Assessment of the Contamination Level of Persistent Organic Pollutants in Breast Milk of Ghanian Women from from a polluted Area in Accra

Asamoah, Anita

DOI (link to publication from Publisher):
10.5278/vbn.phd.eng.00011

Publication date:
2017

Document Version
Publisher's PDF, also known as Version of record

ASSESSMENT OF THE CONTAMINATION LEVEL OF PERSISTENT ORGANIC POLLUTANTS IN BREAST MILK OF GHANAIAN WOMEN FROM A POLLUTED AREA IN ACCRA

BY
ANITA ASAMOAH

DISSERTATION SUBMITTED 2017

AALBORG UNIVERSITY
DENMARK

ASSESSMENT OF THE CONTAMINATION LEVEL OF PERSISTENT ORGANIC POLLUTANTS IN BREAST MILK OF GHANAIAN WOMEN FROM A POLLUTED AREA IN ACCRA

Ph.D. Dissertation

by

Anita Asamoah

AALBORG UNIVERSITY
DENMARK

Aalborg University

Department of Chemistry and Bioscience

Neils Bohrs Vej 8a, DK-6700 Esbjerg

May, 2017

Dissertation submitted: June 20, 2017

PhD supervisor: Prof. Erik Gydesen Søgaard,
Aalborg University, Denmark

Assistant PhD supervisors: Associate Prof. David Kofi Essumang,
University of Cape Coast, Ghana

Associate Prof. Jens Muff
Aalborg University, Denmark

PhD committee: Professor Henriette Giese (chairman)
Aalborg University, Denmark

Godfred Darko, Senior Lecturer
Kwame Nkrumah University of Science and Technology, Ghana

Ole Stig Jacobsen, Adjunct Senior, Biogeochmemist
Geological Survey of Denmark and Greenland (GEUS), Denmark

PhD Series: Faculty of Engineering and Science, Aalborg University

ISSN (online): 2446-1636
ISBN (online): 978-87-7112-980-9

Published by:
Aalborg University Press
Skjernvej 4A, 2nd floor
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

Printed in Denmark by Rosendahls, 2017

ABSTRACT

The chemical evolution, industrialization, and advancement of technology have brought much relief to humans. The production of pesticides brought victory in the fight against crop pest and diseases which save the ever growing population from starvation. Industrialization and the expansion in technology have made life easier. Polychlorinated biphenyls (PCBs) were used in several industrial and commercial applications due to its unique properties such as non-flammability, chemical stability, high boiling point and electric insulations. Years on, it has come to bare that these chemicals are not only helping to solve challenges they are hailed for but are causing deleterious effects on humans and wildlife. Industrial activities and urbanization have also compounded the problems through the release of unintentionally produced toxic chemicals such as Polycyclic aromatic hydrocarbons (PAHs).The production of intentionally produced chemicals such as PCBs and PAHs have been halted but persist in the environment. This thesis involves the assessment of Persistent Organic Pollutants in the human breast milk samples from a hotspot and non-hot spot areas in the Greater Accra regions of Ghana.

A preliminary site study on soil sample from an e-waste recycling site revealed PCB and PAHs contamination in soil. It was based on this finding that curiosity of its influence in the surrounding initiated the assessment of PCBs and PAHs in humans.

A total of 128 human milk samples from Primiparae and Multiparae donor mothers have been used in the PCBs and PAHs monitoring process. The donors from the host spot area live and work in an e-waste recycling vicinity whereas the donors from the non-hot spot area reside and work in a residential area with no industrial activities. Another set of 42 Primiparae mothers from rural and urban communities in the Greater Accra region were used for the monitoring of the Organochlorine pesticides (OCPs) residue in breast milk study. Donor Mothers completed a questionnaire which provided information on demographic conditions such as parity, BMI, age, and food preference. The breast milk samples from the donor mothers were extracted and analyzed in the laboratory for PAHS, PCB, and OCPs. A to total of 18 different PAHs (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene , acenaphthylene ,fluorene, anthracene, phenanthrene, pyrene, fluoranthene, chrysene, benzo[a]anthracene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[g,h,i]perylene, dibenz[a,h]anthracene,and indeno[1,2,3,c,d]pyrene) were found the breast milk samples. Also, seven PCB congeners were found in the milk samples. They include PCB-18, PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180. The total mean concentrations of PAHs in the hotspot area and the non-hotspot area were 1304 ng/g lipid wt. and 199 ng/g lipid w,t respectively. They were between the ranges 0.302 ng/g lipid wt. and 15800 ng/g lipid wt. for human milk samples from the hot spot area and between below the limit of detection (<LOD) and 687ng/g lipid wt. for human milk samples

from the non-hot spot area, respectively. The mean concentrations of 1026 ng/g lipid wt. and 78 ng/g lipid wt. for naphthalene were highest in both the human milk samples from the hot spot and non-hot spot areas, respectively. Most of the high molecular weight PAHs such as chrysene, benzo[a]pyrene, benzo[k]fluoranthene and Benzo [g,h, i] Perylene which are possible carcinogens were detected in the breast milk samples from the hot spot area but were below limit of detection in the breast milk samples from non-hot spot area. The possible sources of the PAHs in the breast milk samples were assessed the PAHs diagnostic ratio test. The results from the test predicted pyrogenic origin for the PAHs in the milk samples from hot spot area. Carcinogenic and mutagenic risk assessment of infants based on the result of this study were 1.1×10^{-5} and 1.9×10^{-5}. This means that approximately 1 out of 100000 and 2 out of 100000 infants may have cancer and other non-cancer related adverse diseases such as pulmonary diseases or low IQ during their lifetime as a result of taking carcinogenic PAHs in breast milk.

PCB-28 was detected in all the breast milk samples from the hot spot area. Only one PCB congener, PCB-28 was recorded in the milk samples from the breast milk samples from the non-hot spot area ad was recorded in just one sample. PCB-28 made the highest contribution of 29.5% and PCB-101 contributed the least of 1.704% out of the total PCBs in the breast milk samples. The Estimated Daily Intake (EDI) of PCBs contaminated milk by infants in this study was 0.02 µg/kg body wt. /day and ranges between <0.001- 0.03µg/kg body wt. /day. The risk assessment on infants reveled no risk, but it is worth mentioning that some were at the threshold limit.

Correlation test revealed no association between PCB concentration and demographic conditions such as age, parity, body mass index or food preference.

A total of 14 different organochlorine pesticide residues were detected in the human milk breast samples from both the rural and urban communities. They include Gamma-HCH, Delta-HCH, Heptachlor, Aldrin, Gamma-Chlordane, Alpha-Endosulfan, Endosulfan-Sulphate, p,p'-DDT, p,p'-DDE, Dieldrin, Endrin, Endrin-Aldehyde, Endrin Ketone and Methoxychlor. The mean concentrations for Endosulphan of 91.1 µg/g lipid and 63.8 µg/g lipid was highest for both breast milk samples from the rural and urban communities respectively. Except for the mean concentrations for Endosulphan-Sulphate, the mean concentrations of all the organochlorine pesticide residues detected in the breast milk samples from both rural and urban communities were below the Australian Maximum Residue Limit for breast milk.

SYNOPSIS

Den kemiske udvikling, industrialisering og fremskridt inden for teknologi i almindelighed har bragt bedre livsbetingelser for mennesker. Produktionen af pesticider frembragte en sejr i kampen mod skadedyr og sygdomme i afgrøder, og de redder den stadigt voksende befolkning fra sult. Industrialisering og udvidelse i teknologien har dermed gjort livet lettere. Polychlorerede biphenyler (PCB'er) er blevet anvendt i flere industrielle og kommercielle aktiviteter på grund af deres unikke egenskaber, såsom ikke-brændbarhed, kemisk stabilitet, højt kogepunkt og som elektriske isolatorer. Gennem årene er det kommet til samfundenes bevidtshed, at disse kemikalier ikke kun hjælper med at løse de udfordringer, de er frembragt til, men også forårsager skadelige virkninger på mennesker og dyreliv. Industrielle aktiviteter og urbanisering har desuden forværret problemerne ved utilsigtet frigivelse af producerede giftige kemikalier såsom polycykliske aromatiske carbonhydrider (PAH'er). Produktionen af forsætligt fremstillede kemikalier som PCB og PAH er stoppet, men den fortsætter i miljøet. Denne afhandling involverer vurdering af vedvarende organiske forurenende stoffer i humane modermælksprøver fra et hotspot- og et referenceområde (non-hot spot) i Greater Accra-regionerne i Ghana.

En foreløbig undersøgelse af jorden på jordprøver fra et genbrugsplads for affald afslørede PCB og PAHs forurening af jorden. Det var baseret på denne konklusion, at nysgerrigheden om dens indflydelse i omgivelserne påbegyndte undersøgelsen af tilstedeværelsen af PCB og PAH'er hos mennesker.

I alt 128 humane mælkeprøver fra første- og fleregangsfødende donormødre er blevet anvendt i PCB'ernes og PAHs overvågningsproces. Donorerne fra hot-spot området lever og arbejder i nærheden af en elektronikaffald genbrugsplads uden særlige miljømæssige foranstaltninger, mens donorerne fra det ikke-hot spot-område bor og arbejder i et beboelsesområde uden industrielle aktiviteter. Et andet sæt af 42 førstegangsfødende mødre fra landdistrikter og bysamfund i Greater Accra-regionen blev brugt til overvågning af organiske chlorpesticidrester i brystmælkestudier. Donormødrene udfyldte et spørgeskema, der gav information om demografiske forhold som paritet, BMI, alder og madpræference. Brystmælksprøverne fra donormødrene blev ekstraheret og analyseret i laboratoriet for PAH'er, PCB og OCP'er. I alt 18 forskellige PAH'er (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene , acenaphthylene ,fluorene, anthracene, phenanthrene, pyrene, fluoranthene, chrysene, benzo[a]anthracene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[g,h,i]perylene, dibenz[a,h]anthracene,and indeno[1,2,3,c,d]pyrene) blev fundet brystmælksprøverne. Der blev også fundet syv PCB-kongenere i mælkeprøverne. De omfatter PCB-18, PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 og PCB-180. De samlede gennemsnitlige koncentrationer af PAH'er i hotspot-området og ikke-

hotspot-området var henholdsvis 1304 ng / g lipid og 199 ng / g lipid. Ekstremerne var 0,302 ng / g lipid og 15800 ng / g lipidvægt for humane mælkeprøver fra hot spot-området, og mellem under detektionsgrænsen (<LOD) og 687 ng / g lipid for humane mælkeprøver fra non-hot spot-området. De gennemsnitlige koncentrationer for naphthalen på 1026 ng / g lipid og 78ng / g lipidvægt i de to områder var den højeste forekomst af PHA'erne. De fleste af de højmolekylære PAH'er, såsom chrysen, benzo [a] pyren, benzo [k] fluoranthen og benzo [g, h, i] perylen, som er mulige kræftfremkaldende stoffer, blev påvist i brystmælksprøverne fra hot spotområdet, men blev ikke detekteret i brystmælksprøverne fra ikke-hot spot-området. De mulige kilder til PAH'erne i modermælksprøverne blev vurderet ved hjælp af PAHs diagnostiske forholdstest. Resultaterne fra testen præciserede en pyrogen oprindelse for PAH'erne i mælkeprøverne fra hot spot-området. Kræftfremkaldende og mutagen risikovurdering af spædbørn baseret på resultatet af denne undersøgelse var $1,1 \times 10^{-5}$ og $1,9 \times 10^{-5}$. Det svarer til, at ca. 1 ud af 100.000 og 2 ud af 100.000 spædbørn kan have kræft og andre ikke-kræftrelaterede sygdomme som lungesygdomme eller lavt IQ i løbet af deres levetid som følge af indtagelse af kræftfremkaldende PAH'er i modermælk.
PCB-28 blev detekteret i alle brystmælksprøverne fra hot spot-området. Netop kun denne ene PCB-congener, PCB-28, blev registreret i mælkeprøverne fra brystmælksprøverne fra ikke-hot spot-området, og det blev registreret i blot en prøve. PCB-28 havde det højeste bidrag på 29,5%, og PCB-101 bidrog mindst med 1,704% af de samlede PCB'er i modermælksprøverne fra hot-spot området. Den estimerede daglige indtagelse (EDI) af PCB forurenet mælk af spædbørn i denne undersøgelse var 0,02 µg / kg legemsvægt pr. dag. Intervallerne lå mellem <0,001 og 0,03 µg / kg legemsvægt pr. dag. Risikovurderingen af spædbørn har dermed ikke givet nogen risiko i forhold til de vedtagne grænser for tilstedeværelse og indtagelse af de forurenende stoffer, men det er værd at nævne, at nogle af prøverne lå netop ved grænsen.
Korrelationstest afslørede ingen sammenhæng mellem PCB-koncentration og demografiske forhold såsom alder, paritet, body mass indeks eller fødevarepræference.

I alt blev der fundet 14 forskellige organiske chlorpesticidrester i humane mælkebrystprøver fra både landdistrikterne og bysamfundene. De omfatter Gamma-HCH, Delta-HCH, Heptachlor, Aldrin, Gamma-Chlordan, Alfa Endosulfan, Endosulfansulfat, p,p'-DDT, p,p'-DDE, Dieldrin, Endrin, Endrin-Aldehyd, Endrin Keton Og methoxychlor. De gennemsnitlige koncentrationer for Endosulfan på 91,1 µg / g lipid og 63,8 µg / g lipid var højest for begge brystmælksprøver fra henholdsvis landdistrikterne og byerne. Bortset fra de gennemsnitlige koncentrationer for endosulfansulfat var de gennemsnitlige koncentrationer af alle organiske chlorpesticidrester, der blev opdaget i brystmælksprøverne fra både landdistrikter og bysamfund under den australske maksimalgrænseværdi for modermælk.

PREFACE

This thesis is submitted in partial fulfillment of the requirements for the Ph.D. degree at the Department of Chemical Engineering and Bioscience, Aalborg University, Denmark. The Ph.D. project was performed under the supervisions of Professor Erik G. Søgaard from the section of chemical engineering at Aalborg as the principal supervisor and Co-supervisors Professors David Kofi Essumang and Jens Muff from University of Cape Coast, Ghana and Aalborg University, Denmark, respectively. The project deals with the assessment of persistent organic pollutants in the human breast milk of some nursing mothers from the hotspot and non-hotspot areas in Accra, Ghana.

In our quest to develop as humans, we undertake different paths that can solve the problems of today but we do not take into consideration the consequences of what today's satisfaction will have on tomorrow. Chemical production and human activities have resulted in the release of toxic chemicals into the environment.

"For the first time in the history of the world, every human being is now subject to contact with dangerous chemicals from the moment of conception until death" (Rachel Carson).

The thesis is designed as a collection of scientific papers. The chapter one of the thesis is the introduction part. It talks about the background to the study, the problem statement and the objectives of the study. Chapter two reviewed the relevant literature pertaining to the study. Chapter three describes the various methodologies such as sampling protocols, sample extractions and analytical procedures used in the study. Chapter four, five and six covers the extent of contamination of PAHs, PCBs and organochlorine pesticides in human breast milk. Chapter seven talks about the preliminary studies conducted on soil at e-waste hotspot. At the end, conclusion summarizes the findings from the study and provides answers to the research objectives.

I end with a quote from Nathaniel H. Egleston 1882

"Nature bears long with those who wrong her. She is patient under abuse. But when the abuse has gone wrong too far, when the time of reckoning comes she is equally slow to be appeased and turn away her wrath" (Nathaniel H. Egleston 1882).

I hope you enjoy reading this thesis.

Anita Asamoah 2017

ACKNOWLEDGEMENTS

This Ph.D. study has not been a lonely journey; it has been fruitful due to the efforts of some individuals, groups, and organizations.

To begin with, I would like to express my utmost gratitude to the principal supervisor, Professor Erik G. Sogaard for the opportunity given me to pursue a Ph.D., you have been supportive and contributed immensely for the successful fruition of this Ph.D. I am highly indebted to my two co-supervisors, Professors David Kofi Essumang and Jens Muff, you have always been there and have directed in all possible ways to ensure the success of this Ph.D. study.

I sincerely thank my colleagues Hulya U.Sokoli, Katarzyna R. Arturi and Mahdi Nikbakht Fini and Adeel N. Sohal for the academic discussions and the nice times we shared together, you made the Ph.D. journey fun and less stressful. Many thanks go to Heidi Thompson (Section secretary), Dorte Spangsmark, Linda Madson, Lisbeth Skou and Morten Strandgaard (Laboratory technicians, Aalborg University Esbjerg). I am grateful to Professor Sergey Kucheryavskiy for the statistical input in this Ph.D. study.

My sincerest thanks go to all the lactating mothers who donated their breast milk samples, without you this research would not have been a reality.

I would like to thank Sarah Blankson-Arthur and colleagues at Ghana Atomic Energy Commission's environmental research group; you have been a great team.

I thank Duke Henry Nii Amon Ashie and Dr.Paul Osei-Fosu of the Ghana Standard Authority for their assistance.

My heartfelt gratitude goes to my dear husband, Joseph Kwadwo Asamoah for the support, love, and encouragement throughout this Ph.D. journey. I am also grateful to my two daughters, Nhyira and Aseda for tolerating and coping with my often absence from home.

I thank all family and friend Ernestina Botchey and the Adofos and my dear Uncle Nana Akwasi Baah Sarpong who never stopped believing in me and always kept encouraging me to soar high. I am also grateful to my mother in blessed memory, Dora Darkoah, It was always your dream to see me climb higher up the academic ladder, I hope I have made you proud.

I thank God for the strength for each day and bright hope for tomorrow.

Finally, I would like to thank the International Atomic Agency for sponsoring this Ph.D. study.

THESIS DETAILS

Thesis Tiltle: Assessment of the contamination level of persistent organic pollutants in breast milk of Ghanaian women from a polluted area in Accra

Ph.D.Student: Anita Asamoah

Supervisor: Professor Erik Gydesen Søgaard

Co-supervisors: Associate Professor David Kofi Essumang
Associate Professor Jens Muff

The main body of thesis is provided based on the following papers:

Paper I: Anita Asamoah, Erik Gydesen Søgaard, David Kofi Essumang, Jens Muff and Mahdi Nikbakht Fini, "Assessment of PAHs contamination levels, possible sources and infants carcinogenic and mutagenic risks in human breast milk of some Ghanaian women from a hot spot and non-hot spot areas", Submitted to *International Journal of Hygiene and Environmental Health*

Paper II: Anita Asamoah, Erik Gydesen Søgaard, David Kofi Essumang, Jens Muff and Sergey V. Kucheryavskiy, " Assessment of PCBs and exposure risk to infants in the breast milk of primiparae and multiparae mothers in an electronic waste hot spot and non-hot spot areas in Ghana", *Science of Total Environment, Under review*

Paper III: Anita Osei Tutu, P.O. Yeboah, A.A. Golow, D. Denutsui and S. Blankson-Arthur, "Assessment Organochlorine Pesticides Residues in the Breast Milk of Some Primiparae Mothers in La Community, Accra, Ghana", *Research Journal of Environmental and Earth Sciences* 3(2): 153-159, 2011

Paper IV: Anita Osei Tutu, Philip Owiredu Yeboah, A. A. Golow, Samuel Adu- Kumi, Edith. Clarke and Paul Osei-Fosu, "Levels of Organochlorine pesticide residues found in the breast milk of some first-birth mothers from a rural community (Ada) in Ghana", *Elixir Pollution* 54 (2013)

12668-12672

Paper V: Assessment of PCBs and PAHs in soil samples from Agbogbloshie e-waste site, Accra Ghana, "Anita Asamoah, Erik Gydesen Søgaard, David Kofi Essumang", under preparation

Besides, the following oral presentation has also been made.

Oral presentation:

Organochlorine Pesticide Residues In the breast milk of some primiparae mothers in La community, Accra, Ghana, presented at *40th Anniversary Scientific Forum* 18-20th November, 2014. *Ghana Environmental Protection Agency*

TABLE OF CONTENTS

Paper I: Assessment of PAHs contamination levels, possible sources and infants carcinogenic and mutagenic risks in human breast milk of some Ghanaian women from a hot spot and non-hot spot areas

Paper II: Assessment of PCBs and exposure risk to infants in the breast milk of primiparae and multiparae mothers in an electronic waste
hot spot and non-hot spot areas in Ghana

Paper III: Assessment Organochlorine Pesticides Residues in the Breast Milk of Some Primiparae Mothers in La Community, Accra, Ghana

Paper IV: Levels of Organochlorine pesticide residues found in the breast milk of some first-birth mothers from a rural community (Ada) in Ghana

Paper V: Assessment of PCBs and PAHs in soil samples from Agbogbloshie e-waste site, Accra Ghana

TABLE OF FIGURES

ABBREVIATIONS

WHO	World Health Organization
POPs	Persistent Organic Pollutants
PAHs	Polycyclic Aromatic Hydrocarbons
PCDD/Fs	polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans
PCBs	Polychlorinated biphenyls
PBDE	polybrominated diphenyl ethers
UNEP	United Nations Environmental Programme
OCP	Organochlorine pesticide
DDT	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethene
ATSDR	Agency for Toxic Substances and Disease Registry
GEPA	Ghana Environmental Protection Agency
USEPA	United States Environmental Protection Agency
ACGIH	American Conference of Governmental Industrial Hygienists
EEE	electric and electronic equipment
GHS	Ghana Health Service
QuECHERS	Quick Effective Cheap Effective and Rugged and Safe
Naph	naphthalene
2-Met	2-methylnaphthalene
1-Met	methylnaphthalene
Acy	acenaphthylene
Ace	acenaphthalene
Flu	fluorene
Ant	anthracene
Phe	phenanthrene
Pyr	pyrene
Flt	fluoranthene
Chr	chrysene
BaA	benzo[a]anthracene
BaP	benzo[a]pyrene
BkF	benzo [k]fluoranthene
BbF	benzo[b]fluoranthene
BghiP	benzo[g,h,i]perylene
DahA	dibenz[a,h]anthracene
IndP	indeno[1,2,3,c,d]pyrene
TEQ	Toxicity equivalent
MEQ	Mutagenic equivalent
BMI	Body Mass Index
GEPA	Ghana Environmental Protection Agency
HCHs	Hexachlorocyclohexanes
EDI	Estimated Daily Intake

CHAPTER 1. INTRODUCTION

1.1. BACKGROUND OF STUDY

Human breast milk is the first meal most babies are introduced to before any other food. It has most of the essential nutrients, vitamins, enzymes and protein binding in their right proportions for child's growth and also provides immunological and anti-inflammatory protection against many diseases (LaKind et al., 2004; WHO, 2001; Schanler, 2001; Lawrence and Lawrence, 2010). It is therefore not surprising that the World Health Organization (WHO) has recommended that babies must exclusively be breastfed with breastmilk in the first six months of their lives (WHO, 2001). Breast milk continues to be the best meal for a baby especially in the first six months of their lives and anything that affects its quality is of great concern because babies are more susceptible to infections and sicknesses due to the fact that their system is still in the developing stage. Sadly, the safety of breast milk for babies cannot be guaranteed; they may not only be having nourishment and parental affection but also significant doses of poisonous chemicals. Nursing mothers get rid of the hauled lipophilic contaminants in their breast milk to breastfed infants, aiding in the trans-generational movement of contaminants (Bordajandi et al., 2008; Munoz-de-Toro et al., 2006; Kanja et al., 1992). Breast milk is now contaminated with persistent organic pollutants (POPs), heavy metals, polycyclic aromatic hydrocarbons (PAHs) and other pollutants (Munoz-de-Toro et al., 2006; Kanja et al., 1992).The chemical revolution brought to birth the production of all forms chemicals to solve most of the challenges humans were confronted with. Pesticides Manufacturing become essential as human pollution continued to increase and the territory of pests kept expanding. The need to produce more to feed the growing population became a necessity, and crop pest became a hindrance to this agenda. The era of the production of organochlorine pesticides became a significant relief in the fight against crop pest and disease control in public health (Clarke et al., 1997; Hogstedt et al., 1992; WHO, 2011; Ross, 2005). Most of these chemicals were banned due to their deleterious effect on humans and wildlife (Carson, 1962; UNEP, 2009; Dunlap, 1981). Even though they are prohibited and are no more in use they still pose serious concerns due to their persistent in the environment, ubiquitous nature, toxicity, ability to accumulate and biomagnify in fatty tissues (Beyer and Biziuk, 2009; Kelly et al., 2007; Namiki et al., 2013; Wang et al., 2013). Aside the problems with pesticides, Ghana, like many developing nations has become the hub for most second-hand electrical equipment and electronic gadgets. Most of these electrical and electronic gadgets arrive as completely obsolete. This has resulted in e-waste recycling becoming a fast growing business in Ghana by some individuals with the aim of obtaining precious metals which they sell for their upkeep. Unfortunately, unacceptable ways of recycling processes are employed in obtaining these valuable metals, and unskilled individuals are doing them (Tang et al., 2010;

Wang et al., 2012). Eventually, they end up contaminating themselves, neighbors and the environment at large with heavy metals, Polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers PBDE and other pollutants (Caravanos et al., 2011; Kyere et al., 2016; Feldt et al., 2014; J. Wang et al., 2012; Q. Wang et al., 2016). Ghana has one of the biggest e-waste dump sites in Africa. Agbogbloshie electronic waste site is located in the heart of Accra, the capital of Ghana and it is surrounded by a cluster of schools, a hospital, offices, churches, and a mosque. Adjacent to the Agbogbloshie e-waste site is one of the largest retail and wholesale market in Accra where all forms of foods and vegetables from all parts of Ghana are brought to be sold. The whole vicinity is sometimes covered with thick a cloud of smoke emanating from the e-waste site, yet people continue to go through their normal daily activities just like it was a normal healthy environment. People residing and working around this e-waste site may be exposing themselves to POPs as a result of the e-waste activities. People of all ages and forms including the more susceptible ones such as pregnant women and children are in the area, and this creates much worry on the level of exposure of these individuals. Breast milk is used as the main matrix in this research work because of its significant role in growing infant hence anything that compromises its wholesomeness is a serious concern (Lawrence and Lawrence 2011). Aside from this, research has shown that contaminants in breast milk correlate with the levels of contaminants in blood and adipose tissue, therefore using breast milk which is non-invasive gives a reflection the body burden of these contaminants in humans (Malish and Van Leeuwen, 2003).

1.2. PERSISTENT ORGANIC POLLUTANTS (POPS)

Persistent Organic Pollutants (POPs) are a group of organic chemicals (that is carbon based) which were produced intentionally or unintentionally. When they are released into the environment, tend to persist, concentrate and biomagnify through the food chain and have the potential of causing an adverse effect on the environment, wildlife and humans at large (UNEP, 2001). POPs can be classified as legacy POPs and non-legacy POPs. For instance, organochlorine pesticides, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are examples of legacy POPs. They were intentionally produced by industries to curb challenges of the time. Organochlorine pesticides were very useful in controlling crop pest and public health sector for disease control. DDT, an organochlorine pesticide played a tremendous role in the fight against malaria. PCBs were also produced and used as dielectric fluids in capacitors and transformers, as hydraulic fluids, flame retardants, lubricating fluids and other broad range uses (Erickson and Kaley, 2011; WHO, 1993). Emerging POPs such as polychlorinated alkanes (PCAs), perfluorinated organic compounds (PFCs) and Polybrominated Diphenyl Ethers are also examples of non-legacy POPs (UNEP, 2002). POPs such as PAHs are also unintentionally released into the environment when there is incomplete combustion of carbon-containing compounds.

POPs are stable compounds which are highly resistance to degradation and hence tend to persist for a longer period in the environment. POPs have a semi-volatile characteristic which easily facilitates their circulation far and near in every part of the environment. The long range transport nature of POPs has resulted in making them ubiquitous in the environment; they are even found in the Arctic and Antarctic where they have never been used (Hung et al., 2016; De March et al., 1998 Koziol and Pudykiewicz, 2001). POPs are lipophilic and therefore can bioaccumulate in the fatty tissues of organisms and biomagnify along the apex of the food chain (Porta et al., 2008; UNEP, 2001).

Food is considered as the main source of exposure of humans to POPs (Brauner et al., 2011; Hedley et al., 2010; Llobet et al., 2003). Other sources of exposure are through direct contact with these chemicals through occupation, polluted air and water (Feldt et al., 2014; Asante et al., 2012; Brigden et al., 2008; J. Wang et al., 2012).

