecsedi-angyal et al., 2019).

Evaluation and Assessment of the Extent of Toxic Heavy Metals Exposure to Infants and Levels of Risk Associated with Breast Milk as a Principal Source of Infants Nutrition

In order to evaluate and assess the extent of toxic heavy metals exposure to infant to ascertain the level of risks posed to them through breastfeeding, the hazard quotient (HQ) and Cancer risk (CRs) management strategies were employed. The results of the risk assessment using HQ for TBM is shown in Table 56 while the risk assessment results during the stages of lactation are presented in appendix H. The calculated child cancer risk (CRs) for heavy metals is s presented in Table 58.

Table 56: EDIs and Hazards Quotients (HQs) for Toxic Heavy Metals in Human Breast Milk

Heavy Metals	Average Lower Consumption		Average Upper Consumption		Total Mean	
	EDI	HQ	EDI	HQ	Mean EDI	Mean HQ
Al	0.0119	0.0298	0.0223	0.0558	0.0171	0.0428
As	0.0041	0.0137	0.0076	0.0253	0.0059	0.0197
Cd	0.0003	0.0003	0.0006	0.0006	0.0005	0.0005
Pb	0.0019	0.0005	0.0037	0.0010	0.0028	0.0008
Hg	0.0007	0.0044	0.0012	0.0075	0.0009	0.0056
$\sum EDI_{5HMs}$	0.0189	0.0443	0.0354	0.0827	0.0272	0.0638

Source: Laboratory Data (2022)

The calculation of the hazard quotient took into account the estimated daily intakes (EDIs) of toxic heavy metals through breastfeeding and the reference dose (RfD) values of ingestion for each toxic heavy metal. Due to

the generally low concentrations of the toxic heavy metals in the breast milk, the EDIs were low for both the average and upper limits of consumption. The HQs of the heavy metals calculated ranged from 0.0005 (minimum) to 0.04288 (maximum). Since the hazard quotients (HQ) of Al, As, Cd, Pb and Hg were all << 1, it implied negligible health risks to the nursing infants.

Moreover, the results obtained for the cancer risk assessment is shown in Table 57.

Table 57: Child Cancer Risk (CR_s) Values for Heavy Metals in Human Breast Milk

Heavy metal	Al	AS	Cd	Pb	Hg
CR Child	-	5.90E-04	4.16E-04	1.61E-06	-

Source: Laboratory Data (2022)

The values for As, Cd and Pb were all within the acceptable range of 1.0×10^{-6} to 1.0×10^{-4} specified by the US EPA but with minimal risk implications By interpretation, approximately 6 infants out of 1,000,000 may suffer cancer risks due to ingestion of As through breastfeeding; about 4 infants out of 10,000 may suffer cancer related risks as a result of ingesting Cd through breastfeeding and approximately 2 infants out of 10,000 may suffer from cancer risks through ingestion of Pb in breast milk. The cancer risk for Al and Hg could not be calculated because the cancer slope factor (CSF) for these heavy metals could not be found in the literature. Mercury, in particular, does not have a cancer slope factor because it is not considered to create cancer in humans (ATSDR, 1999a; Gnonsoro et al., 2022)

Thus the potential health risks posed by the toxic heavy metals to breastfeeding infants in the study were quite minimal compared to those found in some studies conducted in Ghana (Bansa et al., 2017; Bentum et al., 2010;

Koka et al., 2011), Southern Spain (Motas et al., 2021), Pretoria (Olowoyo et al., 2021) and Zanzibar (Khamis et al., 2017). Therefore, based on the cancer and non-cancer risk assessment results of the study, it can be concluded that the breast milk of the mothers analysed in this study were not highly contaminated by toxic heavy metals and so may not pose any significant health risks to nursing infant.

Chapter Summary

This chapter presented and discussed the results of the study. Four types of PFAS were detected in human breast milk. The concentration of the PFAS detected were not too high and followed the ascending order: PFHxA < PFHpA < PFOA < PFOS.

None of the OCPs and PCBs were detected in the breast milk at any of the stages of lactation. As a result, no statistical analysis nor risk assessment were performed on the results.

The levels of heavy metals detected during three stages of lactation followed the ascending order:

- Colostrum: Cd < Hg < Pb < As Al
- Transitional milk: Cd < Hg < As < Pb < Al
- Mature milk: Cd < Hg < Pb < As < Al

The results of risk assessment on the heavy metals indicated that they might not pose any significant health risks to breastfeeding infants.

All the ten essential elements were detected in human breast milk in varying concentrations. The concentrations of Ca, P, Na, K and S decreased across lactational stages from colostrum to mature milk whereas the others did not exhibit any meaningful variation acros lactational stages. The levels of some heavy metals and essential elements were related to maternal factors.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Overview

This chapter presents summary of the study and provide some recommendation. The study was conducted at the Ho Teaching Hospital to analyse human breast milk for the presence of POPs (PFAS, OCPs, and PCBs), heavy metals (Al, As, Cd, Pb & Hg) and essential elements (Ca, Cu, Fe, Mg, P, Na, K, S, Se & Zn). Twenty-nine (29) breast milk samples were analysed for PFAS while one hundred and forty-one (141) breast milk samples were analysed for the OCPs, PCBs, toxic heavy metals and the essential elements.

Summary

The total levels of the individual PFAS detected, ∑PFAS, were PFHxA (76.80 ± 2.65 ng/L), PFHpA (91.00 ± 3.14 ng/L), PFOA (1,775.60 ± 61.23 ng/L) and PFOS (2,411.00 ± 83.14 ng/L). PFOS and PFOA were the predominant PFAS found in human breast milk. Among the four PFAS quantified in human breast milk, a significant and strong positive correlation was found between PFOS and PFOA ($r = 0.784$; $P<0.0001$), indicating that they were of the same anthropogenic origin and are often found together. Furthermore, the study found negative and significant correlations between PFHxA levels and maternal weight ($r = -0.524$; $p = 0.004$) as well as PFHxA levels and maternal BMI ($r = -0.516$; $p = 0.004$). These correlations suggested inverse relationships between the concentrations of PFHxA in breast milk and maternal body weight and BMI implying that, as maternal weight or BMI increases, levels of PFHxA would decrease and vice versa.

The study did not find any significant relationship between levels of PFAS in breast milk and infant's sex, maternal characteristics and lifestyle. The values of the tolerable daily intakes (TDIs) of PFAS in human breast milk did not show any significant health risks of PFAS for breastfeeding infants. In summary, the results the study compared favourably well with those conducted in developed countries. All the six POPs (6 OCPs - Aldrin, Chlordane, Dieldrin, DDT, Endrin & Heptachlor) and 14 PCB congeners determined in the study were, however, below limits of detection, implying that even if some were present in the breast milk at all, they might not be able to cause any significant health risk to breastfeeding infants.

The minimum and maximum levels of the toxic heavy metals measured in colostrum ranged from 0.002 µg/L (Cd) – 0.823 µg/L (Al), transitional milk from 0.002 µg/L (Cd) – 0.997 µg/L (Pb) and in mature milk from 0.002 µg/L (Cd) - 0.564 µg/L (Al). Though the concentrations of all the heavy metals measured in breast milk during the three stages of lactation were generally low, Al recorded the highest concentration in all the lactation stages while Cd recorded the least concentration. Thus, the general pattern of levels of heavy metals in breast milk during the three stages of lactation in ascending order was:

Colostrum: Cd < Hg < Pb < As < Al

Transitional milk: Cd < Hg < As < Pb < Al

Mature breast milk: Cd < Hg < Pb < As <Al

Besides, no significant correlation was established among the five toxic heavy metals during the three stages of lactation.

The study identified some significant factors relating to levels of toxic heavy metals in mothers' milk. First, the study found a significant relationship between Pb levels in colostrum with parity (p = 0.03, Cl = 0.05). Multiparous mothers had higher levels of Pb in their colostrum milk than primiparous mothers. Again, the study established a significant relationship (p = 0.038, Cl = 0.05) between Cd levels in transitional milk and maternal education. Mothers without formal education had higher concentrations of Cd in their transitional milk than mothers with formal education. Furthermore, there was a significant relationship (p = 0.026, Cl = 0.05) between Pb levels in mature milk and parity. Primiparous mothers were found to have higher concentrations of Pb in their mature milk than multiparous mothers. Finally, a significant relationship (p = 0.037, Cl = 0.05) was found between Cd levels in total breast milk and parity. Primiparous mothers were found to have higher levels of Cd in their total breast milk than their multiparous counterparts.

Exploratory factor analysis revealed two components causing variation in the data for heavy metals in colostrum milk. Components one (PC1) which essentially concerned association between Al and Pb in breast milk accounted for 48.04% of the total variation while component two (PC2) which concerned association among Hg, As and Cd also accounted for 26.06% of the variation. In the TM, a total of two (2) components were extracted. Component one (PC1) concerned association among As, Cd, Al and Hg accounted for 73.87% of the variation while component two (PC2) representing 25.08% was mainly due to Pb. In the total breast milk, three (3) factors were identified to cause variation in the data. Component one (PC1) which accounted for 37.81% was attributed to Al and Pb; Hg and As also caused a variation of 32.08% in

component two (PC2) while Cd contributed to component three (PC3) representing 22.03% of the total variance.

The mean levels of essential elements determined in CM, TM and MM ranged from 0.11 ± 0.03 mg/L (Se) – 602.63 ± 77.62 mg/L (K); 0.10 ± 0.03 mg/L (Se) – 530.15 mg/L (K) and 0.09 ± 0.05 mg/L (Se) – 456.34 ± 76.45 mg/L (K) respectively. With exception of Mg which did not show any meaningful variability, the levels of all the essential elements measured in breast milk were noticed to occur with a pattern of steady decline at each stage of lactation. The concentrations of all the essential elements were highest in the CM and lowest in the MM.

The study also observed some factors which caused significant variations in the levels of essential element in breast milk of mothers. Firstly, it observed a significant difference ($p = 0.04$, Cl – 0.05) between K levels in CM and maternal employment. Self-employed mothers were found to have higher levels of K in their CM than other categories of mothers. It also found a significant relationship ($p = 0.028$, Cl = 0.05) between Zn levels in CM and parity. Multiparous mothers had higher levels of Zn in their CM than their primiparous counterparts. Secondly, it observed a significant relationship ($p = 0.030$, Cl = 0.05) between Cu levels in TM and maternal age. Mothers who were around 38 years had higher levels of Cu in their TM than mothers who were younger or older. Thirdly, it found a very significant relationship ($p = 0.008$, Cl = 0.05) between Zn levels in MM and parity. Multiparous mothers were observed to have higher concentrations of Zn in their mature milk than primiparous mothers. Finally, it observed significant difference between levels of Ca in TBM with infants' sex and parity ($p = 0.032$; Cl = 0.05). Mothers

who gave birth to females had higher concentrations of Ca in their TBM than those gave birth to males. Moreover, with respect to parity, the study found a significant difference between levels of Se ($p = 0.031$, Cl = 0.05) and Zn ($p = 0.018$, Cl = 0.05) with parity. Multiparous mothers had higher levels of Se and Zn in their TBM than primiparous mothers.

Exploratory factor analysis revealed factors which caused variations in the mean concentrations of the essential elements. In CM of mothers, three main factors were identified: Component one (PC1) which accounted for 27.77% variation was concerned with the association among Na, Fe, Ca and K; component two (PC2) contributed to 14.52% of the variation and concerned with the association among Se, Zn and S while component three (PC3) contributed to 13.52% of the variation concerned with the association among Ca, P and Mg.

In TM of mothers, a total of four (4) components were extracted. Component one (PC1) was concerned with variables such as Ca, Fe and K, accounting for 26.15%; component two (PC2) dealt with association among Cu, S and Na accounting for 18.53% of total variation, component three (PC3) was concerned with the association among Z, P and Mg accounting for 12.75% variation while component four (PC4) which accounted for 11.49% of the variation was concerned with Se.

In MM of mothers, four (4) factors were extracted. Component one (PC1) which concerned association among Cu, P and S accounted for 30.39% of the total variation; component two was concerned the association between Na and Fe accounted for 16.19% variation, component 3 (PC3) was concerned with association among K, Ca, Zn and Mg accounted for 14.56% variation

while component four (PC4) which accounted for 10.66% of the variation was attributed to Se. In the TBM of mothers, three components were revealed to cause variations in the data. PC1 was concerned with association among K, Fe, Ca and Na which accounted for 28.56% variation, PC2 concerned with association among S, Cu and P accounting for 16.53% of variation in the data while PC3 was attributed to association among Mg, Se and Zn and accounted for 13.11% of the variation.

Conclusions

The detection of PFAS, especially, PFOS and PFOA in mother's breast milk is an indication of moderately high exposure and contamination of both mothers and infants with PFAS in the study. Currently, there is no established PFAS level for breast milk at which a health risk for an infant or mother is expected (ATSDR, 2020); thus making it difficult to interpret test results. The most predominant PFAS in mothers' breast milk were PFOS and PFOA. The results of this first study of levels of PFAS in the breast milk of mothers may serve as a preliminary evidence of the extent of exposure of the general population to PFAS in Ghana. The study is very significant because it would serve as a baseline data for further studies on PFAS in human breast milk in Ghana. PFAS were not examined across lactational due to limited funds and lack of instrumentation for PFAS analysis in Ghana.

Furthermore, since none of the OCPS and PCBs were detected in the breast milk samples, no statistical analysis and risk assessment were performed.

Generally, the highest concentrations of toxic heavy metals did not follow a definite pattern. The highest concentrations of both Al and Pb occurred in the TM, that of As in the MM, while those of Cd and Hg did exhibit any meaningful variations accros lactational stages. However, in the case of the essential elements (i.e. Ca, P, Na, K and S) had highest concentration in the colostrum, followed by the transitional milk, with the mature milk recording the lowest concentrations. Mg, Cu, Fe, Se and Zn did not show any meaningful variations across lactational stages. Levels of almost all the toxic heavy metals determined were found to be below the regulatory dietary guidelines set out by the WHO and were within the acceptable limits recommended in human breast milk (Table 56). Levels of Al were found to be the highest in all the three stages of lactation, those of Pb were higher during the TM stage while those of As were higher in the MM.

No significant difference was observed in the levels of all the toxic heavy metals during the stages of lactation. However, in the case of the essential; elements, with exception of Mg and Se, very significant differences were observed in their levels during the stages of lactation. Moreover, their concentrations were within the recommented limits found in breast milk

Recommendations

1. Despite the large volume of literature on the harmful effects of PFAS on humans and the environment, scanty information exists on research on PFAS in Ghana, especially, on human breast milk. There is therefore, the need for more studies to be carried out on the levels of PFASs in human breast milk in the other regions in Ghana.

2. Considering the adverse effects of PFAS on humans, especially on infants, there is the need for the government to provide research funding on PFAS in human breast milk.
3. The mode of exposure of the general population to PFAS in the environment is widespread and diverse. Thus, there is the need for more public education on the sources and harmful effects of PFAS and how to minimize exposure to these chemicals.
4. The detection of some levels of toxic heavy metals in human breast milk in the studied area suggests the presence of toxic heavy metals in the Ghanaian environment. There is, therefore, the need for more public education on the sources of toxic heavy metals in the environment and how to avoid or minimize exposure. In this regard, further studies should be conducted in other facilities as well as in other regions of Ghana to determine levels of potential toxic heavy metals in human breast milk at lactational stages.
5. This study was probably the first conducted in Ghana to determine levels of essential elements in human breast milk during the three stages of lactation - colostrum, transitional milk and mature milk. Further studies should, therefore, be conducted in the other regions of Ghana to get adequate data on levels of essential elements in colostrum, transitional milk and mature milk in Ghana to form a database.
6. It is important to emphasize that the determination of xenobiotics in human breast milk is of public health importance because it has the potential to promote maternal and neonatal health. However, the cost

of analysing samples for xenobiotics such as toxic heavy metals and persistent organic pollutants (POPs) in human breast milk is very expensive not only in Ghana but worldwide. It is therefore, recommended that research on pollutants in human milk should, therefore, attract government funding.

REFERENCES

ABA. (2023). Unusual colours (appearance) and smells of breastmilk. In *Australian Breastfeeding Association*. Retrieved from https://www.breastfeeding.asn.au/resources/unusual-colours-smells-breastmilk on May 10, 2024.

Abballe, A., Ballard, T. J., Dellatte, E., Ferri, F., Rita, A., Grisanti, G., Iacovella, N., Maria, A., Malisch, R., Miniero, R., Grazia, M., Risica, S., Ziemacki, G., & Felip, E. De. (2008). Chemosphere Persistent Environmental Contaminants in Human Milk : Concentrations and Time Trends in Italy. *Chemosphere*, *73*(1), 220–227. https://doi.org/10.1016/j.chemosphere.2007.12.036

Abdulrazzaq, Y. M., Osman, N., Nagelkerke, N., Kosanovic, M., & Adem, A. (2008). Trace Element Composition of Plasma and Breast Milk of Well-Nourished Women. *Journal of Environmental Science and Health , Part A: Toxic / Hazardous Substances and Environmental Engineering*, *43*(3), 329–334. https://doi.org/10.1080/10934520701792878

Abunada, Z., Alazaiza, M. Y. D., & Bashir, M. J. K. (2020). An Overview of Per- and Polyfluoroalkyl Substances (PFAS) in the Environment: Source, Fate, Risk and Regulations. *Water*, *12*(12), 3590. https://doi.org/10.3390/w12123590

Aceti, A., Barbarossa, A., Gazzotti, T., Zironi, E., Pagliuca, G., Vitali, F., Beghetti, I., & Corvaglia, L. (2021). Exposure to perfluoroalkyl substances through human milk in preterm infants. *European Journal of Pediatrics*, *180*(9), 3047–3051. https://doi.org/https://doi.org/10.100

7/s00431-021-04073-4

Ahrens, L., Felizeter, S., Sturm, R., Xie, Z., & Ebinghaus, R. (2009). Polyfluorinated compounds in waste water treatment plant effluents and surface waters along the River Elbe , Germany. *Marine Pollution Bulletin*, *58*(9), 1326–1333. https://doi.org/10.1016/j.marpolbul.2009.04.028

Akamori, M. N., Inh, N. X. N., Somura, H. I., Oshiike, N. Y., Hien, V. T. T., Nhug, B. T., Nhien, N. Van, Nakano, T., Khan, N. C., & Yamamoto, S. (2009). Nutritional Status of Lactating Mothers and Their Breast Milk Concentration of Iron , Zinc and Copper in Rural Vietnam. *Journal of Nutritional Science and Vitaminology*, *55*(4), 338–345. https://doi.org/10.3177/jnsv.55.338

Akanle, O. A., Balogun, F. A., Owa, J. A., & Spyrou, N. M. (2001). Variations in Trace Element Concentrations in Breast Milk with Stages of Lactation. *Journal of Radioanalytical and Nuclear Chemistry*, *249*(1), 71–75. https://akjournals.com/downloadpdf/journals/10967/249/1/article-p71.xml

Al-Saleh, I., Shinwari, N., & Mashhour, A. (2003). Heavy Metal Concentrations in the Breast Milk of Saudi Women. *Biological Trace Element Research*, *96*(1–3), 21–37. https://doi.org/https://doi.org/10.1385/bter:96:1-3:21

Albers, J. M. C. L., Kreis, I. A., Liem, A. K. D., & Zoonen, E. Van. (1996). Factors that Influence the Level of Contamination of Human Milk with Poly-chlorinated Organic Compounds. *Archives of Environmental Contamination and Toxicology*, *30*, 285–291. https://doi.org/https://

doi.org/10.1007/bf00215810

Algarín, C., Peirano, P., Garrido, M., & Pizarro, F. (2003). Iron Deficiency Anemia in Infancy : Long-Lasting Effects on Auditory and Visual System Functioning. *Pediatric Research*, *53*(2), 217–223. https://doi.org/10.1203/01.PDR.0000047657.23156.55

Almeida, A. A., Lopes, C. M. P. V, Silva, A. M. S., & Barrado, E. (2008). Trace elements in human milk : Correlation with Blood Levels , Inter-element Correlations and Changes in Concentration During the First Month of Lactation. *Journal of Trace Elements in Medicine and Biology*, *22*(3), 196–205. https://doi.org/10.1016/j.jtemb.2008.03.007

Altun, S. K., Dinc, H., Temamogullari, F. K., & Paksoy, N. (2018). Analyses of Essential Elements and Heavy Metals by Using ICP-MS in Maternal Breast Milk from F anl J urfa, Turkey. *International Journal of Analytical Chemistry*, *2018*, 1–5. https://doi.org/10.1155/2018/1784073

Ameen, K., & Keizer, A. (2023). Exposure to hazardous chemicals at work and resulting impact: A global review. In *Encyclopedia of Human Resource Management, Second Edition*. https://doi.org/10.4337/9781800378841.i.19

American Cancer Society. (2020). *Arsenic and Cancer Risk*. Retrieved from https://www.cancer.org/healthy/cancer-causes/chemicals/arsenic.html on June 9, 2022.

American Public Health Association. (2007). *A Call to Action on Breastfeeding : A Fundamental Public Health Issue* (Vol. 200714, Issue Nov 2007). Retrieved from https://www.apha.org/policies-and-

advocacy/public-health-policy-statements/policy-database/2014/07/29/13/23/a-call-to-action-on-breastfeeding-a-fundamental-public-health-issue?msclkid=0d3d93e9cd4911ecbc2b7b1356ed9a86 on April 11, 2021.

Amin-Zaki, M. D., Elhassani, S., Majeed, M. A., Clarkson, T. W., Doherty, R. A., & Greenwood, M. (1979). Prenatal methylmercury, clinical observation over five years. *American Journal of Diseases of Children*, *133*(2), 172–177. https://doi.org/10.1001/archpedi.1979.02130020064013

Anderson, H. A., & Wolff, M. S. (2000). Environmental contaminants in human milk 1. *Journal of Exposure Science and Environmental Epidermiology*, *10*(6), 755–760. https://doi.org/https://doi.org/10.1038/sj.jea.7500133

Andrade, M. T. S., Del Ciampo, A. L., Del Ciampo, L. R. I., Ferraz, I. S., & Barbosa Junior, F. (2014). Breast Milk Micronutrients in Lactating Mothers from Ribeirão Preto (SP), Brazil. *Food and Nutrition Sciences*, *5*(July), 1196–1201. https://doi.org/http://dx.doi.org/10.4236/fns.2014.513130

Andrae, J., Gallini, R., & Betsholtz, C. (2008). Role of platelet-derived growth factors in physiology and medicine. *Genes and Development*, *22*(10), 1276–1312. https://doi.org/10.1101/gad.1653708.revealing

Andreas, N. J., Kampmann, B., & Le-doare, K. M. (2015). Early Human Development Human breast milk : A review on its composition and bioactivity. *Early Human Development*, *91*(11), 1–7. https://doi.org/10.1016/j.earlhumdev.2015.08.013

Apelberg, B. J., Goldman, L. R., Calafat, A. M., Herbstman, J. B., Kuklenyik, Z., Heidler, J., & Witter, F. R. (2007). Determinants of Fetal Exposure to Polyfluoroalkyl Compounds in Baltimore , Maryland. *Environmental Science and Technology*, *41*(11), 3891–3897. https://doi.org/https://doi.org/10.1021/es0700911

Arnaud, J., & Favier, A. (1995). Copper, Iron, Manganese and Zinc Contents in Human Colostrum and Transitory Milk of French Women. *Science of the Total Environment*, *159*(1), 9–15. https://doi.org/10.1016/0048-9697(94)04314-D

Arnaud, J., Prual, A., Preziosi, P., Cherouvrier, F., Favier, A., Galan, P., & Hercberg, S. (1993). Effect of Selenium Supplementation during Pregnancy on Trace Element (Cu, SE, Zn) Concentrations in Serum and Breast Milk from Nigerien Women. *Annals of Nutrition and Metabolism*, *37*(5), 362–371. https://doi.org/10.1159/000177776

Aronson, K. J., Miller, A. B., Woolcott, C. G., Sterns, E. E., Mccready, D. R., Lickley, L. A., Fish, E. B., Hiraki, G. Y., Holloway, C., Ross, T., Hanna, W. M., Sengupta, S. K., & Weber, J. (2000). Breast Adipose Tissue Concentrations of Polychlorinated Biphenyls and Other Organochlorines and Breast Cancer Risk 1. *Cancer Epidemiology Biomarkers & Prevention*, *9*(January), 55–63.

Asamoah, A., Essumang, K. D., Muff, J., Kucheryavskiy, S. V, & Sogaard, E. G. (2018). Assessment of PCBs and exposure risk to infants in breast milk of primiparae and multiparae mothers in an electronic waste hot spot and non-hot spot areas in Ghana. *Science of the Total Environment*, *612*, 1473–1479. https://doi.org/10.1016/j.scitotenv.

2017.08.177

ATSDR. (1993). *ToxFAQs Aldrin / Dieldrin* (Issue April 1993). Retrieved from https://www.nativeknowledge.org/db/files/tfacts1.htm on April 27, 2021.

ATSDR. (1999a). Public Health Statement for Mercury. In *Agency for Toxic Substances and Disease Registry* (Issue March 1999). Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp46-c1-b.pdf on April 22, 2021.

ATSDR. (1999b). *Toxicolgical Profile for Mercury* (Issue March). Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp46.pdf on April 22, 2021.

ATSDR. (2000). Toxicological profile for polychlorinated biphenyls (PCBs). *Agency for Toxic Substances and Disease Registry*. Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp17.pdf on April 22, 2021.

ATSDR. (2002). Toxicological Profile for Aldrin / Dieldrin. In *Agency for Toxic Substances and Disease Registry*. Retrieved from https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/dieldrin.pdf on April 27, 2021.

ATSDR. (2007a). 2007 ATSDR Substance Priority List. In *Agency for Toxic Substances and Disease Registry*. Retrieved from https://www.atsdr.cdc.gov/spl/resources/2007_atsdr_substance_priority_list.html on April 27, 201.

ATSDR. (2007b). Public Health Statement: Lead. In *Agency for Toxic Substances and Disease Registry*. Retrieved from https://www.atsdr.cdc.gov/ToxProfiles/tp13-c1-b.pdf on April 22, 2021.

ATSDR. (2007c). Toxicological Profile for Arsenic. In *Agency for Toxic Substances and Disease Registry* (Issue August). Retrieved from https://www.atsdr.cdc.gov/ToxProfiles/tp2-a.pdf on Marc 20, 2021.

ATSDR. (2007d). Toxicological Profile for Heptachlor and Heptachlor Epoxide. In *United States Department of Health and Human Services* (Issue November). Retrieved from https://www.atsdr.cdc.gov/Tox Profiles/tp12.pdf on March 20, 2021.

ATSDR. (2008a). *Public Health Statement for Aluminum. Agency for Toxic Substances and Disease Registry, Atlanta, GA* (Issue September 2008). Retrieved from https://www.atsdr.cdc.gov/phs/phs.asp?id=1076&tid= 34 on April 30, 2021.

ATSDR. (2008b). Toxicological Profile for Aluminium. In *Agency for Toxic Substances and Disease Registry* (Issue September). Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp22-p.pdf on April 20, 2021.

ATSDR. (2011a). Addendum To the Toxicological Profile for Polychlorinated Biphenyls. In *Agency for Toxic Substances and Disease Registry, US Department of Health and Human Resources* (Issue April). Retrieved from https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=142 &tid=26 on July 20, 2021.

ATSDR. (2011b). What Are the Standards and Regulation for Arsenic Exposure ? In *Agency for Toxic Substances and Disease Registry, Environmental Health and Medicine Education* (Issue October 1, 2011). Retrieved from https://www.atsdr.cdc.gov/csem/arsenic/ standards.html on July 20, 2021.