Research works have linked POPs with a variety of adverse health problems such as birth defects, immunological and neurological challenges, infertility, learning disorders, behavioral discrepancies, different forms of cancers and various forms of health detriments (Sweetman et al ., 2005; Katsoyiannis et al., 2005; Pauwels et al., 2000; Roots et al 2005; Bolt and Degen G ., 2002; UNEP, 2002).

1.3. ACCUMULATION OF PERSISTENT ORGANIC POLLUTANTS IN BREAST MILK

Some research works have linked food as a major source of exposure of POPs in humans, especially people who do not engage in activities which have no direct exposure to these POPs, and other sources include air and water. (Brauner et al., 2011; Hedley et al., 2010; Llobet et al., 2003). Some of these pollutants, especially industrial chemicals such as PCBs, pesticides and organochlorines were massively produced in the past and used to solve most of the problems at the time (ATSDR, 2000; Llobet et al., 2003; Clarke et al., 1997). These chemicals get into the soil, water, and other environments and they are taken up by plants, animals and other aquatic living bodies. Humans, who are mainly on top of the food chain, end up receiving higher doses of these chemicals since these chemicals have the ability to bioaccumulate and biomagnify.

POPs are lipophilic and hence tend to accumulate in the fat of the fatty tissues in the body, and human breast which is also fatty serves a perfect deposit for these pollutants (Steingraber, 2001; Rogan and Gladen, 1986). During lactation fat is being mobilized to form milk; 60% of milk fat is sourced from the mother's fatty tissues, 30% of mother's diet and 10% is produced within the breast (Lawrence and Lawrence 2011). If the fats from the mother contain any contaminants during breast milk formation, there is a greater chance of the breast milk being polluted by these

pollutants (Steingraber, 2001). Interestingly, breastfeeding is the potent ways mothers get rid of their body burden of these pollutants but unfortunately; they are being transferred to young lives that are even more vulnerable. This can lead to the trans-generational transfer of contaminants (Bordajandi et al., 2008; Fenton et al., 2005; Munoz-de-Toro et al., 2006; Kanja et al., 1992).

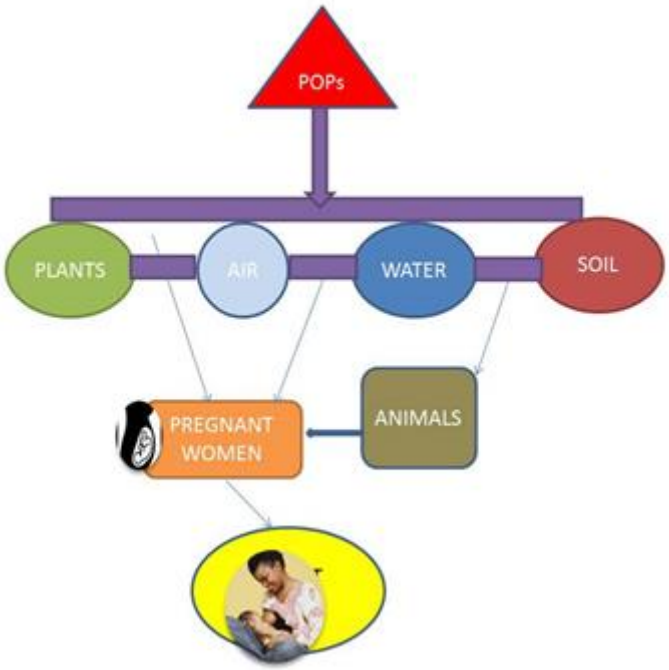


Figure 1-1 Showing possible pathways of how POPs get to breastfed infants.

1.4. BREAST MILK AS A MATRIX FOR POPS MONITORING

POPs are highly lipophilic in nature and therefore tend to stay comfortably in the fatty tissues and fluids of organisms anytime there is an opportunity (Steingraber, 2001). The fatty nature of the breast also makes it a good deposit for POPs as mentioned above. During lactation mothers negatively reduce their body burden of these contaminants to their babies through breastfeeding (Steingraber, 2001). It is known from research that breastfed infants happen to ingest higher doses of contaminants that they might have accumulated in their whole lives (Colborn et al., 1996). Therefore, using breast milk as a biomarker for POPs assessment gives information on not only nursing mothers but also a possible dietary exposure of breastfed infants (WHO, 2001). Also, the non-invasive sampling method of breast milk makes it a better choice of the matrix in assessing body burden POPs as compared to blood or adipose tissues. It is also worth mentioning that there is a good

correlation between levels of POPs in breast milk and other matrices such as blood and adipose tissue (Anda et al., 2007; Darnerud et al., 2010, Schechter et al., 1998). Therefore the use of breastmilk as a matrix also gives approximately the levels of POPs to be expected in fats in the whole body (Schechter et al., 1998). Monitoring of POPs in breast milk is essential for earlier detection of body burden of POPs and alert health officials to put in measures to avoid adverse health effects occurring shortly (Hedley et al., 2010).

1.5. PROBLEM STATEMENT

Activities in the Ghanaian environment in the past and present have contributed to the presence of persistence of Organic pollutants in humans and wildlife. Organochlorine pesticides were extensively used both in agriculture in public health (Clarke et al., 1997; Ntow, 2005). Old transformer oils and plasticizers used in Ghana contained PCBs (GEPA, 2007). Fast growing unauthorized e-waste recycling and with its attendant indiscriminate burning are contributing immensely to the release of POPs into the environment (Feldt et al., 2014; J. Wang et al., 2012; Q. Wang et al., 2016). Some studies have been conducted in Ghana, but there is still a paucity of information especially the levels of these POPs in humans, in particular, using breastmilk as a matrix in monitoring these POPs.

Before the start of the PCB and PAHs assessment in human breast milk, a preliminary site investigation study was conducted at the Agbogbloshie e-waste site. The purpose was to ascertain whether the unlawful e-waste recycling activities are polluting the area. Soil samples from the site were screened for PAHs and PCBs. Soil samples were also analyzed from a residential area as a control. Soil samples from the e-waste site contained significant levels of PAHs and PCBs. A study was then conducted to determine the degree of contamination in humans by using breast milk as the matrix for assessment.

To the best of our knowledge, no research work has been done on Polycyclic Aromatic Hydrocarbons (PAHs) in human breast milk in Ghana. The present study is investigating the presence of PCBs and PAHs in the breast milk of some women residing or working in and around Accra's biggest e-waste recycling site. Also, breast milk from rural farming and urban communities will be screened for Organochlorine pesticide residues. The present study also seeks to find the sources of exposure to PAHs in breast milk and the health risk babies are likely to suffer.

1.6. RESEARCH OBJECTIVE

1.6.1. MAIN OBJECTIVE

The aim of this study is to monitor and assess the human burden of Persistent Organic Pollutant (POPs) [polychlorinated biphenyls (PCBs), polycyclic aromatic

hydrocarbons (PAHs) and organochlorine pesticide (OCPs) residues in some Ghanaian communities including hot spot areas using human breast milk as the main matrix for the investigation. The study also seeks to investigate the possible sources of exposure of these POPs and the effects on maternal health.

1.6.2. SPECIFIC OBJECTIVE

(i) To measure the levels of (POPs) [PCBs, PAHs, and OCs] in human breast milk
(ii) To assess if the levels of these POPs pose any risk to the health of breastfed infants
(iii) To conduct a site study of a possible hotspot point area (Electronic waste site) to assess its influence on levels of PCBs and PAHs in nursing mothers who live and work around the area.
(iv) To find out if there is a possible correlation between the levels of polychlorinated biphenyls and polycyclic aromatic hydrocarbon, demographic and maternal anthropometric characteristics, (age of lactating mothers, parity, body mass index and fish, and meat consumption and the mode of cooking). This is to identify the factors that could potentially influence the concentration of the contaminants in breast milk.
(v) Recommendations for policy makers based on the findings of this research work.

CHAPTER 2. RELEVANT LITRETURE

2.1. HUMAN BREAST MILK COMPOSITION

Human breast milk can be categorized as colostrum, transitional or matured milk. The first milk produced in the first week of delivery is the colostrum, the second week the milk is the transitional milk, and after the second week, the milk is the considered as the matured milk (Lawrence and Lawrence 2011). Human milk consists of both macro and micronutrients. Human breast milk is made up of 88% water, 3.8% fat, 7% lactose, 1% Protein and 0.2% vitamins and minerals. (Prentice, 1995; Nommsen et al., 1991; Lawrence and Lawrence 2011). The micronutrients in breast milk are Minerals and Vitamins. The vitamins are A, D, E, K and some fat soluble vitamins like vitamin C, niacin, riboflavin and as well as pantothenic acid (Prentice 1995). The minerals consist of potassium, iron, sodium, chlorine, magnesium, and phosphorus (Lakind et al., 2004; Prentice 1995, 1996). Aside from the nutritional components, breast milk is also made up of bioactive components. The bioactive components include growth factors, antimicrobial factors, cytokines and anti-inflammatory factors, digestive enzymes, hormones and immunological factors. The immunological factor plays an essential role in the health and sustenance or enhancement of infant's life (Ballard and Morrow, 2013; Kramer & Kakuma 2012; Prentice 1995, 1996).The proteins in breast milk are grouped as the whey and casein complexes with each of them associated with specific proteins and peptides (Liao et al., 2011; Gao et al., 2012). The protein levels in the breast milk of preterm mothers are usually higher than that of full-term mothers. The protein levels are reduced after the first four to six weeks of delivery irrespective of a preterm or full term delivery (Bauer and Gerss 2011). The carbohydrate component of human breast milk is lactose (Lawrence and Lawrence 2011). Lactose facilitates the growth of healthy bacteria in the stomach (Riordan, 2004).

Human milk fat is highly made up of 21% palmitic and 36% oleic acids (Lawrence and Lawrence 2011; Ballard and Morrow 2013). Fat is considered as the most variable macronutrient of human milk. This is because the fat concentration in a first feed (foremilk) may be approximately two to three times lesser than that in the last feed (also called hind milk) which is after the first feed and it is often thicker than foremilk. (Kent et al., 2006). Matured human milk contains a total fat level between 20 g/L and 50 g/L (approximately 3 to 5% fat by weight) which plays an essential role in the development of babies. The fat content in human breast milk aids in the cognitive development of the infants. It also aids in neonatal growth and retinal function of the infant (Lonnerdal, 2003). The lipophilic nature of breast milk makes it a better reservoir to harbor and accumulate organic pollutants such as PCBs, PAHs, OCs and others.

2.2. PERSISTENT ORGANIC POLLUTANTS IN THE ENVIRONMENT

2.2.1. POLYCHLORINATED BIPHENYLS (PCB)

Polychlorinated Biphenyls (PCBs) are synthetic organic chemical made up of Carbon, Hydrogen and Chlorine atoms. PCBs consist of a biphenyl in which chlorine atoms have replaced some or all the hydrogen atoms. PCBs have a general formula of C12H10-nCln, with n ranging from 1(one) to 10 (ten) (Storelli et al., 2003). Based on the number and position of chlorine atoms on the biphenyl about 209 different PCB congeners can be produced. PCBs with little chlorination are more volatile as compared to those with higher chlorination (Breivik et al., 2007). The positions of the chlorine of the PCBs contributes to its toxicity. The chemical structure for PCB is shown in Figure 2-1 below.

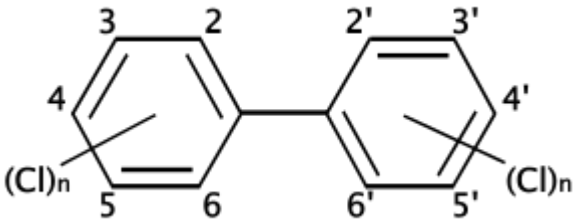


Figure 2-1 Chemical Structure of PCBs

The world's PCBs manufacturing mostly occurred in the US, Europe and Russia (Gioia et al., 2014). The Swann Chemical Company in Anniston, AL. is known to be the premier producers of commercial PCBs in 1929 (Erickson and Kaley, 2011). Theodore Swann established a commercially feasible method for producing biphenyl by bubbling benzene into the molten lead (Erickson and Kaley, 2011). Chlorination of the biphenyl was one of the numerous ways considered in developing commercial uses of biphenyl (Penning, 1930). In 1935, Monsanto Company purchased Swann Chemical Company and manufactured PCBs through direct chlorination of biphenyl (Hubbard 1964). PCBs can also be unintentionally produced during combustion activities consisting of organic matter and chlorine (UNEP, 2001). PCBs chemical stability, low flammability, electrical insulating properties and other fascinating physical properties enhanced its principal production and patronage by most industries (Erickson and Kaley, 2011). It is projected that about 1.3 million tons of PCBs have been produced globally (Breivik et al., 2007).

WHO (1993) classifies the use of PCB under three broad areas. They include totally closed systems as it is in electric equipment such as capacitors and transformers. The

next one is the nominally closed systems, a typical example is hydraulic and heats exchange systems. The final one is the open-ended applications as in adhesives, pesticides extenders, plasticizer, paints, inks, carbonless copy paper, some others (WHO (1993); EPRI (1999); Durfee et al., (1976)).

PCBs production was halted in 1977 and was banned by the US EPA in May 1979 as a result of the concerns raised regarding the possible harmful effects posed on wildlife, humans and the environment (Erickson and Kaley, 2011). Years after the ban on production and use, PCBs continue to be in the environment due to high stability against degradation of the previously used PCBs. An appreciable amount of PCBs can still be found mainly in electronic equipment circulating in the environment. (Penteado and Moreira Vaz, 2001; ATSDR, 2000; UNEP, 2001). Exposure of PCBs in the environment is enhanced as a result of maintenance or repairs and decommission or destruction of PCB-containing equipment. Aged PCB-containing electronic and electrical gadgets such as television sets, refrigerators and old fluorescents lighting system and open burning of halogen containing materials also contribute to PCBs in the environment (ATSDR, 2000).

Ghana, like many African nations, never commercially produced PCBs but patronized PCB materials (GEPA, 2007). PCBs are found in the Ghanaian environment through its application in electric utilities, industrial facilities, also in residential and commercial buildings (GEPA, 2007). According to the Ghana EPA, there are four hundred and fifty-five (455) PCB-containing transformers throughout Ghana and these transformers were brought into Ghana before 1972 (GEPA, 2007). There are also One hundred and forty-seven (147) PCB-containing capacitors, and some of these capacitors have their cans broken and leaking (GEPA, 2007). There is evidence that in the past some empty transformer oil tanks which were used as water reservoirs and also some PCB oils have been smuggled into some small scale industries for making pomade sold in local markets (GEPA, 2007). As mentioned another primary source of PCBs in the Ghanaian environment is from the use of plasticizers, lubricants, certain paints, adhesives, fire retardants among others (GEPA, 2007). In addition, another probable major route of PCB exposure to PCBs in Ghana is the influx of old electric and electronic equipment which is feeding most illegal electronic waste recycling facilities. Ghana like most African countries has become a hub for most electronic waste from the western world (Gioia et al., 2011). These e-wastes are openly dismantled and burnt to obtain precious metals. Some research works have linked PCB emission and exposure e-waste recycling processes (Tang et al., 2010; Caravanos et al., 2011; Gioia et al., 2011). In Ghana, PCBs have been measured in environmental and biota samples such as breast milk and fish (Asante et al., 2011; Adu-Kumi et al., 2010).

2.2.2. ORGANOCHLORINE PESTICIDES (OCPS)

Organochlorine pesticides are a class of human-made or synthetic organic compounds consisting of Carbon, Hydrogen Chlorine and sometimes Oxygen. They are broadly classified as; Chlorinated Cyclodienes (endosulfan, aldrin, endrin, dieldrin, heptachlor, and chlordane), dichloro diphenyl ethanes (DDT, DDE, DDD, methoxychlor) and chlorinated benzenes (toxaphene, mirex, HCB, HCHs, and chlordecone) (UNEP, 2001). Organochlorine pesticides are persistent, semi-volatile, long range transportable in air, lipophilic, highly toxic, and able to bioaccumulate and biomagnify along the food chain (Chen et al., 2008). Organochlorine pesticides were found to exhibit a deleterious effect on wildlife and humans, and this led to their complete ban and others resolve to restricted use (Carson, 1962; Dunlap, 1981). Organochlorine pesticides are associated with many health effects such as congenital disabilities, low sperm count, cancers, reproductive and developmental effects (Weltman and Norback, 1983).

The use of Organochlorine pesticides in Ghana started in the 1940s (GEPA, 2007). Organochlorine pesticides were massively due to their efficacy, affordability and their broad range of activity in controlling crop pest and diseases (Gerken et al., 2001; Wang, 1991) and this boosted large yield in the agriculture sector (Malina et al., 2005). They were used in the agriculture sector to curb crop pest and in the area of public health for disease control (Clarke et al., 1997; Ntow, 2005). For instance, DDT has used to control pest on farm animals, on cocoa and cotton farms to eradicate crop pest (Ntow et al., 2001, 2006). Lindane was also used on cocoa farms, vegetable, and maize farms to eradicate crop pest (Ntow et al., 2006). It is worth mentioning that, DDT contributed immensely in the public health sector in the fight against malaria caused by Anopheles mosquitoes, Aedes aegypti mosquitoes; the causative organism for yellow fever, and black flies which causes Onchocerciasis in humans (Ntow et al., 2001, 2006).

Organochlorine pesticide residues in the Ghanaian environment is as a result of its massive use in the past, unintentional spills, and unlawful disposal of these chemicals and their containers (Ntow, 2006).

In Ghana, Organochlorine pesticides have been recorded in different matrice. This matrice include fish, water, sediment, cow milk, human fluids, fruits and vegetables and others (Ntow et al., 2001; Darko and Acquah, 2007a; Darko, and Acquah, 2007b; Darko et al., 2008).

2.2.3. POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds made up of two or more merged aromatic rings. PAHs are ubiquitous environmental contaminants which are the aftermath of pyrolysis or partial combustion of organic

matter through natural and anthropogenic means (WHO, 2003). Natural activities such as forest fires, decomposition of organic substances by bacteria and volcanic eruptions are some few natural sources of PAHs in the environment (Zhang and Tao, 2009). PAHs are also released into the environment through anthropogenic activities such incomplete burning of fuels, garbage, tobacco smoking, coke ovens, e-waste recycling activities among others (Zhang and Tao, 2009, Armstrong et al., 2004; See et al., 2006)

Figure 2-2 A possible source of pollution emanating from an e-waste burning site[1]

Also, Food processing activities such as roasting, grilling, and frying contribute to PAHs formation and food contamination (Chen and Lin, 2001, Essumang et al., 2012). Due to the numerous activities leading to PAHs formation, they have become ubiquitous in the environment and have been measured in indoor and outdoor air, soil, water and food (ACGIH, 2005).

PAHs are lipophilic in nature and hence tend to be soluble in organic solvents. PAHs also exhibit characteristics like high melting and boiling points, poorly soluble in water and low vapour pressure among others (Masih et al.,2010, 2012; Akyuz and Cabuk, 2010; CCME,2010).

PAHs are known to pose serious health problems depending on the duration and level of exposure, the toxicity of the PAHs, exposure ways and many others (ACGIH, 2005). PAHs can result in skin irritation and inflammation, eye irritation

[1] http://www.testigo.pl/stories/the-worlds-largest-e-waste-dump/

(Unwin et al., 2006). PAHs can also cause all forms of cancers such as skin, gastrointestinal, lung, and bladder (Bach et al., 2003; Boffetta et al.,1997; Olsson et al., 2010; Diggs et al., 2011). Also, PAHs are associated with congenital disabilities, low IQ, cell damage, DNA alteration. PAHs can result in Reproductive problems, neurological defects, preterm delivery and many other serious health challenges. (Garcia-Suastegui et al.,2011; Gunter et al., 2007; John et al., 2009; Kuo et al., 2003; Jong et al., 1999; Latif et al., 2010; Kristensen et al., 1995; Perera et al., 2005; Edwards et al., 2010).

In Ghana, PAHs have been recorded in different matrices such as air, soil, fish, urine, water and many others (Essumang et al., 2016, 2009, 2010, 2012; Feldt et al., 2014). However, to the best of our knowledge, there is no data on PAHs level in human breast milk even though breast milk plays a critical role in the life of developing children. The result from this thesis work will, therefore, serve as a baseline data in Ghana.

2.3. ELECTRONIC WASTE AS POSSIBLE SOURCE OF ENVIRONMENTAL POLLUTANTS

The era of technology has brought to birth the manufacturing of all forms of electrical and electronic equipment (EEE). Their benefits are enormous, and in fact, they have contributed in making life simple. New and improved products are invented and produced almost each passing day, and the old products become a waste since people naturally have the flare for new and improved gadgets. It is therefore not surprising that about 20-50 million tons of electronic waste (e-waste) is generated yearly worldwide by industrialized countries (UNEP, 2005). Unfortunately, a large chunk of these obsolete or partially functioning devices ends up in less developed countries that have no authorized means of dealing with these electronic wastes.

Ghana like many developing nations has become a hub for most old and semi-functioning electric and electronic gadgets. In 2009, about 215,000 tons of brand new and used EEE were brought into Ghana, and 129,000 tons of e-waste was generated in that same year (E-Waste Africa Programme, 2011). The obsolete electric and electronic equipment finally end up in the hands of some unskilled individuals who try to use every means available to them to extract some precious minerals such as gold, iron, copper and others embedded in the obsolete equipment. Ghana has one of the largest e-waste dumps in West Africa. Agbogbloshie, a 6.2-hectare site (15 acres), formally a wetland located on the west of Odaw River in the heart of Accra, the capital city of Ghana is the largest electronic waste site in the country (Brigden et .al., 2007). At Agbogbloshie, anything electrical or electronic from automobile to tiny cables is openly dismantled and burnt by people with or barely any knowledge in e-waste recycling to recuperate precious minerals (Brigden et al., 2007). These e-waste workers carry out their activities without any safety

measures, and they work without any safety apparels. The working site also serves as a home for some of these employees and their families. Unfortunately, e-waste recycling activities do not only lead to obtaining precious metals which generate money but also toxic substances. Examples of these pollutants are PCBs, PAHs, polybrominated diethers (PBDEs) and heavy metals (Asante et al., 2012; Caravanos et al., 2011; Kyere et al., 2016; Feldt et al., 2014; J. Wang et al., 2012; Q. Wang et al., 2016).

Figure 2-3 E-waste workers dismantling obsolete electronic materials[2]

Figure 2-4 An open burning of electronic materials[3]

[2] https://motherboard.vice.com/en_us/article/inside-the-worlds-biggest-e-waste-dump

Some works by researchers are associating e-waste activities as the emergence source of POPs in the environment after their ban from production and active use (Gioia et al., 2014). E-waste dismantling and unlawful recycling activities are resulting in exposing embedded industrially manufactured POPs such as PCBs in old electric and electronic equipment (EEE). The incomplete combustions associated in these e-waste recycling processes is a primary source of production of PAHs, Dioxin and furans, PBDEs and others (Eckhardt et al., 2007; Sepulveda et al. 2010; Gioia et al. 2011; Breivik et al. 2011; Lau et al. 2012).

Figure 2-5 The picture of metals obtained from old electronic materials[4]

[3] https://www.pinterest.com/pin/474355773226684689/

[4] http://www.themalaysiantimes.com.my/india-emerging-as-worlds-largest-e-waste-dumping-ground/

CHAPTER 3. MATERIALS AND METHODS

3.1. ETHICAL CLEARANCE

Ethical approval was sought from the Research and Development Division of the Ghana Health Service (GHS), Ministry of Health (MoH) before the commencement of this research work since the project involved the use of human specimen. The ethical approval was granted after the committee reviewed the research proposal, survey questionnaire, sampling, and sample preservation protocols were in accordance with World Health Organisation's (WHO) Basic Principles for Sampling of Human Breast Milk (WHO, 2007). Among some of the general principles was to ensure that: the Research work will by no means compromise breastfeeding. The importance of breastfeeding to both mother and child must be emphasized. Also, the sampling of breast milk will by no means put undue pressure on nursing mothers or compromise with the basic nutritional requirement of babies, and finally, mothers willingly donate breast milk, and their identities kept confidentially.

The Ghana Ethical Review Committee's approval letter is attached as Appendix 1.

3.2. STUDY AREAS FOR THE RESEARCH

Four areas in the greater Accra region of Ghana were selected for this Research work. They include:

a) Agbogbloshie e-waste site and environs
b) Kwabenya and environs (Residential area) without any major economic activity which is being used as a control)
c) Ada and its surroundings (Rural)
d) La and its surroundings (Urban)

Child welfare centers within these vicinities were selected as locations for breast milk sampling.

Human breast milk samples from Agbogbloshie and Kwabenya were screened for PCBs and PAHs. The donors were both primiparae and multiparae mothers.

Breast milk from Ada and La were used to assess levels of organochlorine pesticide residues. All the milk donors selected for the study in these areas were first birth mothers.

3.2.1. DESCRIPTION OF THE STUDY AREA

3.2.1.1 Agbobgloshie and environs

Agbogbloshie is a 15 acre formally wetland located in the heart of Accra, the capital city of Ghana. It is situated on the west side of the Odaw River and adjacent to one of the largest food markets in Accra. Agbogbloshie is also surrounded by offices, schools, hospitals, churches and mosques, recreational centers and residential facilities. The former wetland has gradually become the largest e-waste dump and recycling site in Ghana (Brigden et al., 2008). The site also serves as a hub for most of these unskilled e-wastes recycling workers and their families who are engaged in dismantling and recycling of everything electrical or electronic, from automobile to tiny cables is dismantled and recycled in the quest to obtain precious metals.

Figure 3-1 Some e-waste workers at Agbogbloshie[5]

[5] http://www.scidev.net/global/digital-divide/multimedia/electronic-waste-dump-supplies-ghana.html

Figure 3-2 Open burning activities by e-waste recycling workers[6]

3.2.1.2 Kwabenya and its environs

Kwabenya and its surrounding areas (Taifa, Dome, Old and New Ashongman, Haatso, Atomic, and Agbogba) are solely residential settlements without any major economic activity that can pose any significant in pact on the environment and the inhabitants.

3.2.1.3 Ada and its environs

Ada is a rural community in the greater Accra region of Ghana. The majority of the general populace are involved in vegetable farming and fishing activities. A detailed description of this study area is paper IV.

[6] http://arthag.typepad.com/arthag/2011/10/pieter-hugo-yossi-milo.html

3.2.1.4 La and it's environ

La is an urban community and a suburb of Accra the capital of Ghana. It is a normal busy city settlement with inhabitants from all walks of life. A map of the area can be found in paper III.

3.3. SAMPLING OF BREAST MILK SAMPLES

3.3.1. EDUCATION, SELECTION AND ADMINISTRATION OF QUESTIONNAIRE

Potential donors were educated on the significant of the project, and those who fall within the selection criteria and were willing to participate were enrolled in the study by first filling a prior informed consent form. Mothers were then made to fill a questionnaire which was designed in accordance with WHO guidelines for Sampling Human Breast Milk (WHO, 2007). The questionnaire was to help provide information on the nursing mother's occupation, health status, dietary, smoking habits. Also, the questionnaire was to provide information on the age, number of birth and other factors capable of influencing the presence of POPs in breast milk (Minh et al., 2013; Croes et al., 2012; Lee et al., 2013; Chovancova et al., 2011; Tue et al., 2010; Li et al., 2008; WHO, 2007). A copy of the questionnaire can be found in Appendix 2. The education, selection, administration of questionnaire and donation of breast milk samples were done in child welfare health centers within the selected study areas.

Figure 3-3 Potential breast milk donors at a child welfare health center

Figure 3-4 A picture showing a mother donating milk sample for the study

Donated milk samples were kept in dry ice and stored at Ghana Standard Authority Pesticide Residues laboratory where they were kept frozen until extraction and analysis.

3.4. LABORATORY METHODS USED IN THE STUDY

Credible laboratory methods were employed in the assessing these POPs. The QuECHERs (Quick Effective Cheap Effective and Rugged and Safe) method which is one of the efficient and widely used techniques was used in this study. (Anastassiades et al., 2003; Castillo et al.,2011; Smoker et al., 2010; Li et al., 2013; Jeong et al.,2012; Luzardo et al., 2013).

All the methods used in the extraction, clean-up and analytical including quality procedures used in assessing the contaminants are explained in details in the list of papers.

Figure 3-5 is a flow chart representing the QuECHERS method.

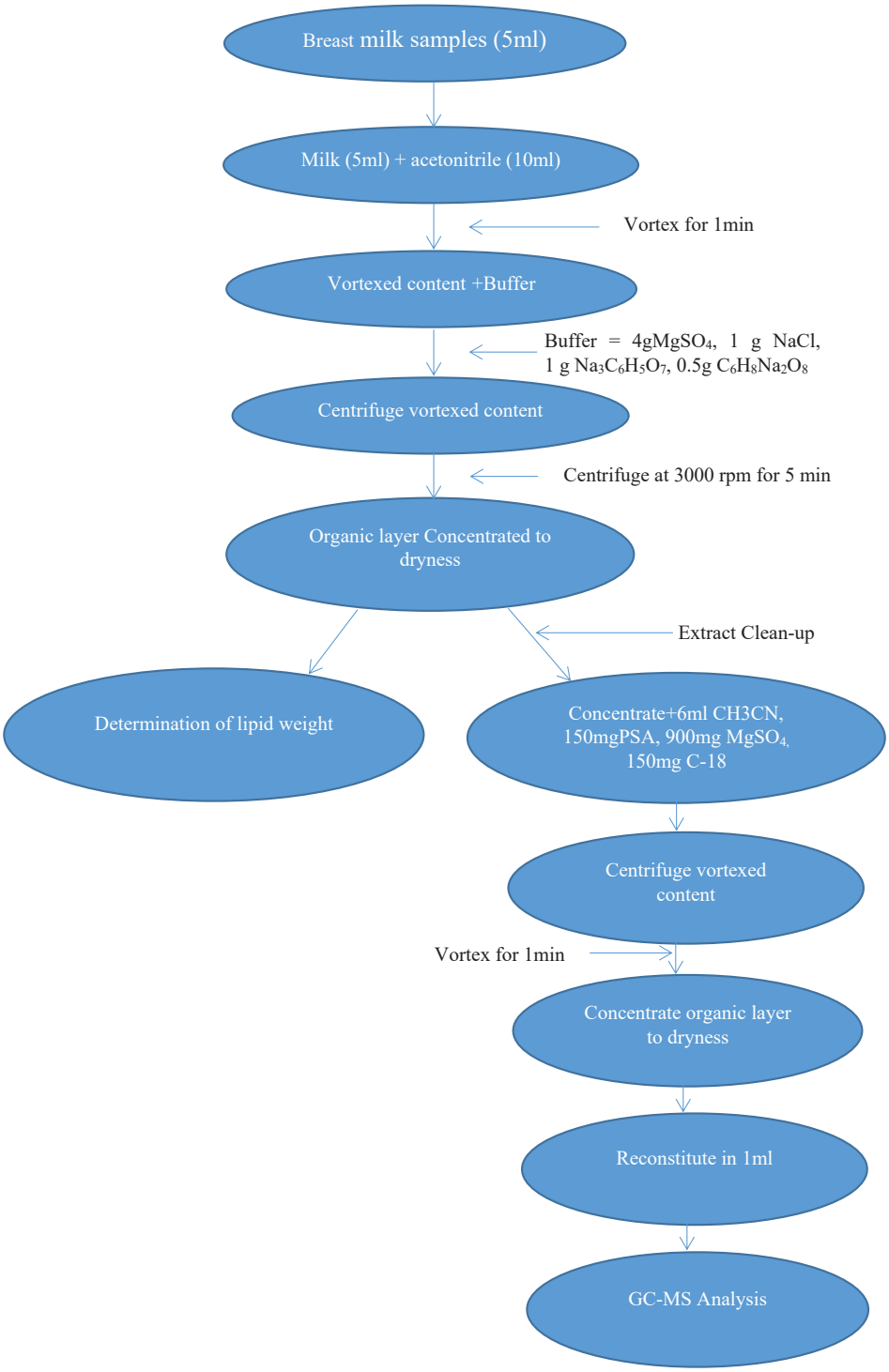


Figure 3-5 The experimental procedure for PCBs and PAHs analysis in Breast milk

3.4.1. DETERMINATION OF LIPID CONTENT OF BREAST MILK

Lipid content was determined gravimetrically according to the method described by Wu et al. (2001); Binelli et al. (2001); Wells and Hess (2000) and also (AOAC, 1990). The empty round bottomed flasks were first weighed (W_1) before used for the POPs extraction. Again, the flask with the concentrate was weighed (W_2) after evaporating the extracts to near dryness using the rotary evaporator or under a gentle Nitrogen stream. Percentage lipid content of breast milk samples was calculated using the equation:

$$\%\ \boldsymbol{Lipid} = \frac{(\boldsymbol{W_2} - \boldsymbol{W_1})}{\boldsymbol{Mass\ of\ breast\ milk\ used}(\boldsymbol{5g})} \qquad \text{Eq. 3-1}$$

3.5. STATISTICAL ANALYSIS

R version 3.4.0 and STATA® /IC version 14.2 (Stata Corporation, College Station, TX, USA) were used for the data analysis.

3.6. RISK ASSESSMENT

3.6.1. RISK ASSESSMENT FOR PCBS

The Daily intake (DI) of PCBs by infants was calculated on the assumption that average weight of a baby is 5 kg and average milk consumption of a 5 kg infant is 700g/day (Oostdam et al., 1999)

$$\boldsymbol{DI} = \frac{\boldsymbol{C_{milk}} \times \boldsymbol{700\ g\ milk/day} \times \boldsymbol{C_{lipid}/100}}{\boldsymbol{5\ Kg\ body\ weight}} \qquad \text{Eq. 3-2}$$

Where

C_{milk} is the concentrations of chemicals in milk (μg/g lipid wt.);

C_{lipid} is the lipid content in milk (%).

The hazard quotient (HQ) is used in assessing Infants health risk as a result of ingesting PCB-contaminated breast milk. Hazard quotient (HQ) is the ratio of the estimated daily intake of a compound through breastfeeding to the maximum acceptable dose for humans or a reference dose (RFD). Hazard Quotient (HQ) greater than one (>1) suggests a potential risk (Oostdam et al., 1991). Hazard

Quotients of PCBs were calculated using a reference dose (RF) values of 1 (Oostdam et al., 1999) and also 0.03 µg/kg body wt. /day by (ATSDR, 2000)

3.6.2. CARCINOGENIC AND MUTAGENIC RISK ASSESSMENT IN PAHS

$$\boldsymbol{TEQ_{BaP} = \Sigma(TEF_i \times C_I)} \quad \text{Eq. 3-3}$$

$$MEQ_{BaP} = \Sigma(MEF_i \times C_I) \quad \text{Eq. 3-4}$$

C_i represents the different PAHs concentration with its corresponding Toxicity Equivalent Factor (TEF_i) or Mutagenic Equivalent Factor (MEF_i) value.

The benzo[a]pyrene (BaP) equivalent dose is calculated using equation 4

$$\boldsymbol{BaP\ equivalent\ dose\ of\ carcinogenic\ (mutagenic) PAHs\ (BaPEQ)} = \frac{\boldsymbol{TEQ(MEQ) \times IR \times EF \times ED}}{\boldsymbol{BW \times AT}} \quad \text{Eq. 3-5}$$

The default values on exposure and intake assumptions were made in agreement with US EPA guidelines (USEPA, 1991).

Where *IR* is the intake rate of breast milk in g per day; *EF* is the Exposure frequency to carcinogenic or mutagenic PAHs in days per year; *ED* is exposure duration in years; *BW* is the average body weight of a baby in kg and *AT* is the average life expectancy.

Default values used are:

IR=700 g milk/day; *ED*= 1 year; *BW*=5 kg; *EF*= 350 days/year and *AT* =2 years

Cancer or mutagenic risk is calculated based on equation 5 as follows:

$$\boldsymbol{Risk(carcinogenic\ or\ mutagenic) = SF_{BaP} \times BaP\ equivalent\ dose\ of\ mixture\ of\ PAHs} \quad \text{Eq.3-6}$$

SF_{BaP} is the oral carcinogenic slope factor for benzo[a]pyrene (BaP) which is 7.3 per mg/kg/day.

Table 3-1 Recommended benzo[a]pyrene equivalent factors for carcinogenic (TEF) and mutagenic toxicity (MEF)

	BaA	Chr	BbF	BkF	BaP	IndP	DahA
TEF USEPA (1993)	0.100	0.001	0.100	0.010	1.000	0.100	1.000
MEF Durant et al. (1996, 1999)	0.017	0.082	0.250	0.110	1.000	0.310	0.290

3.7. SOIL SAMPLING

A total of forty surface soil samples were collected randomly from both e-waste dismantling and burning sites at Abgogbloshie. The soil samples were homogenously mixed and air dried at room temperature. They were then sieved through 100µm mesh.

3.7.1. SAMPLE EXTRACTION AND ANALYSIS

3.7.1.1 Reagents and materials

It was ensured that all chemicals used in this research work were of high purity standard. PCB –Mix 3 from Dr. Ehrenstorfer GmbH, 99%, PAHs standard mix 16 from Dr. Ehrenstorfer GmbH, 95.9-99.9% purity. Pesticide grade Acetonitrile, Analytical grade ethyl acetate, separating flask (100ml), round-bottomed flask (50ml) and 50ml pear shaped flask were used.

3.7.1.2 Equipment

The main equipment used in this study included a sonicator, a mechanical shaker, a rotary evaporator and the GC-MS/MS.

3.7.1.3 Extraction of soil sample

Approximately 10g of the comminuted homogenous soil sample was weighed into 100ml separating flask. A10ml volume of acetonitrile was added to the soil in the separating flask. The flask with its content was corked and sonicated for 5 minutes. An additional 10ml of acetonitrile was added to the content in the separating flask, corked and placed on a horizontal mechanical shaker continuously for 30 minutes.

The separating funnel and its content were allowed to stand for 10 minutes for the separation of the layers. An aliquot of the organic layer was pipetted into a 50ml round-bottomed flask and evaporated to ca. 2ml (RFE 35°C) for extract clean-up.

3.7.1.4 Extract Clean-Up

A silica (1000mg/ 6ml) cartridge with a 1cm thickness layer of anhydrous magnesium sulphate on top was conditioned with 10ml of acetonitrile. The extract was loaded onto the cartridge and the eluate collected into a 50ml pear shaped flask. The cartridge with the extract was further eluted with 10ml acetonitrile and the eluate concentrated just to dryness at 35°C on the rotary evaporator. It was finally re-dissolved in 1ml ethyl acetate. The extract was transferred into a 2ml standard opening vial prior to quantification by GC-MS/MS.

The extraction and clean-up processes was repeated for all the other soil samples including beach sand which was used as sample blank.

3.7.1.5 Instrumental Analysis and Quantification

The following GC conditions were used in the analysis and quantification of PCBs and PAHs in the soil samples. The samples were analyzed for PCBs using Agilent Technologies 7890B 7000C GC-MS/MS Triple Quard with auto sampler 80 and Helium as Carrier gas. Injection temperature was 280 ˚C, splitless mode and 2.0 µl injection volume. The ion source was EI mode, source temperature of 300 ˚C and MSD transfer line of 325 ˚C. The column type HP-5ms (30 m x 0.25 mm x 0.25 um) was used with column flow of 1.25 ml/min. The column temperature was first set at 70 ˚C and held for 2 minutes ramped to 150 ˚C at 25 ˚C/min and then to 200 ˚C at 3 ˚C/min) and then finally to 280 ˚C and held at 3.133 minutes for PCBs and 12.133 minutes for PAHs. The solvent delay time was 4mins and a total run time of 35 minutess for PCBs and 44 minutes for PAHs.

3.7.1.6 Analytical Quality Controls

Quality was ensured in this study by performing procedural blanks alongside every batch of sample. This was to ascertain that no contamination was emanating from solvents and glassware used during the sample preparation. Also, blank beach sand were spiked with known concentrations of PCBs and PAHs for recovery testing. Repeatability was also employed in the study.

CHAPTER 4. ASSESSMENT OF PAHS IN BREAST MILK

4.1. BACKGROUND

This chapter presents the summary of the results in paper I. The aim of the study was to access the levels of contamination in the breast milk samples of some Ghanaian mothers from a hot spot and non- hotspot areas. A total of 128 women of which 105 were donors from Agbogbloshie e-waste site and 23 from Kwabenya participated in this study.The 128 human breast milk samples were screened for 18 different polycyclic aromatic hydrocarbons (PAHs). The level of contamination of the various PAHs in the milk samples from both Agbogbloshie e-waste site which is a hotspot area and Kwabenya a non- hot spot area was assessed. The influence of parity, age, body weight on the PAHs concentrations levels in the milk samples was evaluated. The possible sources of the PAHs in the milk samples have been predicted using the PAHs diagnostic ratio test. Cancer and mutagenic risk in infants as a result of being breastfed with PAHs contaminated milk were estimated.

4.2. LEVEL OF CONTAMINATION OF PAHS IN THE HUMAN MILK SAMPLES

In entirety, 18 different PAHs; naphthalene (Naph), 2-methylnaphthalene (2-Met), 1-methylnaphthalene (1-Met), acenaphthylene (Acy) , acenaphthalene (Ace) ,fluorene (Flu), anthracene (Ant), phenanthrene (Phe), pyrene (Pyr), fluoranthene (Flt), chrysene (Chr), benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), benzo[b]fluoranthene(BbF), benzo[g,h,i]perylene (BghiP), dibenz[a,h]anthracene (DahA),and indeno[1,2,3,c,d]pyrene (IndP) were recorded in the 128 human breast milk samples from Agbogbloshie e-waste site (a hot spot area) and Kwabenya (a non-hot spot area) in Accra, Ghana. Table 4-1, presents the mean concentrations (ng/g lipid wt.) of the 18 PAHs found in the milk samples of the 128 donor mothers.

Table 4-1 Mean concentrations (ng/g lipid wt.) of 18 PAHs in human milk 128 human milk samples

PAHs	Mean±SD	Range	Positive Samples	IARC[a]	EPA[b]
Naph	856.21±1782.04	< LOD* - 14320.67	115	2B*	D*
2-Met	131.84±108.58	< LOD - 537.77	125		
1-Met	76.36±104.93	< LOD - 773.80	116		
Acy	1.37±2.70	< LOD - 5.92	125		D
Ace	1.76±4.77	0.036 - 28.91	123	3	N/A
Flu	3.41±4.00	< LOD - 25.90	75	3	N/A
Ant	12.76±11.20	< LOD- 96.01	109	3	D
Phe	9.51±8.88	< LOD - 45.58	84	3	D
Pyr	2.77±2.08	< LOD - 14.47	114	3	D
Flt	4.63±3.17	< LOD - 16.62	115	3	D
Chr	0.30±0.38	< LOD - 2.77	100	2B	B2
BaA	0.22±0.32	< LOD - 2.21	67	2B	B2
BaP	0.08±0.17	< LOD - 1.66	97	1	B2
BkF	0.04±0.07	< LOD - 0.50	66	2B	B2
BbF	2.94±4.89	< LOD - 32.41	72	2B	B2
BghiP	0.60±1.03	< LOD - 7.64	103	3	N/A
DahA	0.14±0.64	< LOD - 6.12	110	2A	B2
IndP	0.69±1.06	< LOD - 7.66	116	2B	B2
Total	1105.629	<LOD – 15936.57			

LOD: the limit of detection.

a: IARC classification: 1: Carcinogenic to human; 2A: Probably carcinogenic to human;

2B: possible carcinogenic to human; 3: Not classifiable to human.

b: US-EPA classification: B2: Probable Human Carcinogen; D: Not Classifiable; N/A.: Not Available

The means concentrations for the 18 PAHs in the 128 breast milk samples as shown in Table 2 was 1105.629ng/g lipid and ranges from <LOD to 15936.57 ng /g lipid wt. The mean values were higher compared to what was obtained by Cok et al., 2012.The total mean of 16 PAHs in 47 milk samples in work done by Cok et al., 2012 was 84.42 ng/g lipid wt. It can also be observed from Table 2 that the mean values for low molecular weight PAHs were higher than those recorded by the high molecular weight PAHs. The molecular weights of 128 g/mol for naphthalene, and 142 g/mol for both 2-methylnaphthalene, and 1-methylnaphthalene had their mean concentrations as 856.21 ng/g lipid wt., 131.84 ng/g lipid wt., and 76.36 ng/g lipid wt. respectively. The mean concentrations recorded for the high molecular weight PAHs such as dibenz[a,h]anthracene (278g/mol), indeno[2,1,3,c,d]pyrene

(276g/mol) and benzo[k]fluoranthene (252g/mol) were much lower as can be seen clearly in Table 4-1. A similar trend was observed in studies conducted in other countries in the world (Pulkrabova et al., 2016; Cok et.al, 2012).

Figure 4-1 shows the various mean concentrations by the seven probable carcinogenic PAHs from the Agbogbloshie hot spot area and Kwabenya the non-hot spot area. It can be observed from the figure that apart from the mean concentrations of dibenz[a,h]anthracene (DahA), all the mean concentrations of all the others were greater in the milk samples from Agbogbloshie than in Kwabenya. The mean concentrations of chrysene (Chr), benzo[a]pyrene (BaP), benzo[a]anthracene (BaA) and benzo[k]fluoranthene (BkF) were not detected in the milk samples from Kwabeyna. The mean concentration of benzo[b]fluoranthene(BbF), was the highest in the milk samples from both Agbogbloshie and Kwabenya even though the average concentration in the milk samples from Agbogbloshie were greater than that of Kwabenya. Almost all these seven probable PAH have high molecular weights and are less soluble in water. They have high LogK_{ow} and therefore are not easily metabolized and hence tend to accumulate in fatty tissues and remain for a long time (Feng et al., 2015). High molecular weight PAHs are predicted to be originating from a Pyrogenic source (Inam et al., 2015; Malik et al., 2011). The e-waste activities at Agbogbloshie might be a contributing factor to the presence of this high molecular weight and probable carcinogen PAHs in the milk samples of donors from Agbogbloshie and its surrounding areas. A study by Yunker et al., (2002) emphasized that the prevalence of high molecular weight PAHs could be as a result of high-temperature combustion from pyrogenic sources (coal, biomass combustion, or traffic) in an area.

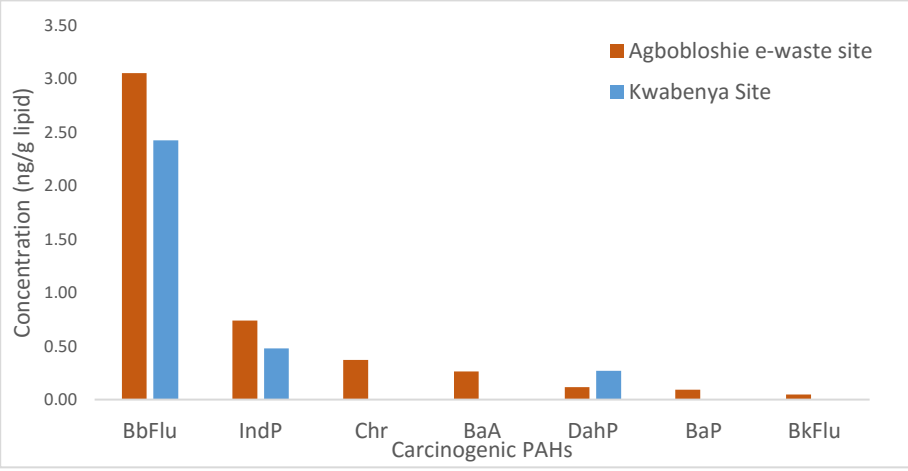


Figure 4-1 the concentrations of the seven probable carcinogenic PAHs in the breast milk samples of donors from Agbogbloshie e-waste and Kwabenya sites.

Figure 4-2 below shows the percentage contributions of the individual PAHs to the total PAHs in the milk samples. Naphthalene contributed the maximum of 77.4 % out of the overall PAHs in the breast milk samples. The least contributors of approximately 0.01% and 0% were dibenz[a,h]anthracene and benzo[k]fluoranthene, respectively.

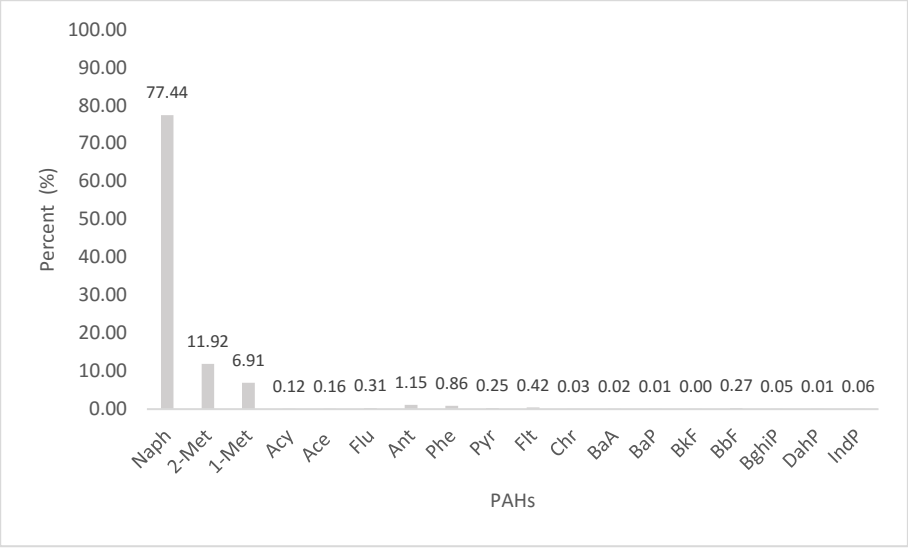


Figure 4-2 The contributions of each PAHs to the total PAHs in the breast milk samples

Table 4-2 below shows the general demographic characteristic of mothers used in the study. There was no correlation between these demographic features and the PAHs concentrations in the milk sample.

Table 4-2 General demographic features of the breast milk Donors

	Agbogbloshie e-Waste site		**Kwabenya**	
	Mean	Range	Mean	Range
Age (Years)	27.6	18-41	28.6	19-35
Weight (Kg)	74.3	60.3-92	56	53-69
Height (m)	1.63	1.43-1.92	1.4o	1.90
BMI (Kg/m^2)	28.02	22.24-36.59	22.5	18-25
Diet	Mixed		Mixed	
Primiparae	47 Samples (44.76%)		8 (34.78%)	
Multiparae	58 Samples (55.24%)		15 (65.22%)	

4.3. SOURCE APPORTIONMENT

The diagnostic ratio test is useful in predicting the sources or source of PAHs in a sample matrix. The ratios of different PAHs suggest whether the PAHs in the sample is from petrogenic, pyrogenic and burning of biomass or coal origins (Tobiszewski, 2014; Yunker et al., 2002). The test could be a ratio of single PAHs as Phe/Ant or a combination of two PAHs, and an example is Ant/(Ant+Phe). Studies conducted by researchers in different parts of the world have suggested the coupling of two PAHs to give a better result. This is because of the effects of factors such as volatility, aqueous solubility and degradation on individual PAH (Chen et al., 2012; Malik et al., 2011). A ratio of Ant/(Ant+Phe) aids in classifying PAHs from petrogenic origins, and BaA/(BaA+Chr), FLT/(Flt/+Pyr), as well as IndP/(IndP+BghiP), are suitable in categorizing PAHs from pyrolytic origins (Yunker et al., 2002). Table 4-3 below presents the results obtained from the diagnostic study.

Table 4-3 Diagnostic ratios for PAHs with their various ranges

	Petrogenic	Pyrogenic	Mixed (Petrogenic+ Pyrogenic)	Agbogbloshie Site	Kwabenya Site	Reference
Phe/Ant	>10	<10	-	0.805	0.223	Chen et al., 2012
Ant/(Ant+Phe)	<0.10	>0.01	-	0.554	0.818	Inam et al., 2015; Yunker et al., 2002
BaA/(BaA+Chr)	<0.2	>0.35	0.2-0.35	0.415	NA	Chen et al., 2012
Flt/(Flt+Pyr)	<0.5	>0.5	-	0.606	0.709	Inam et al., 2015; Yunker et al., 2002
Ind/(Ind+BghiP)	<0.5	>0.5	-	0.503	NA	Inam et al., 2015; Chen et al., 2012

N/A: Not Applicable

As can be seen from the table 4-3 above, the PAHs diagnostic test performed on the samples from Agbogbloshie predicted that the PAHs in the milk samples are from pyrogenic sources. This may be attributed to the influence of the e-waste recycling activity at Agbogbloshie

Research works have suggested e-waste recycling processes as a source of PAHs in the environment since the activity involves the combustion of all forms of materials (Feldt et al., 2014; Wang J. et al., 2012; Wang Qi et al., 2016).

4.4. CANCER AND NON-CANCER RISK ASSESSMENT

Cancer and mutagenic risks to infants based on ingestion of PAHs contaminated milk were performed. The details on the equations and assumptions in the used cancer and mutagenic risk assessments are shown in details in the paper I and chapter 3 as well. cancer and mutagenic risk assessment based on using the seven PAHs considered by the USEPA (2002) as probable carcinogens. They include , benzo[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[k]fluoranthene (BkF), benzo[b]fluoranthene (BbF), dibenz[a,h]anthracene (DahA), indeno[1,2,3,c,d]pyrene (IndP) and chrysene (Chr).

Table 4-4, presents the results for results for cancer and mutagenic risk test. The equations and the assumptions used in the risk assessment are shown in section 3.6.2.

The toxicity equivalent (TEQ) and Mutagenic equivalent (MEQ) were calculated to be 1.462 and 2.645 ng/kg/day, respectively.The TEQ and MEQ values were used to calculate Benzo[a]pyrene Calculated BaPEQ daily dose for an infant with a daily intake of 700 g milk per day based. The corresponding risk values for carcinogenicity and mutagenicity have been computed to be 1.1×10^{-5} and 1.9×10^{-5}respectively. This means that approximately 1 out of 100000 and 2 out of 100000 infants may have cancer and other non-cancer related adverse diseases in a lifetime.

Table 4-4 Carcinogenic and mutagenic risk assessment based on BaP equivalency for human breast milk

	mean concentration (ng/g milk)	TEF	TEQ	BaPEQ Daily dose (ng/kg/day)	PAHs carcinogenic risk	MEF	MEQ	BaPEQ Daily dose (ng/kg/day)	PAHs carcinogenic risk
BaA	0.008	0.100	0.00078	0.052	3.8E-07	0.082	0.00064	0.043	3.1E-07
Chr	0.011	0.001	0.00001	0.001	5.4E-09	0.017	0.00019	0.012	9.1E-08
BbFlu	0.106	0.100	0.01058	0.710	5.2E-06	0.250	0.02645	1.775	1.3E-05
BkFlu	0.001	0.010	0.00001	0.001	7.0E-09	0.110	0.00016	0.010	7.7E-08
BaP	0.003	1.000	0.00276	0.186	1.4E-06	1.000	0.00276	0.186	1.4E-06
IndP	0.025	0.100	0.00249	0.167	1.2E-06	0.310	0.00772	0.518	3.8E-06
DahA	0.005	1.000	0.00515	0.346	2.5E-06	0.290	0.00149	0.100	7.3E-07
Total	**0.159**		**0.02178**	**1.462**	**1.1E-05**		**0.03940**	**2.645**	**1.9E-05**

CHAPTER 5. ASSESSMENT OF PCBS IN THE BREAST MILK

5.1. BACKGROUND

This chapter presents a summary of Paper II. PCBs have never been produced in Ghana but have been used in transformer oil, plasticizers which are have been identified as the largest release of PCBin the Ghanaian environment (EPA, Ghana2007). This study aims at assessing the current levels of PCBs in Accra, Ghana after its ban since the 1970s. Levels of PCBs were measured from 128 donor mothers from Abgogbloshie e-waste site which is a hot spot area and Kwabenya and its surrounding communities (a non-hot spot area). Donor mothers from Agbogbloshie (hot-spot area) were 105 of which 44.74% and 55.24% were primiparae and multiparae mothers respectively. Participating mothers from Kwabenya (non-hot spot) area were 23 of which 34.78% and 65.22% were primiparae and multiparae mothers respectively. Infants risk to ingesting PCB contaminated milk was also assessed. The effect of mother's age, body mass index (BMI), parity and eating habit on the levels of PCBs in the milk sample was also evaluated.

5.2. RESULTS AND DISCUSSIONS

5.2.1. LEVEL OF CONTAMINATION OF PCBS IN THE MILK SAMPLES

Seven indicators PCBs were found in the breast milk samples. The include PCB-18, PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180. The mean, standard deviation, median and range values of the PCBs in milk is shown in Table 5-1.