ATSDR. (2012). Toxicological Profile for Cadmium. In *Agency for Toxic Substances and Disease Registry* (Issue September). Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp5-p.pdf on April 27, 2021.

ATSDR. (2015). *Perfluoroalkyls Division of Toxicology and Human Health Sciences Perfluoroalkyls*. Retrieved from https://www.emerging contaminants.eu/index.php/background-info/Factsheets-PFOS-intro/ Factsheets-PFOS-properties On July 20, 2021.

ATSDR. (2017). Lead Toxicity Case Studies in Environmental Medicine. *Agency for Toxic Substances and Disease Registry, Environmental Health and Medicine Education, June 12, 2017*, 1–185. Retrieved from https://www.atsdr.cdc.gov/csem/lead/docs/lead.pdf on July 20, 2021.

ATSDR. (2018). Toxicological Profile for Perfluoroalkyls. *Agency for Toxic Substances and Disease Registry*, *June*, 1–852. Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp200-p.pdf July 20, 2021.

ATSDR. (2019). Support Document to the 2019 Substance Priority List. In *Agency for Toxic Substances and Disease Registry* (Issue January, 2020). Retrieved from https://www.atsdr.cdc.gov/spl/resources/ ATSDR_2019_SPL_Support_Document-508.pdf on July 22, 2021.

ATSDR. (2020). Toxicological Profile for Lead. In *Agency for Toxic Substances and Disease Registry* (Issue August). Retrieved from https://www.atsdr.cdc.gov/ToxProfiles/tp13.pdf on July 22, 2022.

ATSDR. (2021a). Endrin ToxGuide. In *Agency for Toxic Substances and Disease Registry*. Retrieved from https://www.atsdr.cdc.gov/toxguides/ toxguide-89.pdf on July 22, 2023

ATSDR. (2021b). *Per - and Polyfluoroalkyl Substances (PFAS) and Your Health: PFAS and Breastfeeding*. Retrieved from https://www.atsdr.cdc.gov/pfas/health-effects/pfas-breastfeeding.html#:~:text=Forinfants%2C breast milk can,effect in infants or children. on July 22, 2023.

ATSDR. (2022a). Toxicological Profile for DDT, DDE and DDD. In *United States Department of Health and Human Services* (Issue April). Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp35.pdf on May 10, 2024

ATSDR. (2022b). Toxicological Profile for Mercury. In *Agency for Toxic Substances and Disease Registry* (Issue April). Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp46.pdf on May 10, 2024.

Aumeistere, L., Technology, F., Safety, F., Health, A., Bior, E., Ciprovica, I., Technology, F., Zavadska, D., Bavrins, K., Borisova, A., Safety, F., Health, A., & Bior, E. (2019). Essential elements in mature human milk. *FoodBalt, May*. https://doi.org/10.22616/FoodBalt.2019.005

Backe, W. J., Day, T. C., & Field, J. A. (2013). Zwitterionic, Cationic, and Anionic Fluorinated Chemicals in Aqueous Film Forming Foam Formulations and Groundwater from U.S. Military Bases by Nonaqueous Large-Volume Injection HPLC-MS/MS. *Environmental Science and Technology*, *47*(10), 5226–5234. https://doi.org/10.1021/es3034999

Bakir, F., Damluji, S. F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N. Y., Tikriti, S., Dhahir, H. I., Clarkson, T. W., Smith, J. C., & Doherty, R. A. (1973). Methylmercury Poisoning in Iraq. *Science*, *181*(4096), 230–241. https://doi.org/https://doi.org/10.1126/science.1

81.4096.230

Bansa, D. K., Awua, A. K., Boateng, R., Adom, T., Brown-appiah, E. C., Amewosina, K. K., Diaba, A., Datoghe, D., & Okwabi, W. (2017). Cross-sectional assessment of infants' exposure to toxic metals through breast milk in a prospective cohort study of mining communities in Ghana. *BMC Public Health*, *17*(505), 1–12. https://doi.org/10.1186/s 12889-017-4403-8

Barbarossa, A., Masetti, R., Gazzotti, T., Zama, D., Astol, A., Veyrand, B., Pession, A., & Pagliuca, G. (2013). Perfluoroalkyl substances in human milk: A first survey in Italy. *Environment International*, *51*, 27–30. https://doi.org/10.1016/j.envint.2012.10.001

Barltrop, D., & Hillier, R. (1974). Calcium and Phosphorus Content of Transitional and Mature Human Milk. *Acta Paediatr*, *63*, 347–350. https://doi.org/https://doi.org/10.1111/j.1651-2227.1974.tb04808.x

Barrett, J. (2013). POPs vs . Fat Persistent Organic Pollutant Toxicity Targets and is modulated by adipose tissue. *Environmental Health Perspectives*, *121*(2). https://doi.org/https://doi.org/10.1289%2Fehp. 121-a61

Bassil, M., Daou, F., Hassan, H., Yamani, O., & Kharma, J. A. (2017). Lead, cadmium and arsenic in human milk and their socio-demographic and lifestyle determinants in Lebanon. *Chemosphere*, *191*, 911–921. https://doi.org/10.1016/j.chemosphere.2017.10.111

Bates, C. J., & Prentice, A. (1994). Breast Milk as a Source of Vitamins, Essential Minerals and Trace Elements. *Pharmacology and Therapeutics*, *62*(1–2), 193–220. https://doi.org/10.1016/0163-7258(94

)90011-6

Becker, A. M., Gerstmann, S., & Frank, H. (2008). Perfluorooctane surfactants in waste waters, the major source of river pollution. *Chemosphere*, *72*(1), 115–121. https://doi.org/10.1016/j.chemosphere.2008.01.009

Begum, A., Harikrishna, S., & Khan, I. (2009). Analysis of Heavy metals in Water, Sediments and Fish samples of Madivala Lakes of Bangalore , Karnataka. *International Journal of ChemTech Research*, *1*(2), 245–249.

Bentum, J. K., Sackitey, O. J., Tuffuor, K. J., Essumang, D. K., Koranteng-Addo, E. J., & Owusu-Ansah, E. (2010). Lead, Cadmium and Arsenic in breast milk of lactating mothers in Odumase-Atua community in Manya Krobo District of Eastern Region of Ghana. *Journal of Chemical and Parmaceutical Research*, *2*(5), 16–20.

Berry, M. R., Robinson, C., & Frankl, F. E. K. (2013). Unexpected Clinical Sequelae of Gitelman Syndrome: Hypertension in Adulthood is Common and Females have Higher Potassium Requirements. *Nephrology Dialysis Transplantation*, *28*(6), 1533–1542. https://doi.org/10.1093/ndt/gfs600

Bertazzi, P. A., Riboldi, L., & Pesatori, A. (1987). Cancer mortality on capacitor manufacturing workers. *American Journal of Industrial Medicine*, *11*, 165–176.

Better Health Channel. (2023). Calcium: What is Calcium? In *Better Health Channel*.

Bhuiyan, N. H., Habiburrahmanbhuiyan, K. K., Nath, Ahmed, K., Hassan, T., & Bhuiyan, N. I. (2009). Organochlorine insecticides (DDT and Heptachlor) in dry fish available in Bangladesh : Seasonal Trends and Species Variability. *Journal of the Chilean Chemical Society*, *54*(3), 1–10. https://scielo.conicyt.cl/scielo.php?script=sci_arttext&pid=S0717-97072009000300016

Bianchi, M. L. P., Cruz, A., Zanetiti, M. A., & Dorea, J. G. (1999). Dietary Intake of Selenium and Its Concentration in Breast Milk. *Biological Trace Element Research*, *70*(2), 273–277. https://doi.org/10.1007/BF02783836

Björklund, K. L., Vahter, M., Palm, B., Grandér, M., Lignell, S., & Berglund, M. (2012). Metals and TraceElement Concentrations in Breast Milk of First Time Healthy Mothers : A Biological Monitoring Study. *Environmental Health*, *11*(92), 1–8. https://doi.org/10.1186/1476-069X-11-92

Bloxam, D. L., Williams, N. R., Waskett, R. J. D., Pattinson-green, P. M., Morarji, Y., & Stewart, S. G. (1989). Maternal Zinc during Oral Iron Supplementation in Pregnancy : A Preliminary Study. *Clinical Science*, *76*(1), 59–65. https://doi.org/https://doi.org/10.1042/cs0760059

Bode, L., Mcguire, M., Rodriguez, J. M., Geddes, D. T., Hassiotou, F., Hartmann, P. E., & Mcguire, M. K. (2014). It ' s Alive : Microbes and Cells in Human Milk and Their Potential Benefits to Mother and Infant 1 – 3. *Advances in Nutrition*, *5*, 571–573. https://doi.org/10.3945/an.114.006643.proposed

Bossi, R., Strand, J., Sortkjær, O., & Larsen, M. M. (2008). Perfluoroalkyl compounds in Danish wastewater treatment plants and aquatic environments. *Environment International*, *34*(4), 443–450. https://doi.org/10.1016/j.envint.2007.10.002

Boudreau, H. T., Wilson, J. C., Cheong, J. W., Sibley, K. P., Mabury, A. S., Muir, C. . D., & Solomon, R. K. (2003). Response of the Zooplankton Community and Environmental Fate of Perfluorooctane Sulfonic Acid in Aquatic Microorganisms. *Environmental Toxicology and Chemistry*, *22*(11), 2739–2745. https://doi.org/doi.10.1897/02-394

Bratter, P., Bratter, V. E. N. D. E., Recknagel, S., & Brunetto, R. (1997). Trace Elements Maternal Selenium Status Influences the Concentration and Binding Pattern of Zinc in Human Milk *. *Journal of Trace Elements in Medicine and Biology*, *11*(4), 203–209. https://doi.org/10.1016/S0946-672X(97)80014-4

Brown, K. H., Engle-stone, R., Krebs, N. F., & Peerson, J. M. (2009). Dietary Intervention Strategies to Enhance Zinc Nutrition: Promotion and Support of Breastfeeding for Infants and Young Children. *Food Nutrition Bulletin*, *30*(1), 144–171. https://doi.org/https://doi.org/10.1177%2F15648265090301S108

Butte, N. F., Lopez-Alarcon, M. G., & Garza, C. (2002). Nutrient Adequacy of Exclusive Breastfeeding for the Term Infants during the First Six Months of Life. In *World Health Organization*. https://apps.who.int/iris/bitstream/handle/10665/42519/9241562110.pdf

Butts, C. A., Hedderley, D. I., Herath, T. D., Paturi, G., Id, S. G., Wiens, F., Stahl, B., & Gopal, P. (2018). Human Milk Composition and Dietary Intakes of Breastfeeding Women of Different Ethnicity from the Manawatu-Wanganui Region of New Zealand. *Nutients*, *10*(9), 1231-. https://doi.org/10.3390/nu10091231

Calabrese, E. J. (1982). Human Breast Milk Contamination in the United States and Canada by Chlorinated Hydrocarbon Insecticides and Industrial Pollutants : Current Status. *Journal of the American College of Toxicology*, *1*(3), 91–98.

Callaban, A., Leonard, H., & Powell, T. (2020). Classification of Nutrients. In *Nutrition: Science and Everyday Application* (Vol. 10, pp. 1–19). Retrieved from https://openoregon.pressbooks.pub/nutritionscience/ on July 20, 2022.

Canfield, R. L., & Jusko, T. A. (2008). Lead Poisoning. *Encyclopedia of Infants and Early Childhood Development*, 200–213. https://doi.org/ https://doi.org/10.1016/B978-012370877-9.00091-8

Cao, Y., Cao, X., Wang, H., Wan, Y., & Wang, S. (2015). Assessment on the distribution and partitioning of perfluorinated compounds in the water and sediment. *Environmental Monitoring and Assessment*, *187*(10), 611-. https://doi.org/10.1007/s10661-015-4831-9

Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., Pollono, C., Marchand, P., Leblanc, J., Antignac, J., & Le, B. (2015). Per fl uoroalkyl acid (PFAA) levels and pro fi les in breast milk , maternal and cord serum of French women and their newborns. *Environment International*, *84*, 71–81. https://doi.org/10.1016/j.envint.

2015.07.014

Carratu, B., Boniglia, C., Scalise, F., Ambruzzi, A. M., & Sanzini, E. (2003). Nitogenous Components of Human Milk: Nitrogen , True Protein and Free Amino Acids. *Food Chemistry*, *81*(3), 357–362. https://doi.org/ https://doi.org/10.1016/S0308-8146(02)00430-2

Carroquino, M. J., Posada, M., & Landrigan, P. J. (2012). Environmental Toxicology : Children at Risk. In *Environmental Toxicology: Selected Entries from Encyclopedia of Sustainability Science and Technology*. https://doi.org/10.1007/978-1-4419-0851-3

Casey, C. E., Neville, M. C., & Hambidge, Michael, K. (1989). Studies in Human Lactation: Secretion of Zinc, Copper and Manganese in Human Milk. *The American Journal of Clinical Nutrition*, *49*(5), 773–785. https://doi.org/10.1093/ajcn/49.5.773

CDC. (2017). Arsenic Factsheet. In *Centers for Disease Control and Prevention*. Retrieved from https://www.cdc.gov/biomonitoring/ Arsenic_FactSheet.html#:~:text=Inorganic arsenic compounds are in,mainly in fish and shellfish. 0n April 22, 2021.

Cecil, K. M., Brubaker, C. J., Adler, C. M., Dietrich, K. N., Altaye, M., Egelhoff, J. C., Wessel, S., Elangovan, I., Hornung, R., Jarvis, K., & Lanphear, B. P. (2008). Decreased Brain Volume in Adults with Childhood Lead Exposure. *PLOS Medicine*, *5*(5), 741–750. https://doi.org/10.1371/journal.pmed.0050112

Cerná, M., Krsková, A., & Cejchanová, M. (2012). Human biomonitoring in the Czech Republic: An overview. *International Journal of Hygiene and Environmental Health*, *215*, 109–119. https://doi.org/10.1016/j.ij

heh.2011.09.007

Chao, H.-H., Guo, C., Huang, C., & Chou, Y. (2014). ScienceDirect Arsenic, Cadmium, Lead, and Aluminium Concentrations in Human Milk at Early Stages of Lactation. *Pediatrics and Neonatology*, *55*(2), 127–134. https://doi.org/10.1016/j.pedneo.2013.08.005

Chao, H., Wang, S., Lin, T., & Chung, X. (2006). Levels of organochlorine pesticides in human milk from central Taiwan. *Chemosphere*, *62*(11), 1774–1785. https://doi.org/10.1016/j.chemosphere.2005.07.036

Chen, H., Zhang, C., Han, J., Yu, Y., & Zhang, P. (2012). PFOS and PFOA in influents , effluents , and biosolids of Chinese wastewater treatment plants and effluent-receiving marine environments. *Environmental Pollution*, *170*, 26–31. https://doi.org/10.1016/j.envpol.2012.06.016

Chen, L., Min, J., & Wang, F. (2022). Copper homeostasis and cuproptosis in health and disease. *Signal Transduction and Targeted Therapy*, *7*(378), 1–16. https://doi.org/10.1038/s41392-022-01229-y

Chen, Y., Yu, M., Rogan, W., Gladen, B., & Hsu, C. (1994). A 6-Year Follow-Up of Behaviour and Activity Disorders in the Taiwan Yu-cheng Children. *American Journal of Public Heal*, *84*(3), 415–421. https://doi.org/https://doi.org/10.2105/ajph.84.3.415

Cherkani-hassani, A., Ghanname, I., & Mouane, N. (2016). Assessment of Cadmium Level in Human Breast Milk and the Affecting Factors : A Systematic Critical Reviews in Food Science and Nutrition. *Critical Reviews in Food Science and Nutrition*, *57*(11), 2377–2391. https://doi.org/10.1080/10408398.2015.1057633

Chierici, R., Saccomandi, D., & Vigi, V. (1999). Dietary supplements for the lactating mother : influence on the trace element content of milk. *Acta Paediatr*, *88*(s430), 7–13. https://doi.org/https://doi.org/10.1111/j.1651-2227.1999.tb01294.x

Chilimba, A. D. C., Young, S. D., Black, C. R., Rogerson, K. B., Louise Ander, E., Watts, M. J., Lammel, J., & Broadley, M. R. (2011). Maize Grain and Soil Surveys Reveal Suboptimal Dietary Selenium Intake is Widespread in Malawi. *Scientific Reports*, *1*(72), 1–9. https://doi.org/10.1038/srep00072

Chung, J., Yu, S.-D., & Hong, Y. (2014). Environmental Source of Arsenic Exposure. *Journal of Preventive Medicine and Public Health*, *47*(5), 253–257. https://doi.org/https://doi.org/10.3961%2Fjpmph.14.036

Clarkson, T. W. (2002). The Three Modern Faces of Mercury Methyl Mercury in Fish History of Human Exposure. *Environmental Health Persperctives*, *110*(February), 11–23.

Combs, G. F. (2001). Selenium in Global Food Systems. *British Journal of Nutrition*, *85*(5), 517–547. https://doi.org/10.1079/bjn2000280

Cotruvo, J. A. (2017). 2017 Who Guidelines for Drinking Water Quality: First Addendum to the Fourth Edition. *Journal - American Water Works Association*, *109*(7), 44–51. https://doi.org/10.5942/jawwa.2017.109.0087

Counter, S. A., & Buchanan, L. H. (2004). Mercury exposure in children : a review. *Toxicology and Appliied Pharmacology*, *198*, 209–230. https://doi.org/10.1016/j.taap.2003.11.032

CRC CARE. (2013). CRCCARE Technical Report 24: Analytical methods for priority and emerging contaminants - a literature review. In *CRC for Contamination Assessment and Remediation of the Environment.* (Issue February 2013). Retrieved from https://crccare.com/wp-content/uploads/2022/12/CRCCARETechReport24-Analyticalmethodsforpriorityandemergingcontaminants_aliteraturereview6.pdf on May 8, 2023.

CRC CARE. (2017). Guidance on managing PFAS contamination in Australia. In *Australian Government Department of Industry, Innovation and Science* (Issue March). Retrieved from https://www.battelle.org/docs/default-source/conferences/chlorinated-conference/proceedings/2018-chlorinated-conference-proceedings/a7-pfas-risk-assessment-and-toxicity/a7_-1188_poster_jit.pdf?sfvrsn=1a148188_0 on May 8, 2023.

Daniels, L., Gibson, R. S., Diana, A., Haszard, J. J., Rahmannia, S., & Luftimas, D. E. (2019). Micronutrient Intakes of Lactating Mothers and their Association with Breast Milk Concentrations and Micronutrient Adequacy of Exclusively Breastfed Indonesian Infants. *The American Journal of Clinical Nutrition*, *110*(2), 391–400. https://doi.org/https://doi.org/10.1093/ajcn/nqz047

Davidson, P. W., Myers, G. J., & Weiss, B. (2004). Mercury Exposure and Child Development Outcomes. *Pediatrics*, *113*(3), 1–16.

de Laeter, J. R., Bohlke, J. K., De Bievre, P., Hidaka, H., Peiser, H. S., Rosman, K. . . . R., & Taylor, P. D. P. (2009). Atomic weights of the elements : Review 2000 (IUPAC Technical. *Pure and Applied Chemistry: IUPAC Technical Report*, *81*(8), 1535–1536.

https://doi.org/10.1351/PAC-REP-09-06-03

Demirgoren, S. B., Ozbek, A., Ormen, M., Kavurma, C., Ozer, E., & Aydın, A. (2017). Do Mothers with High Sodium Levels in their Breast Milk have High Depression and Anxiety Scores? *Journal of International Medical Research*, *45*(2), 843–848. https://doi.org/10.1177/0300060517700013

DEPA. (2015). Perfluoroalkylated substances: PFOA, PFOS and PFOSA - Evaluation of health hazards and proposal of a health based quality criterion for drinking water, soil and ground water. *Danish Environmental Protection Agency.1665*, 1–90. Retrieved from https://www2.mst.dk/Udgiv/publications/2015/04/978-87-93283-01-5.pdf on May 11, 2023.

Dewailly, E., Ayotte, P., Bruneau, S., Laliberte, C., Muir, D. C. G., & Norstrom, R. J. (1993). Inuit Exposure to Organochlorines through the Aquatic Food Chain in Arctic Quebec. *Environmental Health Perspectives*, *101*(7), 618–620.

Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin*, *24*(1), 5–28. https://doi.org/10.1177/156482650302400102

Dewey, K. G., Finley, D. A., & Lonnerdal, B. (1984). Breast Milk Volume and Composition During Lactation (7 - 20 Months). *Journal of Pediatric Gastroenterology and Nutrition*, *3*(5), 713–720. https://doi.org/https://doi.org/10.1097/00005176-198411000-00014

Dillon, K. H., Wilson, D. J., Schaffner, W., & Labo-, H. (1974). Lead Concentrations in Human Milk Recently ,. *American Journal of Diseases of Childrenf Children*, *128*(4), 491–492. https://doi.org/10.1001/archpedi.1974.02110290061010

Doguer, C., Ha, J., & Collins, J. F. (2018). Intersection of Iron and Copper Metabolism in the Mammalian Intestine and Liver. *Comprehensive Physiology*, *8*(October), 1433–1461. https://doi.org/10.1002/cphy.c170045

Domellöf, M., Lönnerdal, B., Dewey, K. G., Cohen, R. J., & Hernell, O. (2004). Iron , Zinc , and Copper Concentrations in Breast Milk are Independent of Maternal Mineral Status. *The American Journal of Clinical Nutrition*, *79*(1), 111–115. https://doi.org/10.1093/ajcn/79.1.111

Donovan, S. M., & Odie, J. (1994). Growth factors in milk as mediators of infant development. In *Annual Review of Nutrition* (Vol. 14, Issue 122, pp. 147–167). https://doi.org/https://doi.org/10.1146/annurev.nu.14.070194.001051

Dorea, Jose, G. (1999). Calcium and Phosphorus in Human Milk. *Nutrition Research*, *19*(5), 709–739.

Dorea, J. G. (1999). Calcium and Phosphorus in Human Milk. *Nutrition Research*, *19*(5), 709–739. https://doi.org/10.1016/S0271-5317(99)00035-4

Dorea, J. G. (2000). Iron and Copper in Human Milk. *Nutrition*, *16*(3), 209–220. https://doi.org/https://doi.org/10.1016/s0899-9007(99)00287-7

Dorea, J. G. (2002). Selenium and Breast-feeding. *British Journal of Nutrition*, *88*(5), 443–461. https://doi.org/10.1079/BJN2002692

Dorea, J. G. (2004). Review article Mercury and lead during breast-feeding. *British Journal of Nutrition*, *92*(01), 21–40. https://doi.org/10.1079/BJN20041163

Dorea, J. G. (2014). Aluminium Concentrations in Human Milk : Additional Comments on Exposure Issues in the Neonate. *Pediatrics and Neonatology*, *55*(2), 81–82. https://doi.org/10.1016/j.pedneo.2014.03.001

Dórea, J. G. (2000). Magnesium in Human Milk. *Journal of the American College of Nutrition*, *19*(2), 210–219. https://doi.org/10.1080/07315724.2000.10718919

Drasch, G., Aigner, S., Roider, G., Staiger, F., & Lipowsky, G. (1998). Mercury in Human Colostrum and Early Breast Milk . *Journal of Trace Elements in Medicine and Biology*, *12*(1), 23–27. https://doi.org/10.1016/S0946-672X(98)80017-5

Dribben, W. H., Creeley, C. E., & Farber, N. (2011). Neurotoxicology and Teratology Low-level lead exposure triggers neuronal apoptosis in the developing mouse brain. *Neurotoxicology and Teratology*, *33*(4), 473–480. https://doi.org/10.1016/j.ntt.2011.05.006

Dror, D. K., & Allen, L. H. (2018). Overview of Nutrients in Human Milk. *ASN Supplement*, *June*, 2785–2945. https://doi.org/10.1093/advances/nmy022

Du, G., Hu, J., Huang, H., Qin, Y., Han, X., Wu, D., Song, L., Xia, Y., & Wang, X. (2013). Perfluorooctane Sulphonate (PFOS) Affects Hormone Receptor Activity, Steroidogenesis, and Expression of Endocrine-Related Genes in Vitro and in Vivo. *Environmental Toxicology and Chemistry*, *32*(2), 353–360. https://doi.org/10.1002/etc.2034

Dursun, A., Yurdakok, K., Yalcin, S. S., Tekinalp, G., Aykut, O., Orhan, G., & Morgil, G. K. (2016). Maternal risk factors associated with lead, mercury and cadmium levels in umbilical cord blood, breast milk and newborn hair. *Journal of Maternal-Fetal and Neonatal Medicine*, *29*(6), 954–961. https://doi.org/10.3109/14767058.2015.1026255

Ecsedi-angyal, M., Tatár, E., Óvári, M., Kurin-csörgei, K., Záray, G., & Mihucz, V. G. (2019). Determination of Low-Level Arsenic , Lead , Cadmium and Mercury Concentration in Breast Milk of Hungarian Women. *International Journal of Environmental Analytical Chemistry*, 1–18. https://doi.org/10.1080/03067319.2019.1637429

EFSA. (2020). Exposures - Statement on the EFSA Opinion on the risks to human health related to the presence of perfluoroalkyl substances in food Dietary. In *Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment*. https://cot.food.gov.uk/Exposures - Statement on the EFSA Opinion on the risks of perfluoroalkyl substances in food

EFSA NDA Panel. (2016). Scientific opinion on Dietary Reference Values for potassium. *EFSA Journal*, *xx*(x), xxxx. https://doi.org/10.2903/j.efsa.2016.xxxx

Eick, F., Maleta, K., Govasmark, E., Duttaroy, A. K., & Bjune, A. G. (2009). Food Intake of Selenium and Sulphur Amino Acids in Tuberculosis Patients and Healthy Adults in Malawi. *International Journal of Tuberculosis and Lung Disease*, *13*(12), 1579. https://pubmed.ncbi.nlm.nih.gov/19793440/

El-farrash, R. A., Abdel, E., Ismail, R., & Nada, A. S. (2012). Cord Blood Iron Profile and Breast Milk Micronutrients in Maternal Iron Deficiency Anemia. *Pediatric Blood Cancer*, *58*(2), 233–238. https://doi.org/10.1002/pbc

El-Kady, A. A., & Abdel-Wahhab, M. A. (2018). Occurrence of trace metals in foodstuffs and their health impact. *Trends in Food Science and Technology*, *75*, 36–45. https://doi.org/10.1016/j.tifs.2018.03.001

El-saeid, M. H., Hassanin, A. S., & Bazeyad, A. Y. (2021). Levels of Pesticide Residues in Breast Milk and the Associated Risk Assessment. *Saudi Journal of Biological Sciences*, *28*(7), 3741–3744. https://doi.org/10.1016/j.sjbs.2021.04.062

El-Shahawi, M. S., Hamza, A., Bashammakh, A. S., & Al-Saggaf, W. T. (2010). An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. *Elsevier*, *80*(5), 1587–1597. https://doi.org/10.1016/j.talanta.2009.09.055

Elbeltagi, R., Al-beltagi, M., Saeed, N. K., & Bediwy, A. S. (2023). Cardiometabolic effects of breastfeeding on infants of diabetic mothers. *World Journal of Diabetes*, *14*(5), 617–631. https://doi.org/10.4239/wjd.v14.i5.617

Elmastas, M., Can, M., Uzun, S., & Hassan, Y. A.-E. (2005). Determinations of Copper, Zinc, Cadmium, and Nickel in Cows', Goats', Ewes', and Human Milk Samples Using Flame Atomic Absorption Spectrometry (FAAS) Microwave Digestion. *Analytical Letters*, *38*(1), 157–165. https://doi.org/10.1081/AL-200043473

Emmett, E. A., Shofer, F. S., Zhang, H., Freeman, D., Desai, C., & Shaw, L. M. (2006). Community Exposure to Perfluorooctanoate : and Exposure Sources. *Journal of Occupational and Environmental Medicine*, *48*(8), 759–770. https://doi.org/10.1097/01.jom.0000232486.07658.74

FAO/WHO. (2023). Joint FAO/WHO Expert Committee on Food Additives Ninety-Sixth Meeting (Safety Evaluation of CertainFood Additives) 27 June - July 6, 2023. In *The Food and Agriculture Organization and World Health Organization* (Issue April). Retrieved from https://cdn.who.int/media/docs/default-source/food-safety/jecfa/summary-and-conclusions/jecfa93-summary-and-conclusions-april2022.pdf?sfvrsn=33db6aca_3&download=true on May 20, 2024.