The total means of PCBs in Agbogbloshie and Kwabenya were found to be 4.428 ng/g lipid weight and 0.02 ng/g lipid wt respectively. The PCB concentrations range between 0.002ng/g lipid and 6.069 ng/g lipid for Agbogbloshie and <LOD and 0.670 ng/g lipid for Kwabenya respectively. Apart from PCB-28, all the other indicator PCBs in the breast milk samples from donors in Kwabenya were below the detection limit. The mean concentration for PCB-28 in the milk samples from Kwabenya was 0.02ng/g lipid wt, which is much lower as compared to the mean concentration of 1.33ng/g lipid wt. for PCB-28 in the milk samples from Agbogbloshie. PCB-28 was recorded in all the 105 milk samples from Agbogbloshie. The presence of PCBs in the milk samples of donors from Agbogbloshie might be as a result of their closeness to the Agbogbloshie e-waste site. E-waste activities have been cited as one of the ways through which PCBs get into the environment (Gioia et al., 2014; Chan and Wong, 2012; Wong et al., 2007).

Comparing the mean concentrations of PCBs in breast milk in this study to earlier works done in Ghana and other parts of the world it was observed that the mean values obtained in this work are is much lower. In 2009, Asante et al conducted a study in Accra, Ghana and had PCB-153 recording the highest concentration of 22 ng/g lipid wt. but 0.415ng/g lipid wt was recorded for PCB-153 in this study. The situation is different in this work; the PCB with the highest mean concentration was PCB-28.In this study, PCB-28 recorded mean concentration of 1.33ng/g lipid wt. The mean concentrations for PCB-28 were 0.61 ng/g lipid wt. in work done by Asante et al., 2009 and it is lower than what was observed in this study.

Table 5-1 Mean concentrations (ng/g lipid wt.) of indicator PCBs in breast milk samples

	Agbogbloshie e-Waste site, n=105				Kwabenya, n=23			
PCB	Mean±SD	Range	Median	Positive Samples	Mean±SD	Range	Median	Positive Samples
PCB-18	1.056±0.976	<LOD -6.069	0.84636	104	<LOD	<LOD	NA	0
PCB-28	1.303±1.247	0.002-5.750	0.79075	105	0.0291±0.139	<LOD -0.670	NA	1
PCB-52	0.185±0.201	<LOD -1.031	0.11074	105	<LOD	<LOD	NA	0
PCB-101	0.077±0.105	<LOD -0.532	0.04227	104	<LOD	<LOD	NA	0
PCB-138	0.863±0.928	<LOD -4.424	0.61653	104	<LOD	<LOD	NA	0
PCB-153	0.415±0.919	<LOD -5.428	0.10653	104	<LOD	<LOD	NA	0
PCB-180	0.529±0.778	<LOD -5.969	0.36057	105	<LOD	<LOD	NA	0

The various contributions of the individual PCBs to the total PCBs for the milk samples is shown in Figure 5-1, below. PCB-28 made the highest contribution of 29.4%, and PCB-101 contributed the least of 1.74% out of the total PCBs in the milk samples.

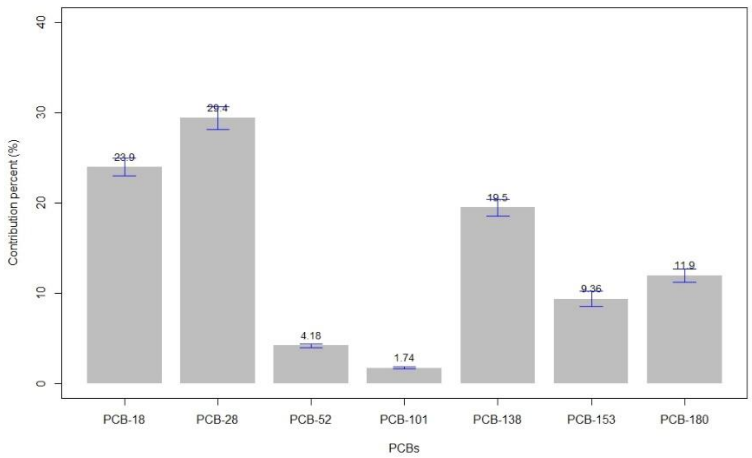


Figure 5-1 Contribution (in percentages) of each PCB congener to the total PCBs (error bars represent standard deviation of the mean concentrations)

5.2.2. MEAN CONCENTRATIONS OF PCBS IN PRIMIPARAE AND MULTIPARAE MOTHERS

The mean concentrations of the various PCBs in the breast milk samples from both Primiparae and Multipara mothers from Agbogbloshie are compared in Fig 13. below. There were no significant variations between the mean concentrations of the PCBs in the breast milk samples of both Primiparae and Multiparae mothers. However, it was observed that the mean concentrations of PCB-18 and PCB-28 were slightly higher in Primiparae mothers than in Multiparae. The opposite was observed for PCBs 153 and 180; the concentrations in Multiparae mothers were rather higher.

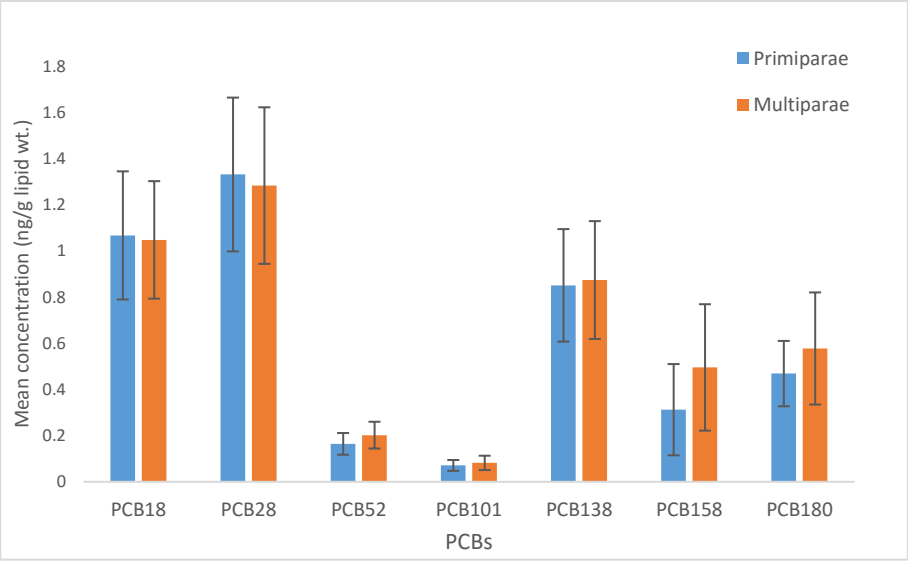


Figure 5-2 The percentage contributions of the various PCBs in Primiparae and Multiparae mothers (Error bar represent 95% confidence interval)

5.2.3. DAILY INTAKE AND HEALTH RISK ASSESSMENT

The equation and assumptions for calculating the daily intake and hazard quotients is shown in eq. 3-2 in subsection 3.6.1.

The value for Estimated Daily Intake (EDI) of PCBs by infants was 0.02 µg/kg body wt./day and ranges from <0.001 to 0.03µg/kg body wt./day. The Hazard Quotient (HQ) which is the ratio of the Acceptable Daily Intake (ADI) or the Tolerable Daily Intake (TDI) is less than one by using the health Canada's standard of 1 µg/kg body wt. /day. This indicates babies are under no risk. When the minimal risk value of 0.03 µg/kg body wt. /day by (ATSDR, 2000) was used, the total upper range value

was just at the threshold limit of 0.03 µg/kg body wt. /day, therefore, continuous monitoring of PCBs in humans is very necessary.

Table 5-2 represents the general demographic characteristics of the donor mothers of the milk samples. Some studies have reported some factors such as Body mass index (BMI), age, parity, specific food intakes by nursing mothers could influence the concentrations of contaminants in the breast milk (Harris et al.,2001). No correlation was observed between the PCB concentrations and parity, mothers age, diet, and BMI in this study. A similar trend was observed in other works (Minh et al., 2004; Asante et al., 2011; Yang et al., 2002).

Table 5-2 General demographic characteristics of the breast milk Donors

	Agbogbloshie e-Waste site		**Kwabenya**	
	Mean	Range	Mean	Range
Age (Years)	27.6	18-41	28.6	19-35
Weight (Kg)	74.3	60.3-92	56	53-69
Height (m)	1.63	1.43-1.92	1.4o	1.90
BMI (Kg/m^2)	28.02	22.24-36.59	22.5	18-25
Diet	Mixed		Mixed	
Primiparae	47 Samples (44.76%)		8 (34.78%)	
Multiparae	58 Samples (55.24%)		15 (65.22%)	

CHAPTER 6. ORGANOCHLORINE PESTICIDE RESIDUES IN THE BREAST MILK

6.1. BACKGROUND

This chapter is a summary of Paper III and IV. Organochlorine pesticides were used in Ghana since the 1940s to curb crop pest and the public health sector for disease control (GEPA, 2007). The properties of organochlorine pesticides such as persistence in the environment, lipophilicity, highly toxic to humans and wildlife, long range transport, and the ability to bioaccumulate and biomagnify along the food chain led to their ban (Chen et al., 2008; Carson, 1962; Dunlap, 1981). Organochlorine pesticides are associated with health problems like cancers and congenital disabilities, and low sperm (Weltman and Norback, 1983).

In this study, the levels of organochlorine pesticides were measured in the human breast milk samples of 42 first birth mothers in Ada (rural community) and La (urban community) in the Greater Accra regions of Ghana.

6.2. RESULTS AND DISCUSSION

6.2.1. LEVEL OF CONTAMINATION

A total of fourteen (14) different organochlorine pesticide residues were detected in the human breast milk samples from La and Ada. These include Gamma-HCH, Delta-HCH, Heptachlor, Aldrin, Gamma-Chlordane, Alpha-Endosulfan, Endosulfan-Sulphate, p,p'-DDT, p,p'-DDE, Dieldrin, Endrin, Endrin-Aldehyde, Endrin Ketone and Methoxychlor.

The mean concentrations of the various Organochlorine pesticides are shown in Table 6-1 below.

Table 6-1 Mean concentrations (µg/kg lipid weight) of OCP residues

	La		Ada	
OCP Residues	Mean±SD	Range	Mean±SD	Range
γ-HCH	4.2 ± 0.6	<0.001– 6.3	5.4 ± 1.6	<0.001– 11.4
δ-HCH	13.9 ±2.0	<0.001– 21.2	6.7 ± 3.5	<0.001- 10.3
Heptachlor	11.8 ± 1.2	<0.001– 17.4	0.7 ± 0.2	<0.001 – 1.2
Aldrin	3.0 ± 0.2	<0.001- 6.3	2.4 ± 0.6	<0.001
γ-Chlordane	1.8 ± 0.2	<0.001– 3.3	1.3 ± 0.4	<0.001 – 2.3
α-Endosulfan	4.7 ± 0.5	<0.001– 8.2	2.6 ± 0.7	<0.001– 4.1
Endosulfan Sulfate	99.1 ± 10.7	<0.001– 102.4	63.8 ± 11.2	<0.001– 119.7
p,p'-DDT	3.1 ± 0.4	<0.001– 7.3	6.3 ± 2.0	<0.001 – 17.2
p,p'-DDE	23.4 ± 3.2	9.5 – 42.1	24.2 ± 7.6	<0.001 – 55.3
Dieldrin	2.4 ± 0.3	<0.001– 5.5	2.2 ± 0.5	<0.001 – 4.2
Endrin	7.7 ± 1.0	<0.001– 12.2	3.5 ± 1.3	0.3 – 10.5
EndrinAldehyde	7.8 ± 2.7	<0.001– 13.7	<0.001	<0.001
Endrin Ketone	63.8 ± 33.1	<0.001– 98.2	1.4 ± 0.3	<0.001 – 8.2
Methoxychlor	20.1 ± 4.1	<0.001– 32.9	4.8 ± 0.7	<0.001 – 16.9
Total	266.8	9.5 – 337.0	125.3	0.3 – 261.3

The mean concentrations for Endosulphan of 91.1 µg/g lipid and 63.8 µg/g lipid were highest for both Accra and Ada, respectively.

The mean concentrations for Delta-HCH, heptachlor, Aldrin, Gamma Chlordane, Alpha-Endosulphan, Dieldrin, Endrin, Endosulfan-Sulphate, Endrin Ketone and Methoxychlor were higher in the human milk samples from Accra than Ada, whereas the mean concentrations for Gamma-HCH, p,p'-DDE and p,p'-DDT were higher in the human milk samples from Ada as compared to those from Accra. Endrin Aldehyde was detected in the human milk samples from Accra but not Ada.

Except for the mean concentrations of Endosulphan-Sulphate for human breast milk samples from both Ada and Accra, the average concentrations of all the organochlorine pesticide residues detected were below the Australian Maximum Residue Limit for human breast milk.

Figure 6-1 below shows the Organochlorine pesticides contribution for each area

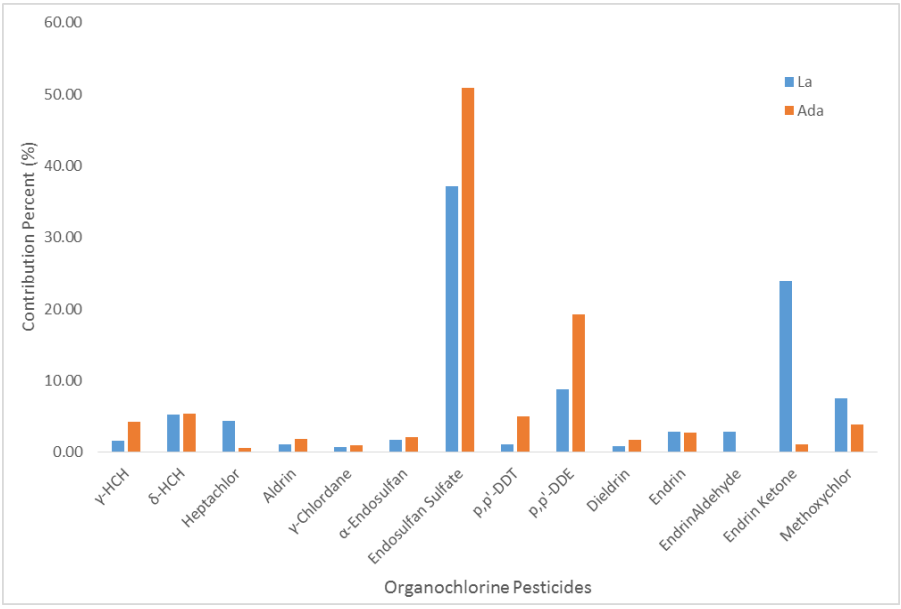


Figure 6-1 The contributions of the organochlorine pesticides from both La and Ada.

CHAPTER 7. PAHS AND PCBS CONTAMINATIONS IN SOIL SAMPLES

7.1. BACKGROUND

This chapter talks about the preliminary site studies that were conducted at the Agbogbloshie e-waste and recycling site before the commencement of the human breast milk study. We suspected the unauthorized activities at the e-waste site to be a probable source of PAHs and PCBs released into the environment. Soil from the e-waste site was sampled and taken to the laboratory where it was analyzed for PCBs and PAHs. Soil samples were also sampled from Kwabenya a non- industrial area as a control. Paper V which is still in the preparatory stage will provide full results of the study.

7.2. PRELIMINARY RESULTS

Tables 7-1 and 7-2 show the mean concentrations of both PCBs and PAHs in soil samples from Agbogbloshie e-waste site and Kwabenya.

Table 7-1 Mean concentrations in (µg/g) of PAHs in soil samples from Agbogbloshie e-waste site and Kwabenya.

	Agbogbloshie e-waste Site			Kwabenya Site		
	Mean	Range	Median	Mean	Range	Median
Naph	2.518±2.616	<LOD-8.694	1.943	0.164±0.086	0.077-0.364	0.137
2-Met	5.918±5.530	<LOD-15.964	5.664	0.090±0.023	0.048-0.124	0.097
1-Met	0.294±0.347	<LOD -1.134	0.186	0.096±0.092	0.011-0.312	0.069
Acy	0.047±0.040	2.5E-05-0.112	0.045	0.008±0.009	0.000-0.031	0.005
Ace	0.045±0.034	1.6E-04-0.087	0.057	0.066±0.125	0.000-0.352	0.004
Flu	0.588±0.561	3.7E-04-1.630	0.455	0.100±0.073	0.031-0.279	0.072
Ant	1.517±1.667	0.001-4.521	0.927	0.195±0.074	0.103-0.382	0.187
Phe	2.541±2.583	0.001-6.907	1.977	0.049±0.053	0.003-0.142	0.015
Pyr	1.134±1.252	3.7E-04-3.791	0.702	0.109±0.099	0.021-0.324	0.063
Flt	0.937±1.210	0.001-3.574	0.369	0.109±0.099	0.021-0.324	0.063
Chr	0.858±0.784	1.8E-05-1.951	0.680	0.087±0.056	0.013-0.185	0.077
BaA	0.836±0.830	5.3E-05-2.178	0.656	0.062±0.034	0.018-0.134	0.058
BaP	0.359±0.341	2.2E-05-0.887	0.264	0.039±0.026	0.003-0.107	0.033
BkF	0.357±0.339	2.2E-05-0.881	0.263	0.033±0.020	0.004-0.061	0.033
BbF	0.296±0.323	8.7E-06-0.922	0.188	0.015±0.016	0.003-0.057	0.007
BghiP	0.076±0.059	4.0E-05-0.169	0.081	0.048±0.023	0.018-0.108	0.044
DahA	0.076±0.059	4.0E-05-0.169	0.081	0.050±0.023	0.019-0.112	0.044
IndP	0.077±0.060	0.000-0.171	0.082	0.050±0.023	0.019-0.112	0.044
Total	18.475	0.003-53.744		1.369	0.411-3.508	

Table 7-2 Mean concentrations in (µg/g) of PCBs in soil samples from Agbogbloshie e-waste site and Kwabenya

	Agbogbloshie e-waste Site			Kwabenya Site		
	Mean	Range	Median	Mean	Range	Median
PCB-18	0.234±0.603	0.009 - 1.947	0.037	1.3E-05±3.3E-05	<LOD-1.1E-04	<LOD
PCB-28	0.125±0.352	0.001 - 1.126	0.014	6.5E-03±1.1E-02	<LOD-3.9E-02	4.3E-03
PCB-52	0.049±0.081	0.000 - 0.235	0.006	2.0E-02±2.1E-02	1.4E-03-6.4E-02	9.9E-03
PCB 101	0.240±0.217	0.045 - 0.735	0.176	2.7E-02±3.7E-02	3.9E-04-1.2E-01	9.3E-03
PCB 138	0.227±0.180	0.042 - 0.480	0.168	1.8E-02±1.1E-02	1.0E-04-3.7E-02	1.8E-02
PCB 153	0.338±0.289	0.037 - 0.770	0.262	4.2E-02±4.2E-02	6.1E-04-1.4E-01	3.2E-02
PCB 180	0.025±0.031	0.000 - 0.084	0.014	2.9E-03±3.4E-03	<LOD -1.1E-02	1.2E-03
Total	1.238	0.134 - 5.377		1.2E-01	2.5E-03-4.1E-01	

The results of the preliminary study showed PAHs and PCBs concentration in the soil from Agbogbloshie to be higher than those from Kwabenya.

The total mean concentration of PCB in the soil sampled at Agbogbloshie was 1.238 µg/g. This may be probably due to the e-waste dismantling and recycling activities taking place at Agbogbloshie.

However, the mean concentration of PCBs at Kwabenya was much lower (1.2E-01). This may be attributed to the fact that Kwabenya is a residential area without any economic activity that impact significantly in the environment.

CONCLUSION

This study involves the assessment of PCBs and PAHs in the breast milk of Primiparae and multiparae mothers living and working in Agbogbloshie, an e-waste recycling area which is considered as a hotspot. The other donor mothers in the PCBs and PAHs assessment live and work in Kwabenya, a residential area with virtually no economic activity and it is considered as a non-hot spot area.

Also in this same study, levels of organochlorine pesticide residues were assessed in the breast milk samples of some 42 Primiparae mothers from Ada, a rural and La urban communities in the Greater Accra regions of Ghana.

Results from the preliminary study indicated the presence of PAHs and PCBs at Agbogbloshie and Kwabenya. However, the mean concentrations of both PAHs and PCBs were significantly higher in the soil sampled at Agbogbloshie than that from Kwabenya.

In this study Levels of Polychlorinated biphenyls were assessed in 128 donor mothers. A total of 105 of these donor mothers lived or and worked in an e-waste hot spot area, and the other 23 live or work in a non-hot spot area where there is almost nothing industrial or commercial.

Based on the result from this study, 18 different congeners of PAHs were found in the human breast milk samples analyzed. The low molecular weight PAHs such as Naphthalene were higher than the high molecular weight PAHs in the milk samples from both sites. The mean values of most of the low molecular weight PAHs congeners were higher in the breast milk samples from Agbogbloshie, the hot spot area than in the Kwabenya, the non-hot spot area. Most of the high molecular weight PAHs which are considered as probable carcinogens were recorded in the human milk samples from the hot spot area but were below the limit of detection in the milk samples from Kwabenya, the non-hot spot area.The diagnostic ratio test which is used in predicting the possible origins of a PAH suggested pyrogenic source as the possible origin for the PAHs in the breast milk samples from Agbogbloshie, the hot spot area. The carcinogenic and mutagenic risk on infants in relation to the ingestion of PAHs contaminated breast milk in this study was found to be very minimal.

The results from this study on the assessment of PCBs found seven different PCB congeners in the milk samples. They include PCB-18, PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180. All the seven PCBs were found in the milk samples from Agbogbloshie hot spot area. Only one PCB congener, PCB-28 was identified in the breast milk samples from Kwabenya, the non-hot spot area. It is worth mentioning that, the one congener that was recorded in the milk samples from

Kwabenya was from just one donor. PCB-28 made the highest contribution of 29.4% of the total PCBs in the breast milk samples, and the least contribution of 1.74% was made by PCB 101. The estimated daily intake (EDI) of PCBs in the breast milk samples in this study was 0.02 µg/kg body wt. /day and they range between of <0.001- 0.03µg/kg body wt. /day. In assessing the potential risk of infants using the health Canada's guideline, of a maximum daily intake 1 µg/kg body wt. /day, the hazard quotient was found to be less than 1 hence babies are at no risk. However, when the when, infants risk was accessed using the Agency for Toxic substances and disease registry standards (ATSDR, 2000) of 0.03 µg/kg body wt. /day, some of the values obtained for the hazard quotient was just at the threshold limit.

The results from Ada, a rural and farming community and La, an urban community indicates the presence of fourteen different organochlorine pesticides in the breast milk samples. These Organochlorine pesticides are Aldrin, Gamma-Chlordane, Endrin, Endrin-Aldehyde, Endrin Ketone, Delta-HCH, p,p'-DDT, p,p'-DDE, Dieldrin, Alpha-Endosulfan, Endosulfan-Sulphate, Heptachlor, Methoxychlor, and Gamma-HCH. The mean concentrations of eleven out of the fourteen organochlorine pesticides detected in the breast milk samples were greater in the breast milk samples from La, an urban community than the breast milk samples from Ada which is a rural and farming community. Endosulfan-sulphate was the only pesticides that exceeded the Australian Maximum residue limit.

Results from this work showed no association between the concentrations of the various persistent organic pollutants and demographic factors such as age, Parity, Body Mass Index and choice of food.

RECOMMENDATIONS

Agbogbloshie e-waste site must be reconstructed to a properly engineered e-waste recycling facility with all safety mechanisms to avoid the release of toxic contaminants into the environment.

There should be regular training programs and workshops for e-waste recyclers so that they will follow right principles in the discharge of their duties.

FUTURE PERSPECTIVES

This study is just a small part of a huge problem that has been identified. There is still a lot to investigate. Among the areas for investigation are:

- To continue on a full site study on the Abgobgloshie e-waste site. This will include site investigation, monitoring of other Persistent Organic Pollutants (POPs) and collaborate for land remediation if the need be.
- Monitoring of persistent organic pollutants and heavy metals in baby foods
- Assessment of POPs in the blood and milk of the occupationally exposed women
- Relationship between serum concentration of polychlorinated biphenyls, and pesticide and dietary habits of pregnant women
- Assessment of the levels of POPs in the mother's blood and cord blood of neonates.

LITERATURE LIST

ACGIH (American Conference of Governmental Industrial Hygienists) (2005), Polycyclic aromatic hydrocarbons (PAHs) biologic exposure indices (BEI) Cincinnati. OH: American Conference of Governmental Industrial Hygienists; 2005.

Adu-Kumi, S., Kawano, M., Shiki, Y., Yeboah, P. O., Carboo, D., Pwamang, J., Morita, M. and Suzuki, N. (2010). Organochlorine pesticides (OCPs), dioxin-like polychlorinated biphenyls (dl-PCBs), polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo furans (PCDD/Fs) in edible fish from Lake Volta, Lake Bosumtwi and Weija Lake in Ghana. *Chemosphere* 8: 675–684.

Akyuz M, Cabuk H. Gas-particle partitioning and seasonal variation of polycyclic aromatic hydrocarbons in the atmosphere of Zonguldak, Turkey. Sci Total Environ 2010; 408: 5550–8.

Anastassiades M, Lehotay SJ, Stajnbaher D, Schenck FJ (2003) Fast and easy multi-residue method employing acetonitrile extraction/ partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J AOAC Int 86(2):412–431

Anda EE, Nieboer E, Dudarev AA, Sandanger TM, Odland JO (2007): Intra– and intercompartmental associations between levels of organochlorines in maternal plasma, cord plasma and breast milk, and lead and cadmium in whole blood, for indigenous peoples of Chukotka, Russia. Journal of Environmental Monitoring 9, 884893.

AOAC, (1990). Official methods of analysis of the association of analytical chemists,15th ed. Kenneth Arlington, Virginia, USA.

Armstrong B.G., Hutchinson E, Unwin J, Fletcher T. (2004). Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis. Environ Health Perspect112 (9):970–8.

Asante KA, Agusa T, Biney CA, Agyekum WA, Bello M, Otsuka M, (2012). Multi-trace element levels and arsenic speciation in urine of e-waste recycling workers from Agbogbloshie, Accra in Ghana. Sci Total Environ 2012; 424:63–73.

Asante Ansong Kwadwo, Adu-Kumi Sam, Nakahiro Kenta , Takahashi Shin , Isobe Tomohiko, Sudaryanto Agus , Devanathan Gnanasekaran , Clarke Edith , Ansa-Asare Duodu Osmund, Dapaah-Siakwan Stephen, Tanabe Shinsuke (2011). Human exposure to PCBs, PBDEs and HBCDs in Ghana: Temporal variation, sources of

exposure and estimation of daily intakes by infants: Sci Total Environ 20011; 928: 921–.37.

ATSDR [Agency for Toxic Substances and Disease Registry] (2000). Toxicological Profile for Polychlorinated Biphenyls (PCBs). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Available at: http://www.atsdr.cdc.gov. Accessed on: October 20, 2014

Bach PB, Kelley M.J, Tate R.C, McCrory D.C. (2003). Screening for lung cancer: a review of the current literature. Chest 2003; 123:72–82.

Ballard Olivia and Morrow Ardythe L. (2013) Human Milk Composition: Nutrients and Bioactive Factors. Pediatr Clin North Am. 2013 February; 60(1): 49–74.

Bauer J. and Gerss J (2011). Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clinical nutrition (Edinburgh, Scotland). 2011; 30(2):215–220.

Beyer A, Biziuk M. (2009) Environmental fate and global distribution of polychlorinated biphenyls. In: Whitacre DM, editor. Reviews of environmental contamination and toxicology, vol. 201. US: Springer; 2009. p. 137–58

Binelli, A., Bacchetta, R., Vailati, G., Galassi, S. and Provini A. (2001). DDT contamination in Lake Maggiore (N. Italy) and effects on zebra mussel spawning. *Chemosphere* 45:409-415.

Bolt, H., & Degen, G. (2002). Comparative assessment of endocrine modulators with oestrogenic activity. II. Persistent organochlorine pollutants. *Arch. Environ. Contam. Toxicol., 76*, 187.

Boffetta P, Jourenkova N, Gustavsson P. (1997) Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control 1997; 8(3):444–72.

Bordajandi, L. R., Abad, E. and Marıa Jose´ Gonzalez, M. J. (2008). Occurrence of PCBs, PCDD/Fs, PBDEs and DDTs in Spanish breast milk: Enantiomeric fraction of chiral PCBs. *Chemosphere* 70: 567-575.

Brauner, E.V., Raaschou-Nielsen, O., Gaudreau, E., Leblanc, A., Tjonneland, A., Overvad, K. and Sorensen, M. (2011). Predictors of polychlorinated biphenyl concentrations in adipose tissue in a general Danish population. *Environ. Sci. Technol.* 45: 679–685.

Breivik K, Gioia R, Chakraborty P, Zhang G, Jones KC (2011) Are reductions in industrial organic contaminants emissions in rich achieved partly by export of toxic wastes? Environ SciTechnol 45:9154–9160

Breivik, K., Sweetman, A., Pacyna, J. M. and Jones, K. C. (2007). Towards a global historical emission inventory for selected PCB congeners—A mass balance approach 3. An Update, *Science of the Total Environment* 377: 296–307.

Brigden K, Labunska I, Santillo D, Johnston P. Chemical contamination at e-waste recycling and disposal sites in Accra and Korforidua, Ghana. Research Laboratories Technical Note; 10/2008

Caravanos Jack, Clark Edith, Fuller Richard, Lambertson Calah, (2011), AssessingAssessing Worker and Environmental Chemical Exposure Risks at an e-Waste Recycling and Disposal Site in Accra, Ghana. J Health Pollution 1:16-25.

Carson, R., (1962). Silent Spring. Houghton Mifflin, pp: 378.

Castillo M, Gonzalez C, Miralles A (2011) An evaluation method for determination of non-polar pesticide residues in animal fat samples by using dispersive solid-phase extraction clean-up and GC-MS.Anal Bioanal Chem 400(5):1315–1328. doi:10.1007/s00216-011-4656-5

CCME (Canadian Council of Ministers of the Environment). (2010), Canadian soil quality guidelines for potentially carcinogenic and other PAHs: scientific criteria document.Winnipeg: CCME.

Chen B.H. and Lin Y.S. (2001) Formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids. J Agric Food Chem;49:5238-43

Chen Jiawei, Liu Chen, Yang Zhongfang and Wang Jiyuan (2008). Residues and Characteristics of Organochlorine Pesticides in the Surface Water in the Suburb of Beijing. Earth Science Frontiers, 15(5): 242–247.

Chovancová, J., Conka, K., Kocan, A. and Sejáková, Z. S. (2011). PCDD, PCDF, PCB and PBDE concentrations in breast milk of mothers residing in selected areas of Slovakia. *Chemosphere* 83:1383–1390.

Clarke E. E. K., Levy L. S., Spurgeon A. and Calvert I. A. (1997). The problems associated with pesticide use by irrigation workers in Ghana. Occup. Mod. Vol. 47, No. 5, pp. 301-308, 1997

Colborn, T., Dumanoski, D. and Myers, J. (1996). Our stolen future. New York: Plume. pp. 215-216.

Cok, I., Mazmanci, B., Mazmanci, M.A., Turgut, C., Henkelmann, B., Schramme, K.-W., (2012), Analysis of human milk to assess exposure to PAHs, PCBs and organochlorine pesticides in the vicinity Mediterranean city Mersin, Turkey. Environ. Int. 40, 63–69.

Croes, K., Colles, A., Koppen, G., Govarts, E., Bruckers, L., Mieroop, E. Van De, and Baeyens, W. (2012). Persistent organic pollutants (POPs) in human milk: A biomonitoring study in rural areas of Flanders (Belgium). *Chemosphere 89*(8): 988–994.

Darko, G., Akoto, O., and Oppong, C. (2008). Persistent organochlorine pesticide residue in fish, sediment and water from Lake Bosomtwi, in Ghana. *Chemosphere* 72: 21-24.

Darko, G., and Acquah, S. (2007a). Levels of organochlorine pesticide residues in meat. *Int. J. Environ. Sci Tech.* 4 (4): 521-524

Darko, G., and Acquah, S. (2007b). Levels of organochlorine pesticide residues in dairy products in Kumasi, Ghana. *Chemosphere* 71(2): 294-298

Darnerud PO, Lignell S, Glynn A, Aune M, Törnkvist A, Stridsberg M (2010): POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. Environment International 36, 180-187.

De March, B.G.E., De Wit, C.A., Muir, D.C.G., Braune, B.M., Gregor, D.J., Norstrom, R.J., Olsson, M., Skaare, J.U. and Stange, K. (1998). Persistent organic pollutants. AMAP assessment report: Arctic pollution issues, 859.

Diggs D.L., Huderson A.C., Harris K.L., Myers J.N., Banks L.D., Rekhadevi P.V., *et al*.(2011) Polycyclic aromatic hydrocarbons and digestive tract cancers: a perspective. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2011; 29(4):324–57.

Dunlap, T.R., (1981). DDT: Scientists, Citizens, and Public Policy. Princeton University Press, Princeton, NJ.

Durant, J., Busby, W., Lafleur, A., Penman, B., Crespi, C., 1996. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. Mutat. Res.-Genet. Tox. 371, 123–157.

Durant, J., Lafleur, A., Busby, W., Donhoffner, L., Penman, B., Crespi, C., 1999.Mutagenicity of C24H14 PAH in human cells expressing CYP1A1. Mutat. Res.-Genet. Toxicol. E. M. 446, 1–14.

Durfee, R L, Contos G, Whitmore FC, Barden JD, Hackman EE III, Westin RA (1976) PCBs in the United States - industrial use and environmental distributions, Report No. EPA 560/6-76-005 (NTIS No. PB-252 012). US Environ Prot Agency, Washington, DC

Eckhardt S, Breivik K, Mano S, Stohl A (2007) Record high peaks inPCB concentrations in the Arctic atmosphere due to long-range transport of biomass burning emissions. Atmos Chem Phys7:4527–4536

Edwards SC, JedrychowskiW, Butscher M, Camann D, Agnieszka Kieltyka A,Mroz E, et al.(2010) Prenatal exposure to airborne polycyclic aromatic hydrocarbons and children's intelligence at 5 years of age in a prospective cohort study in Poland. Environ Health Perspect; 118(9):1326–31.

EPRI (Electr Power Res Inst) (1999) The PCB information manual: production, uses, characteristics, and toxicity of PCBs. TR- 114091-Vl. Electr Power Res Inst, Palo Alto, CA

Erickson D. Mitchell and Kaley G. Robert. (2011). Applications of polychlorinated biphenyls. Environ Sci Pollut Res 18:135−151.

Essumang D.K. Dodoo D. K., Adjei J.K. Adjei (2012). Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish product. Journal of Food Composition and Analysis 27; 128-138.

Essumang K.D., Dodoo D. K., Hadzi G. (2010). Distribution, levels, and risk assessment of polycyclic aromatic hydrocarbons in the soot of some kitchens in the Cape Coast Metropolis of Ghana Toxicological & Environmental Chemistry Vol. 92, No. 9, 1633–1647

Essumang Kofi David, Adokoh Kweku Christian, Afriyie Joseph, Mensah Esther (2009). Source Assessment and Analysis of Polycyclic Aromatic Hydrocarbon (PAH's) in the Oblogo Waste Disposal Sites and Some Water Bodies in and around the Accra Metropolis of Ghana. *J. Water Resource and Protection*, 1, 456-468.

Essumang K. D., Dodoo D. K., Obiri S. and Oduro A. K. (2006). Analysis Of Polycyclic Aromatic Hydrocarbons In Street Soil Dust In Kumasi Metropolis Of Ghana Environmental Monitoring And Assessment 121: 401–408

E-waste Africa Programme. Where is WEee in Africa? Findings from the BaselConvention. Châtelaine: Secretariat of the Basel Convention; 2011.

Feldt Torsten , Fobil Julius N., Wittsiepe Jürgen , Wilhelm Michael , Till Holger, Zoufaly Alexander , Burchard Gerd , Göen Thomas (2014). High levels of PAH-

metabolites in urine of ewaste recycling workers from Agbogbloshie, Ghana. Science of the Total Environment 466–467, 369–376.

Feng, J., Xi, N., Zhang, F., Zhao, J., Hu, P., & Sun, J. (2015). Distributions and potential sources of polycyclic aromatic hydrocarbons in surface sediments from an emerging industrial city (Xinxiang). *Environmental Monitoring and Assessment*, *188*(1), 1-14.

Fenton, S.E., Condon, M., Ettinger, A. S., LaKind, J. S., Mason, A., McDiarmid, M., Qian, Z. and Selevan, S. G. (2005). Collection and use of exposure data from human milk biomonitoring in the United States. *J Toxicol Environ Health A* 68(20): 1691-1712.

Gao X, McMahon RJ, Woo JG, Davidson BS, Morrow AL, Zhang Q. (2012) temporal changes in milk proteomes reveal developing milk functions. J Proteome Res. 2012; 11(7):3897–3907. [PubMed: 22676802]

Garcia-Suastegui W.A, Huerta-Chagoya A., Carrasco-Colın K.L., Pratt M.M., John K., Petrosyan P., et al., (2011) Seasonal variations in the levels of PAH-DNA adducts in young adults living in Mexico City. Mutagenesis; 26:385–91.

Gerken, A., Suglo, J. V., and Braun, M. (2001). Pesticide Policy in Ghana. MOFA PPRSD, ICD Project. Pesticide Policy Project. GTZ Accra, Ghana. Pesticide Policy Project Publication Series, No. 10, pp 26-28.

Gunter MJ, Divi RL, Kulldorff M, Vermeulen R, Haverkos KJ, KuoMM, et al., (2007) Leukocyte polycyclic aromatic hydrocarbon-DNA adducts formation and colorectal adenoma. Carcinogenesis; 28:1426–9.

GEPA [Ghana Environmental Protection Agency] (2007). National implementation plan of the Stockholm convention on persistent organic pollutants. pp. 1-267.

Gioia Rosalinda, Abidemi James Akindele, Adekunle Adebusoye Sunday, Asante Kwadwo Ansong, Tanabe Shinsuke, Buekens Alfons and Sasco J. Annie (2014). Polychlorinated biphenyls (PCBs) in Africa: a review of environmental levels. Environ Sci Pollut Res (2014) 21:6278–6289.

Gioia R, Eckhardt S, Breivik K, Jaward FM, Prieto A, Nizzetto L,Jones KC (2011) Evidence for major emissions of PCBs in the West African region. Environ Sci Technol 45:1349–1355

Harris CV, Woolridge MW, Hay AWM. (2001), Factors affecting the transfer of organochlorine pesticide residues to breast milk. Chemosphere; 43:243–56.

Hedley, A. J., Hui, L. L., Kypke, K., Malisch, R., van Leeuwen, F.X.R. Moyd, G. Wong, I. T. W. and Nelson, E.A.S. (2010). Residues of persistent organic pollutants (POPs) in human milk in Hong Kong. *Chemosphere* 79: 259–265.

Hogstedt C. Pesticides. In: JeyaratnamJ, ed. Occupational Health in Developing Countries. Oxford University Press. 1992: 242- 243.

Hubbard HL (1964) Chlorinated biphenyl and related compounds. In: Kirk-Othmer Encycl Chem Tech 5:289–297

Hung, H., Katsoyiannis, A.A., Brorström-Lundén, E., Olafsdottir, K., Aas, W., Breivik, K., Bohlin-Nizzetto, P., Sigurdsson, A., Hakola, H., Bossi, R., Skov, H., Sverko, E., Barresi, E., Fellin, P., Wilson, S., (2016). Temporal trends of Persistent Organic Pollutants (POPs) in arctic air: 20 years of monitoring under the Arctic Monitoring and Assessment Programme (AMAP). Environ. Pollut. 217, 52–61.

Inam, E., Offiong, N.-A., Essien, J., Kang, S., Kang, S.-Y., Antia, B., (2016). Polycyclic aromatic hydrocarbons loads and potential risks in freshwater ecosystem of the Ikpa River Basin, Niger Delta-Nigeria. Environ. Monit. Assess. 188, 49.

Jeong IS, Kwak BM, Ahn JH, Jeong SH (2012), Determination of pesticide residues in milk using a QuEChERS-based method developed by response surface methodology. Chemosphere 133(2):473–481

John K., Ragavan N, Pratt M.M., Singh P.B., Al-Buheissi S, Matanhelia S.S., et al.,(2009). Quantification of phase I/II metabolizing enzyme gene expression and polycyclic aromatic hydrocarbon-DNA adduct levels in human prostate. Prostate; 69:505–19.

Jong W.H., Kroese E.D., Vos J.G., Loveren H.V.(1999). Detection of immunotoxicity of benzo[a]pyrene in a subacute toxicity study after oral exposure in rats. Toxicol Sci; 50(2):214–20.

Kanja, L., Skaare, U. J., Ojwang, S. B. O. and Maitai, K. C. (1992). A comparative study of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood and human milk from mother/infant pairs in Kenya. *Arch. Environ. Contam. Toxicol.* 22: 21-24.

Katsoyiannis Athanasios, Samara Constantini (2005) Persistent organic pollutants (POPs) in the conventional activated sludge treatment process: fate and mass balance. Environ. Res. 97: 245-251

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. (2007) Food web-specific biomagnification of persistent organic pollutants. Science ;317(5835):236–9

Kent JC, Mitoulas LR, Cregan MD, Ramsay DT, Doherty DA, Hartmann PE (2006). Volume and frequency of breastfeedings and fat content of breast milk throughout the day. Pediatrics. 117(3):e387–395. [PubMed: 16510619]

Kramer MS, Kakuma R (2012): Optimal duration of exclusive breastfeeding. Cochrane Database of Systematic Reviews, 132.

Kristensen P, Eilertsen E, Einarsdóttir E, Haugen A, Skaug V, Ovrebo S.(1995) Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. Environ Health Perspect ;103:588–90.

Koziol, A., and Pudykiewicz, J. (2001). Global-Scale environmental transport of persistent organic pollutants. *Chemosphere* 45: 118-120.

Kuo C.Y., Hsu Y.W., Lee H.S.(2003). Study of human exposure to particulate PAHs using personal air samplers. Arch Environ Contam Toxicol; 44:454–9.

Kyere Nartey Vincent, Klaus Greve, Atiemo M. Sampson (2016): Spatial assessment of soil contamination by heavy metals from informal electronic waste recycling in Agbogbloshie, Ghana. Environmental Health and Toxicology 2016; 31:e2016006.

LaKind, J. S., Wilkins, A. A. and Berlin Jr., C. M. (2004). Environmental chemicals in human milk: a review of levels, infant exposures and health, and guidance for future research. *Toxicology and Applied Pharmacology* 198 (2): 184–208.

Latif IK, Karim AJ, Zuki ABZ, Zamri-Saad M, Niu JP, Noordin MM. (2010), Pulmonary modulation of benzo[a]pyrene-induced hemato- and hepatotoxicity in broilers. Poult Sci 2010;89(7): 1379–88.

Lau MHY, Leung KMY, Wong SWY, Wang H, Yan Z-G (2012) Environmental policy, legislation, and management of persistent organic pollutants (POPs) in China. Environ Pollut 165:182–192

Lawrence R.A., Lawrence R.M. (2010). Breastfeeding: a guide for the medical profession. 7th ed. Philadelphia: Saunders.

Lee, S., Kim, S., Lee, H., Lee, I., Park, J., Kim, H., Lee, J. J., Choi, G., Choi, S., Kim, S., Kim, S.Y., Choi, K., Kim, S. and Moon, H. (2013). Contamination of polychlorinated biphenyls and organochlorine pesticides in breast milk in Korea: Time-course variation, influencing factors, and exposure assessment. *Chemosphere* 93:1578–1585

Li N, Lei L, Nian L, Zhang R,Wu S, Ren R,Wang Y, Zhang H, Yu A (2013), A modified QuEChERS method for the determination of some herbicides in yogurt and milk by high-performance liquid chromatography. Talanta 105:219–228. doi:10.1016/j.talanta.2012.11.057

Li, J., Yu, H., Zhao, Y., Zhang, G. and Wua, Y. (2008). Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from Beijing, China. *Chemosphere* 73:182–186.

Liao Y, Alvarado R, Phinney B, Lonnerdal B. (2011), Proteomic characterization of human milk whey proteins during a twelve-month lactation period. J Proteome Res. 10(4):1746–1754.[PubMed: 21361340]

Llobet, J. M., Bocio, A., Domingo, J. L., Teixidó, A., Casas, C. and Müller, L. (2003). Levels of polychlorinated biphenyls in foods from Catalonia, Spain: Estimated dietary intake. *J. Food Protect*. 66: 479–484.

Lonnerdal, B., (2003). Nutritional and physiologic significance of human milk proteins. Am. J. Clin. Nutr. 77, 1537S–1543S.

Luzardo, O.P., Ruiz-Suárez, N., Almeida-González, M., Henríquez-Hernández, L.A., Zumbado, M., Boada, L.D., (2013). Multi-residue method for the determination of 57persistent organic pollutants in human milk and colostrum using a QuEChERS based extraction procedure. Anal Bioanal Chem DOI 10.1007/s00216-013-7377-0.

Malik, A., Verma, P., Singh, A.K., Singh, K.P., (2011). Distribution of polycyclic aromatic hydrocarbons in water and bed sediments of the Gomti River, India. Environ. Monit. Assess. 172, 529–545.

Malina, C., Falcon, M., Barba, A., Ange, L, C., Oiva, J. and Luna, A. (2005). HCH and DDT Residues in Human fat. *Ann Agricultural Environmental Med*. 12: 133-136

Malish, R., and Van Leeuwen, F. X. R. (2003). Results of the WHO coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. *Organohal. Comp*. 64: 140–143.

Masih J, Masih A, Kulshrestha A, Singhvi R, Taneja A. (2010), Characteristics of polycyclic aromatic hydrocarbons in indoor and outdoor atmosphere in the north central part of India. J Hazard Mater 2010; 177:190–8.

Masih J, Singhvi R, Kumar K, Jain VK, Taneja A. (2012) Seasonal variation and sources of polycyclic aromatic hydrocarbons (PAHs) in indoor and outdoor air in a semi-arid tract ofNorthern India. Aerosol Air Qual Res 2012; 12:515–25.

Minh, N. H., Someya, M. Minh, T. B., Kunisue, T., Iwata, H., Watanabe, M., Tanabe, S., Viet P. H. and Tuyen, B. C. (2004). Persistent organochlorine residues in human breast milk from Hanoi and Hochiminh City, Vietnam: contamination, accumulation kinetics and risk assessment for infants. *Environ. Pollut*. 129:431–441.

Munoz-de-Toro, M., Beldomenico, H. R, Garcia, S. R., Stoker, C., De Jesus, J.J., Beldomenico, P. M, et al. (2006). Organochlorine levels in adipose tissue of women from a littoral region of Argentina. *Environ Res* 102:107–112.

Namiki S, Otani T, Seike N. (2013) Fate and plant uptake of persistent organic pollutants in soil. Soil Sci Plant Nutr. ;59(4):669–79.

Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, Dewey KG (1991). Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. The American journal of clinical nutrition; 53(2):457–465.

Ntow, W. J., Gijzen, H. J., Kelderman, P. and Dresshsel, P. (2006). Farmer Perceptions and Pesticide use practices in vegetable production in Ghana. *Society of Chemical Industry Pest Manag Sci.* 62: 356-365.

Ntow, W, J. (2005). Pesticide Residue in the Volta Lakes and Reservoirs*; Res Manage*. 10: 243-248.

Ntow, W. J. (2001). Organochlorine pesticide residues in water, sediments, crops and human fluids in a farming community in Ghana. *Arch. Environ. Contam. Toxicol*. *Liv* 557-663.

Olsson A.C., Fevotte J., Fletcher T., Cassidy A., Mannetje A., Brennan P., et al. (2010) Occupational exposureto polycyclic aromatic hydrocarbons and lung cancer risk: amulticenter study in Europe. Occup Environ Med; 67:98–103.

Oostdam JV, Gilman A, Dewailly E, Usher P, Wheatley B, Kuhnlein H. (1999), Human health implications of environmental contaminants in Arctic Canada: a review. Sci Total Environ; 230:1-82.

Pauwels A. , Covaci A., Weyler J. Delbek , Dhont M., Sutter P., Haoghe T., Schepens P. (2000) Comparison of Persistent Organic Pollutant Residues in Serum and AdiposeTissue in a Female Population in Belgium, 1996–1998. Arch. Environ. Contam. Toxicol. 39, 265–270.

Penning C. (1930) Physical characteristics and commercial possibilities of chlorinated diphenyl. Ind Eng Chem 22:1180−1182.

Penteado, J.C.P. and Moreira Vaz, J. (2001). O legado das bifenilas policloradas (PCBs). *Quımica Nova* 24: 390–398.

Perera F, Tang D, Whyatt R, Lederman S.A, JedrychowskiW.(2005). DNA damage from polycyclic aromatic hydrocarbons measured by benzo[a]pyrene-DNA adducts in mothers and newborns from Northern Manhattan, the World Trade Center Area, Poland, andChina. Cancer Epidemiol Biomarkers Prev; 14(3):709–14.

Porta, M., Puigdomènech, E., Ballester, F., Selva, J., Ribas-Fitó, N., Domínguez-Boada L., et al. (2008). Studies conducted in Spain on concentrations in humans of persistent toxic compounds. *Gac Sanit* 22:248–266.

Prentice, A. (1996). Constituents of human milk. *Food and Nutrition Bulletin* 17: 1-12.

Prentice, A.(1995). Regional Variations in the Composition of Human Milk. In: Jensen, R.G., editor. Handbook of Milk Composition. Academic Press, Inc.; San Diego, CA: 1995. p. 919

Pulkrabova, J., Stupak, M., Svarcova, A., Rossner, P., Rossnerova, A., Ambroz, A., Hajslova, J. (2016). Relationship between atmospheric pollution in the residential area and concentrations of polycyclic aromatic hydrocarbons (PAHs) in human breast milk. The Science of the Total Environment, 562, 640–647.

Riordan, J. (2004). Breastfeeding and human lactation. The biological specificity of breast milk. Boston, USA: Jones and Bartlett. pp 12-25.

Rogan, W. J., Gladen, B.C., McKinney, J. D., Carreras, N., Hardy, P., Thullen, J., et al. (1986). Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: Effects of maternal factors and previous lactation. *Am J Public Health* 76:172–177.

Roots, O., Zitko, V., Roose, A.(2005). Persistent organic pollutant patterns in grey seals (Halichoerus grypus). Chemosphere 60, 914–921.

Ross G. (2005). Risks and benefits of DDT. The Lancet 366 (9499): 1771.

Sepulveda A, Schluep M, Renaud FG, Streicher M, Kuehr R, Hageluken C, Gerecke AC (2010) A review of the environmental fate and effects of hazardous substances released from electrical and electronic equipment during recycling: examples from China and India. Eur Env Imp Assess30:28–e41

Schechter A., Kassia I., and Papke, O. (1998). Partitioning of dioxins, dibenzofurans, and co-planer PCBs in blood, milk, adipose tissues, placenter and cord blood from five American women. *Chemosphere* 37: 1817-1823.

See S.W., Karthikeyan S., Balasubramanian R. (2006). Health risk assessment of occupational exposure to particulate-phase polycyclic aromatic hydrocarbons associated with Chinese, Malay and Indian cooking. J Environ Monit; 8:369–76

Steingraber, S., (2001) Having Faith: An Ecologist's Journey to Motherhood. Perseus Publishing, Cambridge MA. pp 62-75.

Smoker M, Tran K, Smith RE (2010) Determination of polycyclic aromatic hydrocarbons (PAHs) in shrimp. J Agric Food Chem. doi:10.1021/jf1029652

Storelli, M. M., Giacominelli-Stuffer, R., Storelli, A. and Marcotrigiano, G. O. (2003). Polychlorinated biphenyls in seafood: contamination levels and human dietary exposure. *Food Chemistry* 82: 491–496.

Sweetman, M., Vall, K., Predouros, K., & Tones. (2005). The role of soil organic carbon in the global cycling of persistent organic pollutants (POPs): Interpreting and modelling field data. *Chemosphere, 60*: 959-970.

Tang X, Shen C, Cheema SA, Chen L, Xiao X, Zhang C, et al. (2010) Levels and distributions of polycyclic aromatic hydrocarbons in agricultural soils in an emerging e-waste recycling town in Taizhou area, China. J Environ Sci Health Part A; 45: 1076–84

Tobiszewski, M. (2014). Application of Diagnostic Ratios of PAHs to Characterize the Pollution Emission Sources. *5th International Conference on Environmental Science and Technology (IPCBEE)*, vol.69 IACSIT Press, Singapore DOI: 10.7763/IPCBEE. 2014. V69. 9

Tue, N. M., Sudaryanto, A., Minh T. B, Isobe T, Takahashi S, Viet P H, and Tanabe, S (2010). Accumulation of polychlorinated biphenyls and brominated flame retardants in breast milk from women living in Vietnamese e-waste recycling sites. *Science of the Total Environment* 408: 2155–2162.

UNEP [United Nations Environment Programme] (2009). Stockholm Convention on Persistent Organic Pollutants (POPs) Available at: http://chm.pops.int. Accessed on: March, 14 2017

UNEP (2002). Ridding the World of POPs: A guide to the Stockholm convention on persistent organic pollutants. GE.02-01805/E-6000. Available at: UNEP/Chemicals/2002/3. http://www.pops.int. Accessed on: May 14, 2014.

UNEP (2001). Final Act of the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic Pollutants, Stockholm, Sweden, May 22-23, 2001. Geneva: United Nations Environment Programme. pp 1-43.

Unwin J, Cocker J, Scobbie E, and Chambers H. (2006). An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK. Ann Occup Hyg 2006; 50(4): 395–403.

USEPA. Polycyclic Organic Matter. Washington, D.C.: Environmental Protection Agency; 2002. Available at:http://www.epa.gov/ttnatw01/hlthef/polycycl.html [assessed25 July 2011].

USEPA, 1993. Provisional Guidance for Quantitative Risk Assessment of PAH, EPA/600/R-93/089, United States Environmental Protection Agency.

USEPA, 1991. Risk Assessment Guidance for Superfund contaminants. Human Health Evaluation Manual Supplementary Guidance 'Standard Default Exposure Factors' Interim Final. Office of Emergency and Remedial Response, vol. 1. USEPA, Washington, DC, PB91-921314.

Wang J, Chen S, Tian M, Zheng X, Gonzales L, Ohura T.(2012). Inhalation cancer risk associated with exposure to complex polycyclic aromatic hydrocarbon mixtures in an electronic waste and urban area in South China. Environ Sci Technol 2012a; 46:9745–52.

Wang L. S. (1991). The Chemistry of Organic Contamination. Beijing: Science Press, 1991

Wang Qi, Yuichi Miyake, Takashi Amagai, Go Suzuki, Hedenori Matsukami, Nguyen Minh Tue, Shin Takahashi,Shinsuke Tanabe, Len Hu Teyen, Pham Hung Viet, Hidetaka Takigamy (2016). Halogenated Polycyclic Aromatic Hydrocarbons in Soil and River Sediment from E-Waste Recycling Site in Vietnam. Journal of Water and Environmental Technology 2016; 3: 166-176.

Wang X, Xue Y, Gong P, Yao T. (2013) Organochlorine pesticides and polychlorinated biphenyls in Tibetan forest soil: profile distribution and processes. Environ Sci Pollut Res :1–8

Wells, D. E, and Hess, P. (2000). Separation, clean-up and recoveries of persistent trace organic contaminants from soils, sediment and biological matrices. In: Barceló E (ed) Sample handling and trace analysis of pollutants, techniques, applications and quality assurance. Elsevier, Amsterdam. pp 73–116.

Weltman, R.H. and D.H. Norback, 1983. Lack of hepatocarcinogenic activity after 2,3,6,2_,3_,6_-hexachlorobiphenyl (HCB) exposure in Sprague- Dawley rats: a sequential ultra-structural study.Toxicologist, 3: 101 (abstract 401).

WHO [World Health Organization] (2011). Environmental Health Criteria 241. DDT in Indoor Residual Spraying: Human Health Aspects. World Health Organization, Geneva.

WHO [World Health Organization](2007). Fourth WHO- Coordinated Survey of Human Milk for Persistent Organic Pollutants in cooperation with UNEP. Guidelines for developing National Protocol. Available at http: //www.who.int/foodsafety/chem/POPprotocol.pdf. Accessed on 18th January 2012.