FAO/WHO. (2004). Vitamin and Mineral Requirements in Human Nutrition. In *World Health Organization*. https://doi.org/92 4 154612 3

FAO/WHO. (2011). Aluminium-Containing Food Additives. In *Compendium of Food Additives Specifications. FAO JECFA Mongraphs*. Retrieved from https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/6179 on May 15, 2021.

Farhadi, R., & Roy, P. K. (2017). Induction of Lactation in the Biological Mother After Gestational Surrogacy of Twins : A Novel Approach and Review of Literature. *Breastfeeding Medicine*, *12*(6), 373–376.

https://doi.org/10.1089/bfm.2016.0112

Feeley, R. M., Eitenmiller, R. R., Jr, J. B. J., & Barnhart, H. (1983). Calcium , phosphorus, and magnesium contents of human milk during early lactation. *Journal of Pediatric Gastroenterol Nutrition*, *2*(2), 262–267. https://pubmed.ncbi.nlm.nih.gov/6683754/

Fiorentini, D., Cappadone, C., Farruggia, G., & Prata, C. (2021). Magnesium: Biochemistry, Nutrition, Detection, Social Impact of Diseases Linked to Its Deficiency. *Nutients*, *13*(4), 1136. https://doi.org/involved in more than 300 metabolic reactions

Flax, V. L., Adair, L. S., Allen, L. H., Shahab-ferdows, S., Hampel, D., Chasela, C. S., Tegha, G., Daza, E. J., Corbett, A., Davis, N. L., Kamwendo, D., Kourtis, A. P., Horst, C. M. Van Der, Jamieson, D. J., Bentley, M. E., & Study, B. A. N. (2015). Plasma Micronutrient Concentrations are Altered by Antiretroviral Therapy and Lipid- Based Nutrient Supplements in Lactating HIV- Infected Malawian Women. *The Journal of Nutrition*, *145*(8), 1950–1957. https://doi.org/10.3945 /jn.115.212290.1

Flesch-Janys, D. (1996). Journal of Toxicology and Elimination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans in Occupationally Exposed. *Journal of Toxicology and Environmental Health*, *47*(4), 360–378. https://doi.org/10.1080/009841096161708

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead : A review with recent updates. *Interdisciplinary Toxicology*, *5*(2), 47–58. https://doi.org/10.2478/v10102-012-0009-2

Frank, N. M., Lynch, K. F., Uusitalo, U., Yang, J., Lönnrot, M., Virtanen, S. M., Hyöty, H., Norris, J. M., & Study, T. (2019). The relationship between breastfeeding and reported respiratory and gastrointestinal infection rates in young children. *BMC Pediatrics*, *19*(1), 339-. https://doi.org/https://doi.org/10.1186%2Fs12887-019-1693-2

Fransson, G.-B., & Lonnerdal, B. (1983). Distribution of Trace Elements and Minerals in Human and Cow Milk. *International Pediatric Foundation Inc*, *17*(11), 20–23. https://doi.org/10.1203/00006450-198311000-00015

Friel, J. K., Andrews, W. L., Jackso, S. E., Longerrich, H. P., Mercer, C., McDonald, A., Dawson, B., & Sutradhar, B. (1999). Elemental Composition of Human Milk from Mothers of Premature and Full-Term Infants During the First 3 Months of Lactation. *Biological Trace Element Research*, *67*(3), 225–247. https://doi.org/https://doi.org/10.1007/bf02784423

Frkovic, A., Kras, M., & Alebic-Juretic, A. (1997). Lead and Cadmium Content in Human Milk from the Northern Adriatic Area of Croatia. *Bull. Environ. Contam. Toxicol.*, *58*(1), 16–21. https://doi.org/https://doi.org/10.1007/s001289900294

Fromme, H., Tittlemier, S. A., Vo, W., Wilhelm, M., & Twardella, D. (2009). Perfluorinated compounds – Exposure assessment for the general population in western countries. *International Journal of Hygiene and Environmental Health*, *212*(3), 239–270. https://doi.org/10.1016/j.ijheh.2008.04.007

Fujii, Y., Yan, J., Harada, K. H., Hitomi, T., Yang, H., Wang, P., & Koizumi, A. (2012). Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. *Chemosphere*, *86*(3), 315–321. https://doi.org/10.1016/j.chemosphere.2011.10.035

Funk, M. A., Hamlin, L., Picciano, M. F., Prentice, A., & Milner, J. A. (1990). Milk Selenium of Rural African Women: Influence of Maternal Nutrition, Parity, and Length of Lactation. *American Journal of Clinical Nutrition*, *51*(2), 220–224. https://doi.org/10.1093/ajcn/51.2.220

Furst, P., Furst, C., & Wilmers, K. (1994). Human Milk as a Bioindicator for Body Burden of PCDDs , PCDFs , Organo- chlorine Pesticides , and PCBs. *Environmental Health Persperctives*, *102*(1), 187–193.

Ghana's Ministry of Health. (2019). Ho Teaching Hospital. In *Government of Ghana. Retrieved* https://www.moh.gov.gh/ho-teaching-hospital/ on March 10, 2024.

Gibson, R. S., Bailey, K. B., Ampong Romano, A. B., & Thomson, C. D. (2011). Plasma Selenium Concentrations in Pregnant Women in Two Countries with Contrasting Soil Selenium Levels. *Journal of Trace Elements in Medicine and Biology*, *25*(4), 230–235. https://doi.org/10.1016/j.jtemb.2011.10.001

Giesy, J. P., & Kannan, K. (2001). Global Distribution of Perfluorooctane Sulfonate in Wildlife. *Environmental Science and Technology*, *35*(7), 1339–1342. https://doi.org/https://doi.org/10.1021/es001834k

Gillies, M. E., & Neill, A. E. (1985). Variations in the mineral concentrations in breast milk during a single nursing , diurnally and on consecutive days. *Human Nutrition & Applied Nutrition*, *39*(5), 370–375. https://pubmed.ncbi.nlm.nih.gov/4077574/

Gnonsoro, P. U., Ake Assi, Y. D. E., Sangare, N. S., Kouakou, Y. U., & Trokourey, A. (2022). Health Risk Assessment of Heavy Metals (Pb, Cd, Hg) in Hydroalcoholic Gels of Abidjan, Côte d' Ivoire. *Biological Trace Element Research*, *200*, 2510–2518. https://doi.org/10.1007/s12011-021-02822-y

Goc, Z., Kilian, K., Formicki, G., Stawarz, R., Gren, A., & Muchacka, R. (2012). Calcium and Magnesium Concentration of Breast Milk in Relation with Age and Parity of Nursing Women. *Journal of Microbiology, Biotechnology and Food Sciences*, *2*(1), 329–337. https://www.jmbfs.org/wp-content/uploads/2012/08/Goc_jmbfs_RF.pdf

Godhia, M. L., & Patel, N. (2013). Colostrum - Its Composition , Benefits As A Nutraceutical : A Review. *Current Research in Nutrition and Food Science*, *1*(1), 37–47.

Gomaa, A., Hu, H., Bellinger, D., Schwartz, J., Tsaih, S., Gonzalez-cossio, T., Schnaas, L., Peterson, K., Aro, A., & Hernandez-avila, M. (2002). Maternal Bone Lead as an Independent Risk Factor for Fetal Neurotoxicity : A Prospective Study. *PEDIATRICS*, *110*(1), 110–118. https://doi.org/https://doi.org/10.1542/peds.110.1.110

Gorini, F., Muratori, F., & Morales, M. A. (2014). The Role of Heavy Metal Pollution in Neurobehavioral Disorders : a Focus on Autism. *Review Journal of Autism and Developmental Disorderisorders*, *1*(4), 354–372. https://doi.org/10.1007/s40489-014-0028-3

Goudarzi, M. A., Parsaei, P., Nayebpour, F., & Rahimi, E. (2013). Determination of mercury, cadmium and lead in human milk in Iran. *Toxicology and Industrial Health*, *29*(9), 820–823. https://doi.org/10.1177/0748233712445047

Goudarzi, M., Parsaei, P., Nayebpour, F., & Rahimi, E. (2012). Determination of mercury, cadmium and lead in human milk in Iran. *Toxicology and Industrial Health*, *29*(9), 1–11. https://doi.org/10.1177/074823371244 5047

Goyer, R. A., & Cathryn, R. M. (1972). Susceptibility Lead Toxicity. *Environmental Health Perspectives*, *2*, 73–80. https://doi.org/https:// doi.org/10.2307/3428100

Grandjean, P., Budtz-jergensen, E., White, R. F., Jorgensen, P. J., Weihe, P., Debes, P., & Keiding, N. (1999). Methylmercury Exposure Biomarkers as Indicators of Neurotoxicity in Children Aged 7 Years. *American Journal of Epidermiology*, *150*(3), 301–305. https://doi.org/https://doi. org/10.1093/oxfordjournals.aje.a010002

Grandjean, P., Weihe, P. A. L., White, R. F., & Debes, F. (1997). Cognitive Deficit in 7-Year-Old Children with Prenatal Exposure to Methylmercury. *Neurotoxicology and Teratology*, *19*(6), 417–428.

Gruden, Š., & Ulrih, N. P. (2021). Diverse Mechanisms of Antimicrobial Activities of Lactoferrins, Lactoferricins, and Other Lactoferrin-Derived Peptides. *International Journal of Molecular Sciences*, *22*(20), 11264. https://doi.org/https://doi.org/10.3390%2Fijms222011264

Gu, C., Jiang, G., Szilasie, R., Hassan, S., Zhang, A., Sanders, M., Scientific, T. F., & Jose, S. (2010). Sensitive and Accurate Quantitation of Perfluorinated Compounds in Human Breast Milk using Selected Reaction Monitoring Assays by LC/MS/ MS. *Thermo Fisher Scientific*. https://www.thermofisher.com/content/dam/tfs/ATG/CMD/CMD Documents/Application & Technical Notes/Chromatography/Liquid Chromatography/Liquid Chromatography Accessories/AN51936_E_ 0610M(1).pdf

Guerranti, C., Palmieri, M., Mariottini, M., & Focardi, S. E. (2011). Persistent Organic Pollutants in Human Milk from Central Italy: Levels and Time Trends. *ISRN Toxicology*, *2011*, 1–6. https://doi.org/10.5402/2011/10 7514

Guerranti, C., Perra, G., Corsolini, S., & Focardi, S. E. (2013). Pilot study on levels of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in selected foodstuffs and human milk from Italy. *Food Chemistry*, *140*(1–2), 197–203. https://doi.org/10.1016/j.foodchem. 2012.12.066

Gulec, S., & Collins, J. F. (2014). Molecular Mediators Governing Iron-Copper Interactions. *Annual Review of Nutrition*, *34*, 95–116. https://doi.org/10.1146/annurev-nutr-071812-161215.Molecular

Gundacker, C., Pietschnig, B., Wittmann, K. J., Lischka, A., Salzer, H., Hohenauer, L., & Schuster, E. (2002). Lead and Mercury in Breast Milk. *Pediatrics*, *110*(5), 873–878. http://pediatrics.aappublications.org/content/110/5/873

Gundacker, C., & Zodl, B. (2005). Heavy metals in breast milk: implication for toxicity. *Review of Food, Nutrition and Toxicolgy*, *4*, 1–28. https://doi.org/http://dx.doi.org/10.1201/9781420037524.ch1

Guo, M. (2014). Human Milk Biochemistry and Infant Formula Manufacturing Technology. In *ScienceDirect*. https://doi.org/https://doi.org/10.1016/C2013-0-17349-7

Guo, Y., Lambert, G. H., Hsu, C., & Hsu, M. (2004). Yucheng : health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. *International Archives of Occupational and Environmental Health*, *77*(3), 153–158. https://doi.org/10.1007/s00420-003-0487-9

Guo, Y., Yu, M., Hsu, C., & Rogan, W. J. (1999). Chloracne , Goiter , Arthritis , and Anemia after Polychlorinated Biphenyl Poisoning : 14-Year Follow-Up of the Taiwan Yucheng Cohort. *Environmental Health Perspectives*, *107*(9), 715–719.

Guzman, M. M., Clementini, C., Pérez-cárceles, M. D., Jiménez, S., Cascone, A., Martellini, T., Guerranti, C., & Cincinelli, A. (2016). Per fl uorinated carboxylic acids in human breast milk from Spain and estimation of infant ' s daily intake. *Science of the Total Environment*, *544*, 595–600. https://doi.org/10.1016/j.scitotenv.2015.11.059

Haas, J. D., & Brownlie, T. (2001). Iron Deficiency and Reduced Work Capacity : A Critical Review of the Research to Determine a Causal Relationship. *The Journal of Nutritional*, *131*(2), 676–690. https://doi.org/https://doi.org/10.1093/jn/131.2.676s

Haddow, J. E., Polumaki, G. E., Allan, W. C., Williams, J. R., Knight, G. J., Gagnon, J., & Klein, R. Z. (1999). Neuropsychological development of the child. *The New England Journal of Medicine*, *341*(8), 549–555. https://doi.org/https://doi.org/10.1056/NEJM199908193410801

Hallen, I. P., Jorhemb, L., Lagerkvist, B. J., & Oskarssona, A. (1995). Lead and cadmium levels in human milk and blood. *The Science of the Total Environment*, *166*(3), 149–155. https://doi.org/https://doi.org/10.1016/0048-9697(95)04523-4

Hambidge, K. M. (1976). The importance of trace elements in infant nutrition. *Current Medical Research and Opinion*, *4*(1), 44–52. https://www.tandfonline.com/doi/abs/10.1185/03007997609109351?journalCode=icmo20

Hambidge, K. M., Krebs, N. F., Sibley, L., & English, J. (1987). Acute Effects of Iron Therapy on Zinc Status during Pregnancy. *Obstetrics and Gynecology*, *70*(4), 22–23. https://pubmed.ncbi.nlm.nih.gov/3627628/

Hamm, M. P., Cherry, N. M., Chan, E., & Martin, J. W. (2010). Maternal exposure to perfluorinated acids and fetal growth. *Journal of Exposure Science and Environmental Epidemiology*, *20*(7), 589–597. https://doi.org/10.1038/jes.2009.57

Han, J., Won, E., Lee, M., Soo, J., Lee, S., & Lee, J. (2015). Developmental retardation , reduced fecundity , and modulated expression of the defensome in the intertidal copepod Tigriopus japonicus exposed to BDE-47 and PFOS. *Aquatic Toxicology*, *165*, 136–143. https://doi.org/10.1016/j.aquatox.2015.05.022

Handy, D. E., Joseph, J., & Loscalzo, J. (2021). Selenium, a Micronutrient that Modulates Cardiovascular Health via Redox Enzymology. *Nutrients*, *13*(9). https://doi.org/10.3390/nu13093238

Hannan, M. A., Faraji, B., Tanguma, J., Longoria, N., & Rodriguez, R. C. (2009). Maternal Milk Concentration of Zinc , Iron , Selenium , and Iodine and Its Relationship to Dietary Intakes. *Biological Trace Element Research*, *127*(1), 6–15. https://doi.org/10.1007/s12011-008-8221-9

Hanukoglu, A., Chalew, S., & Kowarski, A. (1988). Late-Onset Hypocalcemia, Rickets, and Hypoparathyroidism in an Infant of a Mother with Hyperparathyroidism. *The Journal of Pediatrics*, *112*(5), 751–754. https://doi.org/10.1016/s0022-3476(88)80696-6

Hardisson, A., Revert, C., González-weller, D., Gutierrez, A., Paz, S., & Rubio, C. (2017). Aluminium Exposure Through the Diet. *HSOA Journal of Food Sciennce and Nutrition*, 1–44. https://doi.org/10.24966/FSN-1076/100020

Harzer, G., Haug, M., & B, J. G. (1986). Ern ~ ihrungswissenschaft Biochemistry of human milk in early lactation. *Z Ernahrungswiss*, *25*(2), 77–90. https://doi.org/https://doi.org/10.1007/bf02020737

Hasballah, A. F., & Beheary, M. S. (2017). Detection of Heavy Metals in Breast Milk and Drinking Water in Damietta Governorate , Egypt. *Asian Journal of Biology*, *1*(2), 1–7. https://doi.org/10.9734/AJOB/2016/30517

Hassine, S. B., Ameur, W. B., Gandoura, N., & Driss, M. R. (2012). Determination of chlorinated pesticides , polychlorinated biphenyls , and polybrominated diphenyl ethers in human milk from Bizerte (Tunisia) in 2010. *Chemosphere*, *89*(4), 369–377. https://doi.org/10.1016/j.chemosphere.2012.05.035

Haug, L. S., Huber, S., Schlabach, M., Becher, G., & Thomsen, C. (2011). Investigation on Per- and Polyfluorinated Compounds in Paired Samples of House Dust and Indoor Air from Norwegian Homes. *Environmental Science and Toxicology*, *45*(19), 7991–7998. https://doi.org/10.1021/es103456h

Hazelton, D. P., Cope, W. G., Pandolfo, J. T., Mosher, S., Strynar, J. M., Barnhart, M. C., & Bringolf, B. R. (2012). Partial Life-Cycle and Acute Toxicity of Perfluoroalkyl acids to Freshwater Mussels. *Environmental Toxicology and Chemistry*, *31*(7), 1611–1620. https://doi.org/10.1002/etc.1866

Hegar, B., Wibowo, Y., Basrowi, R. W., Ranuh, R. G., Sudarmo, S. M., Munasir, Z., Atthiyah, A. F., Widodo, A. D., Kadim, M., Suryawan, A., Diana, N. R., Manoppo, C., & Vandenplas, Y. (2019). The Role of Two Human Milk Oligosaccharides, 2'-Fucosyllactose and Lacto-N-Neotetraose, in Infant Nutrition. *Journal of Pediatric Gastroenterology and Nutrition*, *22*(4), 330–340. https://doi.org/10.5223/pghn.2019.

22.4.330

Higashi, A., Tamari, H., Kuroki, Y., & I., M. (1983). Longitudinal Changes in Selenium Content of Breast Milk. *Acta Paediatr Scand*, *72*, 433–436. https://doi.org/https://doi.org/10.1111/j.1651-2227.1983.tb09742.x

Hoffman, K., Webster, T. F., Weisskopf, M. G., & Weinberg, J. (2010). Exposure to Polyfluoroalkyl Chemicals and Attention Deficit / Hyperactivity Disorder in U.S. Children 12 -15 Years of Age. *Environmental Health Perspectives*, *118*(12), 1762–1768. https://doi.org/10.1289/ehp.1001898

Honda, R., Tawara, K., Nishijo, M., Nakagawa, H., Tanebe, K., & Saito, S. (2003). Cadmium Exposure and Trace Elements in Human Breast Milk. *Toxicology*, *186*(3), 255–259. https://doi.org/10.1016/S0300-483X(03)00002-7

Honeycutt, M., & Jones, L. (2014). Encyclopedia of Toxicology - Endrin. *Acadmic Press*, 344–347. https://doi.org/https://doi.org/10.1016/B978-0-12-386454-3.00142-1

Hooper, K., Chuvakova, T., Kazbekova, G., Hayward, D., Tulenova, A., Petreas, M. X., Wade, T. J., Benedict, K., Cheng, Y., & Grassman, J. (1999). Analysis of Breast Milk to Assess Exposure to Chlorinated Contaminants in Exposures in an Agricultural Region of Southern Kazakhstan. *Environmental Health Perspectives*, *107*(6), 447–457.

Horta, L. B., & Cesar, G. V. (2013). [Long-term health effects of breastfeeding]. *World Health Organisation*, *129*(8–9), 293–298. http://www.ncbi.nlm.nih.gov/pubmed/18198630

Hossain, S., & Mihrshahi, S. (2022). Exclusive Breastfeeding and Childhood Morbidity : A Narrative Review. *International Journal of Environmental Research and Public Health*, *19*(22), 14804. https://doi.org/https://doi.org/10.3390%2Fijerph192214804

Hsueh, Y. M., Lee, C. Y., Chien, S. N., Chen, W. J., Shiue, H. S., Huang, S. R., Lin, M. I., & Chi, S. (2017). Association of blood heavy metals with developmental delays and health status in children. *Scientific Reports*, *7*, 43608. https://doi.org/https://doi.org/10.1038%2Fsrep43608

Hu, H. (1991). Knowledge of Diagnosis and Reproductive History among Survivors of Childhood Plumbism. *American Journal of Public Health*, *81*(8), 1070–1072.

Hu, J., Yu, J., & Tanaka, S. (2011). Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) in Water Environment of Singapore. *Water, Air & Soil Pollution*, *216*(1–4), 179–191. https://doi.org/10.1007/s11270-010-0525-7

Huat, L. H., Zakariya, D., & Eng, K. H. (1983). Archives of Environmental Health : An International Lead Concentrations in Breast Milk of Malaysian Urban and Rural Mothers Lead Concentrations in Breast Milk of Malaysian Urban and Rural Mothers. *Archives of Environmental Health: An International Journal*, *38*(4), 205–209. https://doi.org/10.1080/00039896.1983.10545803

Hunt, S. M., & Schofield, F. A. (1969). Magnesium Level Balance in Adult Human Protein Female. *The American Journal of Clinical Nutrition*, *22*(3), 367–373. https://doi.org/10.1093/ajcn/22.3.367

Iannotti, L. L., Tielsch, J. M., Black, M. M., & Black, R. E. (2006). Iron Supplementation in Early Childhood : Health Benefits and Risks. *The American Journal of Clinical Nutrition*, *84*(6), 1261–1276. https://doi.org/https://doi.org/10.1093/ajcn/84.6.1261

IARC. (1991). Chlordane and Heptachlor. In *International Agency for Research on Cancer*.

IARC. (2016). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 110.* (Vol. 110). Retrieved from https://publications .iarc.fr/Book-And-Report-Series/Iarc-Monographs-On-The-Identifi cation-Of-Carcinogenic-Hazards-To-Humans/Some-Chemicals-Used-As-Solvents-And-In-Polymer-Manufacture-2016 on March 12, 2021.

Igiri, B. E., Okoduwa, S. I. R., Idoko, G. O., Akabuogu, E. P., Adeyi, A. O., & Ejiogu, I. K. (2018). Toxicity and Bioremediation of Heavy Metals Contaminated Ecosystem from Tannery Wastewater : A Review. *Journal of Toxicology*, *2018*, 1–17. https://doi.org/https://doi.org/ 10.1155%2F2018%2F2568038

Illinois EPA. (2016). Chapter 4 Lead/Copper Rule. In *Illinois Environmental Protection Agency* (pp. 1–15). Retrieved from https://epa.illinois.gov/ content/dam/soi/en/web/epa/documents/compliance-enforcement/ drinking-water/sample-collectors-handbook/chapter-4-pbcu.pdf on June 16, 2022.

Inoue, K., Okada, F., Ito, R., Kato, S., Sasaki, S., Nakajima, S., & Uno, A. (2004). Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples : assessment of PFOS exposure in a susceptible population during Pregnancy.

Environmental Health Perspectives, *112*(11), 1204–1207. https://doi.org/10.1289/ehp.6864

Isobe, T., Oda, B. H., Takayanagi, A. N., Kunisue, A. T., Komori, H., Arita, D. N., Ueda, D. N., & Nose, D. M. (2009). Hexabromocyclododecanes in human adipose tissue from Japan. *Environmental Chemistry*, *6*, 328–333. https://doi.org/10.1071/EN09024

Iyengar, V., & Woittiez, J. (1988). Trace elements in human clinical specimens: evaluation of literature data to identify reference values. *Clinical Chemistry*, *34*(3), 474–481.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity , mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, *7*(2), 60–72. https://doi.org/10.2478/intox-2014-0009

Jaraczewska, K., Lulek, J., Covaci, A., Voorspoels, S., Kaluba-skotarczak, A., Drews, K., & Schepens, P. (2006). Distribution of polychlorinated biphenyls , organochlorine pesticides and polybrominated diphenyl ethers in human umbilical cord serum , maternal serum and milk from Wielkopolska region , Poland. *Science of the Total Environment*, *372*, 20–31. https://doi.org/10.1016/j.scitotenv.2006.03.030

Jarjou, L. M. A., Prentice, A., Sawo, Y., Laskey, M. A., Bennett, J., Goldberg, G. R., & Cole, T. J. (2006). Randomized, Placebo-Controlled, Calcium Supplementation Study in Pregnant Gambian Women : Effects on Breast-Milk Calcium Concentrations and Infant Birth Weight, Growth, and Bone Mineral Accretion in the First Year of Life. *The American Journal of Clinical Nutrition*, *83*(3), 657–666. https://doi.org/https:/

/doi.org/10.1093/ajcn.83.3.657

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*(1), 167–182.