WHO [World Health Organization] (2003). Polynuclear aromatic hydrocarbons in drinking-water.Background document for development ofWHO Guidelines for Drinking-water Quality; 2003 [WHO/SDE/WSH/03.04/59].

WHO (2001). The optimal duration of exclusive breastfeeding. Report of an Expert Consultation Geneva, Switzerland: World Health Organization Available at: http://www.who.int/nutrition/publications/infantfeeding/WHO_NHD_01.09/en/. Accessed on April, 25 2013

WHO [World Health Organization] (1993) Polychlorinated biphenyls and terphenyls. In: Dobson S,van Esch GJ (eds) Environ Heal Criteria 140, 2nd edn. World Health Organization (WHO), Geneva

Wu, W.Z., Schramm K.W., Xu, Y. and Kettrup, A. (2001). Accumulation and partition of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in the muscle and liver of fish. *Chemosphere* 43:633-641.

Yang YH, Chan YS, Kim BH, Shin DC, Ikonomou MG. (2002), Congener-distribution patterns and risk assessment of polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans in Korean human milk. Chemosphere;47:1087–95.

Yunker, M. B., Macdonald, R. W., Vingarzan, R., Mitchell, R. H., Goyette, D., & Sylvestre, S. (2002). PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry*, *33*(4), 489-515.

Zhang Y, and Tao S.(2009) Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. Atmos Environ; 43:812–9.

APPENDICES

APPENDIX 1. THE GHANA ETHICAL REVIEW COMMITTEE'S APPROVAL LETTE

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. :GHS-ERC: 3
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Tel: +233-302-681109
Fax + 233-302-685424
Email: nanatuesdaykad@yahoo.com

28th June, 2013

Anita Osei Tutu
Ghana Atomic Energy Commission
NNRI/NCERC

ETHICAL APPROVAL - ID NO: GHS-ERC: 09/03/13

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Use of nuclear and related analytical techniques in studying human health impacts of toxic elements and pesticide residues in some selected mining and farming communities in Ghana"

This approval requires that you inform the Ethical Review Committee (ERC) when the study begins and provide Mid-term reports of the study to the Ethical Review Committee (ERC) for continuous review. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification without ERC approval is rendered invalid.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your sponsor before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
DR. CYNTHIA BANNERMAN
(GHS-ERC VICE-CHAIRPERSON)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX 2. A COPY OF THE QUESTIONNAIRES FILLED BY DONOR MOTHERS

Questionnaire for Breast Milk Donors

QUESTIONNAIRE FOR POTENTIAL BREAST MILK DONORS
Persistent Organic Pollutants in the Breast Milk of Primiparae and Multiparae Mothers from Three Locations in Southern Ghana: Levels of Contamination, Influencing Factors and Infant Risk Assessment. *CONFIDENTIAL!*
Section 1: Personal Information

Name	Phone number	Today's Date (dd/mm/yyyy)
	e-mail	
Address		
History of infectious disease or chronic ill-health within the past 2 years (e.g. Hepatitis, AIDS, Diabetes) Yes □ No □		
Status of donor in regard to the survey Selected □ Reserve □		
Individual Identification Code		

Section 2: Screening Questionnaire: To be completed by interviewer/sample collector

Name of Interviewer/collector:	Date of interview/collection (dd/mm/yyyy):
Place of interview/collection: (dd/mm/yyyy):	Name of Health Facility:

1. Are you breastfeeding your child? Yes □ No □
2. Is this your first child? Yes □ No □
3. Is your child born as a single child? (not twins) Yes □ No □
4. Did you have a normal healthy pregnancy? Yes □ No □
5. Have you lived in your current area for 10 years? Yes □ No □ * If no, actual number of years _____

6. Are you between 25 and 38 years old? Yes □ No □ * If no, date of birth _____(dd/mm/yyyy)

*Note that if the answers to questions 5 or 6 was "no", please ask what the participant's actual residence time and/or birth date.

Instruction to interviewer: If any answers to questions 1-6 were "no" or if the answer to question 7 was "yes", the participant is not eligible for this survey. Please thank the participant for their interest in the survey and end this interview. If all answers are "yes" except question 7, proceed with Section 3.

Section 3: Health History Questionnaire

Date of Birth (dd/mm/yyyy)	Age(yrs)
Height (cm)	Weight before pregnancy (kg)

1. What was your delivery date (dd/mm/yyyy)?

2. What was the type of your delivery?

Natural birth □ Caesarean □

3. Where have you been residing during last 10 years:

urban (city) □ rural (countryside) □

Describe the actual periods of residence in the area _____years

4. How would you describe your dietary habits before pregnancy?

Mixed diet □ Vegetarian but with milk and eggs □

Strictly vegetarian □ Other □

5. How often, on average, did you eat following foods before pregnancy?

	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						

5.1 What types of fish do you consume most often?

Fish from the sea □ Freshwater fish □ Both □

Please state the species if known :

5.2 Were your dietary habits changed after pregnancy? Yes □ No □ If yes, describe major changes briefly:_____
6. Were you born in Ghana? Yes □ No □
7. Was your mother born in Ghana? Yes □ No □
8. Were you breastfed? Yes □ No □ Do not know □ If you know, for how long? _____
9. Were you engaged in dismantling of e-waste before pregnancy? Yes □ No □ If yes, please state the duration : _____
10. What is your present occupation? _____
11. Do you smoke? Yes □ No □
11.1 If yes, how many cigarettes do you smoke per day? _____

11.2 If no, have you ever smoked? Yes □, please state the quitting periods:_____ years No □

Section 4. Postnatal Information
1. Are you prepared to sign the consent form? Yes □ No □ If yes, attach signed consent form. If no, mother is not eligible to participate in survey.
2. How old is your infant? less than 3 weeks* □ 3-4 weeks □ 5-8 weeks □ more than 8 weeks □**
3. What is the gender of your infant? Male □ Female □
4. What was the birth weight of your baby? _____ kg
5. What is the weight of your baby? _____ kg
6. Did you feed colostrum to your baby? Yes □ No □
7. Are you taking any medicine? Yes □ No □ If yes, please describe the medicines: _____

4. Is your current weight different than your weight before pregnancy? Gained □ Lost □ Not changed □
5. Can you provide a sample now? Yes □ Later □ When? _____ At home □

PAPER I

Anita Asamoah, Erik Gydesen Søgaard, David Kofi Essumang, Jens Muff, Mahdi Nikbakht Fini

Assessment of PAHs contamination levels, possible sources and infants carcinogenic and mutagenic risks in human breast milk of some Ghanaian women from a hot spot and non-hot spot areas

Submitted to International Journal of Hygiene and Environmental Health

May 2017

Manuscript Details

Manuscript number	IJHEH_2017_300
Title	Assessment of PAHs contamination levels, possible sources and infants carcinogenic and mutagenic risks in human breast milk of some Ghanaian women from a hot spot and non-hot spot areas
Article type	Full Length Article

Abstract

Contamination levels of polycyclic aromatic hydrocarbons (PAHs) were assessed in breast milk samples of 128 primiparae and multiparae Ghanaian women in a polluted hot spot area and a reference area (non-hot spot) in 2014 and 2016. This research work is aimed at assessing PAHs levels in human milk samples from some Ghanaian mothers, prediction of the possible sources of these PAHs and the probable carcinogenic and mutagenic risks to infants. PAHs in the breast milk were analyzed using a gas chromatography coupled with a mass spectrometer (GC-MS/MS). A total of 18 PAHs congeners were identified in the human milk samples with a total range between ˂0.01 and 7444.50 ng/g lipid wt. and a total mean of 1161.24 ng/g lipid wt. In general, the mean concentrations for low molecular weight PAHs were higher than for high molecular weight PAHs in the milk samples with naphthalene recording the highest mean concentrations of 1026 ng/g lipid wt. and 78 ng/g lipid wt. for both hot spot and non-hot spot areas respectively. Naphthalene contributed 77.4% of the total PAHs in the milk samples. Most of the high molecular weight PAHs were below the limit of detection in milk samples from Kwabenya (non-hot spot area) but were detected in the milk samples from Agbogbloshie (hot spot area). The diagnostic ratio tests in this study suggest that most of the PAHs in the milk samples are originating from pyrogenic sources. Risk assessment for carcinogenicity and mutagenicity on infants based on this study were 1.1×10^-5 and 1.9×10^-5, respectively.

Keywords	PAHs; Human breast milk; Carcinogenic and mutagenic risks; Hot-spot area; Ghana;

Corresponding Author	Anita Asamoah
Corresponding Author's Institution	Aalborg University
Order of Authors	Anita Asamoah, Erik Sogaard, David Kofi Essumang, Jens Muff, Mahdi Nikbakht Fini
Suggested reviewers	Godfred Darko, ISMET COK, Torsten Feldt, Riffat Naseem Malik, Kwadwo A. Asante

Submission Files Included in this PDF

File Name [File Type]

Cover letter PAHs.docx [Cover Letter]

Manuscript.docx [Manuscript File]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Dear Editor,

Please consider the enclosed manuscript entitled "Assessment of PAHs contamination levels, possible sources and infants carcinogenic and mutagenic risks in human breast milk of some Ghanaian women from a hot spot and non-hot spot areas" for publication in International Journal of Hygiene and Environmental Health.

This study involves the monitoring of PAHs in 128 human breast milk samples. Both primiparae and multiparae women residing and working in an electronic-waste dump hot-spot area and also donors from a non-hot spot site were used in this study. This paper not only has reported the level of contamination also diagnostic ratio tests and risk assessment for carcinogenicity and mutagenicity on infants have been studied.

The findings in this study are original. The information has not been published or pending in any other journals. All ethical rules including ethical permission were compiled before the commencement of the study.

We believe our paper fits your journal's scope and hope it will be accepted and published.

Best regards,

Anita Asamoah

PhD student

Section of chemical engineering

Department of chemistry and bioscience

Aalborg University

Esbjerg, Denmark

Assessment of PAHs contamination levels, possible sources and infants carcinogenic and mutagenic risks in human breast milk of some Ghanaian women from a hot spot and non-hot spot areas

Anita Asamoah[a*], Erik Gydesen Søgaard[a], David Kofi Essumang[b], Jens Muff[a], Mahdi Nikbakht Fini[a]

[a] Section of Chemical Engineering, Department of Chemistry and Bioscience, Aalborg University, Denmark
[b] Environmental Research Group, Department of Chemistry, University of Cape Coast, Cape Coast, Ghana

Abstract

Contamination levels of polycyclic aromatic hydrocarbons (PAHs) were assessed in breast milk samples of 128 primiparae and multiparae Ghanaian women in a polluted hot spot area and a reference area (non-hot spot) in 2014 and 2016. This research work is aimed at assessing PAHs levels in human milk samples from some Ghanaian mothers, prediction of the possible sources of these PAHs and the probable carcinogenic and mutagenic risks to infants. PAHs in the breast milk were analyzed using a gas chromatography coupled with a mass spectrometer (GC-MS/MS). A total of 18 PAHs congeners were identified in the human milk samples with a total range between □0.01 and 7444.50 ng/g lipid wt. and a total mean of 1161.24 ng/g lipid wt. In general, the mean concentrations for low molecular weight PAHs were higher than for high molecular weight PAHs in the milk samples with naphthalene recording the highest mean concentrations of 1026 ng/g lipid wt. and 78 ng/g lipid wt. for both hot spot and non-hot spot areas respectively. Naphthalene contributed 77.4% of the total PAHs in the milk samples. Most of the high molecular weight PAHs were below the limit of detection in milk samples from Kwabenya (non-hot spot area) but were detected in the milk samples from Agbogbloshie (hot spot area). The diagnostic ratio tests in this study suggest that most of the PAHs in the milk samples are originating from pyrogenic sources. Risk assessment for carcinogenicity and mutagenicity on infants based on this study were 1.1×10^{-5} and 1.9×10^{-5}, respectively.

Keywords: PAHs; Human breast milk; Carcinogenic and mutagenic risks; Hot-spot area; Ghana;

1.0 Introduction

Increase in polycyclic aromatic hydrocarbons (PAHs) pollution in the environment has been of great concerns. Researchers in most countries in the world have reported of their presence in different matrices such as smoked fish, soot, soil, and urine (Essumang et al., 2011, 2010, 2006; Feldt et al., 2014). PAHs are produced unintentionally as a result of pyrolysis or incomplete combustion of organic matter from anthropogenic and natural sources (WHO, 2003). Human activities such as incomplete combustion of fuel, e-waste recycling processes, incinerations, indiscriminate burning, tobacco smoking among others have resulted in the increase levels of PAHs which have become ubiquitous in the environment (Armstrong et al., 2004; Wei See et al., 2006; Zhang and Tao, 2009). Some characteristics of PAHs include lipophilicity and ability to accumulate in fatty tissues of organisms, persistent, toxic, probable human carcinogen among others (IARC, 2012; USEPA, 1994; WHO, 1998). PAHs are known to pose health problems including various forms of cancers in humans (Bach et al., 2003; Boffetta et al., 1997; Schoket, 1999; Shen et al., 2008; Unwin et al., 2006; IARC, 1999; van der Hel et al., 2003).

Both developed and developing countries are battling with the presence of PAHs. The increase in urbanization, industrialization and other activities of humans have resulted in increasing levels of PAHs in the environment. The Ghanaian environment has not been spared from this menace; the capital city of Ghana Accra has one of Africa's biggest e-waste sites (Brigden et al., 2007). The e-waste activities are carried out by unskilled individuals who openly dismantle and burn anything electrical and electronic for the precious metals embedded in these items. E-waste recycling processes have been cited to be a major contributor of PAHs in the environment (Feldt et al., 2014; Wang et al., 2012, 2016). Also, the city of Accra is fast growing with increasing number of vehicles, and the springing up of industries and these can also facilitate the generation of PAHs in the Ghanaian environment (Dong and Lee, 2009; Morillo et al., 2007). There is a paucity of information on PAHs levels in humans in Ghana. A report by the Cancer Control Division of the Ghana Health Services indicates a rise in the number of cancer cases and young people are in the majority (GNS, 2011). It is, therefore, essential to continuously monitor the levels of Persistent Organic Pollutants (POPs) and other contaminates which may be contributing to the escalation in cases of cancer and other diseases in humans. This will allow intervention measures to be put in place before things get out of control. To the best of our knowledge, there has not been any work done on the levels of PAHs in human breast milk in Ghana. This work will, therefore, serve as a baseline data for PAHs levels in breast

milk in Ghana. Again, Breast milk was selected as a matrix for the motoring PAHs because of its importance for the life of babies. Breast milk is still the best food for babies especially in the first six months of their lives, and the World Health Organization (WHO) recommends that babies be exclusively breastfed within this period (WHO, 2001). The safety of breast milk has now been compromised; mothers are not only giving their babies nourishment and affection but also unknowingly excreting doses of toxic contaminants from their bodies to their innocent babies (Bordajandi et al., 2008; Kanja et al., 1992; Munoz-de-Toro et al., 2006). Research on environmental pollution has shown that babies take in the highest doses of contaminants that they would have otherwise accumulated in their whole lives during breastfeeding (Colborn et al., 1996).

The aim of this study is to access the level of PAHs in the breast milk of some Ghanaian women. The study involves women living and working in and around e-waste recycling site and also from women working and residing in areas where there is virtually no industrial or economic activity. In addition, this study seeks to investigate the possible sources of these PAH in the breast milk samples and also ascertain if the levels of the PAHs pose any risk to breastfed infants.

2.0 Materials and Method

2.1 Study Area

Agbogbloshie is a 6.2 hectare (15 acres) former wetland at the west side of the Odaw River located in the Ghana's capital city of Accra. It is the largest e-waste recycling and dump site in Ghana where everything electrical and electronic both big and small are openly dismantled and recycled to obtain the precious metals embedded in these items. Adjacent to the e-waste disposal and recycling dump is the Agbogbloshie food market which one of the biggest markets in Accra where food items are brought from all over Ghana to be sold at wholesale and retail. Agbogbloshie is a busy economic area with large dense populace and vehicles. The e-waste site also serves as a hub for some individuals and their families in the e-waste business. Schools, offices, churches and other neighboring communities surround Agbogbloshie. The second study area is Kwabenya which is mainly residential area without any industrial or commercial activity. A map of the study area is shown in Fig 1 below.

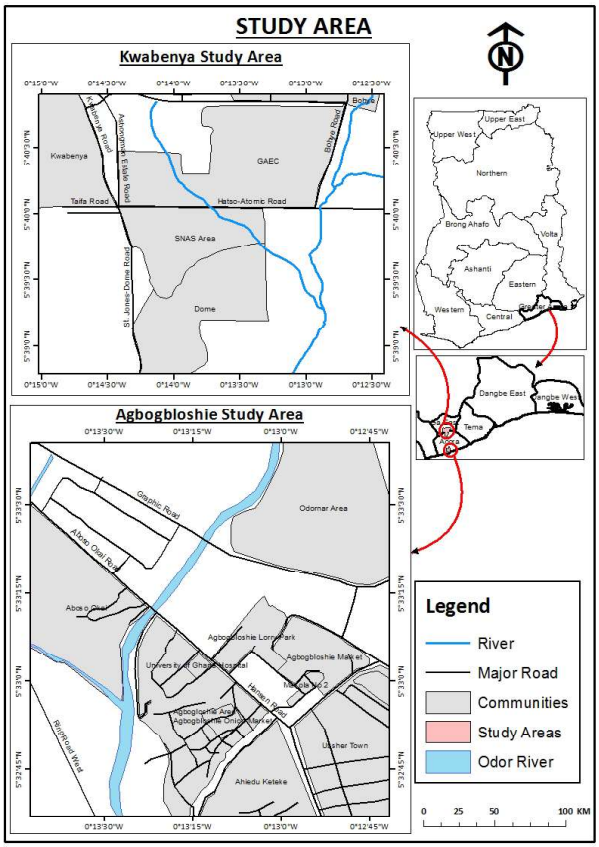


Fig 1: Map showing the study areas.

2.2 Ethical Clearance

Ethical approval was obtained from the Research and Development Division of the Ghana Health Service (GHS), Ministry of Health (MoH) before the commencement of this research. The ethical committee accessed the research proposal to ensure that the research complies with all ethic rules before granting approval for the research to proceed.

2.3 Sampling

2.3.1 Education, selection and administration of Questionnaire

Potential donors were educated on the relevance of the study. Selection of potential donors was based on residing or working in or around Abgobgloshie for five years or more, not having any serious

health challenges such as HIV, hepatitis, cancer and this was done not to add up to the stress they are already having as a result of these health challenges. Also, potential donors should be exclusively breastfeeding their babies for the first six months as recommended by World Health Organization. Qualified mothers who were ready to participate in the study signed an informed consent form. Participants filled a questionnaire to provide information on their occupation, dietary habit, age, weight, height, and other relevant information capable of influencing the levels of contaminants in their breast milk.

2.3.2 Sample Collection

The entire study involved one hundred and twenty-eight (128) individual women. The sampling of breast milk took place between September 2014 and July 2016. The breast milk samples were collected manually by the mothers into previously cleaned 100 ml amber bottles. The milk samples were retained in an ice chest containing dried ice and were conveyed to the pesticide residue laboratory of the Ghana Standard Authority where they remained frozen at −25°C until analysis. Some of the milk samples were airlifted in an ice chest with dried ice to Aalborg University laboratory, Esbjerg campus in Denmark for PAHs analysis

2.4 Chemical Extraction and GC Analysis

2.4.1 Reagents and materials

Chemicals and reagents used in this study were of maximum purity. An 18 component PAH -18-mix in 10ng/µg acetonitrile standard of 95.9-99.9% purity from Dr. Ehrenstorfer, 99% Reagent Plus (R) Sodium Hydrogen Citrate Sesquihydrate from Sigma-Aldrich in Germany, 99% tri-sodium citrate dehydrate with batch number B160812 from Glass world South Africa. Magnesium Sulphate Anhydrous 97% reagent grade 20809 from Sigma-Aldrich, Sodium Chloride (Pesticide grade) Acetonitrile (analytical grade), and ethyl acetate all from VWR International (West Chester, PA, USA), 50ml Polypropylene (PP) Centrifuge tubes with screw caps VWR Cat.No.525-0155, and 15 Centrifuge Tubes with screw caps VWR European Cat. No. 525-0149.

2.4.2 Equipment

The main equipment used in this study was the following: a vortex, a centrifuge, a rotary evaporator and the GC-MS/MS equipment.

2.4.3 Extraction of PCBs in Breast Milk

The Polychlorinated Biphenyls in the human breast milk samples was extracted using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method with slight modification (Luzardo et al., 2013).

The individual 128 frozen human breast milk samples was liquefied at room temperature. A 5ml of the each homogenized human milk sample was transferred into already cleaned 50ml PP bottle. A 10ml of acetonitrile was added to 5ml human breast milk samples and vortexed for a minute. A mixture of 4g Magnesium Sulphate anhydrous (to remove water), 1g of Sodium Chloride (for separation of water phase from organic phase), 1g of Trisodium citrate dehydrate and 0.5g of Disodium hydrogen citrate sesquihydrate (as a buffer) were added to the breast milk in the acetonitrile. The milk was vortexed instantly to avoid agglomeration of the salts. The mixture was then centrifuged at 3000rpm for 5mins. The organic phase was collected concentrated to dryness under a gentle nitrogen stream, and fat weight measured gravimetrically (AOAC, 1990). Clean up was done by adding 6 ml of acetonitrile into the concentrate and the slurry was transferred into an already cleaned 15 ml PP centrifuge bottle containing 150 mg primary secondary amine (PSA), 900 mg of magnesium sulphate and 150 mg of C-18. The PP centrifuge tube with its content was closed and vortexed for 30s and centrifuged for 5min at 3000 rpm. 4 ml of the cleaned up was transferred to a clean glass tube, and 40 µLof 5% formic acid in acetonitrile was added to adjust the pH. It was then evaporated to dryness under a gentle stream of nitrogen. The extract was reconstituted in 1ml of ethyl acetate and 2040 µL of 1% polyethylene glycol in ethyl acetate (v/v). The extract was transferred into 2ml GC vial for analysis.

2.4.4. Instrumental Analysis and Quantification

The samples were analyzed using Agilent Technologies 7890B 7000C GC-MS/MS Triple Quad with autosampler 80 and Helium as a carrier gas. Injection temperature was 280 ˚C, splitless mode, and 2.0-µl injection volume. The ion source was EI mode, source temperature of 300 ˚C and MSD transfer line of 325 ˚C. The column type HP-5ms (30 m x 0.25 mm x 0.25 um) was used with column flow of 1.25 ml/min. The column temperature was first set to 70 ˚C and held for 2 mins ramped to 150 ˚C at 25 ˚C /min and then to 200 ˚C at 3 ˚C /min) and then finally to 280 ˚C and held at 12 minutes. The solvent delay time was 4 minutes and a total time of 44mins.

2.4.5 Analytical Quality Controls

Quality assurance and quality control measures such as procedural blanks were analyzed alongside every batch of ten samples. The concentration of PAHs determined in the procedural blank was subtracted from the respective samples. Also, a blank powdered milk baby formula was spiked with known concentrations of PCBs for recovery testing. In addition, quantification procedure included verification of retention times and isotope ratios of labeled standards and specified analytes. The mass fragment having the highest intensity of the molecular or fragment ion cluster was used for quantification. Concentrations were obtained based on the signal heights of the analyte with its specific labeled analog.

2.5. Statistical evaluation

R version 3.4.0 was used in the data analysis. ANOVA test was performed for investigation of statistically significant difference of different variables. In the present study probability value (p-value) of smaller than 0.05 ($p<0.05$) was taken into consideration as statistically significant.

2.6. Cancer evaluation calculation method

Toxicity equivalent factors (*TEF*) have been defined for seven PAHs considered by the USEPA (2002) as probable carcinogens, and the potency of these factors is dependent on BaP, which is a known carcinogen with a *TEF* value of 1. Its corresponding *TEF* value multiplies each PAH concentration and the sum of these values gives the BaP equivalent concentrations, TEQ_{BaP} (toxicity equivalent quotient). Mutagenicity of each PAH to BaP can be also calculated using Mutagenic equivalent factor (*MEF*) recommended by Durant et al. (Durant et al., 1999, 1996). The total of each concentration multiplied by its equivalent value represents the mutagenic equivalent quotient (MEQ).

$$TEQ_{BaP} = \Sigma(TEF_i \times C_i) \quad \text{Eq. (1)}$$

$$MEQ_{BaP} = \Sigma(MEF_i \times C_i) \quad \text{Eq. (2)}$$

Where C_i is the individual PAHs concentration with its corresponding TEF_i or MEF_i value.

The BaP equivalent dose is calculated using equation 3:

$$BaP\ equivalent\ dose\ of\ carcinogenic\ (mutagenic) PAHs\ (BaPEQ) = \frac{TEQ(MEQ) \times IR \times EF \times ED}{BW \times AT} \quad \text{Eq. (3)}$$

Where *IR* is the intake rate of breast milk in g per day; *EF* is the Exposure frequency to carcinogenic or mutagenic PAHs in days per year; *ED* is exposure duration in years; *BW* is the average body weight of a baby in kg and *AT* is the average life expectancy.

The default values on exposure and intake assumptions were made in consistent US EPA guidelines (USEPA, 1991). Default values used are:

IR=700 g milk/day; *ED*= 1 year; *BW*=5 kg; *EF*= 350 days/year and *AT* =2 years

Cancer or mutagenic risk is calculated based on equation 4 as follows:

$$Risk(carcinogenic\ or\ mutagenic) = SF_{BaP} \times BaP\ equivalent\ dose\ of\ mixture\ of\ PAHs \quad \text{Eq. (4)}$$

Where SF_{BaP} is the oral carcinogenic slope factor for benzo[a]pyrene (7.3 per mg/kg/day).

3.0. Result and discussion

Screening of breast milk samples for PAHs is important for assessing the level of contamination and the possible risks to breastfed infants.

PAHs concentrations were assessed in 128 primiparae and multiparae nursing mothers. A total of 105 of the 128 nursing mothers live and or work around Ghana's largest electronic waste dump and recycling site, and 23 reside in and around Kwabenya, a suburb of Accra. Kwabenya is mainly a residential area without industrial or economic activities.

3.1. Anthropometric characteristics of the breast milk donors

The anthropometric characteristics of donor mothers used in the study are shown in Table 1 below.

Table 1. General demographic characteristics of the breast milk Donors.

	Agbogbloshie e-waste site		Kwabenya	
	Mean	Range	Mean	Range
Age (Years)	27.6	18-41	28.6	19-35
Weight (Kg)	74.3	60.3-92	56	53-69
Height (m)	1.63	1.43-1.92	1.4o	1.90
BMI (Kg/m^2)	28.02	22.24-36.59	22.5	18-25
Diet	Mixed		Mixed	
Primiparae	47 Samples (44.76%)		8 (34.78%)	
Multiparae	58 Samples (55.24%)		15 (65.22%)	

As can be seen from Table 1, donor mothers residing and working in and around Agbogbloshie e-waste site were between the ages of eighteen and forty-one (18-41), and those from Kwabenya and its neighborhood were within the age range nineteen to thirty-five (19-35). Body mass index (BMI) ranged from 22.24 kg/m^2 to 36.59 kg/m^2 for donors from Agbogbloshie e-waste site and from 18kg/m^2 to 28kg/m^2 for those from Kwabenya respectively. A total of 44.76% of donor mothers from Agbogbloshie e-waste site was first birth mothers (primiparae) and 55.24% multiple birth (Multiparae) mothers and 65.22% and 34.78% for multiple mothers and first birth mothers respectively for donors from Kwabenya. The mean lipid content in the lipid was 3.7% and ranged from 2.2 to 5.1%.

3.2. Level of contamination

A total of 18 individual PAHs (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, acenaphthalene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, chrysene, benzo[a]anthracene, benzo[a]pyrene, benzo[k]fluoranthene, benzo[b]fluoranthene benzo[g,h,i]perylene, dibenz[a,h]anthracene, indeno[1,2,3,c,d]pyrene) were detected in the 128

human breast milk samples from Agbogbloshie e-waste site (a hot spot area) and Kwabenya (a non-hot spot area) in Accra, Ghana. Table 2 shows the mean concentrations of 18 PAHs from 128 women.

Table 2. Mean concentrations (ng/g lipid wt.) and the range of 18 PAHs in human breast milk for 128 mothers.