Jeong, K. S., Ha, E., Shin, Y. J., Park, H., Hong, Y., Ha, M., Kim, S., Lee, S., Lee, Y. K., Kim, H. J., & Kim, Y. (2017). Science of the Total Environment Blood Heavy Metal Concentrations in Pregnant Korean Women and their Children up to Age 5 Years : Mothers ' and Children ' s Environmental Health (MOCEH) birth cohort study. *Science of the Total Environment*, *605–606*, 784–791. https://doi.org/10.1016/j.scitotenv.2017.06.007

Jin, H., Mao, L., Xie, J., Zhao, M., Bai, X., Wen, J., Shen, T., & Wu, P. (2020). Poly- and per fl uoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth. *Science of the Total Environment*, *713*, 136417. https://doi.org/10.1016/j.scitotenv.2019.136417

Jonas, A. J., & Domiguez, B. (1989). Low Breast Milk Phosphorus Concentration in Familial Hypophosphatemia. *Journal of Pediatric Gastroenterology and Nutrition*, *8*(4), 541–543. https://doi.org/10.1097/00005176-198905000-00021

Jong, M. F. C. De, Riphagen, I. J., Kootstra-ros, J. E., & Groenewout, M. (2022). Potassium and Magnesium in Breast Milk of a Woman With Gitelman Syndrome. *Kidney International Reports*, *7*(7), 1720–1721. https://doi.org/10.1016/j.ekir.2022.05.006

Juhaiman, L. A. Al. (2010). Estimating Aluminum leaching from Aluminum cook wares in different meat extracts and milk. *Journal of Saudi Chemical Society*, *14*(1), 131–137. https://doi.org/10.1016/j.jscs.2009.12.020

Kadar, H., Veyrand, B., Barbarossa, A., Pagliuca, G., Legrand, A., Bosher, C., Boquien, C., Durand, S., Monteau, F., Antignac, J., & Le, B. (2011). Chemosphere Development of an analytical strategy based on liquid chromatography – high resolution mass spectrometry for measuring perfluorinated compounds in human breast milk : Application to the generation of preliminary data regarding perinatal exposu. *Chemosphere*, *85*(3), 473–480. https://doi.org/10.1016/j.chemosphere.2011.07.077

Kaiser, H. F., & Rice, J. (1974). Educational and Psychological Measurement. *Educational and Psychological Measurement*, *34*(111), 111–117. https://doi.org/10.1177/001316447403400115

Kantola, M., & Vartiainen, T. (2001). *Changes in Selenium , Zinc , Copper and Cadmium Contents in Human Milk During the Time When Selenium has been Supplemented to Fertilizers in Finland*. *15*(1), 11–17. https://doi.org/10.1016/s0946-672x(01)80020-1

Kärrman, A., Domingo, J. L., Llebaria, X., Nadal, M., Bigas, E., Bavel, B. Van, & Lindström, G. (2010). Biomonitoring perfluorinated compounds in Catalonia, Spain: concentrations and trends in human liver and milk samples. *Environmental Science and Pollution Research*, *17*(3), 750–758. https://doi.org/10.1007/s11356-009-0178-5

Kärrman, A., Ericson, I., Bavel, B. Van, Darnerud, P. O., Aune, M., Glynn, A., Lignell, S., & Lindström, G. (2007). Exposure of Perfluorinated Chemicals through Lactation : Levels of Matched Human Milk and Serum and a Temporal Trend, 1996 – 2004, in Sweden. *Environmental Health Perspectives*, *115*(2), 226–230. https://doi.org/10.1289/ehp.9491

Kawahara, M., & Kato-negishi, M. (2011). Link between Aluminum and the Pathogenesis of Alzheimer' s Disease: The Integration of the Aluminum and Amyloid Cascade Hypotheses. *International Journal of Alzheimer's Disease*, 1–17. https://doi.org/10.4061/2011/276393

Kelleher, S. L., & Lonnerdal, B. (2005). Molecular Regulation of Milk Trace Mineral Homeostasis. *Molecular Aspect of Medicine*, *26*(4–5), 328–339. https://doi.org/10.1016/j.mam.2005.07.005

Kent, B. Y. J. C., Arthur, P. G., Retallack, R. W., & Hartmann, P. E. (1992). Calcium , phosphate and citrate in human milk at initiation of lactation. *Journal of Diairy Reseearch*, *59*(2), 161–167. https://doi.org/https://doi.org/10.1007/BF03032674

Kent, J. C., Gardner, H., & Geddes, D. T. (2016). Breastmilk Production in the First 4 Weeks after Birth of Term Infants. *Nutrients*, *8*(756), 9–14. https://doi.org/10.3390/nu8120756

Khamis, H., Lusweti, K., & Mwevura, H. (2017). Quantification of Heavy Metals in Breast Milk Samples Sampled from Kilimani / Kidoti in Zanzibar. *American Scientific Research Journal for Engineering, Technology and Sciences*, *35*(1), 295–308.

Khanjani, N., Jafari, M., & Mousavi, E. A. (2018). Breast milk contamination with lead and cadmium and its related factors in Kerman , Iran. *Journal of Environmental Health Sciences and Engineering*. https://doi.org/https://doi.org/10.1007%2Fs40201-018-0320-8

Khanjani, N., & Sim, M. R. (2006). Reproductive outcomes of maternal contamination with Cyclodiene insecticides , Hexachlorobenzene and β -Benzene Hexachloride. *Science of the Total Environment*, *368*(2006), 557–564. https://doi.org/10.1016/j.scitotenv.2006.03.029

Kim, S., Park, J. H., Kim, E. A., & Kim, E. A. (2012). Longitudinal Study on Trace Mineral Compositions (Selenium, Zinc, Copper, Manganese) in Korean Human Preterm Milk. *Journal of Korean Medical Science*, *27*(5), 532–536. https://doi.org/https://doi.org/10.3346%2Fjkms.2012.27.5.532

Kim, S. Y., & Yi, D. Y. (2020). Components of human breast milk: From macronutrient to microbiome and microRNA. *Clinical and Experimental Pediatrics*, *63*(8), 301–309. https://doi.org/10.3345/cep.2020.00059

Kishi, R., Araki, A., Minatoya, M., Hanaoka, T., Miyashita, C., Itoh, S., Kobayashi, S., Bamai, Y. A., Yamazaki, K., Miura, R., Tamura, N., & Ito, K. (2017). The Hokkaido Birth Cohort Study on Environment and Children' s Health: cohort profile — updated 2017. *Environmental Health Aand Preventive Medicine*, *22*(1), 1–16. https://doi.org/10.1186/s12199-017-0654-3

Klein, L. D., Breakey, A. A., Scelza, B., Valeggia, C., Jasienska, G., & Hinde, K. (2017). Concentrations of trace elements in human milk : Comparisons among women in States. *PLoS ONE*, *12*(8), 1–16. https://doi.org/https://doi.org/10.1371/journal.pone.0183367

Koka, J. K., Koranteng-Addo, J. E., Bentum, J. K., Koka, D. M., & Kamoah, G. (2011). Analysis of Lead and Cadmium in Human Milk in the Greater Accra Region of Ghana. *Der Chemica Sinica*, *2*(2), 240–246. https://ir.ucc.edu.gh/xmlui/bitstream/handle/123456789/5629/analysis-of-lead-and-cadmium-in-human-milk-in-the-greater-accra-region-of-ghana %281%29.pdf?sequence=1&isAllowed=y

Konwick, J. B., Tomy, T. G., Ismah, N., Peterson, T. J., Fauver, J. R., Higginbotham, D., & Fisk, T. A. (2008). Concentrations and Patterns of Perfluoroalkyl Acids in Georgia , USA Surface Waters Near and Distant to a Major Use Source. *Environmental Toxicology and Chemistry*, *27*(10), 2011–2018. https://doi.org/doi:10.1897/07-659.1

Kosanovic, M., Adem, A., Jokanovic, M., & Yousef, M. (2008). Simultaneous Determination of Cadmium, Mercury, Lead, Arsenic, Copper, and Zinc in Human Breast Milk by ICP - MS / Microwave Digestion. *Analytical Letters*, *41*(3), 37–41. https://doi.org/10.1080/00032710701862910

Kowalski, C. H., Costa, J. G., Godoya, H. T., & Augustoc, F. (2010). Determination of Polychlorinated Biphenyls in Brazilian Breast Milk Samples using Solid-Phase Microextraction and Gas Chromatography-Electron Capture Detection. *Journal of the Brazilian Chemical Society*, *21*(3), 502–509. https://doi.org/10.1590/S0103-50532010000300016

Kuklenyik, Z., Reich, J. A., Tully, J. S., Needham, L. L., & Calafat, A. M. (2004). Automated Solid-Phase Extraction and Measurement of Perfluorinated Organic Acids and Amides in Human Serum and Milk. *Environmental Science and Technology*, *38*(13), 3698–3704. https://doi.org/https://doi.org/10.1021/es040332u

Kumpulainen, J., Salmenpera, L., Siimes, M. A., Koivitoinen, P., & Perheentuga, J. (1985). Selenium Status of Exclusively Breast-fed Infants as Influenced by Maternal Organic or Inorganic Selenium Supplementation. *The American Journal of Clinical Nutrition*, *42*(5), 829–835. https://doi.org/10.1093/ajcn/42.5.829

Kunter, İ., Hürer, N., & Gülcan, H. O. (2017). Assessment of Aflatoxin M1 and Heavy Metal Levels in Mothers Breast Milk in Famagusta , Cyprus. *Biological Trace Element Research*, *175*(1), 42–49. https://doi.org/10.1007/s12011-016-0750-z

Laden, F., Neas, L. M., Spiegelman, D., Hankinson, S. E., Wafter, C., Ireland, K., Wolff, M., & Hunter, D. J. (1999). Predictors of Plasma Concentrations of DDE and PCBs in a Group of. *Environmental Health Perspectives*, *107*(1), 75–81. https://doi.org/https://doi.org/10.1289/ehp.9910775

Lakind, J. S., Berlin, C. M., & Naiman, D. Q. (2001). Infant exposure to chemicals in breast milk in the United States: what we need to learn from a breast milk monitoring program. *Environmental Health Persperctives*, *109*(1), 75–88. https://doi.org/https://doi.org/10.1289/ehp.0110975

Landrigan, P. J. (1999). Risk Assessment for Children and Other Sensitive Populations. *Annals of the New Yorke Academy of Sciences*, *895*(1), 1–9. https://doi.org/https://doi.org/10.1111/j.1749-6632.1999.tb08073.x

Landrigan, P. J., Sonawane, B., Mattison, D., Mccally, M., & Garg, A. (2002). Chemical Contaminants in Breast Milk and Their Impacts on Children's Health: An Overview Mini-Monograph. *Environmental Health Perspectives*, *110*(6), 313–315. https://doi.org/https://doi.org/10.1289%2Fehp.021100313

Lankova, D., Lacina, O., Pulkrabova, J., & Hajslova, J. (2013). Talanta The determination of per fl uoroalkyl substances , brominated fl ame retardants and their metabolites in human breast milk and infant formula. *Talanta*, *117*(October 2012), 318–325. https://doi.org/10.1016/j.talanta.2013.08.040

Lasier, J. P., Washington, W. J., Hassan, M. S., & Jenkins, M. T. (2011). Perfluorinated Chemicals in Surface Waters and Sediments from Northwest Georgia , USA , and their Bioaccumulation in Lumbriculus Variegatuus. *Environmental Toxicology and Chemistry*, *30*(10), 2194–2201. https://doi.org/10.1002/etc.622

Layman, D. K., Lo, B., & Fernstrom, J. D. (2018). Applications for a -lactalbumin in human nutrition. *Nutrition Reviews*, *76*(6), 444–460. https://doi.org/10.1093/nutrit/nuy004

Lee, B., Ahn, J., Kim, N., Lee, C. B., Park, J., & Kim, Y. (2016). Association of Blood Pressure with Exposure to Lead and Cadmium : Analysis of Data from the 2008 – 2013 Korean National Health and Nutrition Examination Survey. *Biological Trace Element Research*, *174*(1), 40–

51. https://doi.org/10.1007/s12011-016-0699-y

Leotsinidis, M., Alexopoulos, A., & Kostopoulou-Farri, E. (2005). Toxic and essential trace elements in human milk from Greek lactating women : Association with dietary habits and other factors. *Chemosphere*, *61*(2), 238–247. https://doi.org/10.1016/j.chemosphere.2005.01.084

Lerner, S. (2018). *3M Knew About the Dangers of PFOA and PFOS Decades Ago, Internal Documents Show*. https://theintercept.com/2018/07/31/3m-pfas-minnesota-pfoa-pfos/

Letinić, J. G., Sarić, M. M., Piasek, M., Jurasović, J., Varnai, V. M., Grgec, A. S., & Orctc, T. (2016). Use of human milk in the assessment of toxic metals exposure and essential element status in breastfeeding women and their infants in coastal Croatia. *Journal of Trace Elements in Medicine and Biology*, *38*, 117–125. https://doi.org/10.1016/j.jtemb.2016.08.002

Levander, A., Moser, B., & Morris, C. (1987). Dietary Selenium Intake and Selenium Concentrations of Plasma , Erythrocytes , and Breast Milk in Pregnant and Postpartum Lactating and Nonlactating Women. *The American Journal of Clinical Nutrition*, *46*(4), 694–698. https://doi.org/10.1093/ajcn/46.4.694

Li, C., Solomons, N. W., Scott, M. E., & Koski, K. G. (2016). Minerals and Trace Elements in Human Breast Milk Are Associated with Guatemalan Infant Anthropometric Outcomes within the First 6 Months. *Journal of Nutrition ASN*, *5*, 2067–2074. https://doi.org/10.3945/jn.116.232223.plasma-atomic

Li, G., Xiong, C., Xu, W., Mei, R., Cheng, T., & Yu, X. (2021). Factors Affecting the Aluminum, Arsenic, Cadmium and Lead Concentrations in the Knee Joint Structures. *Frontiers in Public Health*, *9*(December), 1–11. https://doi.org/10.3389/fpubh.2021.758074

Lidsky, T. I., & Schneider, J. S. (2003). Lead neurotoxicity in children : basic mechanisms and clinical correlates. *Guarantors of Brain*, *126*, 5–19. https://doi.org/10.1093/brain/awg014

Lignell, S., Aune, M., Darnerud, P. O., Hanberg, A., Larsson, S. C., & Glynn, A. (2013). Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure : a cross-sectional study. *Environmental Health*, *12*(44), 1–9.

Lin, T., Jong, Y., & Chiang, C. (1998). Longitudinal Changes in Ca , Mg , Fe , Cu , and Zn in Breast Milk of Women in Taiwan over a Lactation Period of One Year. *Biological Trace Element Research*, *62*(1–2), 31–41. https://doi.org/https://doi.org/10.1007/bf02820019

Lipsman, S., Dewey, K. G., & Lonnerdal, B. (1985). Breast-Feeding Among Teenage Mothers : Milk Composition , Infant Growth , and Maternal Dietary Intake. *Journal of Pediatr Gastroenterology and Nutrition*, *4*(3), 426–434. https://pubmed.ncbi.nlm.nih.gov/4020574/

Liu, J., Leung, P., & Yang, A. (2014). *Breastfeeding and Active Bonding Protects against Children's Internalizing Behavior Problems*. *6*(1), 76–89. https://doi.org/10.3390/nu6010076

Liu, J., Li, J., Liu, Y., Man, H., Zhao, Y., Cai, Z., & Wu, Y. (2011). Comparison on gestation and lactation exposure of per fl uorinated compounds for newborns. *Environment International*, *37*(7), 1206–1212. https://doi.org/10.1016/j.envint.2011.05.001

Liu, J., Li, J., Zhao, Y., Wang, Y., Zhang, L., & Wu, Y. (2010). The occurrence of per fl uorinated alkyl compounds in human milk from different regions of China. *Environment International*, *36*(5), 433–438. https://doi.org/10.1016/j.envint.2010.03.004

Liu, J., & Mejia, S. A. (2013). Microbial degradation of polyfluoroalkyl chemicals in the environment: A review. *Environment International*, *61*, 98–114. https://doi.org/10.1016/j.envint.2013.08.022

Llorca, M., Farré, M., Picó, Y., Lopez, M., Álvarez, J. G., & Barceló, D. (2010). Infant exposure of per fl uorinated compounds : Levels in breast milk and commercial baby food. *Environment International*, *36*(6), 584–592. https://doi.org/10.1016/j.envint.2010.04.016

Longnecker, M. P., Rogan, W. J., & Lucier, G. (1997). The Human Health Effects of DDT (Dichlorodiphenyl- Trichloroethane) and PCBS (Polychlorinated Biphenyls) and an Overview of Organochlorines in Public Health. *Annu. Rev. Public Health*, *18*, 211–244.

Lonnerdal, B. (2000). Regulation of Mineral and Trace Elements in Human Milk: Exogenous and Endogenous Factors. *Nutrition Reviews*, *58*(8), 223–229. https://doi.org/10.1111/j.1753-4887.2000.tb01869.x

Lonnerdal, B., Hoffman, B., & Hurley, L. S. (1982). Zinc and Copper Binding Proteins in Human. *The American Journal of Clinical Nutrition*, *36*(6), 1170–1176. https://doi.org/10.1093/ajcn/36.6.1170

Lopes, B. R., Barreiro, J. C., & Cass, Q. B. (2016). Bioanalytical challenge: A review of environmental and pharmaceuticals contaminants in human milk. *Journal of Pharmaceutical and Biomedical Analysis*, *16*, 1–32. https://doi.org/10.1016/j.jpba.2016.06.012

Lozoff, B., Jemenez, E., & Wolf, A. W. (1991). Long-Term Developmental Outcome of Infants with Iron Deficiency. *The New England Journal of Medicine*, *325*(10), 687–694. https://doi.org/10.1056/NEJM199109053251004

Lozoff, B., Jimenez, E., Hagen, J., Mollen, E., Wolf, A. W., Objective, A., Periurban, S., & Jose, S. (2000). After Treatment for Iron Deficiency in Infancy. *Pedaiatrics*, *105*(4), 1–11. https://doi.org/https://doi.org/10.1542/peds.105.4.e51

Ma, L. H., Alexander, M., You, D., Alkema, L., Group, U. N. I., & Estimation, M. (2019). Articles National , regional , and global levels and trends in neonatal mortality between 1990 and 2017 , with scenario-based projections to 2030 : a systematic analysis. *The Lancet Global Health*, *7*(6), e710–e720. https://doi.org/10.1016/S2214-109X(19)30163-9

Macheka-Tendenguwo, L. R., Ojowoyo, J. O., Mugivhisa, L. L., & Abafe, O. A. (2018). Per- and polyfluoroalkyl substances in human breast milk and current analytical methods. *Environmental Science and Pollution Research International*, *25*(36), 36064–36086. https://doi.org/10.1007/s11356-018-3483-z

Macheka, L. R., Abafe, O. A., Mugivhisa, L. L., & Olowoyo, J. O. (2022). Occurrence and infant exposure assessment of per and polyfluoroalkyl substances in breast milk from South Africa. *Chemosphere*, *288*(Pt 2), 1–9. https://doi.org/https://doi.org/10.1016/j.chemosphere.2021.132601

Macy, Icie, G. (1949). Composition of Human Colostrum and Milk. *American Journal of Diseases of Children*, *78*(4), 589–603. https://doi.org/https://doi.org/10.1001/archpedi.1949.02030050604009

Mahdavi, R., Nikniaz, L., & Gayemmagami, S. J. (2010). Association Between Zinc, Copper, and Iron Concentrations in Breast Milk and Growth of Healthy Infants in Tabriz , Iran. *Biological Trace Element Research*, *135*(1–3), 174–181. https://doi.org/10.1007/s12011-009-8510-y

Mandia, N., Bermejo-barrera, P., Herbello, P., Olalla, L., Fraga, J. M., Fern, C., & Couce, L. (2021). Human Milk Concentrations of Minerals, Essential and Toxic Trace Elements and Association with Selective Medical, Social Demographic and Environmental Factors. *Nutrients*, *13*(1885), 1–21. https://doi.org/https://doi.org/10.3390%2Fnu13061885

Mandic, M. L., Grgic, J., Serugaa, Z. G. M., & Hasenay, D. (1995). Aluminium Levels in Human Milk. *The Science of the Total Environment*, *170*(3), 165–170. https://doi.org/https://doi.org/10.1016/0048-9697(95)04702-4

Manganaro, R., Marseglia, L., Mamì, C., Palmara, A., Paolata, A., Loddo, S., Gargano, R., Mondello, M., & Gemelli, M. (2007). Breast Milk Sodium Concentration, Sodium Intake and Weight Loss in Breast-Feeding Newborn Infants. *British Journal of Nutrition*, *97*(2), 344–348. https://doi.org/10.1017/S0007114507280572

Mannan, S., & Picciano, F. (1987). Influence of Maternal Selenium Status on Human Milk Concentration and Glutathione Peroxidase Activity. *The American Journal of Clinical Nutrition*, *46*(1), 95–100. https://doi.org/10.1093/ajcn/46.1.95

Manz, F. (1992). Why is the phosphorus content of human milk exceptionally low ? *Monatsschr Kinderheilkd*, *140*(1), S35–S39. https://pubmed.ncbi.nlm.nih.gov/1435825/

Marta, I. S. V., Oliveira, A. B. P., & Gomes, M. T. S. R. (2006). Leaching of Aluminium from Cooking Pans and Food Containers. *Sensors and Actuators B*, *118*(2006), 192–197. https://doi.org/10.1016/j.snb.2006.04.061

Martin, C. R., Ling, P. R., & Blackburn, G. L. (2016). Review of infant feeding: Key features of breast milk and infant formula. *Nutrients*, *8*(5), 1–11. https://doi.org/10.3390/nu8050279

Maru, M., Birhanu, T., & Tessema, D. A. (2013). Calcium , Magnesium , Iron, Zinc and Copper, Compositions of Human Milk from Populations with Cereal and 'ENSET' BASED Diets. *Ethiopian Journal of Health Science*, *23*(2), 90–97. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3742886/

Massart, F., Gherarducci, G., Marchi, B., & Saggese, G. (2008). Chemical Biomarkers of Human Breast Milk Pollution. *Biomarker Insights*, *3*, 159–169. https://doi.org/https://doi.org/10.4137%2Fbmi.s564

Massart, F., Harrell, J. C., Federico, G., & Saggese, G. (2005). Human Breast Milk and Xenoestrogen Exposure : A Possible Impact on Human Health. *Journal of Perinatology*, *25*(4), 282–288. https://doi.org/10.1038/sj.jp.7211251

Massart, F., Parrino, R., Seppia, P., Federico, G., & Saggese, G. (2006). How do environmental estrogen disruptors induce precocious puberty ? *Minerva Pediatr*, *58*(3), 247–254.

Mastorakou, D., Ruark, A., Weenen, H., Stahl, B., & Stieger, M. (2019). Sensory characteristics of human milk : Association between mothers ' diet and milk for bitter taste. *Journal of Dairy Science*, *102*(2), 1116–1130. https://doi.org/10.3168/jds.2018-15339

McNally, M. E., Atkinson, S. A., & Cole, D. E. C. (1991). Contribution of Sulfate and Sulfoesters to Total Sulfur Intake in Infants Fed Human Milk. *Minerals and Trace Elements*, *121*(8), 1250–1254. https://doi.org/http://dx.doi.org/10.1093/jn/121.8.1250

Mead, M. N. (2008). Contaminants in human milk: Weighing the risks against the benefits of breastfeeding. *Environmental Health Perspectives*, *116*(10), A426–A434. http://www.scopus.com/inward/record.url?eid=2-s2.0-55949129821&partnerID=40&md5=5977c4633d4738043f242c50bfb6e58b

MedlinePlus. (2016). *Definitions of Health Terms : Minerals*. National Library of Medicine.

MedlinePlus. (2023a). *Magnesium in diet*. National Library of Medicine. Retrieved from https://nationalpeanutboard.org/news/magnificent-magnesium/ on May 19, 2024.

MedlinePlus. (2023b). Magnesium in diet. In *Medical Encyclopedia*. Retrieved from https://medlineplus.gov/ency/article/002423.htm#:~:text=Magnesium%20is%20needed%20for%20more,production%20of%20energy%20and%20protein. on May 19, 2024.

Meironyte, D., Noren, K., & Bergman, A. (1999). Analysis of Polybrominated Diphenyl Ethers in Swedish Human Milk: A Time-Related Trend Study, 1972 - 1997. *Journal of Toxicology and Environmental Health*, *56*(6), 329–341. https://doi.org/https://doi.org/10.1080/009841099157197

Micetic-Turk, D., Rossipal, E., Krachler, M., & Li, F. (2000). Maternal Selenium Status in Slovenia and its Impact on the Selenium Concentration of Cmbilical Cord Serum and Colostrum. *European Journal of Clinical Nutrition*, *54*(6), 522–524. https://doi.org/10.1038/sj.ejcn.1601050

Michalke, B., & Schramel, P. (1997). Selenium Speciation in Human Milk with Special Respect to Quality Control. *Biological Trace Element Research*, *59*(1–3), 45–56. https://doi.org/10.1007/BF02783229

Mielech, A., Puscion-Jakubit, A., & Socha, K. (2021). Assessment of the Risk of Contamination of Food for Infants and Toddlers. *Nutrients*, *13*(9), 2358-. https://doi.org/https://doi.org/10.3390%2Fnu13072358

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. Bin, Nainu, F., Khusro, A., Idris, A. M., Khandaker, M. U., Osman, H., Alhumaydhi, F. A., & Simal-Gandora, J. (2022). *Impact of heavy metals on the environment and human health : Novel therapeutic insights to counter the toxicity* (Vol. 34, Issue 3).

Modak, A., Ronghe, V., & Gomase, K. P. (2023). The Psychological Benefits of Breastfeeding: Fostering Maternal Well-Being and Child Development. *Cureus*, *15*(10), e46730. https://doi.org/10.7759/cureus.46730

Mohammad, F. S., Zubaidy, E. A. H. Al, & Bassioni, G. (2011). Effect of Aluminum Leaching Process of Cooking Wares on Food. *International Journal of Electrochemical Science*, *6*, 222–230. https://doi.org/.

Momodo, M. A., & Anyakora, C. A. (2010). Heavy Metal Contamination of Ground Water : The Surulere Case Study Heavy Metal Contamination of Ground Water: The Surulere Case Study. *Research Journal Environmental and Earth Sciences*, *2*(1), 39–43.

Moser, B., & Reynolds, D. (1993). Dietary zinc intake and zinc concentrations plasma, erythrocytes, and breast milk in antepartum and postpartum lactating and nonlactating women: a longitudinal. *American Soceiiy for Clinical Nutrition*, *38*(March), 101–108.

Motas, M., Jiménez, S., Oliva, J., Cámara, M. Á., Dolores, M., & Cárceles, P. (2021). Heavy Metals and Trace Elements in Human Breast Milk from Industrial / Mining and Agricultural Zones of Southeastern Spain. *International Journal of Environmental Research and Public Health*, *18*(17), 9289. https://doi.org/https://doi.org/10.3390/ijerph18179289

Motee, A., & Jeewon, R. (2014). Importance of Exclusive Breast Feeding and Complementary Feeding Among Infants. *Current Research in Nutrition and Food Science*, *2*(2), 56–72. https://doi.org/http://dx.doi.org/10.12944/CRNFSJ.2.2.02

Moukarzel, S., & Bode, L. (2016). Human Milk Oligosaccharides and the Preterm Infant. *Clinics in Perinatology*, *44*(1), 193–207. https://doi.org/10.1016/j.clp.2016.11.014

Murata, K., Weihe, P., Renzoni, A., Debes, P., Vasconcelos, R., Zino, F., Araki, S., Jorgensen, P. J., White, R. F., & Grandjean, P. (1999). Delayed Evoked Potentials in Children Exposed to Methylmercury from Seafood. *Neurotoxicology and Teratology*, *21*(4), 343–348. https://doi.org/Weihe, Pal

Muro-Valdez, J. C., Meza-Rios, A., Aguilar-Uscanga, B. R., Lopez-Roa, R. I., Medina-Díaz, E., Franco-Torres, E. M., & Zepeda-Morales, A. S. M. (2023). Breastfeeding-Related Health Benefits in Children and Mothers: Vital Organs Perspective. *Medicina (Lithuania)*, *59*(9), 11–12. https://doi.org/10.3390/medicina59091535

National Center for Biotechnology Information. (2024). Chlordane: PubChem Compound Summary for CID 5993. In *National Library of Medicine*.

Needham, L. L., & Wang, R. Y. (2002). Mini-Monograph Analytic Considerations for Measuring Environmental Chemicals in Breast Milk Incorporation of Environmental Chemicals into Breast Milk. *Environmental Health Perspectives*, *110*(6), 317–324.

Neville, C., Keller, P., Seacat, J., Casey, E., & Allen, C. (1984). Studies on human lactation . I . Within-feed and between-breast variation in selected components of human milk. *The American Journal of Clinical Nutrition*, *40*(3), 635–646. https://doi.org/https://doi.org/10.1093/ajcn/40.3.635

Niaz, B., Saeed, F., Ahmed, A., Imran, M., Aslam, A., Kashif, M., Khan, I., Tufail, T., Muhammad, F., Hussain, S., Ansar, H., Suleria, R., Niaz, B., Saeed, F., Ahmed, A., Imran, M., Aslam, A., Ahmed, A., & Imran, M. (2019). Lactoferrin (LF): a natural antimicrobial protein. *International Journal of Food Properties*, *22*(01), 1626–1641. https://doi.org/10.1080/10942912.2019.1666137

Nickkho-Amiry, M., Prentice, A., Ledi, F., Laskey, M. A., Das, G., Berry, J. L., & Mughal, M. (2008). Maternal Vitamin D Status and Breast Milk Concentrations of Calcium and Phosphorus. *Archives of Disease in Childhood*, *93*(2), 179. https://doi.org/10.1136/adc.2007.124008

Nishijo, M., Nakagawa, H., Honda, R., Tanebe, K., Saito, S., & Tawara, K. (2002). Effects of Maternal Exposure to Cadmium on Pregnancy Outcome and Breast Milk. *Occupational and Environmental Medicine*, *59*(6), 394–398. https://doi.org/https://doi.org/10.1136/oem.59.6.394

Noh, S., & Lee, E. (2021). Relationship Between Selected Trace Elements in Human Milk and Psychosocial Characteristics in Korean Early Postpartum Women. *International Journal of Environmental Research and Public Health*, *18*(350), 1–12. https://doi.org/https://www.mdpi.com/1660-4601/18/1/350/htm#

Nokes, C., van den Bosch, C., & Bundy, D. A. (1998). The Effects of Iron Deficiency and Anemia on Mental and Motor Performance, Educational Achievement, and Behavior in Children: The Effects of Iron Deficiency and Anemia on Mental and Motor Performance, Educational Achievement, and Behavior in Children: A R. In *An Annotated Bibliography*.

Noren, K., & Meironyt, D. (2000). Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20 - 30 years. *Chemosphere*, *40*(2000), 1111–1123.

Norouzi, E., Bahramifar, N., & Ghasempouri, S. M. (2010). Determination Concentration of Lead in Breast in Lactating Women in Region Industrial Zarinshahr and Effect on Infant. *Journal of Isfahan Medical School*, *28*(112), 640–646. https://www.sid.ir/paper/50864/en#downloadbottom

NRDC. (2001). *"Healthy Milk, Healthy Baby: Chemical Pollution and Mother's Milk."* The Natural Reouces Defence Council. Retrieved from https://www.nrdc.org/press-releases/healthy-milk-healthy-baby-chemical-pollution-and-mothers-milk on April 12, 2021.

Oakes, D. K., Sibley, K. P., Martin, W. J., MacLean, D. D., Solomon, R. K., Mabury, A. S., & Van Der Kraak, J. G. (2005). Short-Term Exposures of Fish to Perfluorooctane Sulfonate: Acute Effects on Fatty-Acyl – COA Oxidase Activity, Oxidative Stress, and Circulating Sex Steroids. *Environmental Toxicology and Chemistry*, *24*(5), 1172–1181. https://doi.org/https://doi.org/10.1897/04-419.1

OECD. (2005). *Results of Survey on Production and Use of PFOS, PFAS and PFOA, Related Substanes and Products/Mixtures Containing These Substances. OECD, Environment, Health and Safety Publications. Series on Risk Management No. 19* (Issue ENV/JM/MONO(2005)1). Retrieved from http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2005)1&doclanguage=en on April 12, 2021.

Ojovan, M. I., & Lee, W. E. (2005). Heavy Metal: An introduction to Nuclear Waste Immobilisation. In *ScienceDirect* (pp. 1–12). https://doi.org/ https://doi.org/10.1016/B978-008044462-8/50006-5

Okada, E., Sasaki, S., Kashino, I., Matsuura, H., Miyashita, C., Kobayashi, S., Itoh, K., Ikeno, T., Tamakoshi, A., & Kishi, R. (2014). Prenatal exposure to Perfluoroalkyl Acids and Allergic Diseases in Early Childhood. *Environment International*, *65*, 127–134. https://www.ncbi.nlm.nih.gov/pubmed/17805419

Oliveira, M. M., Trevilato, T. M. B., Segura-Muñoz, S. I., Aragon, D. C., Alves, L. G., Nadal, M., Marquès, M., Domingo, J. L., Sierra, J., & Camelo, J. S. (2020). Essential and toxic elements in human milk concentrate with human milk lyophilizate: A preclinical study. *Environmental Research*, *188*, 109733. https://doi.org/10.1016/j.envres.2020.109733

Olowoyo, J. O., Macheka, L. R., & Mametja, P. M. (2021). Health Risk Assessments of Selected Trace Elements and Factors Associated with Their Levels in Human Breast Milk from Pretoria, South Africa. *International Journal of Environmental Research and Public Health*,

18(18), 9754. https://doi.org/https://doi.org/10.3390/ijerph18189754

Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A. M., Butenhoff, J. L., & Zobel, L. R. (2007). Half-Life of Serum Elimination of Perfluorooctanesulfonate , Perfluorohexanesulfonate , and Perfluorooctanoate in Retired Fluorochemical Production Workers. *Environmental Health Perspectives*, *115*(9), 1298–1305. https://doi.org/10.1289/ehp.10009

Orun, E., Yalcin, S. S., Aykut, O., Orhan, G., & Morgil, G. K. (2012). Zinc and Copper Concentrations in Breastmilk at the Second Month of Lactation. *Indian Pediatrics*, *49*(2), 133–135. https://doi.org/10.1007/s13312-012-0021-9.

Oskarsson, A., Hallcn, I. P., & Sundberg, J. (1995). Exposure to Toxic Elements via Breast Milk *. *The Analyst*, *120*(3), 765–770. https://doi.org/https://doi.org/10.1039/AN9952000765

Oskarsson, A., Hallen, I. P., Sunberg, J., & Grawe, K. P. (1998). Risk assessment in relation to neonatal metal exposure †. *The Analyst*, *123*(1), 19–23. https://doi.org/https://doi.org/10.1039/a705136k

Oskarsson, A., Schütz, A., Skerfving, S., Hallén, I. P., & Lagerkvist, B. J. (1996). Archives of Environmental Health : An International Total and Inorganic Mercury in Breast Milk and Blood in Relation to Fish Consumption and Amalgam Fillings in Lactating Women Total and Inorganic Mercury in Breast Milk and Blood in Relation to Fish Consu. *Arcihives of Environmental Health: An International Journal Journal*, *51*(3), 234–241. https://doi.org/10.1080/00039896.1996.9936021

Pajewska-szmyt, M., Sinkiewicz-darol, E., & Gadza, R. (2019). The impact of environmental pollution on the quality of mother's milk. *Environmental Science and Pollution Research*, *26*(8), 7405–7427. https://doi.org/10.7363/040223

Pala, K., & Dundar, N. (2008). Prevalence & risk factors of anaemia among women of reproductive age in Bursa, Turkey. *Indian Journal of Medical Research*, *128*(3), 282–286. https://pubmed.ncbi.nlm.nih.gov/19052339/

Parcell, S. (2002). Sulfur in Human Nutrition and Applications in Medicine. *Alternative Medicine Revision*, *7*(1), 44–45. https://www.mdpi.com/2304-8158/9/5/659/htm

Parr, R. M., Demaeyer, E. M., Iyengar, V. G., Byrne, A. R., Kirkbright, G. F., Schoch, G., Niinistc, L., Pineda, O., Vis, H. L., Hofvander, Y., & Omollolu, A. (1991). Minor and Trace Elements in Human Milk from Guatemala, Sweden, and Zaire Results from a WHO / IAEA Joint Project. *Biological Trace Element Research*, *29*(1), 51–75. https://doi.org/doi:10.1007/bf03032674

Patton, S., Canfieldb, L. M., Hustonc, G. E., Ferrisd, A. M., & Jensend, R. G. (1990). Carotenoids of Human Colostrum. *Lipids*, *25*(3), 159–165. https://doi.org/10.1007/BF02544331

Peixoto, A. R. R., Codo, B. C. R., Sanches, V. L., Guiraldelo, C. T., da Silva, F. F., Ribessi, L. R., Marba, T. S. M., & Cadore, S. (2019). Trace Mineral Composition of Human Breast Milk from Brazilian Mothers. *Journal of Trace Elements in Medicine and Biology*, *54*(2019), 199–205. https://doi.org/10.1016/j.jtemb.2019.05.002

Pennington, J. A. T. (1988). Aluminium content of foods and diets. *Food Additives & Contaminants*, *5*(2), 37–41. https://doi.org/10.1080/02652038809373696

Perrin, M. T., Fogleman, A. D., Newburg, D. S., & Allen, J. C. (2016). A longitudinal Study of Human Milk Composition in the Second Year Postpartum : Implications for Human Milk Banking. *Maternal and Child Nutrition*, *13*(1), e12239. https://doi.org/10.1111/mcn.12239

Perrone, L., Palma, L. D. I., Toro, R. D. I., Gialanella, G., Moro, R., Pediatria, O., Andrea, V. S., & Civile, O. (1994). Interaction of Trace Elements in a Longitudinal Study of Human Milk from Full-Term and Preterm Mothers. *Biological Trace Element Research*, *41*(3), 321–330. https://doi.org/10.1007/BF02917432

Pirsaheb, M., Limoee, M., Namdari, F., & Khamutian, R. (2015). Organochlorine pesticides residue in breast milk: A systematic review. *Medical Journal of the Islamic Republic of Iran*, *29*(228), 1–10.

Playford, R. J., Macdonald, C. E., & Johnson, W. S. (2000). Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. *American Journal of Clinical Nutrition*, *72*(1), 5–14.

Pluim, H. J., Boersma, E. R., & Olie, K. (1994). Influence of Short-Term Dietary Measures on Dioxin Concentrations in Human Milk. *Environmental Health Persperctives*, *102*(11), 968–971. https://doi.org/https://doi.org/10.1289/ehp.94102968

Pohl, H. R., Wheeler, J. S., & Murray, H. E. (2013). Sodium and Potassium in Health and Disease. In *Metal Ions Life Science* (pp. 29–47). https://doi.org/10.1007/978-94-007-7500-8

Polder, A., Gabrielsen, G. W., Odland, J. Ø., Savinova, T. N., Tkachev, A., Løken, K. B., & Skaare, J. U. (2008). Spatial and temporal changes of chlorinated pesticides , PCBs , dioxins (PCDDs / PCDFs) and brominated flame retardants in human breast milk from Northern Russia. *Science of the Total Environment*, *391*(1), 41–54. https://doi.org/10.1016/j.scitotenv.2007.10.045

Post, G. B., Cohn, P. D., & Cooper, K. R. (2012). Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: A critical review of recent literature. *Environmental Research*, *116*, 93–117. https://doi.org/10.1016/j.envres.2012.03.007

Powe, C. E., Knott, C. D., & Conklin-brittain, N. (2010). Infant Sex Predicts Breast Milk Energy Content. *American Journal of Human Biology*, *22*(1), 50–54. https://doi.org/10.1002/ajhb.20941

Pratt, I. S., Anderson, W. A., Crowley, D., Daly, S. F., Evans, R. I., Fernandes, A. R., Fitzgerald, M., Geary, M. P., Keane, D. P., Malisch, R., Mcbride, J., Morrison, J. J., Reilly, A., & Tlustos, C. (2012). Chemosphere Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in breast milk of first-time Irish mothers : Impact of the 2008 dioxin incident in Ireland. *Chemosphere*, *88*(7), 865–872. https://doi.org/10.1016/j.chemosphere.2012.03.095

Prentice, A., & Barclay, D. V. (1991). Breast-Milk Calcium and Phosphorus Concentrations of Mothers in Rural Zaire. *European Journal of Clinical Nutrition*, *45*(12), 611–617. https://pubmed.ncbi.nlm.nih.gov/1810721/

Prentice, A., Jarjou, L. M. A., Stirling, D. M., Buffenstein, R., & Fairweather-tait, S. (1998). Biochemical Markers of Calcium and Bone Metabolism during 18 Months of Lactation in Gambian Women Accustomed to a Low Calcium Intake and in Those Consuming a Calcium Supplement. *The Journal of Clinical Endocrinology and Metabolism*, *83*(4), 1059 1066. https://doi.org/10.1210/jcem.83.4.4737

Prentice, A., Jarjou, L. M., Cole, T. J., Stirling, D. M., Dibba, B., & Fairweather-Tait, S. (1995). Calcium requirements of lactating Gambian mothers : effects of a calcium supplement on breast-milk calcium concentration , maternal bone mineral content , and urinary calcium excretion1-3. *American Journal of Clinical Nutrition*, *62*(1), 58–67. https://doi.org/10.1093/ajcn/62.1.58

Prohaska, T., Irrgeher, J., Benefield, J., Bohlke, J. K., Chesson, L., Coplen, T. B., Ding Tiping, Philip, J. H., Dunn, M. G., Holden, N. E., Meijer, H. A. J., Moossen, H., Possolo, A., Takahashi, Y., Vogl, J., Walczyk, T., Wang, J., Wieser, M. E., SYoneda, S., Zhu, X.-K., & Meija, J. (2022). Standard atomic weights of the elements 2021 (IUPAC Technical Report). *Pure and Applied Chemistry: United States Geological Survey*, *94*(5). https://doi.org/10.1515/pac-2019-0603

Quansah, R., Armah, F. A., Essumang, D. K., Luginaah, I., & Clarke, E. (2015). Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis. *Environmental Health Perspectives*, *123*(5), 412–421. https://doi.org/https://doi.org/10.1289/ehp.1307894

Radisch, B., Luck, W., & Nau, H. (1987). Cadmium Concentrations in Milk and Blood of Smoking Mothers. *Toxicology Letters*, *36*(2), 147–152. https://doi.org/https://doi.org/10.1016/0378-4274(87)90178-0

Rahimi, E., Hashemi, M., & Torki Baghbadorani, Z. (2009). Determination of cadmium and lead in human milk. *International Journal of Environment, Science and Technology*, *6*(4), 671–676.

Ramakrishnan, U., & Yip, R. (2002). Experiences and Challenges in Industrialized Countries : Control of Iron Deficiency in Industrialized Countries. *The Journal of Nutrition*, *134*(4), 820–824. https://doi.org/https://doi.org/10.1093/jn/132.4.820S

Rawn, D. F. K., Dufresne, G., Clément, G., Fraser, W. D., & Arbuckle, T. E. (2022). Science of the Total Environment Per fluorinated alkyl substances in Canadian human milk as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) study. *Science of the Total Environment*, *831*(March), 1–8. https://doi.org/10.1016/j.scitotenv.2022.154888

Ray, C., Kerketta, J. A., Rao, S., Patel, S., Dutt, S., Arora, K., Pournami, F., & Bhushan, P. (2019). Human Milk Oligosaccharides: The Journey Ahead. *International Journal of Pediatrics*, *2019*, 12–14.

Rayman, M. P. (2012). Selenium and Human Health. *The Lancet*, *379*(9822), 1256–1268. https://doi.org/10.1016/S0140-6736(11)61452-9

Rebelo, F. M., & Caldas, E. D. (2016). Arsenic , lead , mercury and cadmium : Toxicity , levels in breast milk and the risks for breastfed infants. *Environmental Research*, *151*, 671–688. https://doi.org/10.1016/j.envres.2016.08.027

Rodriguez, C. E., Setzer, R. W., & Barton, H. A. (2009). Pharmacokinetic modeling of perfluorooctanoic acid during gestation and lactation in the mouse ☪. *Reproductive Toxicology*, *27*(3–4), 373–386. https://doi.org/10.1016/j.reprotox.2009.02.009

Rodriguez Rodriguez, E. M., Sanz, A. M., & Diaz, R. C. (2000). Concentrations of Iron, Copper and Zinc in Human Milk and Powdered Infant Formula. *International Journal of Food Sciences and Nutrition*, *51*(5), 373–380. https://doi.org/10.1080/096374800426966

Rogan, W. J., Bagniewska, A., & Damstra, T. (1980). Pollutants in breast milk. *The New England Journal of Medicine*, *302*(26), 1450–1453. https://doi.org/10.1056/NEJM198006263022604

Roosens, L., Hollander, W. D., Bervoets, L., Reynders, H., Campenhout, K. Van, Cornelis, C., Heuvel, R. Van Den, Koppen, G., & Covaci, A. (2010). Brominated fl ame retardants and per fl uorinated chemicals , two groups of persistent contaminants in Belgian human blood and milk. *Environmental Pollution*, *158*(8), 2546–2552. https://doi.org/10.1016/j.envpol.2010.05.022

Rossipal, E., & Krachler, M. (1998). Pattern of Trace Elements in Human Breast Milk During the Course of Lactation. *Nutrition Research*, *18*(1), 11–24. https://doi.org/https://doi.org/10.1016/S0271-5317(97)00196-6

Rothenberg, S. J., Khan, F., Manalo, A. M., Jiang, J., Cuellar, R., Reyes, S., Acosta, S., Jauregui, M., Diaz, M., Sanchez, M., Todd, A. C., & Johnson, C. (2000). Maternal Bone Lead Contribution to Blood Lead during and after Pregnancy. *Environmental Research*, *82*(1), 81–90. https://doi.org/https://doi.org/10.1006/enrs.1999.4007

Roy, S., Basu, A., Dhar, P., & Ghosh, M. (2014). Calcium, iron and Essential fatty acid Composition of Bengali Mother's Milk: A Population Based Cross-Sectional Study. *Indian Journal of Community Health*, *26*(2), 310–317. https://www.iapsmupuk.org/journal/index.php/IJCH/article/view/511

Ryan, J. J., & Rawn, D. F. K. (2014). Chemosphere Polychlorinated dioxins , furans (PCDD / Fs), and polychlorinated biphenyls (PCBs) and their trends in Canadian human milk from 1992 to 2005. *Chemosphere*, *102*, 76–86. https://doi.org/10.1016/j.chemosphere.2013.12.065

Sabatier, M., Garcia-rodenas, C. L., Castro, C. A. De, Kastenmayer, P., Vigo, M., Andrey, D., Nicolas, M., Payot, J. R., Bordier, V., Thakkar, S. K., Beauport, L., Tolsa, J., Fumeaux, J. F., & Michael, A. (2019). Longitudinal Changes of Mineral Concentrations in Swiss Women. *Nutrients*, *11*(8), 1885-. https://doi.org/https://doi.org/10.3390%2Fnu11081855

Saleh, A. M., Ragab, A. A., Kamel, A., Jones, J., & El-Sebae, K. A. (1996). Regional Distribution of Lead in Human Milk from Egypt. *Chemosphere*, *32*(9), 1859–1867. https://doi.org/https://doi.org/10.1016/0045-6535(96)00079-3

Salem, D. A., & Ahmed, M. M. (2002). Evaluation of Some Organochlorine Pesticides in Human Breast Milk and Infants ' Dietary Intake in Middle and Upper Egypt Abstract : Introduction : Subjects and Methods : Results : *Alexandria Journal of Pediatrics*, *16*(2), 259–265. http://www.emro.who.int/imemrf/Alexandria_j_Pediatrics/2002_16_2_259.pdf

Salmenpera, L., Perheentuga, J., Pakarinen, P., & Siimes, M. (1986). Copper Nutrition in Infants During Prolonged Exclusive Low Intake but RisingSerum Concentrations of Copper and Ceruloplasmin. *The American Journal of Clinical Nutrition*, *43*(2), 251–257. https://doi.org/10.1093/ajcn/43.2.251

Samiee, F., Leili, M., Faradmal, J., Torkshavand, Z., & Asadi, G. (2019). Exposure to arsenic through breast milk from mothers exposed to high levels of arsenic in drinking water: infant risk assessment. *Food Control*. https://doi.org/10.1016/j.foodcont.2019.05.034

Sanchez, C., Fente, C., Barreiro, R., Lopez-Racamonde, O., Cepeda, A., & Regal, P. (2020). Association between Breast Milk Mineral Content and Maternal Adherence to Healthy Dietary Patterns. *Foods*, *9*(5), 659. https://doi.org/https://doi.org/10.3390/foods9050659

Savage, E. P., Keefe, T. J., Tessari, J. D., Wheeler, H. W., Applehans, F. M., Goes, E. A., Ford, S. A., U, C. S., Collins, F., Keefe, T. J., Tessarl, J. D., Applehans, F. M., Goes, E. A., Epidemiologic, C., Studies, P., Hall, S., Col-, F., Ep-, C., Hall, S., … Rhea, T. (1981). National study of chlorinated hydrocarbon insecticide residues in human milk, usa. *American Journal of Epidermiology*, *113*(4), 413–422.

Schade, G., & Heinzow, B. U. (1998). Organochlorine pesticides and polychlorinated biphenyls in human milk of mothers living in northern Germany : Current extent of contamination , time trend from 1986 to 1997 and factors that influence the levels of contamination. *The Science of the Total Environment*, *215*, 31–39.

Schafer, K. S., & Kegley, S. E. (2002). Persistent toxic chemicals in the US food supply*. *J Epidermiol Community Health*, *56*, 813–817.

Schoeters, G., Hond, E. Den, Zuurbier, M., Hazel, P. Van Den, Stilianakis, N., Ronchetti, R., & Koppe, J. G. (2006). Cadmium and children : Exposure and health effects. *Acta Paediatricia*, *95*(453), 50–54. https://doi.org/10.1080/08035320600886232

Schramel, P., Lill, G., Hasse, S., & Klose, B. (1988). Mineral- and Trace Element Concentrations in Human Breast Milk , Placenta , Maternal Blood , and the Blood of the Newborn. *Biological Trace Element Research*, *16*(1), 67–75. https://doi.org/10.1007/BF02795335

Schrenk, D., Bignami, M., Bodin, L., Chipman, J. K., Grasl-kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barreg, L., Ceccatelli, S., Knutsen, H. K., Rose, M., … Schwerdtle, T.

(2020). Risk to human health related to the presence of per fl uoroalkyl substances in food. *EFSA Journal*, *18*(9), 6223. https://doi.org/10.2903/j.efsa.2020.6223

Sears, M. E., Kerr, K. J., & Bray, R. I. (2012). Arsenic , Cadmium , Lead , and Mercury in Sweat : A Systematic Review. *Journal of Environmental and Public Health*, *2012*, 184745. https://doi.org/10.1155/2012/184745

Semwal, A. D., Pamashree, A., Khan, M. A., Sharma, G., & Bawa, A. S. (2006). Leaching of aluminium from utensils during cooking of food. *Journal of the Science of Food and Agriculture*, *86*(14), 2425–2430. https://doi.org/https://doi.org/10.1002/jsfa.2635

Seow, J. (2013). *Fire Fighting Foams with Perfluorochemicals - Environmental Review*. *June*, 1–76. https://cswab.org/wp-content/uploads/2018/03/Fire-Fighting-Foams-with-PFAS-Env-Review-June-2013-Australia-.pdf

Sharma, R., & Pervez, S. (2005). Toxic Metals Status in Human Blood and Breast Milk Samples in an Integrated Steel Plant Environment in Central India. *Environmental Geochemistry and Health*, *27*(1), 39–45. https://doi.org/https://doi.org/10.1007/s10653-004-1628-0

Shawahna, R., Zyoud, A., Dwikat, J., El-Helo, M., Yacoub, B., & Hilal, H. (2016). Breast Milk Lead Levels in 3 Major Regions of the West Bank of Palestine. *Journal of Human Lactation*, *32*(3), 455–461. https://doi.org/10.1177/0890334416646566

Shenker, N. (2013). The mysteries of milk. *The Biologist: Royal Society of Biology*, *64*(3), 18–20.

Sherman, P. O., & Smith, S. (1971). Block and Graft Copolymers containing Water-Solvatable Polar Groups and Fluoroaliphatic Groups. In *United States Patent Office*. Retrieved from https://patentimages.storage.googleapis.com/9c/20/74/e0dbd23ecdd80b/US3574791.pdf on March 10, 2021.

Shi, Y., Sun, G., Zhang, Z., Deng, X., Kang, X., Liu, Z., & Ma, Y. (2011). The Chemical Composition of Human Milk from Inner Mongolia of China. *Food Chemistry*, *127*(3), 1193–1198. https://doi.org/10.1016/j.foodchem.2011.01.123

Shrestha, N. (2021). Factor Analysis as a Tool for Survey Analysis. *American Journal of Applied Mathematics and Statistics*, *9*(1), 4–11. https://doi.org/10.12691/ajams-9-1-2

Silbergeld, E. K. (1991). Lead in Bone : Implications for Toxicology during Pregnancy and Lactation. *Environmental Health Perspectives*, *91*, 63–70. https://doi.org/https://doi.org/10.1289/ehp.919163

Sillanpää, M., & Jansson, H. (1992). Status of Cadmium, Lead, Cobalt and Selenium in Soils and Plants of Thirty Countries. *FAO Soils Bulletin*, *65*, xii + 195pp. Retrieved from https://login.research4life.org/tacsgr1www_cabdirect_org/cabdirect/abstract/19941900070 on March 10, 2021.

Silvestre, D. M., Lagarda, J. M., Farre, R., Martinez-Costa, C., Brines, J., Molina, A., & Clemente, G. (2000). A Study of Factors that May Influence the Determination of Copper , Iron , and Zinc in Human Milk During Sampling and in Sample Individuals. *Biological Trace Element Research*, *76*(3), 217–227. https://doi.org/10.1385/BTER:

76:3:217

Silvestre, D., Martinez-Costa, C., Lagarda, M. J., Brines, J., Farre, R., & Clemente, G. (2001). Copper , Iron , and Zinc Contents in Human Milk During the First Three Months of Lactation: A Longitudinal Study. *Biological Trace Element Research*, *80*(1), 1–11. https://doi.org/10.1385/BTER:80:1:01

Sipahi, H., Eken, A., Aydın, A., Şahin, G., & Baydar, T. (2014). Safety Assessment of Essential and Toxic Metals in Infant Formulas. *The Turkish Journal of Pediatrics*, *56*(4), 385–391.

Skroder, H. M., Hamadani, J. D., Tofail, F., Persso, Å. L., Vahter, M. E., & Kippler, M. J. (2015). Selenium Status in Pregnancy Influences Children's Cognitive Function at 1. 5 Years of Age. *Clinical Nutrition*, *34*(5), 923–930. https://doi.org/10.1016/j.clnu.2014.09.020

So, M. K., Yamahita, N., Taniyasu, S., Jiang, Q., Giesy, J. P., Chen, K., & Lam, K. S. P. (2006). Health Risks in Infants Associated with Exposure to Perfluorinated Compounds in Human Breast Milk from Zhoushan, China. *Environmental Science and Technology*, *40*(9), 2924–2929. https://doi.org/https://doi.org/10.1021/es060031f

Soleimani, S., Shahverdy, M. R., Mazhari, N., & Abdi, K. (2014). Lead Concentration in Breast Milk of Lactating Women who Were Living in Tehran , Iran. *Acta Medica Iranica*, *52*(1), 56–59.

Solomon, G. M., & Weiss, P. M. (2002). Mini-Monograph Chemical Contaminants in Breast Milk : Time Trends and Regional Variability. *Environmental Health Perspectives*, *110*(6), 339–347.

Sonawane, B. R. (1995). Chemical Contaminants in Human Milk : An Overview. *Environmental Health Perspectives*, *103*(6), 197–205.

Sood, S., & Gupta, A. (2017). Colostrum-Miraculous Fluid. *International Journal of Management and Humanities*, *04*(01 January 2017), 1–5.

Specker, L. (1984). Nutritional Vegetarian Concerns Diets ' of Lactating Women Consuming. *The American Journal of Clinical Nutrition*, *59*(5), 1182S-1186S. https://doi.org/10.1093/ajcn/59.5.1182S

SPS. (2021). Scotchgard vs Scotchguarding. In *Stain Protection Services*. Retrieved from https://stainprotect.ca/scotchgard-vs-scotchguarding/#:~:text=Scotchgard%20was%20the%20first%20stain,stain%20protecting%20a%20fabric%20furnishing. on June 8, 2023.