PAHs	Mean±SD	Range	Positive Samples	IARC[a]	EPA[b]
Naph	856.21±1782.04	□LOD* - 14320.67	115	2B*	D*
2-Met	131.84±108.58	□LOD - 537.77	125		
1-Met	76.36±104.93	□LOD - 773.80	116		
Acy	1.37±2.70	□LOD - 5.92	125		D
Ace	1.76±4.77	□LOD - 28.91	123	3	N/A
Flu	3.41±4.00	□LOD - 25.90	75	3	N/A
Ant	12.76±11.20	□LOD - 96.01	109	3	D
Phe	9.51±8.88	□LOD - 45.58	84	3	D
Pyr	2.77±2.08	□LOD - 14.47	114	3	D
Flt	4.63±3.17	□LOD - 16.62	115	3	D
Chr	0.30±0.38	□LOD - 2.77	100	2B	B2
BaA	0.22±0.32	□LOD - 2.21	67	2B	B2
BaP	0.08±0.17	□LOD - 1.66	97	1	B2
BkF	0.04±0.07	□LOD - 0.50	66	2B	B2
BbF	2.94±4.89	□LOD - 32.41	72	2B	B2
BghiP	0.60±1.03	□LOD - 7.64	103	3	N/A
DahA	0.14±0.64	□LOD - 6.12	110	2A	B2
IndP	0.69±1.06	□LOD - 7.66	116	2B	B2
Total	1105.63	□LOD – 15936.57			

Naph=naphthalene, 2-Met=2-methylnaphthalene, 1-Met=1-methylnaphthalene, Acy=acenaphthylene, Ace= acenaphthalene Flu= fluorene, Ant= anthracene, Phe= phenanthrene, Pry= pyrene, Flt=fluoranthene, Chr=chrysen, BaA= Benzo[a]anthracene, BaP= benzo[a]pyrene, BkF=Benzo [k]fluoranthene, BbF= Benzo[b]fluranthene BghiP= benzo[g,h,i]perylene, DahA=dibenz[a,h]anthracene, IndP= indeno[1,2,3,c,d]pyrene

LOD: the limit of detection.

a: IARC classification; 1: Carcinogenic to human; 2A: Probably carcinogenic to human; 2B: possible carcinogenic to human; 3: Not classifiable to human. B: US-EPA classification; B2: Probable Human Carcinogen; D: Not Classifiable; N/A: Not Available

The sum of the 18 PAHs in the human milk samples ranges between □LOD and 15936.57 ng /g lipid wt. and having a mean sum level of 1105.63 ng/g lipid wt. Cok et al., 2012, recorded a mean sum of 84.42ng/g for the16 PAHs in 47 breast milk samples. The low molecular weight PAHs such as naphthalene (128g/mol), 2-methylnaphthalene (142g/mol), 1-methylnaphthalene (142g/mol) and

anthracene (178g/mol) recorded the highest mean of 856.21 ng/g lipid wt., 131.84ng/g lipid wt., and 76.36 ng/g lipid wt. and 12.76 ng/g lipid wt. respectively. High molecular weight PAHs such as dibenz[a,h]anthracene (278g/mol), indeno[1,2,3,cd]pyrene (276g/mol) and benzo[k]fluoranthene (252g/mol) recorded the least mean concentrations of 0.14ng/g lipid, 0.69ng/g lipid and of 0.04 ng/g lipid wt. respectively. A similar trend was observed in a study from the Czech Republic with the low molecular weight PAHs recording higher concentrations even though the mean concentrations in the present work are relatively higher than what they recorded (Çok et al., 2012; Pulkrabova et al., 2016).

As shown in Table 2, 98% of the mothers recorded 2-methylnaphthalene and Acenaphthene in their breast milk whereas 52% of the mothers also had benzo[a]anthracene and benzo[k]fluoranthene in their breast milk. The most carcinogenic PAH, benzo[a]pyrene, was recorded in 76% of the breast milk samples. The contributions of the individual PAHs to the total PAHs concentration are shown in Fig 2. naphthalene contributed 77.4 % of the total PAHs in the breast milk samples, and dibenz[a,h]anthracene and benzo[k]fluoranthene contributed approximately 0.01%, and 0% respectively. Benzo[a]pyrene, the most carcinogenic PAH contributed approximately 0.01% of the total mean PAHs in the milk samples. A similar trend of low molecular weight PAHs contributing with a higher percentage out of the total mean PAHs than high molecular weight PAHs in the breast milk samples is seen in similar works done in other parts of the world (Çok et al., 2012; Pulkrabova et al., 2016).

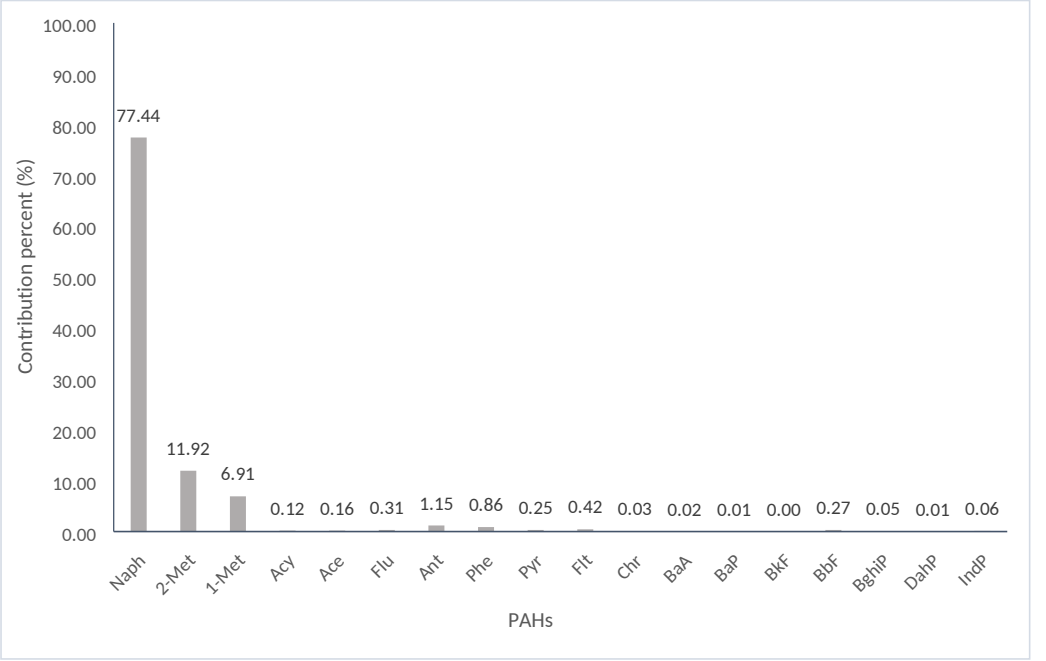


Fig 2. A bar chart showing the contributions of each PAHs to the total PAHs in the milk samples.

According to Table 3, comparing the level of contamination of PAHs in the breast milk samples from both Agbogbloshie (hot spot area) area Kwabenya (non-hot spot area), the ranges were between 0.302 and 15820.30 ng/g lipid wt. and from □LOD to 687.44 ng/g lipid wt. for Agbogbloshie and Kwabenya respectively. The total mean concentrations were 1304.163ng/g lipid wt. and 199.273ng/g lipid wt. for Agbogbloshie and Kwabenya respectively. Most high molecular weight PAHs such as chrysene, benzo[a]pyrene, benzo[k]fluoranthene and Benzo[g,h, i]Perylene which are probable carcinogens were all below limit of detection in the milk samples from Kwabenya, but were detected in the breast milk samples from Agbogbloshie. Apart from acenaphthylene, acenaphthalene, fluorene, anthracene, fluoranthene, and dibenz[a,h]anthracene all the mean concentration levels of PAHs in the breast milk samples from Agbogbloshie e-waste site were higher than their respective mean concentrations from Kwabenya.

Acenaphthene and anthracene were detected in all the milk samples from Agbogbloshie. Also, 99% percent of the milk samples from donors Agbogbloshie recorded 2-methylnaphthalene, acenaphthylene, and pyrene. benzo[g,h,i]perylene, dibenz[a,h]anthracene and indeno[1,2,3,c,d]pyrene were detected in 98% in the breast milk samples from Agbogbloshie. The

most carcinogenic of all the PAHs, benzo[a]pyrene was detected in 92% in the milk samples from Agbogbloshie but were below the limit of detection in all the samples from Kwabenya. The most detected PAHs in the milk samples from Kwabenya was naphthalene; it was recorded in 95% in the breast milk sample.

Table 3. PAHs concentrations (ng/g lipid wt.) in the breast milk samples of donor mothers from Agbogbloshie e-waste site and Kwabenya.

	Agbogbloshie –waste site				Kwabenya			
PAHs	Mean±SD	Range	Median	Positive Samples	Mean±SD	Range	Median	Positive Samples
Naph	1026.52±1927.34	□LOD-14320.67	227.35	93	78.73±42.19	□LOD - 184.89	70.06	22
2-Met	156.11±105.11	□LOD - 537.77	141.83	104	21.06±13.96	□LOD - 53.00	21.65	21
1-Met	79.20±112.24	□LOD - 773.80	15.79	95	63.38±61.59	□LOD - 190.61	30.89	21
Acy	0.66±0.65	□LOD - 5.92	0.55	104	4.61±5.18	□LOD - 15.87	2.08	21
Ace	0.56±0.36	0.036 - 3.22	0.53	105	7.27±9.59	□LOD - 28.91	2.22	18
Flu	3.23±3.03	□LOD - 10.79	3.49	65	4.24±6.94	□LOD - 25.90	□LOD	10
Ant	13.97±6.76	0.266 - 52.43	15.05	105	7.25±21.66	□LOD - 96.01	□LOD	4
Phe	11.24±8.55	□LOD - 45.58	14.31	82	1.62±5.44	□LOD - 21.65	□LOD	2
Pyr	2.87±1.29	□LOD - 11.96	2.57	104	2.31±4.10	□LOD - 14.47	□LOD	10
Flt	4.41±2.23	□LOD - 16.62	4.40	97	5.63±5.75	□LOD - 16.40	2.90	18
Chr	0.37±0.39	□LOD - 2.77	0.27	100	□LOD	□LOD	NA	0
BaA	0.26±0.34	□LOD - 2.21	0.18	67	□LOD	□LOD	NA	0
BaP	0.09±0.18	□LOD - 1.66	0.07	97	□LOD	□LOD	NA	0
BkF	0.05±0.07	□LOD - 0.50	0.01	65	□LOD	□LOD	NA	0
BbF	3.05±4.18	□LOD - 13.00	0.37	66	2.42±7.42	□LOD - 32.41	□LOD	6
BghiP	0.73±1.09	□LOD - 7.64	0.02	103	□LOD	□LOD	NA	0
DahA	0.12±0.62	□LOD - 6.12	0.02	103	0.27±0.72	□LOD - 3.42	□LOD	7
IndP	0.74±1.10	□LOD - 7.66	0.02	103	0.48±0.86	□LOD - 3.89	0.07	13
Total	1304.163	0.302-15820.30			199.273	□LOD - 687.44		

Note: LOD= Limit of Detection

PAHs concentration levels in the milk samples of primiparae and multiparae mothers from both Agbogbloshie e-waste site and Kwabenya were compared in Table 4. Primiparae mothers were 53 and multiparae mothers were 75 in number. The range for the total of 18 PAHs was between 1.09 and

15022.57 ng/g lipid wt. and □LOD to 7439.79 ng/g lipid wt. for primiparae mothers and multiparae mothers respectively. The mean concentrations were 951.23 ng/g lipid wt. and 1161.24ng/g lipid wt. for primiparae and multiparae respectively. Both primiparae and multiparae mothers had naphthalene recording the highest concentration of 768.22 ng/g lipid and 918.39 ng/g lipids respectively. The mean levels of all the high molecular weight and the most toxic PAHs were greater in primiparae mothers than in multiparae mothers. The mean concentration levels of the low molecular weight PAHs were higher in the milk samples multiparae mothers than in primiparae mothers.

Table 4. PAHs concentrations (ng/g lipid wt.) in the breast milk samples of Primiparae and Multiparae mothers from all the 128 samples.

	Primiparae				Multiparae			
PAHs	Mean±SD	Range	Median	Positive Samples (out of 53)	Mean±SD	Range	Median	Positive Samples (out of 75)
Naph	768.22±2134.45	□LOD - 14320.67	185.83	47	918.39±1496.41	□LOD - 6399.66	225.9	68
2-Met	140.30±104.66	1.086 - 426.18	138.10	53	125.86±111.58	□LOD - 537.77	76.55	72
1-Met	73.76±124.50	□LOD - 773.80	14.73	45	78.20±89.42	□LOD - 327.19	21.12	70
Acy	1.02±1.79	0.069 - 12.11	0.56	53	1.61±3.18	□LOD - 15.87	0.61	72
Ace	1.94±5.64	□LOD - 28.91	0.58	51	1.64±4.08	□LOD - 25.62	0.56	72
Flu	3.53±4.20	□LOD - 25.90	3.45	34	3.32±3.88	□LOD - 18.68	3.33	42
Ant	14.49±14.29	□LOD - 96.01	14.65	47	11.54±8.24	□LOD - 38.51	11.32	62
Phe	10.85±9.25	□LOD - 45.58	14.14	40	8.57±8.54	□LOD - 23.49	6.69	44
Pyr	2.86±1.86	□LOD - 11.96	2.60	50	2.70±2.23	□LOD - 14.47	2.41	64
Flt	4.68±3.36	□LOD - 16.62	4.33	48	4.59±3.05	□LOD - 13.76	4.49	67
Chr	0.34±0.40	□LOD - 2.26	0.26	44	0.28±0.38	□LOD - 2.77	0.21	56
BaA	0.25±0.35	□LOD - 2.21	0.18	31	0.19±0.29	□LOD - 1.75	0.00	36
BaP	0.09±0.23	□LOD - 1.66	0.06	42	0.07±0.11	□LOD - 0.73	0.04	55
BkF	0.05±0.08	□LOD - 0.50	0.01	31	0.03±0.05	□LOD - 0.27	0.00	34
BbF	2.95±5.56	□LOD - 32.41	0.36	33	2.93±4.39	□LOD - 16.11	0.13	39
BghiP	0.63±1.24	□LOD - 7.64	0.02	46	0.58±0.86	□LOD - 3.14	0.02	57
DahA	0.22±0.95	□LOD - 6.12	0.02	49	0.09±0.24	□LOD - 1.53	0.01	61
IndP	0.75±1.30	□LOD - 7.66	0.02	51	0.65±0.86	□LOD - 3.14	0.02	64
Total	1026.94	1.155 - 15818.20			1161.23	□LOD – 7444.45		

3.3. Statistical analysis

Using statistical tools for the collected data from donor mothers, there was no significant correlation between level of breast milk contamination and age, weight or BMI of those mothers. Even the parity did not cause a significant difference in the level of PAHs in the breast milk samples (p-value=0.687). It might be because of size of the picked samples. However, as previously mentioned in Table 3, the residing area could explain the variance of the samples. The calculated p-value for the total mean of PAHs and studied area using ANOVA test (p-value=0.0089) illustrated there was a highly significant difference in the breast milk PAHs level with respect to the region the samples come from.

3.4. Source Apportionment

Diagnostic ratios of different PAHs have been used to predict the source of PAHs, thus whether the PAHs was emanating from petrogenic, pyrogenic and burning of biomass or coal origins (Tobiszewski, 2014; Yunker et al., 2002). The test can be done by using a single source PAHs ratio, for instance, Phe/Ant and Flt/Pyr or coupling two PAHs, for example, Ant/(Ant+Phe). Coupling two PAHs gives a better prediction of PAHs origins than using a single PAHs and this is due to impact factors such as volatility and aqueous solubility, partitioning and adsorption and also degradation (Chen et al., 2012; Malik et al., 2011). A ratio of Ant/(Ant+Phe) helps in identifying PAHs from petrogenic origins, and BaA/(BaA+Chr), Flt/(Flt/+Pyr), as well as IndP/(IndP+BghiP) are useful in identifying PAHs from pyrogenic origins (Yunker et al., 2002). A ratio of IndP/(IndP+BghiP) value of lower than 0.2 predicts a petrogenic source, a ratio value between 0.2 and 0.5 is ascribed to combustion of liquid fuels origin and a ratio of above 0.5 indicates solid fuel combustion (Chen et al., 2012; Inam et al., 2016). An Ant/(Ant+Phe) ratio value below 0.1 and above 0.1 suggests petrogenic and pyrogenic sources of PAHs respectively. Also, a ratio of Flt/(Flt+Pyr) lower than 0.4 indicates an unburned petroleum origin. Also, a ratio between 0.4 and 0.5 for Flt/(Flt+Pyr) suggests a combustion from liquid fuels source, and a ratio value of 0.5 and above suggests that the PAHs are emanating from coal, grass and wood burning (Inam et al., 2016; Yunker et al., 2002). In addition a ratio of BaA/(BaA+Chr) value below 0.2 may suggest a petrogenic origin, values above 0.35 predicting a pyrogenic source and a ratio value between 0.2 and 0.35 suggest both petrogenic and pyrogenic sources (Chen et al., 2012).

The diagnostic ratio was used to predict the sources of PAHs in the breast milk samples from the two locations Agbogbloshie and Kwabenya) in this study. Table 3 below shows the results obtained from the diagnostic ratio test.

Table 5. PAHs source assessment using diagnostic ratios

	Petrogenic	Pyrogenic	Mixed (Petrogenic+ Pyrogenic)	Agbogbloshie Site	Kwabenya Site	References
Phe/Ant	>10	<10	-	0.805	0.223	(Chen et al., 2012)
Ant/(Ant+Phe)	<0.10	>0.01	-	0.554	0.818	(Inam et al., 2016; Yunker et al., 2002)
BaA/(BaA+Chr)	<0.2	>0.35	0.2-0.35	0.415	NA	(Chen et al., 2012)
Flt/(Flt+Pyr)	<0.5	>0.5	-	0.606	0.709	(Inam et al., 2016; Yunker et al., 2002)
Ind/(Ind+BghiP)	<0.5	>0.5	-	0.503	NA	(Inam et al., 2016; Yunker et al., 2002)

N/A=Not Applicable

The diagnostic ratio of BaA/(BaA+Chr) for milk samples from donor mothers in Agbogbloshie was found to be 0.42. This suggests that the PAHs in the milk are originating from pyrogenic source(Chen et al., 2012). Also, the diagnostic ratio Ant/(Ant+Phe) for breast milk samples from Agbogbloshie was 0.6; this suggests a pyrogenic origin (Yunker et al., 2002). A ratio of IndP/(IndP+BghiP) value of 0.5 predicts a PAH from the combustion of liquid fuel origin. In addition a proportion of Flt/(Flt+Pyr) value of 0.606 indicates origin from coal, grass or wood combustion. Almost all the diagnostic ratio values obtained indicates a pyrogenic origin for PAHs in the breast milk of donor mothers from Agbogbloshie. The e-waste recycling activities at Agbogbloshie might be a contributing factor to the pyrogenic origin of these PAHs in the breast milk. E-waste recycling at Agbogbloshie involves a great deal of open burning to get access to the precious metals embedded in the electronic gadgets. E-waste recycling processes have been cited in works done in other parts of the world to be a major contributor of PAHs in the environment (Feldt et al., 2014; Wang J. et al., 2012; Wang Q et al., 2016).

Diagnostic ratio test of Ant/(Ant+Phe) and of Flt/(Flt+Pyr) on milk samples from donors in Kwabenya gave ratios of 0.818 and 0.709 respectively indicating the pyrogenic source. Kwabenya is

a residential area without any industrial activities that may be contributing significantly to PAHs. PAHs in the milk sample from Kwabenya may be attributed to the dietary intake of PAHs; smoked fish is a delicacy in Ghana but have found to contain a high level of PAHs due to the processing procedure (Essumang et al., 2012). Another contributing factor may be open burning which is a common practice in Ghana and most developing countries since it has remained one of the cheapest, easiest and most sanitary means of scaling down volumes and discarding combustible materials (UNIDO, 2008).

3.5. Cancer and non-cancer Risk Assessment

There is no acceptable daily intake guideline for PAHs in human milk. Human exposure to PAHs is assessed by using benzo[a]pyrene (BaP) as an indicator. Table 6 provides the calculated toxicity equivalent dose (TEQ) [ng/g milk], Mutagenic equivalent dose (MEQ) [ng/g milk], cancer and non-cancer adverse effects. The values for TEF (USEPA, 1993) and MEF (Durant et al., 1999, 1996), as well as calculated TEQ and MEQ values, are presented in Table 6.

Table 6. Carcinogenic and mutagenic risk assessment based on BaP equivalency for human milk

	mean concentration (ng/g milk)	TEF	TEQ	BaPEQ Daily dose (ng/kg/day)	PAHs carcinogenic risk	MEF	MEQ	BaPEQ Daily dose (ng/kg/day)	PAHs carcinogenic risk
BaA	0.008	0.100	0.00078	0.052	3.8E-07	0.082	0.00064	0.043	3.1E-07
Chr	0.011	0.001	0.00001	0.001	5.4E-09	0.017	0.00019	0.012	9.1E-08
BbFlu	0.106	0.100	0.01058	0.710	5.2E-06	0.250	0.02645	1.775	1.3E-05
BkFlu	0.001	0.010	0.00001	0.001	7.0E-09	0.110	0.00016	0.010	7.7E-08
BaP	0.003	1.000	0.00276	0.186	1.4E-06	1.000	0.00276	0.186	1.4E-06
IndP	0.025	0.100	0.00249	0.167	1.2E-06	0.310	0.00772	0.518	3.8E-06
DahA	0.005	1.000	0.00515	0.346	2.5E-06	0.290	0.00149	0.100	7.3E-07
Total	**0.159**		**0.02178**	**1.462**	**1.1E-05**		**0.03940**	**2.645**	**1.9E-05**

As can be seen in Table 7, calculated BaPEQ daily dose for an infant with a daily intake of 700 g breast milk per day based on TEQ and MEQ values, are 1.462 and 2.645 ng/kg/day respectively. In addition, the corresponding risk values for carcinogenicity and mutagenicity are 1.1×10^{-5} and 1.9×10^{-5}. This means that approximately 1 out of 100000 and 2 out of 100000 infants may have cancer and other non-cancer related adverse diseases such as pulmonary diseases or low IQ during their lifetime as a result of taking carcinogenic PAHs in breast milk.

The mean concentrations of seven PAHs in the present study were also compared with different studies conducted in other parts of the world as shown in Table 7. The mean concentrations of indeno[1,2,3,c,d]pyrene (0.6 ng/g lipid wt.) and benzo[b]fluoranthene (2.94ng/g lipid wt.) in this study were the highest apart from the results from China. The mean concentrations of indeno[1,2,3,c,d]pyrene (2.50ng/g lipid wt.) and benzo[b]fluoranthene (20.5ng/g lipid wt.) respectively as recorded by Yu et al., 2011. The concentration of benzo[k]fluoranthene (0.04) was the least as compared to the mean concentrations of benzo[k]fluoranthene in works done in other parts of the world as shown in Table 7.

Table 7. Comparison of mean concentrations of seven probable carcinogen PAHs from different parts of the world.

Country	Year of sampling	Samples Size	BaA	Chr	BbF	BkF	BaP	IndP	DahA	Ref.
Ghana	2017	128	0.22	0.30	2.94	0.04	0.08	0.69	0.14	This study
Italy	2004-2005	21	0.32	0.281	0.262	ND	ND	ND	ND	(Zanieri et al., 2007)
Italy	2004-2005	11	0.98	1.028	0.531	0.128	0.519	0.417	1.330	(Zanieri et al., 2007)
Italy	2004-2005	10	0.61	0.519	0.550	ND	ND	ND	ND	(Zanieri et al., 2007)
Italy	2004-2005	11	0.07	ND	ND	ND	ND	ND	ND	(Zanieri et al., 2007)
Turkey	2009	47	0.57	1.483	0.458	0.209	0.247	0.532	0.096	(Çok et al., 2012)
Spain	NA	23	0.30	0.17	ND	ND	ND	ND	ND	(Luzardo et al., 2013)
Spain	NA	18	0.61	0.34	ND	ND	0.19	ND	ND	(Luzardo et al., 2013)
China	2005-2006	40	3.97	7.42	20.5	8.62	1.77	2.50	6.39	(Yu et al., 2011)
Czech	2013 Summer	93	0.12	0.27	0.05	0.19	0.17	0.23	ND	Pulkrabova et al., 2016
Czech	2014 Winter	95	0.11	0.21	0.12	0.14	0.14	0.11	ND	Pulkrabova et al., 2016
Czech	2013 Summer	66	0.04	0.11	011	0.06	0.27	0.1	ND	Pulkrabova et al., 2016
Czech	2014 Winter	67	0.06	0.11	0.05	ND	0.24	ND	ND	Pulkrabova et al., 2016

Conclusion

In this study, 18 PAHs levels were assessed in the breast milk of primiparae and multiparae mothers who resides in an e-waste spot area and non-hot spot area. The levels of PAHs contamination were evaluated in the milk from both two locations, from each site, and as primiparae and multiparae. Possible origins for the PAHs in the milk samples were predicted using the PAHs diagnostic ratio test. Cancer and mutagenic risk of PAHs in milk were also performed in this study. The results obtained from this study shows low levels of PAHs in milk. The PAHs levels were moderately higher in the milk samples from the hot spot area as compared to the milk from the non-hot spot area. The diagnostic ratios test predicted that most of the PAHs in the milk samples were originating from pyrogenic sources. The result of carcinogenicity and mutagenicity assessment on PAHs in human milk gave a negligible risk.

This study is a very comprehensive work done on PAHs in human breast milk comparing it with other studies on human milk in different parts of the world. To the best of our knowledge, it is the first survey done in Ghana. The results were compared with some studies done in different countries in the world. This study serves as a baseline study for PAHs in milk in Ghana for future research. It also serves as a major contribution to the paucity of information on PAHs in human milk samples in Africa.

Acknowledgement

We are thankful to the International Atomic Energy Agency for financing this research work. We appreciate all the nursing mothers who provided their milk samples for this research work.

We are again grateful to the Ghana health services and staff of the various health posts centers where milk was sampled for their support. We are also thankful to Ghana Atomic Energy Commission and Ghana Standard Authority. We also appreciate Aalborg University, Esbjerg campus.

References

AOAC, 1990. Official methods of analysis of the association of analytical chemists,15th ed. Kenneth Arlington, Virginia, USA.

Armstrong, B., Hutchinson, E., Unwin, J., Fletcher, T., 2004. Lung Cancer Risk after Exposure to Polycyclic Aromatic Hydrocarbons: A Review and Meta-Analysis. Environ. Health Perspect. 112, 970–978. doi:10.1289/ehp.6895

Bach, P.B., Kelley, M.J., Tate, R.C., McCrory, D.C., 2003. Screening for lung cancer*: A review of the current literature. Chest 123, 72S–82S.

Boffetta, P., Jourenkova, N., Gustavsson, P., 1997. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control 8, 444–472.

Bordajandi, L.R., Abad, E., Gonzalez, M.J., 2008. Occurrence of PCBs, PCDD/Fs, PBDEs and DDTs in Spanish breast milk: enantiomeric fraction of chiral PCBs. Chemosphere 70, 567–575. doi:10.1016/j.chemosphere.2007.07.019

Brigden K, Labunska I, Santillo D, Johnston P. Chemical contamination at e-waste recycling and disposal sites in Accra and Korforidua, Ghana. Research Laboratories Technical Note; 10/2008

Chen, H., Teng, Y., Wang, J., 2012. Source apportionment of polycyclic aromatic hydrocarbons (PAHs) in surface sediments of the Rizhao coastal area (China) using diagnostic ratios and factor analysis with nonnegative constraints. Sci. Total Environ. 414, 293–300.

doi:https://doi.org/10.1016/j.scitotenv.2011.10.057

Çok, I., Mazmanci, B., Mazmanci, M.A., Turgut, C., Henkelmann, B., Schramm, K.-W., 2012. Analysis of human milk to assess exposure to PAHs, PCBs and organochlorine pesticides in the vicinity Mediterranean city Mersin, Turkey. Environ. Int. 40, 63–69. doi:https://doi.org/10.1016/j.envint.2011.11.012

Colborn, T., Dumanoski, D. and Myers, J. (1996). Our stolen future. New York: Plume. pp. 215-216.