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., & Vaccarino, V. (2009). Original Contribution Association of Perfluorooctanoic Acid and Perfluorooctane Sulfonate With Serum Lipids Among Adults Living Near a Chemical Plant. *American Journal of Epidemiology*, *170*(10), 1268–1278. https://doi.org/10.1093/aje/kwp279

Subramanian, A., Ohtake, M., Kunisue, T., & Tanabe, S. (2007). High levels of organochlorines in mothers' milk from Chennai (Madras) city , India. *Chemosphere*, *68*, 928–939. https://doi.org/10.1016/j.chemosphere.2007.01.041

Sudaryanto, A., Kunisue, T., Tanabe, S., Niida, M., & Hashim, H. (2005). Persistent Organochlorine Compounds in Human Breast Milk from Mothers Living in Penang and Kedah , Malaysia. *Archives of Environmental Contamination and Toxicology*, *49*(3), 429–437. https://doi.org/10.1007/s00244-004-0208-8

Sundström, M., Ehresman, D. J., Bignert, A., Butenhoff, J. L., Olsen, G. W., Chang, S., & Bergman, Å. (2011). A temporal trend study (1972 – 2008) of per fl uorooctanesulfonate , per fl uorohexanesulfonate , and per fl uorooctanoate in pooled human milk samples from Stockholm , Sweden. *Environment International*, *37*(1), 178–183. https://doi.org/ 10.1016/j.envint.2010.08.014

Suzuki, K. T., Tamagawa, H., Hirano, S., Kobayashi, E., & Takahashi, K. (1991). Changes in Element Concentration and Distribution in Breast-Milk Fractions of a Healthy Lactating Mother. *Biological Trace Element Research*, *28*(2), 109–121. https://doi.org/https://doi.org/10.10 07/bf02863077

Szyrwinska, K., & Lulek, J. (2007). Exposure to specific polychlorinated biphenyls and some chlorinated pesticides via breast milk in Poland. *Chemosphere*, *66*(2007), 1895–1903. https://doi.org/10.1016/j.chemo sphere.2006.08.010

Taguchi, S., & Yakushiji, T. (1988). contami ~ ion Influence of Termite Treatment in the Home on the Chlordane Concentration in H u m a n Milk. *Arch. Environ. Contam. Toxicol.*, *17*, 65–71.

Tamarp, Y., & Tsuji, H. (1995). Longitudinal Study on Selenium Content in Human Milk Particularly During Early Lactation Compared to that in Infant Formulas and Cow ’ s Milk in Japan. *Journal of Trace Elements in Medicine and Biology*, *9*(1), 34–39. https://doi.org/10.1016/S0946-672X(11)80006-4

Tanabe, S., & Kunisue, T. (2007). Persistent organic pollutants in human breast milk from Asian countries. *Environmental Pollution*, *146*(2007), 400–413. https://doi.org/10.1016/j.envpol.2006.07.003

Tao, L., Kannan, K., Wang, C. M., Arcaro, K. F., & Butenhoff, J. L. (2008). Perfluorinated Compounds in Human Milk from Massachusetts , U . S . A . *Environmental Science and Technology*, *42*(8), 3096–3101. https://doi.org/https://doi.org/10.1021/es702789k

Tao, L., Ma, J., Kunisue, T., Libelo, E. L., Tanabe, S., & Kannan, K. (2008). Perfluorinated Compounds in Human Breast Milk from Several Asian Countries, and in Infant Formula and Dairy Milk from the United States. *Environmental Science and Technology*, *42*(22), 8597–8602. https://doi.org/https://doi.org/10.1021/es801875v

Tapiero, H., Townsend, D. M., & Tew, K. D. (2003). Trace elements in human physiology and pathology. Copper. *BioMed Pharmacother*, *57*(9), 386–398. https://doi.org/https://doi.org/10.1016%2Fs0753-3322(03)00012-x

Terry, N., Zayed, A. M., Souza, M. P., & Tarun, A. (2000). Selenium in Higher Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *51*(1), 401–432. https://pubmed.ncbi.nlm.nih.gov/15012198/

Thacher, T. O. M. D., Pettifor, J. M., Fischer, P. R., Okolo, S. N., & Prentice, A. N. N. (2006). Case-Control Study of Breast Milk Calcium in Mothers of Children with and Without Nutritional Rickets. *Acta Paediatricia*, *95*(7), 826–833. https://doi.org/10.1080/08035250500452613

Theelen, R. M. C., Liem, A. K. D., & Van Wijnen, J. H. (1993). Intake of 2,3,7,8 Chlorine Substituted Dioxins, Furans, and Planner PCBs from Food in the Netherlands: Median and Distribution. *Chemosphere*, *27*(9), 1625–1635. https://doi.org/https://doi.org/10.1016/0045-6535(93)90144-T

Thomsen, C., Haug, L. S., Stigum, H., Frøshaug, M. ., Broadwell, S. L., & Becher, G. (2010). Changes in Concentrations of Perfluorinated Compounds , Polybrominated Diphenyl Ethers , and Polychlorinated Biphenyls in Norwegian Breast-Milk during Twelve Months of Lactation. *Environmental Science and Tox*, *44*(24), 9550–9556. https://doi.org/10.1021/es1021922.

Toms, L. L., Thompson, J., Rotander, A., Hobson, P., Calafat, A. M., Kato, K., Ye, X., Broomhall, S., Harden, F., & Mueller, J. F. (2014). Decline in perfl uorooctane sulfonate and per fl uorooctanoate serum concentrations in an Australian population from 2002 to 2011. *Environment International*, *71*, 74–80. https://doi.org/10.1016/j.envint.2014.05.019

Trafikowska, U., Sobkowiak, E., Butler, J. A., Whanger, P. D., & Zachara, B. A. (1998). Organic and Inorganic Selenium Supplementation to Lactating Mothers Increase the Blood and Milk Se Concentrations and Se Intake by Breast-Fed Infants. *Journal of Trace Elements in Medicine and Biology*, *12*(2), 77–85. https://doi.org/10.1016/S0946-672X(98)80029-1

Turan, S., Saygı, Ş, & Acar, O. (2001). Determination of Heavy Metal Contents in Human Colostrum Samples by Electrothermal Atomic Absorption Spectrophotometry. *Journal of Tropical Pediatrics*, *47*(2), 81–85.

UNEP. (2023). Why do persistent organic pollutants matter ? *United Nations Environment Programme*, 1–8. Retrieved from https://www.unep.org/topics/chemicals-and-pollution-action/pollution-and-health/persistent-organic-pollutants-pops/why#:~:text=Persistent organic pollutants (POPs) are,to both humans and wildlife. on May 8, 2024.

Ursinyova, M., & Masanova, V. (2005). Cadmium , Lead and Mercury in Human Milk from Slovakia. *Food Additives and Contaminants*, *22*(6), 579–589. https://doi.org/10.1080/02652030500135201

US CDC. (2009). Fact Sheet on Arsenic. In *Centers for Disease Control and Prevention* (Issue November 2009). Retrieved from https://www.epa.gov/sites/default/files/2014-03/documents/arsenic_factsheet_cdc_2013.pdf on May 9, 2021.

US CDC. (2023). Breastfeeding Benefits Both Baby and Mom. In *Centers for Disease Control and Prevention*. Retrieved from https://www.cdc.gov/breastfeeding/features/breastfeeding-benefits.html#:~:text=Breast feeding has health benefits for,against certain illnesses and diseases. on April 10, 2024.

US CDC. (2024). Lead and Breastfeeding. In *Centers for Disease Control and Prevention* (Issue February 2024). Retrieved from https://www.cdc.gov/breastfeeding-special-circumstances/hcp/exposures/lead.html#:~:text=Women who are or have,lead level has been identified. on May

18, 2024.

US EPA. (1976). Pesticidal aspect of chlordane in relation to man and the environment. In *United States Environmental Protection Agency*. Retrieved from https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100B9L4.TXT on April 12, 2021.

US EPA. (2002a). A Review of the Reference Dose and Reference Concentration Process. In *Epa/630/P-02/002F* (Issue December). Retrieved from http://www.epa.gov/raf/publications/pdfs/rfd-final.pdf on July 22, 2021.

US EPA. (2002b). Child-Specific Exposure Factors Handbook. In *National Center for Environmental Assessment, Washington D.C; EPA/600/P - 00/002B*. Retrieved from https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=55145 on July 22, 2021.

US EPA. (2008). Child-Specific Exposure Factors Handbook (Final Report). In *US Environmental Protection Agency, Washington DC, EPA/600/R - 06/096F, 2008*. (Issue September). Retrieved from https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=199243 on April 27, 2023

US EPA. (2009). *Persistent Organic Pollutants : A Global Issue , A Global Response*. United States Environmental Protection Agency. Retrieved from https://www.epa.gov/international-cooperation/persistent-organic-pollutants-global-issue-global-response#pops on May 12, 2022.

US EPA. (2012a). Emerging Contaminants – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance. In *United States Environmental Protection Agency* (Issue December). Retrieved fro https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100LTG6.TXT

on May 12, 2022.

US EPA. (2012b). Integrated Science Assessment for Lead. In *US Environmental Protection Agency* (Issue November). Retrieved from https://www.epa.gov/lead/learn-about-lead#:~:text=Lead can be found in,lead-based paint in homes. on April 21, 2022.

US EPA. (2014). Emerging Contaminants – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance. In *United Environmental Protection Agency.* (Issue March). Retrieved from https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100LTG6.TXT on April, 12, 2022.

US EPA. (2016a). FACT SHEET PFOA & PFOS Drinking Water Health Advisories. In *United Environmental Protection Agency* (Issue November). Retrieved from https://www.epa.gov/sites/default/files/2016-06/documents/drinkingwaterhealthadvisories_pfoa_pfos_updated_5.31.16.pdf on May 9, 2022.

US EPA. (2016b). Health Effects Support Document for Perfluorooctanoic Acid (PFOA). *United Environmental Protection Agency. Retrieved on May 12, 2023*, *May*, 1–322. Retrieved from https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final-plain.pdf on May 21, 2022.

US EPA. (2016c). Supporting Documents for Drinking Water Health Advisories for PFOA and PFOS. *US Environmental Protection Agency.* 2016–2017. Retrieved from https://www.epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos on May 12, 2022,

US EPA. (2017). Technical Fact Sheet – Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) At a Glance. In *United Environmental Protection Agency.* (Issue November). Retrieved from https://19january2021snapshot.epa.gov/sites/static/files/2017-12/documents/ffrrofactsheet_contaminants_pfos_pfoa_11-20-17_508_0.pdf on May 12, 2022.

US EPA. (2019). Health Effects of Exposures to Mercury. In *United Environmental Protection Agency* (Issue May 2019). Retrieved from https://www.epa.gov/mercury/health-effects-exposures-mercury#:~:text=High exposure to inorganic mercury,other systems through this route. on May 11, 2022.

US EPA & ATSDR. (2018). Public Health Implications of Exposure to Polychlorinated Biphenyls (PCBs). In *US Environmental Protection Agency and Agency for Toxic Substances and Disease Registry*. Retrieved from https://www.epa.gov/sites/default/files/2015-01/documents/pcb99.pdf on Apri 27, 2021.

US FDA. (2019). Arsenic in Food and Dietary Supplements. In *United States Food and Drug Administration*. Retrieved from https://public4.pagefreezer.com/content/FDA/23-01-2023T07:59/https://www.fda.gov/food/environmental-contaminants-food/arsenic-food-and-dietary-supplements on Hune 24, 2022.

US Institute of Medicine. (2000). Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. In *The National Academies Press* (Vol. 00). https://doi.org/10.17226/9810

US Institute of Medicine. (2001). *Dietary Reference Intakes for Vitamin A , Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc: Washington (DC): National Academies Press (US)* https://doi.org/10 .17226/10026

US Institute of Medicine Standing Committee on the Scientific Evaluation on Dietary Reference Intakes. (1997). Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. In *US National Academies Press, Washington DC* (Vol. 4, Issue Calcium, pp. 1–50).

US NIH. (2022a). Copper: Fact SheetHealth Professional. In *National Institute of Health*. Retrieved from https://ods.od.nih.gov/factsheets/Copper-HealthProfessional/#:~:text=Copper Intakes and Status,-Typical diets in&text=In adults age 20 and,adults age 20 and over. on May10, 2024.

US NIH. (2022b). Zinc: Fact Sheet for Health Professionals. In *National Institute of Health*. Retrieved from https://ods.od.nih.gov/factsheets /Zinc-HealthProfessional/#:~:text=Zinc Intakes and Status,-Most people in&text=The average daily zinc intake from foods and supplements is,12.6 mg%2Fday in women. on May 10, 2024.

US NIH. (2023). Iron: Fact Sheet for Health Professional. In *National Institute of Health*. Retrieved from https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/#:~:text=Iron Intakes and Status,-People in the&text=The average daily iron intake,in women older than 19. on May 10, 2024.

USDA. (2020). Dietary Guidelines for Americans, 2020-2025. In *United States Department of Agriculture* (Issue 9). Retrieved from https://www.dietaryguidelines.gov/sites/default/files/2020-12/Dietary_Guidelines_for_Americans_2020-2025.pdf on July 21, 2022.

Vahidinia, A., Samiee, F., Faradmal, J., Rahmani, A., & Javad, M. T. (2019). Mercury , Lead , Cadmium , and Barium Levels in Human Breast Milk and Factors Affecting their Concentrations in Hamadan , Iran. *Biological Trace Element Research*, *187*(1), 32–40. https://doi.org/ https://doi.org/10.1007/s12011-018-1355-5

Vahter, M. E. (2007). Interactions between Arsenic-Induced Toxicity and Nutrition in Early Life. *The Journal of Nutrition*, *137*(12), 2798–2804. https://doi.org/https://doi.org/10.1093/jn/137.12.2798

Vaughan, L. A., Weber, C. W., & Kemberling, S. R. (1979). Longitudinal of Human in the Mineral Content. *The American Journal of Clinical Nutrition*, *32*(11), 2301–2306. https://doi.org/10.1093/ajcn/32.11.2301

Veschasit, O., Meksumpun, S., & Meksumpun, C. (2012). Heavy Metals Contamination in Water and Aquatic Plants in the Tha Chin River , Thailand. *Nat. Sci*, *46*(6), 931–943.

Victora, C. G., Bahl, R., Barros, A. J. D., França, G. V. A., Horton, S., Krasevec, J., Murch, S., Sankar, M. J., & Walker, N. (2016). Breastfeeding 1 Breastfeeding in the 21st century : epidemiology , mechanisms , and lifelong eff ect. *The Lancet*, *387*(10017), 475–490. https://doi.org/10.1016/S0140-6736(15)01024-7

Vigh, É., Colombo, A., Benfenati, E., Håkansson, H., Berglund, M., Bódis, J., & Garai, J. (2013). Science of the Total Environment Individual breast milk consumption and exposure to PCBs and PCDD / Fs in Hungarian infants : A time-course analysis of the fi rst three months of lactation. *Science of the Total Environment, The*, *449*, 336–344. https://doi.org/10.1016/j.scitotenv.2013.01.024

Visha, A., Gandhi, N., Bhavsar, S. P., & Arhonditsis, G. B. (2018). A Bayesian assessment of polychlorinated biphenyl contamination of fi sh communities in the Laurentian Great Lakes. *Chemosphere*, *210*, 1193–1206. https://doi.org/10.1016/j.chemosphere.2018.07.070

Vitolo, M. R., Soares, L. M. V., Carvalho, E. B., & Cardoso, C. B. (2004). Calcium and Magnesium Concentrations in Mature Human Milk : Influence of Calcium Intake , Age and Socioeconomic Level. *Archives of Latinoam Nutrition*, *54*(1), 118–122. https://pubmed.ncbi.nlm.nih.gov/15332365/

Volkel, W., Genzel-Boroviczeny, O., Demmelmair, H., Gebauer, C., Koletzko, B., Twardella, D., Raab, U., & Fromme, H. (2008). Perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in human breast milk : Results of a pilot study. *International Journal of Hygiene and Environmental Health*, *211*(3–4), 440–446. https://doi.org/10.1016/j.ijheh.2007.07.024

Vuori, B. Y. E. (1979). Intake of Copper, Iron, Manganese and Zinc by Healthy, Exclusively-Breast-fed Infants During the First 3 Months of Life. *British Journal of Nutrition*, *42*(3), 407–411. https://doi.org/10.1079/bjn19790131

Wang, M., Chen, J., Lin, K., Chen, Y., Hu, W., Tanguay, L. R., Huang, C., & Dong, Q. (2011). Chronic Zebra Fish PFOS Exposure Alters Sex Ratio and Maternal Related Effects in F1 Offspring. *Environmental Toxicology and Chemistry*, *30*(9), 2073–2080. https://doi.org/10.1002/etc.594

Wasowicz, W. O. W., Romadzinska, J. O. G., Zram, K. R. S., Ydzynski, K. O. R., Ieslak, J. A. C., & Ietrzak, Z. B. P. (2001). Selenium , Zinc , and Copper Concentrations in the Blood and Milk of Lactating Women. *Biological Trace Element Research*, *79*(3), 221–233. https://doi.org/10.1385/BTER:79:3:221

Webster, L., & Fryer, R. (2022). Status and Trends of Polychlorinated Biphenyls (PCB) in Fish , Shellfish and Sediment: The 2023 Quality Status Report for the North-East Atlantic. In *OSPAR Commission, London*. Retrieved from https://oap- cloudfront.ospar.org/media/filer_public/84/48/8448cdc0-472e-4fbb-90fd-1a3b0bc35626/p00933_qsr2023_pcb.pdf on June 28, 2023.

Werts, H., Schalkwyk, L. C., Taylor, A., Kim-Cohen, J., Williams, B., Moffitt, T. E., Milne, B. J., Poulton, R., Caspi, A., & Craig, I. W. (2007). Moderation of breastfeeding effects on the IQ by genetic variation in fatty acid metabolism. *Proceedings of the National Academy of Sciences*, *104*(47), 18860–18865. https://doi.org/10.1073/pnas.0704292104

WFPHA. (2000). Persistent Organic Pollutants and Human Health. In *World Federation of Public Health Association* (Issue May). Retrieved from https://www.academia.edu/64166089/Persistent_Organic_Pollutants_a

nd_Human_Health on April 27, 2021.

WHO/IAEA. (1989). Minor and Trace Elements in Breast Milk : Report of a Joint WHO/IAEA Collaborative Study. In *World Health Organisation*. Retrieved from https://iris.who.int/handle/10665/39678 on April 27, 2021.

WHO/UNICEF. (2015). *Countdown to 2015 Decade Report (2000- 2010): Taking stock of maternal , newborn , and child survival*. Retrieved from https://data.unicef.org/resources/countdown-2015-decade-report-2000-2010-taking-stock-maternal-newborn-child-survival/ on April 21, 2021.

WHO. (1984). *Environmental Health Criteria 38: HEPTACHLOR*. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/37298/9241540982-eng.pdf on April 21, 2021.

WHO. (1989). IPCE Health and Safety Guide No . 21: Aldrin and Dieldrin Health and Safety Guide. In *World Health Organization, Geneva* (Issue 21). Retrieved from https://iris.who.int/bitstream/handle/10665/39358/9241543434-eng.pdf on April 21, 2021.

WHO. (1993). *Evaluation of Certain Food Additives and Contaminants: WHO 41st Report of the Joint FAO/WHO Experts on Food Additives. Tecnical Report Series No. 837. Geneva*. Retrieved from https://iris.who.int/handle/10665/36981 on April 21, 2021.

WHO. (1998). *Germs/Food International Dietary Survey: Infants Exposure to Certain Organochlorine Contaminants from Breast Milk - A Risk Assessment*. Retrieved from http://apps.who.int/iris/bitstream/10665/64097/1/WHO_FSF_FOS_98.4.pdf?ua=1&ua=1 on April 21, 2021.

WHO. (2003a). Health Risks of Persistent Organic Pollutants from Long-Range. In *World Health Organisation*. Retrieved from https://www.who.int/europe/publications/i/item/EUR-03-5042687 on April 27, 2021.

WHO. (2003b). Polychlorinated biphenyls: human health aspects. In *World Health Organisation*. Retrieved from https://inchem.org/documents/cicads/cicads/cicad55.htm#9.0 on April 27, 2021.

WHO. (2004). Nutrition Counselling, Care and Support for HIV-Infected Women : Guidelines on HIV-Related Care, Treatment and Support for HIV-Infected Women and their Children in Resource-Constrained Settings. In *World Health Organization*. Retrieved from http://whqlibdoc.who.int/publications/2004/9241592125.pdf on April 27, 2021

WHO. (2008). Worldwide Prevalence of Anaemia 1993 - 2005: WHO Global Database on Anaemia. *World Health Organisation*, 1–51. Retrieved from https://www.google.com/search?q=WHO%2C+Centers+for+Disease+Control.+Worldwide+prevalence+of+anaemia+1993–2005%3A+WHO+global+database+of+anaemia.+Geneva%2C+WHO%2C+2008.&oq=WHO%2C+Centers+for+Disease+Control.+Worldwide+prevalence+of+anaemia+1993–2005%3A+WHO on April 27, 2021.

WHO. (2009). *Global Health Risks: Mortality and Burden of Disease Attributable to Selected Major Risks*. Retrieved from https://apps.who.int/iris/handle/10665/44203 on April 27, 2021.

WHO. (2010a). Aluminium in drinking-water: Background Document for Development of WHO Guidelines for Drinking-Water Quality. In *World Health Organisation*. Retrieved from https://cdn.who.int/

media/docs/default-source/wash-documents/wash-chemicals/aluminium.pdf?sfvrsn=e54f4db9_4 on April 27, 2021.

WHO. (2010b). Persistent Organic Pollutants: Impact on Child Health. In *World Health Organisation*. Retrieved from https://www.who.int/publications/i/item/9789241501101 on April 12, 2021.

WHO. (2013). *World Health Statistics 2013: A Wealth of Information on Global Public Health*. Retrieved from https://apps.who.int/iris/bitstream/10665/82058/1/WHO_HIS_HSI_13.1_eng.pdf on April 27, 2021.

WHO. (2017). Mercury and health. In *World Health Organisation* (Issue March 2017). Retrieved from https://www.who.int/news-room/fact-sheets/detail/mercury-and-health on April 12, 2022.

WHO. (2018a). *Arsenic* (Issue February 2018). Retrieved from https://www.who.int/news-room/fact-sheets/detail/arsenic on April 12, 2022.

WHO. (2018b). Arsenic. In *World Health Organisation, Geneva* (Issue February). Retrieved from https://www.who.int/news-room/fact-sheets/detail/arsenic on April 27, 2022.

WHO. (2019). Exposure to arsenic: a major public health concern. *World Health Organisation*. Retrieved from https://iris.who.int/bitstream/handle/10665/329482/WHO-CED-PHE-EPE-19.4.1-eng.pdf?ua=1 on June 24, 2022.

WHO. (2020). Food safety : Persistent organic pollutants (POPs). In *World Health Organisation* (Issue November). Retrieved from https://www.who.int/news-room/questions-and-answers/item/food-safety-persistent-organic-pollutants-(pops) on March 20, 2023.

WHO. (2021). Inventory of evaluations performed by the Joint Meeting on Pesticide Residues (JMPR). In *World Health Organisation, Geneva*. Retrived from https://apps.who.int/pesticide-residues-jmpr-database/Home/Range/All on July 22, 2023.

WHO. (2023). Infant and young child feeding. In *World Health Organisation* (Issue December). Retrieved from https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding on May 25, 2024.

Wickstrom, K., Pyysalo, H., & Siimes, M. A. (1983). Levels of Chlordane , Hexachlorobenzene , PCB and DDT Compounds in Finnish Human Milk in 1982. *Bull. Environ. Contam. Toxicol.*, *256*, 251–256.

Wilhelm, M., Ewers, U., Marczynski, B., & Ranft, U. (2007). Human biomonitoring studies in North Rhine-Westphalia , Germany. *International Journal of Hygiene and Environmental Health*, *210*, 307–318. https://doi.org/10.1016/j.ijheh.2007.01.039

Winfield, S. A., Boyd, N. D., Vimy, M. J., & L., L. F. (1994). Measurement of total mercury in biological specimens by cold vapor atomic fluorescence. *Clin Chem*, *40*(2), 206–210.

Winiarska-Mieczan, A. (2014). Cadmium , Lead , Copper and Zinc in Breast Milk in Poland. *Biol Trace Elem Res*, *157*(1), 36–44. https://doi.org/10.1007/s12011-013-9870-x

Wolff, M. S., Fischbein, A. L. F., & Selikoff, I. J. (1992). Changes in PCB Serum Concentrations among Capacitor Manufacturing Workers. *Environmental Research*, *59*, 202–216.

World Health Organization. (1996). Trace Elements in Human Nutrition and Health. In *World Health Organisation, Geneva*. Retrieved from https://apps.who.int/iris/handle/10665/37931 on August 10, 2021.

Xia, Y., Hill, K. E., Byrne, D. W., Xu, J., & Burk, R. F. (2005). Effectiveness of Selenium Supplements in a Low-Selenium Area of China. *American Journal of Clinical Nutrition*, *81*(4), 829–834. https://doi.org/10.1093/ajcn/81.4.829

Xu, H., Croot, P., & Zhang, C. (2022). Exploration of the spatially varying relationships between lead and aluminium concentrations in the topsoil of northern half of Ireland using Geographically Weighted Pearson Correlation Coefficient. *Geoderma*, *409*(March), 1–10. https://doi.org/10.1016/j.geoderma.2021.115640

Yamawaki, N., Yamada, M., Kan-no, T., Ã, T. K., Kaneko, T., & Yonekubo, A. (2005). Macronutrient , Mineral and Trace Element Composition of Breast Milk from Japanese Women. *Journal of Trace Elements in Medicine and Biology*, *19*(2–3), 171–181. https://doi.org/10.1016/j.jtemb.2005.05.001

Yang, S., Xu, F., Wu, F., Wang, S., & Zheng, B. (2014). Science of the Total Environment Development of PFOS and PFOA criteria for the protection of freshwater aquatic life in China. *Science of the Total Environment*, *470–471*, 677–683. https://doi.org/10.1016/j.scitotenv.2013.09.094

Yoneyama, K., Ikeda, J., & Nagata, H. (1997). Interrelations of the Calcium Concentration in Breast Milk with Maternal Intake of Cow ' s Milk and Milk Products , Bone Resorption and Bone Mineral Density during

Lactation. *Japanese*, *51*(4), 770–779. https://doi.org/10.1265/jjh.51.770

Younes, B., Al-meshari, A. A., Al-hakeem, A., Al-saleh, S., Al-zamel, F., Al-shammari, F., & Alwarthan, A. (1995). Lead Concentration in Breast Milk of Nursing Mothers Living in Riyadh. *Annals of Saudi Medicine*, *15*(3), 249–251. https://pubmed.ncbi.nlm.nih.gov/17590578/

Yu, H., Zhu, Z., Zhao, X., Zhang, X., & Wang, D. (2003). Levels of Organochlorine Pesticides in Beijing Human Milk ,. *Bull. Environ. Contam. Toxicol.*, *70*, 193–197. https://doi.org/10.1007/s00128-002-0176-5

Yurdakök, K. (2015). Lead , mercury , and cadmium in breast milk. *Journal of Paedriatric and Neonatal Individualized Medicine*, *4*(2), 1–11. https://doi.org/10.7363/040223

Zareitalabad, P., Siemens, J., Wichern, F., & Amelung, W. (2013). Dose-dependent reactions of Aporrectodea caliginosa to perfluorooctanoic acid and perfluorooctanesulfonic acid in soil Ecotoxicology and Environmental Safety Dose-dependent reactions of Aporrectodea caliginosa to per fl uorooctanoic acid and per fl uorooc. *Ecotoxicology and Environmental Safety*, *95*(March), 39–43. https://doi.org/10.1016/j.ecoenv.2013.05.012

Zheng, G., Schreder, E., Dempsey, J. C., Uding, N., Chu, V., Andres, G., Sathyanarayana, S., & Salamova, A. (2021). Per- and Poly fl uoroalkyl Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS. *Environmental Science and Technology*, *55*(11), 7510–7520. https://doi.org/10.1021/acs.est.0c06978

Zoroddu, M. A., Aaseth, J., Crisponi, G., Peana, M., & Nurchi, V. M. (2019). The essential metals for humans: A brief overview. *Journal of Inorganic Biochemistry*, *195*, 120–129. https://doi.org/10.1016/j.jinorgbio.2019.03.013

APPENDICES

APPENDIX A

ESTIMATED TIMELINE AND BUDGET FOR THE STUDY AT THE HO TEACHING HOSPITAL

While preparing to conduct a study to determine and compare levels of toxic heavy metals and Persistent Organic Pollutants (POPs) in human breast milk, the researchers will be guided by the timelines below.

Conduct f the Study at Ho Teaching Hospital (January 2020 – December 2020)

Timelines based on 50 x 3 = 150 breast milk samples (for heavy metals analysis and 150 breast milk samples for POPs analysis) and infant formulas.

(50 participants will be recruited for the study; three different portions of breast milk will be collected from each participant at different stages of lactation namely, colostrum, transitional milk and mature milk, making a total of 150 samples).

1 – 3 Months: Basic organization of the study (January 2020 – March 2020)

Planning, organization, selection, training and orientation of research collaborators

4 – 8 months: Selection of participants and sample collection (April 2020 – August 2020)

Selection of participants, educating participants on the importance of breastfeeding, administering of questionnaires to participants, collection of breast milk samples, storage and preservation of samples.

9 – 12 months: Samples Preparation and Analysis (September 2020 – December 2020)

Extraction, cleaning and analysis of samples, data processing, results and discussion

Table of Estimated Human Resources and Costs for the conduct of study at HTH (Estimates based mainly on WHO/UNEP Coordinated Survey of Human Milk for Persistent Organic Pollutants with slight modifications)

Item	Estimated Human Resources and Costs	Justification
Project Personnel	Planning, coordination and training of collaborators of the study 90 hrs. Counselling training on breastfeeding 20 hrs: GH₵3000.00	The need to provide sufficient skills for supporting breastfeeding mothers as well as adequately educating and informing them on the relevance of the study to their wellbeing and that of their baby.
Documentation expenses	Translation, printing and distribution GH₵ 2500.00	Ethical clearance, production and printing of questionnaires, informed consent
Travels	Internal transportation = GH₵800.00	
Supplies/equipment	Collection jars 50 x 3 x12 = GH₵1,800.00 Breast pumps 50 x 3 x10 = GH₵1,500.00 Chemicals and glassware = GH₵3,000.00 Sample tubes 150 x 5 Gh = GH₵750.00	Chemicals and glassware would be required for the collection and extraction of breast milk samples.
Incentives for participants	50 Participants x GH₵25.00 = GH₵1250.00	To facilitate the sampling process.
Analysis	Analysis of Heavy metals from breast milk samples = 150 x 130 Gh = GH₵130 (i.e. analysis of one sample = 150 Gh; number of samples = 150). Analysis of POPs from breast milk samples = 150 x 120 Gh = GH₵18,000.00	Estimates given on analysis of POPs based on Fourth WHO/UNEP Coordinated Survey on Mother's Milk for Persistent Organic Pollutants
Miscellaneous expenses	GH₵1,000.00	
Total Estimate	**GH₵51, 850.00**	

APPENDIX B

INFORMED CONSENT FORM

(Adapted from Fourth WHO/UNEP Coordinated Survey on Mother' Milk for Persistent Organic Pollutants)

We are embarking on a study to determine levels of toxic heavy metals and Persistent Organic Pollutants (POPs) in human breast milk. The purpose and procedures of the study are outlined below.

Purpose of study

Among the contaminants in our natural environments are heavy metals and Persistent Organic Pollutants (POPs). Some heavy metals are useful to man whereas others are harmful. Heavy metals are generated through natural processes and man-made activities. Once released into the environment, these toxic heavy metals find their way into ground water and our food sources. These toxic heavy metals find their way into our bodies through the water we drink and the food we eat. While in our bodies, these toxic heavy metals can cause various health effects. In the case of mothers, these poisonous chemicals can be transferred to their foetus in the womb via the placenta and to the baby through breastfeeding. POPs on the other hand, are man-made lipophilic chemicals which can persist in the environment over a long period of time, often associated with fat-containing food substances, such as human breast milk. The Stockholm Convention has banned the production of POPs because of their ability to bioaccumulate and bio magnify in biological systems causing serious health effects.

The World Health Organization (WHO)/GEMS/Food Programme is helping many countries in the world to conduct a surveys to measures levels of POPs in human breast milk. In line with this objective, we are conducting this study to complement the efforts of the WHO to measure levels of toxic heavy metals and POPs in human breast milk and compare the concentrations in colostrum, transitional milk and mature milk.

While legitimate concerns about toxic heavy metals and POPs in human breast milk are raised, nevertheless, the evidence of the health benefits of

breastfeeding cannot be overemphasised. Therefore, no stones should be left unturned in breastfeeding your baby.

Procedures

We are requesting that you give us 60 ml sample of your breast milk over a period of three weeks, 20 ml at a time. Your breast milk samples will be analysed for the presence of aluminium, arsenic, cadmium, lead, mercury and the POPs. The milk may be collected either by manual expression or by the use of a breast pump.

Risk and Discomfort

The procedure entails no risk but you may experience a little discomfort when you express your milk manually by hand or using a breast pump. All the questions we will asked you will not be personal.

Compensation to Research Participants

There will be no compensation for taking part in the study. However, we will appreciate your time and input into the study by giving you a small gift.

Assurance of Confidentiality

All the information that we will gather from you for the purpose of this study will be held strictly confidential. The information will be stored in a file which will not bear your name but a code exclusively assigned to you. The name associated with the code assigned to your file shall be kept under lock and key and will not be disclosed to anyone except the Principal Investigator (Justice Wiston Amstrong Jonathan). In the event of unintentional disclosure, the consequences are not expected to be significant since your results will not include your name.

APPENDIX C

QUESTIONNAIRE FOR POTENTIAL HUMAN MILK DONORS

Section 1: Mother Personal Information

1. Age (in years)……

2. Have you had any formal education? Yes ☐ No ☐

3. If yes, what level?

☐ Basic ☐ J.S.S ☐ Secondary ☐ Vocational/ Technical

Tertiary ☐

4. Do you work? ☐ Yes ☐ No

5. If yes, what is your occupation?

☐ Civil Servant ☐ Teacher ☐ Lecturer ☐ Trader

Other (Place Specify)…… ……...

6. If you are a trader, what items do trade with?.....

7. Do you deal with any of the following wares? Battery ☐ Metal ☐

wares

Electrical equipment ☐ Other Specify……..... ☐

8. Do you often use any of the following items? Computers ☐

Printers ☐

9. Place of residence ……....

10. Since when have you been residing at your current place of residence?.....

11. Where were you living before your current place of residence.....

……

12. How long did you reside at your old place of residence?.....

13. Have you lived in your current area for 10 years? Yes ☐ No ☐

14. Do you live near incinerators, metal industries or where chemicals are produced?

Yes ☐ No ☐

Section 2: Mother Health History Information

15. Bodyweight during pregnancy (Kg)…… ……

16. Height (cm)…… ……..

17. BMI (Kg/m^2) after pregnancy…….....

18 . How would you describe your dietary habits before pregnancy?

Mixed diet ☐ Vegetarian but with milk and eggs ☐

Strictly vegetarian ☐

Other:…… …….

19. How often, on average, did you eat the following foods before pregnancy?

20. What type(s) of fish do you consume most often?

	Fish and fish products (e.g. tuna salad)	Marine mammals (e.g. whales, dolphins)	Seafood other than fish and marine mammals (e.g. shrimps, mussels)	Milk and milk products (e.g. cheese, butter, cream, yogurt)	Meat and poultry and derived products (e.g. sausage)	Eggs	
Never							
Less than once a week							
Once a week							
Twice a week							
More than twice a week but not every day							
Every day							

Fish from the sea ☐ Freshwater fish ☐ Both Canned fish ☐

Please state the species if known:…… ……

21. Do you smoke or sniff tobacco? Yes ☐ Never ☐

22. If yes, how would you describe your smoking or tobacco sniffing habit?

Occasionally ☐ Frequently ☐ Excessively ☐

23. Have you ever had exposure to tobacco smoke? Yes ☐ No ☐

24. What is the source of your drinking water? Well ☐ Tap water ☐

Other, specify ……

25. Number of Amalgam teeth filling……

26. Do you use lipstick? Yes ☐ No ☐

Section 3: Infant Related Information

27. Is this your first child? Yes ☐ No ☐

28. Parity……...

29. Gestational age (wks)…… ……

30. How old is your infant? less than 3 weeks ☐ 3-4 weeks ☐ 5-8 weeks ☐ more than 8 weeks ☐

31. For how long do you intend to breastfeed your kid?.....

32. Are you aware of the benefits of breastfeeding your kid?

Yes ☐ No ☐

33. Sex of infant……..

34. Bodyweight at birth…….

35.Lactation stage……

APPENDIX D
LEVELS OF PFAS (ug/L) IN BREAST MILK SAMPLES AND SOCIO-DEMOGRAPHIC CHARACTERISTICS

PFHxA	PFHpA	PFOA	PFOS	Age (Yrs)	Mass (kg)	Height (cm)	BMI (kg/m2)	Parity	Education	Employment. Status	Inf. Sex	Gest. Age
LOD	2.90	LOD	LOD	20.00	103.00	165.00	37.83	MP	Tertiary	Civil Servant	F	39
6.40	5.60	78.00	81.00	26.00	61.60	167.00	22.09	MP	JHS	Other	M	40
4.90	4.50	67.00	87.00	33.00	75.00	165.00	27.55	MP	Tertiary	Civil Servant	M	40
LOD	6.50	83.00	92.00	28.00	82.10	163.00	30.90	MP	Tertiary	Civil Servant	M	39
LOD	LOD	68.00	98.00	30.00	90.00	165.00	33.08	MP	Secondary	Other	F	39
5.50	LOD	73.00	97.00	30.00	74.00	158.00	29.64	MP	Tertiary	Teacher	F	40
5.70	4.50	74.00	92.00	38.00	74.00	153.00	31.61	MP	Tertiary	Civil Servant	M	38
LOD	LOD	LOD	LOD	29.00	85.20	156.00	35.01	MP	Tertiary	Teacher	F	40
LOD	6.70	6.60	96.00	32.00	73.00	132.00	41.89	MP	JHS	Other	F	39
LOD	7.10	90.00	95.00	30.00	80.00	144.00	38.58	PP	JHS	Other	M	40
LOD	LOD	LOD	LOD	32.00	86.00	167.00	30.84	MP	Tertiary	Civil Servant	F	39
7.80	5.50	64.00	98.00	26.00	61.60	167.00	22.09	MP	JHS	Other	M	40
8.90	7.80	73.00	92.00	37.00	78.40	164.50	29.01	MP	JHS	Civil Servant	F	41
5.10	9.70	56.00	94.00	22.00	67.00	157.00	27.18	MP	JHS	Other	M	39
7.70	3.40	58.00	88.00	31.00	59.00	159.00	23.34	PP	Tertiary	Teacher	F	39
LOD	LOD	57.00	89.00	35.00	73.00	166.00	26.49	MP	Tertiary	Trader	F	40
LOD	LOD	65.00	90.00	23.00	74.00	165.00	27.18	MP	JHS	Other	M	40
LOD	6.50	67.00	87.00	35.00	86.00	161.50	32.97	MP	Secondary	Trader	M	39
5.80	LOD	68.00	86.00	21.00	75.00	152.00	32.46	MP	JHS	Trader	M	39
6.10	LOD	67.00	93.00	36.00	65.40	168.00	23.17	MP	Secondary	Other	F	41
LOD	4.30	69.00	97.00	26.00	96.80	151.00	42.45	MP	JHS	Trader	M	36
LOD	5.70	72.00	88.00	21.00	69.80	166.50	25.18	PP	Tertiary	Student	M	40
LOD	LOD	LOD	LOD	28.00	90.10	166.00	32.69	MP	JHS	Trader	M	41
5.40	4.90	93.00	89.00	24.00	70.40	155.00	29.30	MP	Secondary	Teacher	M	38
7.50	5.40	79.00	98.00	31.00	78.00	156.00	32.05	MP	Secondary	Other	F	38
LOD	LOD	83.00	97.00	44.00	56.00	147.70	25.67	MP	No	Other	F	38
LOD	LOD	85.00	190.00	31.00	90.00	152.00	38.95	MP	Tertiary	Trader	M	39
LOD	LOD	93.00	99.00	33.00	74.90	164.50	27.68	MP	Secondary	Trader	F	33
LOD	LOD	87.00	98.00	33.00	110.80	163.00	41.70	MP	Tertiary	Civil Servant	M	42

APPENDIX E

LEVELS OF PFAS (NG/ML) IN HUMAN BREAST MILK

Sample ID	PFHxA	PFHpA	PFOA	PFOS
CM12	BDL	0.0029	BDL	BDL
CM20	0.0064	0.0056	0.078	0.081
CM23	0.0049	0.0045	0.067	0.087
CM27	BDL	0.0065	0.083	0.092
CM29	BDL	BDL	0.068	0.098
CM49B	0.0055	BDL	0.073	0.097
CM49H	0.0057	0.0045	0.074	0.092
CM64	BDL	BDL	BDL	BDL
CM65	BDL	0.0067	0.066	0.096
CM66	BDL	0.0071	0.090	0.095
CM67	BDL	BDL	BDL	BDL
MM12	0.0078	0.0055	0.064	0.098
MM20	0.0089	0.0078	0.073	0.092
MM29	0.0051	0.0097	0.056	0.094
MM40	0.0078	0.0034	0.058	0.088
MM46	BDL	BDL	0.057	0.089
MM49	BDL	BDL	0.065	0.090
MM53	BDL	0.0065	0.067	0.087
MM54	0.0058	BDL	0.068	0.086
MM55	0.0061	BDL	0.067	0.093
MM64	BDL	0.0043	0.069	0.097
MM66	BDL	0.0057	0.072	0.088
MM67	BDL	BDL	BDL	BDL
TM12	0.0054	0.0049	0.093	0.089
TM20	0.0075	0.0054	0.079	0.098
TM34	BDL	BDL	0.083	0.097
TM46	BDL	BDL	0.085	0.19
TM54	BDL	BDL	0.093	0.099
TM66	BDL	BDL	0.087	0.098

*BDL = below detection limit;

Internal standard used: ^{13}C-PFOA

Source: Laboratory Data (2022)

APPENDIX F

QUALITY ASSURANCE PROTOCOL FOR THE DETERMINATION OF PFAS IN BREAST MILK

Analyte	LoD (ng/mL)	LoQ (ng/mL)	Linearity (R^2)	Av. Recovery(%) spiked sample at 50 ng/mL
Perfluoroalkyl carboxylic acids (PFCAs)				
Perfluorobutanoic acid (PFBA)	0.010	0.034	0.9990	86
Perfluoropentanoic acid (PFPeA)	0.011	0.036	0.9985	98
Perfluorohexanoic acid (PFHxA)	0.0013	0.0043	0.9971	100
Perfluoroheptanoic acid (PFHpA)	0.0081	0.027	0.9967	110
Perfluorooctanoic acid (PFOA)	0.02	0.065	0.9981	112
Perfluorononanoic acid (PFNA)	0.023	0.076	0.9919	106
Perfluorodecanoic acid (PFDA)	0.10	0.34	0.9971	100
Perfluoroundecanoic acid (PFUA)	0.41	1.38	0.9909	104
Perfluorododecanoic acid (PFDoA)	0.624	2.08	0.9967	110
Perfluorotridecanoic acid (PFTrDA)	0.016	0.053	0.9950	93
Perfluoroalkyl sulfonic acids (PFSAs)				
Perfluorobutane sulfonic acid (PFBS)	0.27	0.89	0.9990	83
Perfluorohexane sulfonic acid (PFHxS)	0.014	0.045	0.9976	97
Perfluorooctane sulfonic acid (PFOS)	0.024	0.079	0.9911	107
Perfluorodecane sulfonic acid (PFDS)	1.13	3.76	0.9923	105

APPENDIX G

RETENTION TIME (RT) OF PFAS IN BREAST MILK ANALYSIS

Analyte	Retention time/min
Perfluoroalkyl carboxylic acids (PFCAs)	
Perfluorobutanoic acid (PFBA)	2.46
Perfluoropentanoic acid (PFPeA)	4.03
Perfluorohexanoic acid (PFHxA)	4.81
Perfluoroheptanoic acid (PFHpA)	5.28
Perfluorooctanoic acid (PFOA)	5.57
Perfluorononanoic acid (PFNA)	5.63
Perfluorodecanoic acid (PFDA)	6.00
Perfluoroundecanoic acid (PFUA)	6.05
Perfluorododecanoic acid (PFDoA)	6.16
Perfluorotridecanoic acid (PFTrDA)	6.38
Perfluoroalkyl sulfonic acids (PFSAs)	
Perfluorobutane sulfonic acid (PFBS)	4.30
Perfluorohexane sulfonic acid (PFHxS)	5.30
Perfluorooctane sulfonic acid (PFOS)	5.85
Perfluorodecane sulfonic acid (PFDS)	6.31

Source: Laboratory Data (2022)

APPENDIX H
RESULTS OF HEALTH RISKS ASSESSMENT OF HEAVY METALS AT LACTATIONAL STAGES

Table H1: Health Risks Assessment of Toxic Heavy Metals in the Colostrum of Mothers

Element	As	Al	Pb	Cu	Hg	Cd
Conc. (mg/L)	3.81E-02	1.04E-01	8.96E-03	5.80E-01	5.80E-03	4.40E-03
Child ADD_{Ing}	4.06E-04	1.11E-03	9.55E-05	6.18E-03	6.18E-05	4.69E-05
HQ Child	1.35E-01	9.22E+00	2.65E-02	2.81E+02	3.86E-01	4.69E-02
CR Child	6.09E-04		8.12E-07			7.03E-04

Table H2: Health Risks Assessment of Toxic Heavy Toxic Metals in Transitional Milk of Mothers

Element	As	Al	Pb	Cu	Hg	Cd
Conc. (mg/L)	3.55E-02	1.23E-01	3.68E-02	5.50E-01	5.60E-03	2.60E-03
Child ADD_{Ing}	3.78E-04	1.31E-03	3.92E-04	5.86E-03	5.97E-05	2.77E-05
HQ Child	1.26E-01	1.09E+01	1.09E-01	2.66E+02	3.73E-01	2.77E-02
CR Child	5.67E-04		3.33E-06			4.16E-04

Table H3: Health Risks Assessment of Toxic Heavy Metals in Mature Breast Milk of Mothers

Element	As	Al	Pb	Cu	Hg	Cd
Conc. (mg/L)	3.83E-02	1.05E-01	8.80E-03	4.50E-01	5.50E-03	2.60E-03
Child ADD_{Ing}	4.08E-04	1.12E-03	9.38E-05	4.80E-03	5.86E-05	2.77E-05
HQ Child	1.36E-01	9.32E+00	2.61E-02	2.18E+02	3.66E-01	2.77E-02
CR Child	6.12E-04		7.97E-07			4.16E-04

Table H4: Health Risks Assessment of Toxic Heavy Metals in the Total Breast Milk of Mothers

Element	**As**	**Al**	**Pb**	**Cu**	**Hg**	**Cd**
Conc. (mg/L)	3.69E-02	1.08E-01	1.78E-02	5.20E-01	5.60E-03	2.60E-03
Child ADD_{Ing}	3.93E-04	1.15E-03	1.90E-04	5.54E-03	5.97E-05	2.77E-05
HQ Child	1.31E-01	9.61E+00	5.27E-02	2.52E+02	3.73E-01	2.77E-02
CR Child	5.90E-04		1.61E-06			4.16E-04

Source: Laboratory Data (2022)

Interpretations:

Child ADD_{Ing} = Average daily dose of toxic heavy metals ingested by a child through breastfeeding

HQ Child = Hazard quotient of toxic heavy metals for a child

CR = Cancer risk for a child through consumption of toxic heavy metals in breast

CR could not be calculated for Al, Cu and Hg because cancer slope factor for these heavy metals could not be found in literature.

HQ < 1 implies no significant non cancer health risks for infants while HQ > 1 means significant non cancer health risks.

$CR \leq 10^{-4}$ means no significant cancer risks of toxic heavy metals to infants through consumption of breast milk.

$CR > 10^{-4}$ means significant cancer risks of toxic heavy metals for infants.

APPENDIX I
CONCENTRATIONS OF TOXIC HEAVY METALS IN COLOSTRUM (µg/L)

	Elements				
Participants	**Al**	**As**	**Cd**	**Pb**	**Hg**
1	0.0729	0.2170	0.0032	0.0075	BDL
2	0.0620	0.0380	BDL	0.0081	BDL
3	0.2670	BDL	BDL	BDL	0.0055
4	0.8723	0.0390	0.0024	0.0079	BDL
5	0.0656	0.0405	BDL	0.0089	0.0058
6	BDL	0.0227	0.0031	0.0077	BDL
7	0.0742	0.0232	BDL	0.0084	BDL
8	0.1246	0.0222	BDL	0.0154	0.0051
9	0.1119	0.0145	0.0031	0.0097	0.0054
10	0.0580	BDL	BDL	0.0076	BDL
11	0.0642	0.0214	0.0025	0.0079	0.0052
12	BDL	0.0350	BDL	0.0322	BDL
13	0.1112	0.0122	0.0035	0.0097	BDL
14	0.0814	0.0215	BDL	0.0083	0.0063
15	0.0767	0.0371	BDL	0.0091	BDL
16	0.0656	BDL	0.0022	0.008	0.0053
17	0.0564	0.0222	BDL	0.0081	0.0056
18	0.1102	0.1223	0.0032	0.0094	0.0058
19	0.0745	0.0111	BDL	0.0074	BDL
20	BDL	0.0412	BDL	0.0096	BDL
21	0.0812	0.0214	0.0027	0.0075	BDL
22	0.1100	0.0128	BDL	0.0087	BDL
23	0.0522	BDL	BDL	0.0093	0.0051
24	0.0912	0.0177	BDL	0.0088	BDL
25	0.0657	0.0390	0.028	0.0075	0.0053
26	BDL	0.0705	BDL	0.0094	BDL
27	0.0814	0.0352	BDL	0.0083	0.0055
28	0.0554	0.0444	BDL	0.0099	0.0077
29	0.0578	0.0407	BDL	0.0076	0.0055
30	0.1224	0.0361	0.0034	0.0082	BDL
31	0.0880	0.0361	BDL	BDL	0.0055
32	0.0675	BDL	0.0026	0.00725	BDL
33	BDL	0.0453	BDL	0.0072	0.0076
34	0.0924	0.0446	BDL	0.0081	BDL
35	0.0584	0.0358	0.0028	0.0075	0.0055
36	0.0798	0.0289	BDL	BDL	0.0075
37	0.1220	0.0343	BDL	0.0088	BDL

38	0.0501	0.0435	0.0032	0.0072	0.0059
39	BDL	0.0245	BDL	BDL	0.0055
40	0.0725	0.0364	BDL	0.0078	BDL
41	0.0827	0.0113	BDL	0.0071	0.0054
42	0.1045	0.0321	0.0031	BDL	0.0075
43	0.1124	BDL	0.0035	0.0091	BDL
44	0.0542	0.0342	BDL	0.0076	0.0055
45	0.0788	0.0246	BDL	0.0073	BDL
46	0.0721	0.0346	0.0024	0.0074	0.0052
47	0.0534	0.0349	0.028	0.0077	BDL
Average	0.1038	0.0381	0.0057	0.0089	0.0058
Maximum	0.8723	0.2170	0.028	0.0322	0.0077
Minimum	0.0501	0.0111	0.0022	0.0071	0.0051

APPENDIX J
CONCENTRATIONS OF TOXIC HEAVY METALS IN TRANSITIONAL MILK (µg/L)

	Elements				
Participants	**Al**	**As**	**Cd**	**Pb**	**Hg**
1	0.0510	0.2900	0.0025	0.0072	BDL
2	0.0570	0.0240	BDL	0.0092	BDL
3	0.1880	BDL	BDL	BDL	0.0052
4	0.0894	0.0406	0.0022	0.0082	BDL
5	BDL	0.0352	BDL	0.0091	BDL
6	0.1546	0.0312	0.0025	BDL	BDL
7	0.0782	BDL	BDL	0.0089	0.0054
8	0.1542	0.0236	0.0029	0.0178	BDL
9	0.1124	0.0209	0.0026	0.0131	0.0053
10	BDL	0.0310	BDL	0.0082	BDL
11	0.7290	BDL	0.0022	BDL	BDL
12	BDL	0.0137	BDL	0.0076	BDL
13	0.1122	0.0124	0.0028	0.0091	BDL
14	0.0822	0.0200	BDL	0.0085	0.0059
15	0.7892	0.0377	BDL	BDL	BDL
16	0.0664	BDL	0.0021	0.0077	0.0052
17	BDL	0.0234	BDL	0.0084	0.0052
18	0.1210	0.0124	0.0029	0.0092	0.0057
19	0.0754	0.0126	BDL	0.0076	BDL
20	0.0612	0.0324	BDL	BDL	BDL
21	0.0822	0.0235	0.0026	0.0077	0.0066
22	0.1210	BDL	BDL	0.0084	0.0055
23	BDL	0.0217	BDL	0.0089	0.0052
24	0.0942	0.0174	0.0022	0.0078	BDL
25	0.0666	0.0406	BDL	0.0079	0.0052
26	BDL	0.0352	BDL	BDL	BDL
27	0.0822	BDL	BDL	0.0078	0.0051
28	0.0563	0.0346	0.0024	0.0097	0.0074
29	0.0668	0.0334	BDL	0.0073	0.0053
30	0.1142	0.0338	BDL	0.0085	BDL
31	0.0867	0.0352	BDL	BDL	0.0053
32	0.0665	BDL	0.0028	0.0071	BDL
33	0.0544	0.0506	BDL	0.0074	0.0066
34	0.0896	0.0458	BDL	0.0079	BDL
35	BDL	0.0432	BDL	BDL	0.0053
36	0.0811	0.0256	0.0023	BDL	0.0064
37	0.1224	0.0320	BDL	0.0082	BDL

38	0.0504	0.0333	BDL	0.0075	0.0055
39	0.0662	0.0157	BDL	BDL	0.0052
40	BDL	0.0351	0.0031	0.0084	BDL
41	0.0867	0.0118	BDL	0.0074	0.0052
42	0.1054	0.0341	BDL	BDL	0.0055
43	0.1223	BDL	0.0029	0.0088	BDL
44	0.0558	0.0354	BDL	0.9974	0.0051
45	0.0798	0.0244	BDL	0.0078	BDL
46	0.0723	0.0313	0.0027	BDL	0.0051
47	0.0544	0.0282	BDL	0.0074	BDL
Average	0.1230	0.0355	0.0026	0.0368	0.0056
Maximum	0.7892	0.2900	0.0031	0.9974	0.0074
Minimum	0.0504	0.0118	0.0021	0.0071	0.0051

APPENDIX K
CONCENTRATIONS OF TOXIC HEAVY METALS IN MATURE MILK (µg/L)

	Elements				
Participants	**Al**	**As**	**Cd**	**Pb**	**Hg**
1	0.0492	0.2930	0.0022	0.0073	BDL
2	0.0550	0.0270	BDL	0.0098	BDL
3	0.2350	0.0520	BDL	BDL	0.0051
4	0.0899	BDL	0.0023	0.0088	BDL
5	BDL	0.0332	BDL	0.0095	0.0054
6	0.1558	0.0136	0.0022	BDL	BDL
7	0.0798	0.0245	BDL	0.0142	0.0053
8	0.1821	0.0221	0.0027	0.0212	BDL
9	0.1211	BDL	0.0022	BDL	0.0052
10	0.0545	0.0172	BDL	0.0121	BDL
11	0.2170	0.0450	0.0021	BDL	0.0055
12	BDL	0.0202	BDL	0.0074	BDL
13	0.1142	0.0184	0.0026	0.0088	BDL
14	0.0842	BDL	0.0021	0.0087	0.0057
15	0.0788	0.0400	BDL	0.0086	0.0055
16	0.0678	0.0132	0.0023	BDL	BDL
17	0.0662	BDL	BDL	0.0087	0.0051
18	BDL	0.0128	0.0027	0.0091	0.0062
19	0.0755	0.0130	BDL	0.0078	BDL
20	0.0622	BDL	BDL	0.0092	BDL
21	0.0834	0.0226	0.0026	0.0078	0.0057
22	0.1221	0.0195	BDL	BDL	0.0052
23	BDL	0.0242	BDL	0.0087	0.0051
24	0.0843	BDL	0.0031	0.0077	BDL
25	0.0674	0.0360	BDL	0.0082	0.0051
26	BDL	0.0332	0.0028	BDL	0.0058
27	0.0825	0.0666	BDL	0.0075	0.0053
28	0.0566	0.0404	BDL	0.0093	0.0069
29	0.0689	BDL	0.0023	0.0072	0.0051
30	0.1254	0.0324	BDL	0.0083	BDL
31	BDL	0.0358	BDL	BDL	0.0051
32	0.0552	0.0344	0.003	0.0076	BDL
33	0.0548	0.0621	BDL	0.0077	0.0061
34	0.0811	BDL	BDL	0.0076	BDL
35	0.0542	0.0445	BDL	0.0074	0.0051
36	BDL	0.0296	0.0034	BDL	0.0058
37	0.1302	0.0359	BDL	0.0074	BDL

38	0.0512	0.0578	BDL	0.0071	0.0053
39	0.0675	BDL	0.0035	BDL	0.0051
40	0.0775	0.0326	BDL	0.0088	BDL
41	BDL	0.0122	BDL	0.0072	0.0051
42	0.1088	0.0344	BDL	BDL	0.0065
43	0.1254	BDL	0.0031	0.0083	BDL
44	0.5642	0.0368	BDL	0.0071	0.0052
45	0.0811	0.0254	BDL	BDL	BDL
46	BDL	0.0277	0.0022	0.0072	0.0053
47	0.0547	0.0291	BDL	0.0076	BDL
Average	0.1049	0.0383	0.0026	0.0088	0.0055
Maximum	0.5642	0.2930	0.0035	0.0212	0.0069
Minimum	0.0492	0.0122	0.0021	0.0071	0.0051

APPENDIX L
CONCENTRATIONS OF TOXIC HEAVY METALS IN TOTAL BREAST MILK (µg/L)

	Elements				
Participants	**Al**	**As**	**Cd**	**Pb**	**Hg**
1	0.0577	0.2667	0.0026	0.0073	BDL
2	0.0580	0.0297	BDL	0.0090	BDL
3	0.2300	BDL	BDL	BDL	0.0053
4	0.3505	0.0385	0.0023	0.0083	BDL
5	0.0681	0.0363	BDL	0.0092	0.0056
6	BDL	0.0225	0.0026	0.0082	BDL
7	0.0774	0.0198	BDL	0.0105	0.0054
8	0.1536	0.0226	0.0028	0.0181	0.0051
9	0.1151	0.0180	0.0026	0.0113	0.0053
10	0.0560	BDL	BDL	0.0093	BDL
11	0.3367	0.0355	0.0023	0.0348	0.0054
12	BDL	0.0230	BDL	0.0157	BDL
13	0.1125	0.0143	0.0030	0.0092	BDL
14	0.0826	0.0244	0.0021	0.0085	0.0060
15	0.3149	0.0383	BDL	0.0089	0.0055
16	0.0666	BDL	0.0022	0.0077	0.0053
17	0.0604	0.0233	BDL	0.0084	0.0053
18	0.1177	0.0492	0.0029	0.0092	0.0059
19	0.0751	0.0122	BDL	0.0076	BDL
20	BDL	0.0297	BDL	0.0094	BDL
21	0.0823	0.0225	BDL	0.0077	0.0062
22	0.1177	0.0165	BDL	0.0084	0.0054
23	0.0515	BDL	BDL	0.0090	0.0051
24	0.0899	0.0174	0.0027	0.0081	BDL
25	0.0666	0.0385	BDL	0.0079	0.0052
26	BDL	0.0463	0.0029	0.0089	0.0058
27	0.0820	0.0454	BDL	0.0079	0.0053
28	0.0561	0.0398	BDL	0.0096	0.0073
29	0.0645	0.0356	BDL	0.0074	0.0053
30	0.1207	0.0341	0.0031	0.0083	BDL
31	0.0875	0.0357	BDL	BDL	0.0053
32	0.0631	BDL	BDL	0.0073	BDL
33	BDL	0.0527	0.0022	0.0074	0.0068
34	0.0877	0.0529	BDL	0.0079	BDL
35	0.0560	0.0412	BDL	0.0075	0.0053
36	0.0808	0.0280	0.0028	BDL	0.0066
37	0.1249	0.0341	BDL	0.0081	BDL
38	0.0506	0.0449	BDL	0.0073	0.0056
39	BDL	0.0211	BDL	BDL	0.0053
40	0.0747	0.0347	BDL	0.0083	BDL
41	0.0865	0.0118	0.0023	0.0072	0.0052
42	0.1062	0.0335	BDL	BDL	0.0065
43	0.1200	BDL	BDL	0.0087	BDL
44	0.2247	0.0355	BDL	0.3374	0.0053

45	0.0799	0.0248	BDL	0.0078	BDL
46	0.0739	0.0312	0.0025	0.0312	0.0052
47	0.0542	0.0307	BDL	0.0076	BDL
Average	0.1082	0.0369	0.0026	0.0178	0.0056
Maximum	0.3505	0.2667	0.0031	0.3374	0.0073
Minimum	0.0506	0.0118	0.0021	0.0072	0.0051

APPENDIX M
LEVELS OF ESSENTIAL ELEMENTS IN THE COLOSTRUM MILK (mg/L)

	Essential Elements									
Participants	**Ca**	**Cu**	**Fe**	**Mg**	**P**	**Na**	**K**	**S**	**Se**	**Zn**
1	325.00	0.81	2.24	23.80	108.00	626.56	815.00	317.00	0.16	5.79
2	234.00	0.65	1.80	26.70	181.00	277.40	673.00	148.00	0.12	3.59
3	226.00	0.44	1.20	26.50	172.00	444.00	675.00	201.00	0.12	5.32
4	251.00	0.37	2.40	27.40	138.00	398.00	643.00	148.00	0.11	7.37
5	286.00	0.59	1.81	23.70	208.00	434.00	528.00	188.00	0.08	2.62
6	172.00	0.89	1.42	36.30	140.00	565.00	540.00	188.00	0.09	2.24
7	221.00	0.56	2.81	24.30	198.00	210.00	680.00	182.00	0.09	2.68
8	148.00	0.92	1.85	21.90	140.00	388.00	640.00	238.00	0.10	5.40
9	149.00	0.59	2.12	18.40	190.00	229.00	548.00	160.00	0.12	2.29
10	278.00	0.54	1.11	18.90	244.00	582.00	609.00	218.00	0.10	2.42
11	242.00	0.76	1.80	23.60	236.00	621.00	721.00	233.00	0.13	3.44
12	311.00	0.58	1.86	23.20	224.00	590.00	645.00	244.00	0.09	4.55
13	322.00	0.67	2.20	24.70	204.00	543.00	604.00	214.00	0.09	2.88
14	256.00	0.76	1.53	29.60	124.00	564.00	712.00	286.00	0.12	4.55
15	324.00	0.63	1.76	28.30	86.00	616.95	624.00	307.00	0.17	5.88
16	342.00	0.68	1.55	22.80	244.00	528.00	664.00	228.00	0.09	4.21
17	241.00	0.58	1.28	26.10	271.00	338.00	509.00	277.00	0.17	5.15
18	228.00	0.43	2.15	27.90	176.00	542.00	645.00	226.00	0.08	2.34
19	286.00	0.72	1.22	18.90	282.00	486.00	587.00	146.00	0.13	3.55
20	288.00	0.49	1.50	23.50	204.00	432.00	518.00	178.00	0.08	2.45
21	302.00	0.81	2.42	20.80	188.00	543.00	698.00	208.00	0.08	6.12
22	248.00	0.44	1.08	18.70	248.00	448.00	684.00	301.00	0.13	2.48
23	221.00	0.39	1.52	25.90	271.00	568.00	616.00	211.00	0.12	3.44
24	246.00	0.67	1.98	23.10	227.00	538.00	588.00	221.00	0.08	5.42
25	202.00	0.11	1.40	20.60	183.00	222.00	564.00	138.00	0.10	3.78
26	444.00	0.47	0.84	23.40	165.00	231.00	703.00	227.00	0.18	4.45
27	228.00	0.40	1.20	22.10	176.00	253.00	651.00	146.00	0.08	2.38
28	121.00	0.47	0.96	19.70	130.00	490.00	498.00	173.00	0.12	3.95
29	191.00	0.52	1.25	26.00	166.00	364.00	444.00	160.00	0.09	3.95
30	190.00	0.56	1.54	24.00	186.00	196.00	608.00	172.00	0.09	2.47
31	212.00	0.55	0.92	28.10	136.00	556.00	530.00	188.00	0.08	2.22
32	149.00	0.75	1.04	22.70	134.00	387.00	604.00	237.00	0.08	5.20
33	219.00	0.82	1.45	28.80	228.00	542.00	609.00	248.00	0.11	2.35
34	216.00	0.45	1.54	21.70	242.00	542.00	568.00	201.00	0.09	4.45
35	233.00	0.56	0.85	27.10	170.00	272.71	637.00	146.00	0.11	4.00
36	285.00	0.67	0.88	28.30	242.00	386.00	648.00	221.00	0.09	3.22
37	150.00	0.57	0.69	18.40	181.00	227.00	543.00	150.00	0.07	2.08
38	188.00	0.39	0.82	20.40	222.00	248.00	544.50	174.00	0.14	2.88
39	202.00	0.67	0.98	23.90	212.00	222.00	442.00	204.00	0.08	4.28
40	133.00	0.48	0.82	18.40	173.00	238.00	511.00	142.00	0.09	1.55
41	188.00	0.45	0.97	21.80	212.00	244.00	588.00	201.00	0.20	2.89
42	308.00	0.72	0.86	19.80	247.00	221.00	578.00	227.00	0.09	5.26
43	221.00	0.66	0.77	21.00	202.00	344.00	448.00	221.00	0.21	4.62
44	590.00	0.79	0.97	23.10	253.00	198.00	558.00	154.00	0.07	1.36
45	188.00	0.54	0.88	28.20	234.00	255.00	575.00	178.00	0.07	3.88
46	242.00	0.30	0.86	26.30	145.00	442.09	672.00	199.00	0.11	5.23
47	242.00	0.36	0.62	27.40	139.00	391.00	634.00	146.00	0.12	5.41
Average	244.45	0.58	1.40	23.96	193.23	403.91	602.63	202.57	0.11	3.79
Maximum	590.00	0.92	2.81	36.30	282.00	626.56	815.00	317.00	0.21	7.37
Minimum	121.00	0.11	0.62	18.40	86.00	196.00	442.00	138.00	0.07	1.36

APPENDIX N
LEVEL OF ESSENTIAL ELEMENTS IN THE TRANSITIONAL MILK (mg/L)

	Essential Elements									
Participants	**Ca**	**Cu**	**Fe**	**Mg**	**P**	**Na**	**K**	**S**	**Se**	**Zn**
1	284.00	0.64	1.81	24.50	148.00	385.25	642.00	281.00	0.12	4.72
2	223.00	0.59	1.50	25.80	168.00	289.45	603.00	134.00	0.11	4.12
3	202.00	0.36	1.40	27.20	163.00	408.00	663.00	161.00	0.11	4.21
4	248.00	0.35	1.40	27.10	139.00	297.00	594.00	104.00	0.10	5.42
5	242.00	0.49	1.31	23.50	161.00	432.00	392.00	142.00	0.07	2.44
6	190.00	0.82	1.10	32.90	156.00	462.00	521.00	196.00	0.08	2.11
7	210.00	0.52	1.72	25.80	174.00	196.00	544.00	156.00	0.08	2.32
8	192.00	0.75	1.24	22.80	190.00	254.00	545.00	194.00	0.09	3.99
9	154.00	0.47	1.86	19.30	166.00	201.00	452.00	122.00	0.09	2.08
10	221.00	0.48	1.45	17.98	201.00	487.00	587.00	181.00	0.09	2.26
11	308.00	0.63	1.87	23.00	211.00	509.00	688.00	198.00	0.12	3.24
12	298.00	0.66	1.52	22.90	201.00	487.00	521.00	201.00	0.08	4.20
13	305.00	0.45	1.44	23.60	188.00	420.00	552.00	164.00	0.08	2.42
14	245.00	0.61	1.33	28.40	158.00	544.00	644.00	184.00	0.11	3.45
15	248.00	0.84	1.24	25.80	138.00	358.69	816.00	181.00	0.21	4.17
16	286.00	0.45	1.31	19.60	163.00	424.00	577.00	174.00	0.08	3.44
17	184.00	0.47	1.44	32.50	222.00	308.00	442.00	148.00	0.19	4.24
18	176.00	0.68	1.84	24.20	148.00	448.00	582.00	217.00	0.09	2.12
19	268.00	0.55	1.85	20.88	155.00	442.00	542.00	124.00	0.09	2.84
20	212.00	0.59	1.21	23.50	165.00	434.00	380.00	132.00	0.07	2.62
21	276.00	0.64	1.81	23.30	153.00	418.00	528.00	187.00	0.07	4.65
22	221.00	0.58	1.22	21.20	218.00	387.00	575.00	284.00	0.09	2.21
23	188.00	0.35	1.65	23.50	186.00	554.00	502.00	174.00	0.11	3.21
24	221.00	0.54	1.24	28.40	189.00	420.00	542.00	206.00	0.07	4.22
25	164.00	0.66	0.75	25.10	130.00	1008.00	321.00	181.00	0.08	2.63
26	220.00	0.33	0.65	20.60	170.00	162.00	552.00	119.00	0.08	3.29
27	188.00	0.45	0.78	21.90	152.00	247.00	507.00	111.00	0.09	1.35
28	246.00	0.45	0.74	24.80	148.00	261.00	563.00	139.00	0.09	3.82
29	184.00	0.58	1.04	19.80	150.00	191.00	359.00	128.00	0.08	3.82
30	210.00	0.50	0.87	25.80	171.00	164.00	543.00	146.00	0.08	2.32
31	211.00	0.81	0.85	36.30	156.00	461.00	511.00	195.00	0.09	2.01
32	217.00	0.56	0.85	21.90	189.00	253.00	552.00	127.00	0.07	2.99
33	201.00	0.65	0.85	24.20	186.00	421.00	578.00	205.00	0.09	2.21
34	182.00	0.47	1.25	22.10	208.00	443.00	502.00	178.00	0.09	3.21
35	231.00	0.55	1.22	27.80	166.00	298.04	630.00	136.00	0.11	4.15
36	219.00	0.58	0.76	27.90	188.00	333.00	555.00	182.00	0.08	2.55
37	149.00	0.49	0.58	18.30	186.00	203.00	447.00	118.00	0.08	1.77
38	148.00	0.32	0.65	21.70	168.00	207.00	480.00	166.00	0.12	2.10
39	155.00	0.58	0.75	22.80	178.00	201.00	388.00	199.00	0.07	3.85
40	180.00	0.54	0.78	21.40	193.00	202.00	452.00	111.00	0.08	2.27
41	167.00	0.42	0.88	22.10	194.00	210.00	452.00	182.00	0.12	2.22
42	211.00	0.68	0.72	21.40	186.00	207.00	465.00	188.00	0.08	3.88
43	189.00	0.45	0.64	20.80	181.00	228.00	421.00	141.00	0.11	3.32
44	213.00	0.91	0.95	16.90	240.00	326.00	565.00	208.00	0.08	0.91
45	226.00	0.63	0.74	22.90	237.00	138.00	455.00	120.00	0.08	2.28
46	286.00	0.41	0.78	27.10	171.00	406.37	636.00	151.00	0.11	3.55
47	248.00	0.30	0.58	26.20	144.00	296.58	549.00	101.00	0.10	7.37
Average	218.02	0.55	1.16	23.99	175.60	349.63	530.15	165.47	0.09	3.16
Maximum	308.00	0.91	1.87	36.30	240.00	1008.00	816.00	284.00	0.21	7.37
Minimum	148.00	0.30	0.58	16.90	130.00	138.00	321.00	101.00	0.07	0.91

APPENDIX O
LEVELS OF ESSENTIAL ELEMENTS IN THE MATURE BREAST MILK (mg/L)

	Essential Elements									
Participants	**Ca**	**Cu**	**Fe**	**Mg**	**P**	**Na**	**K**	**S**	**Se**	**Zn**
1	278.00	0.48	1.50	25.80	152.00	266.43	488.00	142.00	0.09	3.21
2	198.00	0.52	1.11	25.20	142.00	325.26	594.00	128.00	0.09	4.65
3	188.00	0.31	0.98	27.90	146.00	325.00	502.00	114.00	0.10	3.55
4	242.00	0.31	1.20	26.20	144.00	268.00	585.00	98.00	0.09	4.12
5	198.00	0.38	1.02	21.80	142.00	277.00	348.00	101.00	0.08	0.89
6	220.00	0.55	0.99	28.10	240.00	290.00	456.00	198.00	0.08	1.58
7	206.00	0.35	1.21	27.70	112.00	125.00	488.00	101.00	0.08	1.44
8	220.00	0.65	1.11	29.20	202.00	222.00	508.00	155.00	0.08	2.28
9	182.00	0.35	1.02	21.90	158.00	112.00	428.00	115.00	0.08	1.77
10	187.00	0.34	1.82	17.10	142.00	442.00	489.00	138.00	0.08	2.08
11	420.00	0.55	1.32	22.88	181.00	488.00	570.00	166.00	0.10	2.82
12	286.00	0.54	1.15	22.60	165.00	478.00	488.00	182.00	0.08	3.88
13	298.00	0.52	1.21	24.20	145.00	382.00	489.00	144.00	0.08	2.10
14	232.00	0.55	1.08	27.90	165.00	438.00	574.00	165.00	0.10	2.84
15	213.00	0.41	0.89	26.90	117.00	166.34	487.00	89.00	0.10	2.08
16	222.00	0.42	1.02	21.24	121.00	302.00	454.00	161.00	0.07	2.21
17	142.00	0.38	1.54	32.80	182.00	272.00	371.00	141.00	0.20	3.16
18	155.00	0.51	1.25	24.80	114.00	365.00	444.00	189.00	0.09	1.82
19	203.00	0.42	2.21	24.60	122.00	402.56	480.00	97.00	0.08	2.09
20	195.00	0.38	1.04	21.80	151.00	177.00	346.00	76.00	0.07	0.66
21	221.00	0.58	1.58	21.44	114.00	344.00	496.00	126.00	0.07	3.88
22	196.00	0.46	1.80	22.10	186.00	229.00	426.00	186.00	0.08	1.68
23	151.00	0.30	1.20	22.80	145.00	327.00	486.00	156.00	0.09	2.82
24	178.00	0.41	1.11	28.80	172.00	288.00	389.00	181.00	0.08	2.31
25	143.00	0.49	0.94	18.80	128.00	454.00	500.00	153.00	0.08	1.73
26	457.00	0.29	0.58	30.50	124.00	99.00	436.00	75.00	0.07	1.48
27	207.00	0.55	0.62	23.20	150.00	248.00	506.00	102.00	0.17	1.63
28	154.00	0.35	0.66	19.50	158.00	115.00	446.00	76.00	0.09	0.90
29	151.00	0.61	0.82	24.40	139.00	527.00	324.00	143.00	0.09	0.91
30	171.00	0.26	0.55	27.60	138.00	86.00	421.00	78.00	0.07	0.45
31	210.00	0.89	0.79	32.90	220.00	282.00	556.00	197.00	0.09	1.57
32	190.00	0.92	0.82	29.40	200.00	322.00	518.00	195.00	0.08	1.98
33	186.00	0.51	0.77	23.80	141.00	322.00	446.00	188.00	0.08	1.89
34	127.00	0.38	0.98	20.90	181.00	382.00	489.00	142.00	0.08	2.28
35	204.00	0.53	1.65	25.00	181.00	327.62	596.00	128.00	0.10	4.12
36	165.00	0.45	0.71	27.40	121.00	278.00	442.00	122.00	0.07	2.28
37	175.00	0.33	0.56	22.90	159.00	89.00	426.00	101.00	0.07	0.85
38	112.00	0.32	0.54	21.90	144.00	181.00	325.00	121.00	0.09	1.45
39	128.00	0.46	0.66	22.40	141.00	163.00	354.00	137.00	0.07	3.11
40	179.00	0.30	0.61	29.10	134.00	125.00	363.00	62.00	0.07	0.17
41	122.00	0.38	0.55	22.80	145.00	188.00	403.00	128.00	0.09	1.82
42	186.00	0.55	0.65	22.10	121.00	155.00	350.00	131.00	0.07	3.21
43	147.00	0.43	0.58	20.20	142.00	201.00	336.00	108.00	0.10	2.22
44	189.00	0.46	0.78	21.00	158.00	94.00	366.00	84.00	0.07	0.15
45	166.00	0.47	0.66	25.20	119.00	292.00	378.00	133.00	0.35	0.98
46	275.00	0.31	0.65	25.60	163.00	136.52	506.00	80.00	0.08	4.12
47	251.00	0.40	0.55	27.10	138.00	267.75	575.00	98.00	0.09	4.10
Average	202.68	0.45	1.00	24.67	151.17	269.07	456.34	130.45	0.09	2.20
Maximum	457.00	0.92	2.21	32.90	240.00	527.00	596.00	198.00	0.35	4.65
Minimum	112.00	0.26	0.54	17.10	112.00	86.00	324.00	62.00	0.07	0.15

APPENDIX P
LEVELS OF ESSENTIAL ELEMENTS IN TOTAL BREAST MILK (mg/L)

	Essential Elements									
Participant	**Ca**	**Cu**	**Fe**	**Mg**	**P**	**Na**	**K**	**S**	**Se**	**Zn**
1	226.00	0.64	1.85	24.70	136.00	426.08	648.33	246.67	0.12	4.57
2	218.33	0.57	1.47	25.90	163.67	297.37	623.33	136.67	0.11	4.12
3	205.33	0.37	1.19	27.20	160.33	392.33	613.33	158.67	0.11	4.36
4	247.00	0.34	1.67	26.90	140.33	321.00	607.33	116.67	0.10	5.64
5	242.00	0.49	1.38	23.00	170.33	381.00	422.67	110.33	0.08	1.98
6	194.00	0.75	1.17	32.43	178.67	439.00	505.67	194.00	0.08	1.98
7	212.33	0.48	1.91	25.93	161.33	177.00	570.67	146.33	0.08	2.15
8	186.67	0.77	1.40	24.63	177.33	288.00	564.33	195.67	0.09	3.89
9	161.67	0.47	1.67	19.87	171.33	180.67	476.00	132.33	0.10	2.05
10	228.67	0.45	1.46	17.99	195.67	503.67	561.67	179.00	0.09	2.25
11	323.33	0.65	1.66	23.13	209.33	539.33	659.67	199.00	0.12	3.12
12	298.33	0.59	1.51	22.90	196.67	518.33	551.33	209.00	0.08	4.21
13	308.33	0.55	1.62	24.17	179.00	448.33	548.33	174.00	0.08	2.47
14	244.33	0.64	1.31	28.63	149.00	515.33	643.33	211.67	0.11	3.61
15	261.67	0.63	1.30	27.00	113.67	380.66	642.33	192.33	0.16	4.04
16	283.33	0.52	1.29	21.21	176.00	418.00	565.00	187.67	0.08	3.29
17	189.00	0.48	1.42	30.47	225.00	306.00	440.67	188.67	0.19	4.18
18	186.33	0.54	1.75	25.63	146.00	451.67	557.00	210.67	0.09	2.09
19	252.33	0.56	1.76	21.46	186.33	443.33	536.33	122.33	0.10	2.83
20	231.67	0.24	1.25	22.93	173.33	347.67	414.67	128.67	0.07	1.91
21	266.33	0.68	1.94	21.85	151.67	435.00	574.00	173.67	0.07	4.88
22	221.67	0.49	1.37	20.67	217.33	354.67	561.67	257.00	0.10	2.12
23	186.67	0.35	1.46	24.07	200.67	483.00	534.67	180.33	0.11	3.16
24	215.00	0.54	1.44	26.77	196.00	415.33	506.33	202.67	0.08	3.98
25	169.67	0.42	1.03	21.50	147.00	561.33	461.67	157.33	0.09	2.71
26	373.67	0.36	0.81	24.40	153.00	164.00	563.67	140.33	0.11	3.07
27	207.67	0.47	0.87	22.40	159.33	249.33	554.67	119.67	0.11	1.79
28	173.00	0.42	0.79	21.33	145.33	288.67	502.33	129.33	0.10	2.89
29	175.33	0.57	1.04	23.40	151.67	360.67	375.67	143.67	0.09	2.89
30	190.33	0.44	0.99	25.80	165.00	148.67	524.00	132.00	0.08	1.75
31	211.00	0.75	0.85	32.43	170.67	433.00	532.33	193.33	0.09	1.93
32	185.33	0.74	0.90	24.67	174.33	320.67	558.00	186.33	0.08	3.39
33	202.00	0.66	1.02	25.60	218.67	428.33	544.33	213.67	0.09	2.15
34	175.00	0.43	1.26	21.57	210.33	455.67	519.67	173.67	0.09	3.31
35	222.67	0.55	1.24	26.63	172.33	299.46	621.00	136.67	0.11	4.09
36	223.00	0.57	0.78	27.87	183.67	332.33	548.33	175.00	0.08	2.68
37	158.00	0.46	0.61	19.87	175.33	173.00	472.00	123.00	0.07	1.57
38	149.33	0.34	0.67	21.33	178.00	212.00	449.83	153.67	0.12	2.14
39	161.67	0.57	0.80	23.03	177.00	195.33	394.67	180.00	0.08	3.75
40	164.00	0.44	0.74	22.97	166.67	188.33	442.00	105.00	0.08	1.33
41	159.00	0.42	0.80	22.23	183.67	214.00	481.00	170.33	0.14	1.31
42	235.00	0.65	0.74	21.10	184.67	194.33	464.33	182.00	0.08	4.12
43	185.67	0.51	0.66	20.67	175.00	257.67	401.67	156.67	0.14	3.39
44	330.67	0.72	0.90	20.33	217.00	206.00	496.33	148.67	0.07	0.81
45	193.33	0.55	0.76	25.43	196.67	228.33	469.33	143.67	0.17	2.38
46	267.67	0.34	0.76	26.33	159.67	328.33	604.67	143.33	0.10	3.30
47	247.00	0.35	0.58	26.90	140.33	318.44	586.00	115.00	0.10	5.63
Average	220.22	0.52	1.19	24.20	174.05	340.87	529.71	165.45	0.10	3.01
Maximum	373.67	0.77	1.94	32.43	225.00	561.33	659.67	257.00	0.19	5.64
Minimum	149.33	0.24	0.58	17.99	113.67	148.67	375.67	105.00	0.07	0.81