Dong, T.T.T., Lee, B.-K., 2009. Characteristics, toxicity, and source apportionment of polycylic aromatic hydrocarbons (PAHs) in road dust of Ulsan, Korea. Chemosphere 74, 1245–1253. doi:10.1016/j.chemosphere.2008.11.035

Durant, J.L., Busby, W.F., Lafleur, A.L., Penman, B.W., Crespi, C.L., 1996. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. Mutat. Res. Toxicol. 371, 123–157. doi:http://dx.doi.org/10.1016/S0165-1218(96)90103-2

Durant, J.L., Lafleur, A.L., Busby, W.F.J., Donhoffner, L.L., Penman, B.W., Crespi, C.L., 1999. Mutagenicity of C24H14 PAH in human cells expressing CYP1A1. Mutat. Res. 446, 1–14.

Essumang, D.K., Dodoo, D.K., Adjei, J.K., 2012. Polycyclic aromatic hydrocarbon (PAH) contamination in smoke-cured fish products. J. Food Compos. Anal. 27, 128–138. doi:https://doi.org/10.1016/j.jfca.2012.04.007

Essumang, D.K., Dodoo, D.K., Hadzi, G., 2010. Distribution, levels, and risk assessment of polycyclic aromatic hydrocarbons in the soot of some kitchens in the Cape Coast Metropolis of Ghana. Toxicol. Environ. Chem. 92, 1633–1647. doi:10.1080/02772241003694728

Essumang, D.K., Dodoo, D.K., Obiri, S., Oduro, A.K., 2006. Analysis of Polycyclic Aromatic Hydrocarbons in Street Soil Dust in Kumasi Metropolis of Ghana. Environ. Monit. Assess. 121, 401–408. doi:10.1007/s10661-005-9137-x

Essumang, D.K., Kowalski, K., Søgaard, E.G., 2011. Levels, distribution and source characterization of polycyclic aromatic hydrocarbons (PAHs) in topsoils and roadside soils in Esbjerg, Denmark. Bull. Environ. Contam. Toxicol. 86, 438–443. doi:10.1007/s00128-011-0232-0

Feldt, T., Fobil, J.N., Wittsiepe, J., Wilhelm, M., Till, H., Zoufaly, A., Burchard, G., Göen, T., 2014. High levels of PAH-metabolites in urine of e-waste recycling workers from Agbogbloshie, Ghana. Sci. Total Environ. 466–467, 369–376. doi:https://doi.org/10.1016/j.scitotenv.2013.06.097

Ghana Health Service, GHS, 2011. The Cancer Control Division of Ghana Health Service (GHS): Annual Report. Daily Guide News Paper, February 5, 2011, pp. 1, 3.

Inam, E., Offiong, N.-A., Essien, J., Kang, S., Kang, S.-Y., Antia, B., 2016. Polycyclic aromatic hydrocarbons loads and potential risks in freshwater ecosystem of the Ikpa River Basin, Niger Delta-Nigeria. Environ. Monit. Assess. 188, 49. doi:10.1007/s10661-015-5038-9

International Agency for Research on Cancer [IARC] (2012). Agents Classified by the IARC

Monographs, vol. 1–103. Retrieved March 12, 2012 from: http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf

IARC, 1999. Metabolic polymorphisms and susceptibility to cancer. In: Vineis, P., Malats, N., Lang, M., d'Ericco, A., Capaaso, N., Cuzick, J., Boffetta, P. (Eds.), IARC Scientific Publications No. 148. International Agency for Research on Cancer, World Health Organization, Lyon, p. 505.

Kanja, L.W., Skaare, J.U., Ojwang, S.B., Maitai, C.K., 1992. A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood, and human milk from mother/infant pairs. Arch. Environ. Contam. Toxicol. 22, 21–24.

Luzardo, O.P., Ruiz-Suarez, N., Almeida-Gonzalez, M., Henriquez-Hernandez, L.A., Zumbado, M., Boada, L.D., 2013. Multi-residue method for the determination of 57 persistent organic pollutants in human milk and colostrum using a QuEChERS-based extraction procedure. Anal. Bioanal. Chem. 405, 9523–9536. doi:10.1007/s00216-013-7377-0

Malik, A., Verma, P., Singh, A.K., Singh, K.P., 2011. Distribution of polycyclic aromatic hydrocarbons in water and bed sediments of the Gomti River, India. Environ. Monit. Assess. 172, 529–545. doi:10.1007/s10661-010-1352-4

Morillo, E., Romero, A.S., Maqueda, C., Madrid, L., Ajmone-Marsan, F., Grcman, H., Davidson, C.M., Hursthouse, A.S., Villaverde, J., 2007. Soil pollution by PAHs in urban soils: a comparison of three European cities. J. Environ. Monit. 9, 1001–1008. doi:10.1039/b705955h

Munoz-de-Toro, M., Beldomenico, H.R., Garcia, S.R., Stoker, C., De Jesus, J.J., Beldomenico, P.M., Ramos, J.G., Luque, E.H., 2006. Organochlorine levels in adipose tissue of women from a littoral region of Argentina. Environ. Res. 102, 107–112. doi:10.1016/j.envres.2005.12.017

Pulkrabova, J., Stupak, M., Svarcova, A., Rossner, P., Rossnerova, A., Ambroz, A., Sram, R., Hajslova, J., 2016. Relationship between atmospheric pollution in the residential area and concentrations of polycyclic aromatic hydrocarbons (PAHs) in human breast milk. Sci. Total Environ. 562, 640–647. doi:10.1016/j.scitotenv.2016.04.013

Schoket, B., 1999. DNA damage in humans exposed to environmental and dietary polycyclic aromatic hydrocarbons. Mutat. Res. 424, 143–153.

Shen, M., Chapman, R.S., He, X., Liu, L.Z., Lai, H., Chen, W., Lan, Q., 2008. Dietary factors, food contamination and lung cancer risk in Xuanwei, China. Lung Cancer 61, 275–282. doi:https://doi.org/10.1016/j.lungcan.2007.12.024

Tobiszewski, M., 2014. Application of diagnostic ratios of PAHs to characterize the pollution emission sources. Int. Proc. Chem. Biol. Environ. Eng. 69, 41–44.

UNIDO, 2008. Capacity building and public awareness raising programme on unintentionally produced POPs from the open burning of waste at dumpsites in the Kingdom of Cambodia. Project Code: XP/CMB/08/002.

Unwin, J., Cocker, J., Scobbie, E., Chambers, H., 2006. An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK. Ann. Occup. Hyg. 50, 395–403. doi:10.1093/annhyg/mel010

Unwin J, Cocker J, Scobbie E, and Chambers H. (2006). An assessment of occupational exposure to polycyclic aromatic hydrocarbons in the UK. Ann Occup Hyg 2006; 50(4): 395–403.

USEPA. Polycyclic Organic Matter. Washington, D.C.: Environmental Protection Agency;2002. Available at:http://www.epa.gov/ttnatw01/hlthef/polycycl.html [assessed25 July 2011].

USEPA, 1994. US Environmental Protection Agency. Federal Register, 59 FR 1788;1994

USEPA, 1993. Provisional Guidance for Quantitative Risk Assessment of PAH, EPA/600/R-93/089, United States Environmental Protection Agency.

USEPA, 1991. Risk Assessment Guidance for Superfund contaminants. Human Health Evaluation Manual Supplementary Guidance 'Standard Default Exposure Factors' Interim Final. Office of Emergency and Remedial Response, vol. 1. USEPA, Washington, DC, PB91-921314.

van der Hel, O.L., Peeters, P.H.M., Hein, D.W., Doll, M.A., Grobbee, D.E., Kromhout, D., Bueno de Mesquita, H.B., 2003. NAT2 slow acetylation and GSTM1 null genotypes may increase postmenopausal breast cancer risk in long-term smoking women. Pharmacogenetics 13, 399–407. doi:10.1097/01.fpc.0000054106.48725.87

Wang, J., Chen, S., Tian, M., Zheng, X., Gonzales, L., Ohura, T., Mai, B., Simonich, S.L.M., 2012. Inhalation Cancer Risk Associated with Exposure to Complex Polycyclic Aromatic Hydrocarbon Mixtures in an Electronic Waste and Urban Area in South China. Environ. Sci. Technol. 46, 9745–9752. doi:10.1021/es302272a

Wang, Q., Miyake, Y., Amagai, T., Suzuki, G., Matsukami, H., Tue, N.M., Takahashi, S., Tanabe, S., Tuyen, L.H., Viet, P.H., Takigami, H., 2016. Halogenated Polycyclic Aromatic Hydrocarbons in Soil and River Sediment from E-waste Recycling Sites in Vietnam. J. Water Environ. Technol. 14, 166–176. doi:10.2965/jwet.15-053

Wei See, S., Karthikeyan, S., Balasubramanian, R., 2006. Health risk assessment of occupational exposure to particulate-phase polycyclic aromatic hydrocarbons associated with Chinese, Malay and Indian cooking. J. Environ. Monit. 8, 369–376. doi:10.1039/B516173H

WHO [World Health Organization] (2003). Polynuclear aromatic hydrocarbons in drinking water, Background document for development of WHO Guidelines for Drinking water Quality, 2003 [WHO/SDE/WSH/03.04/59].

WHO (2001). The optimal duration of exclusive breastfeeding. Report of an Expert Consultation Geneva, Switzerland: World Health Organization Available at: http://www.who.int/nutrition/publications/infantfeeding/WHO_NHD_01.09/en/. Accessed on April, 25 2013.

WHO (1998). International Programme on chemical safety. Environmental health criteria 202. Selected non-heterocyclic polycyclic aromatic hydrocarbons Available at http://www.inchem.org/documents/ehc/ehc/ehc202.htm

Yu, Y., Wang, X., Wang, B., Tao, S., Liu, W., Wang, X., Cao, J., Li, B., Lu, X., Wong, M.H., 2011. Polycyclic aromatic hydrocarbon residues in human milk, placenta, and umbilical cord blood in Beijing, China. Environ. Sci. Technol. 45, 10235–10242. doi:10.1021/es202827g

Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem. 33, 489–515. doi:https://doi.org/10.1016/S0146-6380(02)00002-5

Zanieri, L., Galvan, P., Checchini, L., Cincinelli, A., Lepri, L., Donzelli, G.P., Del Bubba, M., 2007. Polycyclic aromatic hydrocarbons (PAHs) in human milk from Italian women: Influence of cigarette smoking and residential area. Chemosphere 67, 1265–1274. doi:https://doi.org/10.1016/j.chemosphere.2006.12.011

Zhang, Y., Tao, S., 2009. Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. Atmos. Environ. 43, 812–819. doi:https://doi.org/10.1016/j.atmosenv.2008.10.050

PAPER II

Anita Asamoah, Erik Gydesen Søgaard, David Kofi Essumang, Jens Muff, Sergey V. Kucheryavskiy

Assessment of PCBs and exposure risk to infants in the breast milk of primiparae and multiparae mothers in an electronic waste hot spot and non-hot spot areas in Ghana

Science of Total Environment (Under Review)

May 2017

Elsevier Editorial System(tm) for Science of the Total Environment

Manuscript Draft

Manuscript Number:

Title: Assessment of PCBs and exposure risk to infants in the breast milk of primiparae and multiparae mothers in an electronic waste hot spot and non-hot spot areas in Ghana

Article Type: Research Paper

Keywords: Breast milk, PCBs, Electronic waste, Primiparae and multiparae mothers, Ghana

Corresponding Author: Mrs. Anita Asamoah,

Corresponding Author's Institution: Aalborg University

First Author: Anita Asamoah

Order of Authors: Anita Asamoah; Erik G Søgaard; David K Essumang; Jens Muff; Sergey V Kucheryavskiya

Abstract: A total of 128 individual human breast milk were sampled and collected from both primiparae and multiparae mothers. Some of these mothers (105 individuals) work or reside in and around Agbogbloshie (hot-spot), the largest electric and electronic waste damp and recycling site in Accra, Ghana. Others (23 donor mothers) also reside in and around Kwabenya (non-hotspot) which is a mainly residential area without and industrial activities. The aim of the study was to assess the levels of PCB in the breast milk of these Ghanaian women at suspected hotspot and relatively non-hotspot areas and also to find out if the levels of these PCBs pose any risk to the breastfed infants. The levels of PCBs in the milk samples between primiparae and multiparae mothers were compared. The total mean levels and range of Σ7PCBs were 3.637ng/g lipid wt. and <LOD-29.203 ng/g lipid wt. respectively. Mean concentrations from Agbogbloshie (hot-spot area) and Kwabenya (nonhot-spot areas) were 4.428 ng/g lipid wt. and 0.029 ng/g lipid wt., respectively. PCB 28 contributed the highest of 29.5% of the total PCBs in the milk samples, and PCB 101 contributed the lowest of 1.74%. The estimated daily intake of PCB s and total PCBs concentrations in this work were found to be lower as compared to similar studies across the world. The estimated hazard quotient using health Canada's guidelines threshold limit of 1 showed no potential health risk to babies. But considering Minimum tolerable value of 0.03 µg/kg bw./day defined by agency for toxic substances and disease registry (ATSDR), some mothers were found to be at the threshold limit indicating a potential risk to their babies. Mothers at the threshold levels of the minimum tolerable limits are those who work or reside in and around Agbogbloshie e-waste site.

Suggested Reviewers: Godfred Darko
Kwame Nkrumah University of Science and Technology
godfreddarko@yahoo.com

Kwadwo A Asante
Ehime University

shinsuke@agr.ehime-u.ac.jp

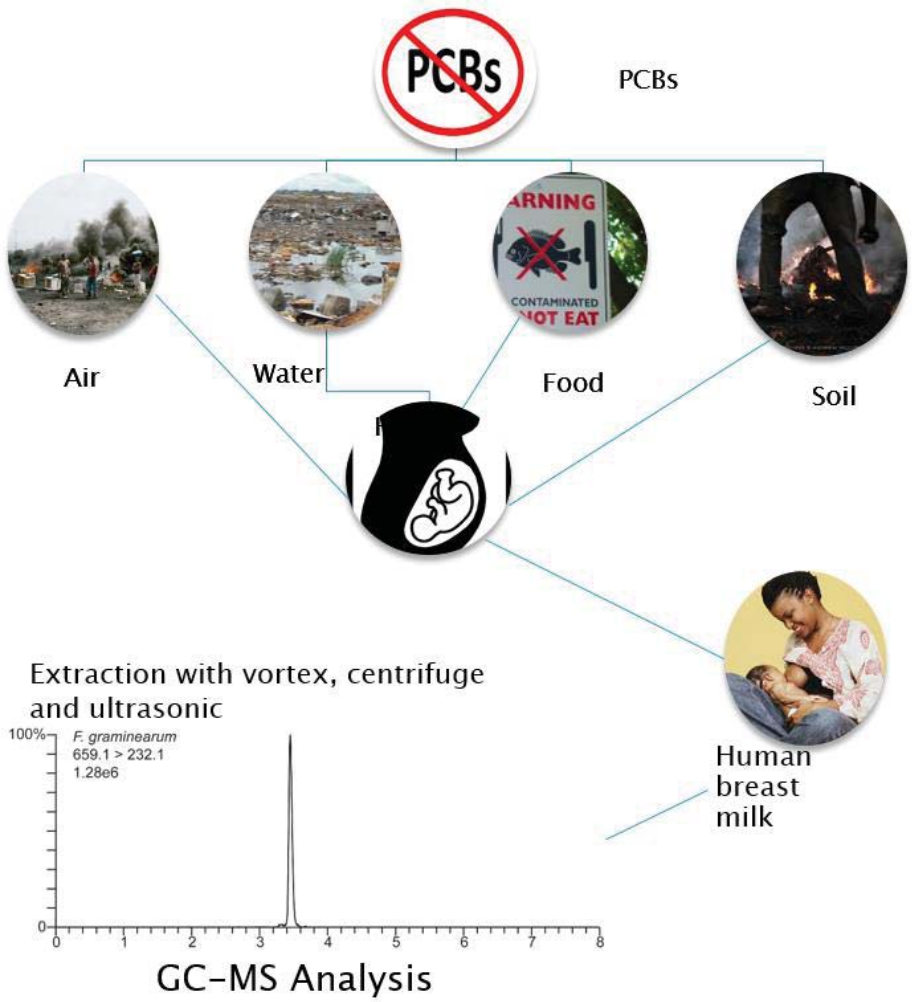
Ismet Cok
Gazi University
ismetc@gazi.edu.tr

Torsten Feldt
University Hospital Dusseldorf
feldt@bni-hamburg.de

Riffat Naseem Malik
Quaid-i-Azam University
r_n_malik2000@yahoo.co.uk

Opposed Reviewers:

*Graphical Abstract



Highlights:

- Breast milk samples were collected from an electronic-waste dump hot spot and non-hot spot area in Ghana.
- 7 indicator PCBs were identified in the breast milk samples.
- Donor mothers from hot spot e-waste area recorded high level of PCBs in their breast milk samples.
- Risk assessment on infants was also performed.

Assessment of PCBs and exposure risk to infants in the breast milk of primiparae and multiparae mothers in an electronic waste hot spot and non-hot spot areas in Ghana

Anita Asamoah[a]*, Erik Gydesen Søgaard[a], David Kofi Essumang[b], Jens Muff[a], Sergey V. Kucheryavskiya[a]

[a] Section of Chemical Engineering, Department of Chemistry and Bioscience, Aalborg University, Esbjerg, Denmark
[b] Environmental Research Group, Department of Chemistry, University of Cape Coast, Cape Coast, Ghana

Abstract

A total of 128 individual human breast milk were sampled and collected from both primiparae and multiparae mothers. Some of these mothers (105 individuals) work or reside in and around Agbogbloshie (hot-spot), the largest electric and electronic waste damp and recycling site in Accra, Ghana. Others (23 donor mothers) also reside in and around Kwabenya (non-hotspot) which is a mainly residential area without and industrial activities. The aim of the study was to assess the levels of PCB in the breast milk of these Ghanaian women at suspected hotspot and relatively non-hotspot areas and also to find out if the levels of these PCBs pose any risk to the breastfed infants. The levels of PCBs in the milk samples between primiparae and multiparae mothers were compared. The total mean levels and range of Σ_7PCBs were 3.637ng/g lipid wt. and <LOD-29.203 ng/g lipid wt. respectively. Mean concentrations from Agbogbloshie (hot-spot area) and Kwabenya (nonhot-spot areas) were 4.428 ng/g lipid wt. and 0.029 ng/g lipid wt., respectively. PCB 28 contributed the highest of 29.5% of the total PCBs in the milk samples, and PCB 101 contributed the lowest of 1.74%. The estimated daily intake of PCB s and total PCBs concentrations in this work were found to be lower as compared to similar studies across the world. The estimated hazard quotient using health Canada's guidelines threshold limit of 1 showed no potential health risk to babies. But considering Minimum tolerable value of 0.03 µg/kg bw./day defined by agency for toxic substances and disease registry (ATSDR), some mothers were found to be at the threshold limit indicating a potential risk to their babies. Mothers at the threshold levels of the minimum tolerable limits are those who work or reside in and around Agbogbloshie e-waste site.

Keywords: Breast milk, PCBs, Electronic waste, Primiparae and multiparae mothers, Ghana

1. Introduction

Polychlorinated Biphenyls (PCBs) have been recorded in various environmental matrices even though there is no clear proof of its natural occurrence based on eco systems (Bordajandi et al., 2008). PCBs are in the environment as a result of their anthropogenic production, usage, and disposal (UNEP, 1999). Industrial use of PCBs started in the early 1930s (Cairns and Siegmund, 1981) and had been used extensively as commercial products and in various industrial applications (Erickson and Kaley, 2011). PCBs have been ranked as human carcinogens by the International Agency for Research on Cancer (Lauby-Secretan et al., 2013). PCBs are associated with immune system disorders, behavioral alterations birth and reproduction defects among others (Grossman, 2013; ATSDR, 2000). PCBs are listed as a persistent Organic pollutant under the Stockholm Convention and are therefore banned from use by many countries (Stockholm Convention, 2015; Anim et al., 2017.). Some research works conducted in Africa acknowledge the increase in the sources of PCBs as a result of leakage and improper disposal of old PCB-containing transformers, increasing importation of e-waste from developed countries, shipwreck and open burning of biomass. E-waste recycling practices to recover precious metals lead to the volatilization of PCBs and other semivolatile organic substances in this waste resulting in high levels of PCBs in the environment (Gioia et al., 2014).

PCB data from a study in the African continent on passive air samplers (Klanova et al., 2009) reported on 100 pg/m3 or more average concentration monthly. Also, research conducted in West African Coast on cruises on board in 2001, 2005 and 2008 revealed a similar increase in levels of PCBs (Gioia et al., 2011). In Ghana, PCBs studies have been conducted using matrices such as fish, cow milk and human breast milk in non-hot spot areas. Relating the facts gotten from 2004 and 2009 (Asante et al., 2012, 2011), there has been a rise in the concentration of PCB. It is, therefore, important to conduct a study at a PCB hot spot area to access the current levels of PCBs in humans. Agbogbloshie, a suburb of Accra-Ghana West Africa use to be a wetland but now the largest e-waste damp, dismantling and recycling sites in Ghana (Brigden et al., 2008). At Agbogbloshie, old electrical appliances are manually dismantled, and some plasticizers and plastic coated wires and cables are openly burnt to recuperate precious metals. It also serves as a disposal site for a broad range of electronic waste (Brigden et al., 2008). The site, also, acts as a habitat for some electronic waste business workers and their families. Adjacent to the Agbogbloshie e-waste site is an open market where wide ranges of foodstuffs are sold on the wholesale and retail basis. There are offices,

schools, a hospital, recreational areas and residential facilities around the Agbogbloshie market. The populace in and around these sectors may be exposed directly or indirectly to possible PCBs emanating from the e-waste dismantling and burning site. Potential sources of human exposure to PCBs are result of direct dermal contact, inhalation of PCB-contaminated indoor or outdoor air, dietary intake and drinking PCB contaminated water (Tiernan et al. 1983; Freels et al., 2007; Fitzgerald et al., 1998). The human breast was considered as one of the best matrices for the assessment of PCBs in humans due to its lipophilic nature and hence it can store and accumulate lipophilic contaminants (Travis et al., 1988). PCBs are fat-loving and hence tend to bioaccumulate in fatty tissues of which breast milk is a good example. Also, the fact that breast milk serves as a primary source of nourishment for babies especially during the first six months of life, it is, therefore, prudent to be sure that babies are fed with healthy breast milk and not poison.

This research aims at measuring PCBs levels in human breast milk samples of some selected nursing mothers working or residing in and around Agbogbloshie. This will provide a current baseline data on people residing and working around Agbogbloshie, the largest e-waste site in Ghana. In addition, some other donor mothers from Kwabenya residential area were studied in order to have a comparison between these two regions. This research seeks to investigate whether the levels of the PCBs in the breast milk pose a potential risk to babies.

2. Materials and Method

2.1 Study area

Agbogbloshie, about a 20-acre scrap yard in the heart of Accra Ghana West Africa used to be a wetland but now it is the largest electronic waste site in Ghana. At Agbogbloshie, tons of obsolete electronics ranging from automobile, old computers, televisions to even USB cables are manually dismantled and openly burnt to in the quest to recover precious metal. The second sampling area is Kwabenya where is also a community in Accra. Kwabenya and its neighboring communities are mainly a residential area with no industrial or commercial activities. Fig 1 below shows a detailed map of the areas of study.

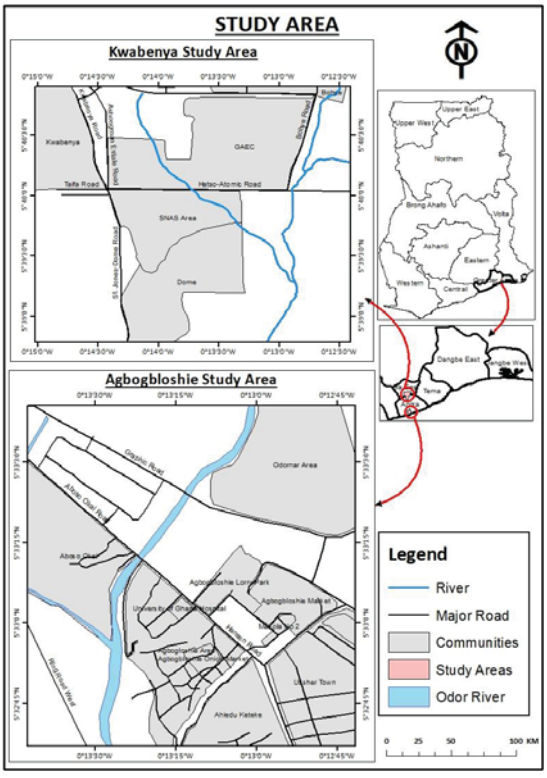


Fig 1. Map of Agbogbloshie and Kwabenya study areas

2.2 Ethical Clearance

Ethical approval was sought from the Research and Development Division of the Ghana Health Service (GHS), Ministry of Health (MoH) before the study. The ethical committee of the Ghana Health service and the Ministry of Health reviewed the Research Proposal for the Study, Survey Questionnaire, Sampling and Sample Preservation Protocols before granting the approval for the research to commence.

2.3 Sampling

2.3.1 Education, Selection and Administration of Questionnaire

Expecting and nursing mothers were educated on the aim benefits of the research. Selection of potential donors was based on residing or working in or around Agbogloshie for five years or more, they being negative to HIV, hepatitis, and other diseases and this was to done not to put too much stress on them aside what the sickness is already having on them. Also, the mother should be practicing the six months exclusive breastfeeding recommended by World Health Organization (WHO). Qualified mothers who were willing to participate in the study were made to sign an informed consent form. Participants were made to also fill a questionnaire on their age, weight, height, occupation and dietary habit to ascertain if other conditions may influence the levels of contaminants in their breast milk.

2.3.2 Sample Collection

A total of one hundred and twenty-eight (128) women participated in the study. Sampling was done between September 2014 and July 2016. Breast milk samples were manually expressed into an already cleaned 100ml amber bottles. The expressed milk was kept in an ice chest filled with dried ice and was transported to the pesticide residue laboratory of the Ghana Standard Authority where it was stored at –25°C. Some of the milk samples were sent to Aalborg University laboratory, Esbjerg campus in Denmark for analysis.

2.4 Chemical Extraction and GC Analysis

2.4.1Reagents and materials

Chemicals and reagents used in this research work were of the highest purity. PCB Mix 3 from Dr. Ehrenstorfer GmbH, 99% Reagent Plus (R) Sodium Hydrogen Citrate Sesquihydrate from Sigma-Aldrich in Germany, 99% trisodium citrate dehydrate with batch number B160812 from Glass world South Africa, Magnesium Sulphate Anhydrous 97% reagent grade 208094- 500G from SIGMA ALDRICH Sodium Chloride(Pesticide grade) Acetonitrile (analytical grade) , 50ml Polypropylene (PP) Centrifuge tubes with screw caps VWR Cat.No.525-0155, and 15 Centrifuge Tubes with Screw Caps VWR European Cat. No. 525-0149.

2.4.2 Equipment

A vortex, a centrifuge, a rotary evaporator and the GC-MS/MS were mostly used as the main equipment in this study.

2.4.3 Extraction of PCBs in Breast Milk

The Polychlorinated Biphenyls in the human breast milk samples was extracted using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method with slight modification (Luzardo et al., 2013).

Each of the frozen one hundred and twenty-eight (128) human breast milk samples was allowed to thaw at room temperature. 5ml of the individual homogenized human milk sample was transferred into already cleaned 50 ml PP bottle. 10 ml of acetonitrile was added to 5 ml human breast milk samples and vortexed for a minute. A mixture of 4 g Magnesium Sulphate anhydrous, 1g of Sodium Chloride, 1g of Trisodium citrate dehydrate and 0.5 g of Disodium hydrogen citrate sesquihydrate (as a buffer) was added to the breast milk in the acetonitrile and vortexed immediately to avoid agglomeration of the salts. The mixture was then centrifuged at 3000 rpm for 5 mins. The organic layer was collected and reduced to dryness by using a rotary evaporator and the fat weight measured gravimetrically. Clean up was done by adding 6ml of acetonitrile into the concentrate and then transferred into an already cleaned 15ml PP centrifuge bottle containing 150 mg primary secondary amine (PSA), 900mg of magnesium sulfate and 150 mg of C-18. The PP centrifuge tube with its content was closed and vortexed for the 30s and centrifuged for 5min at 3000rpm. 4ml of the cleaned up sample was transferred to a clean glass tube, and 40 µL of 5% formic acid in acetonitrile was added to adjust pH. It was then evaporated to dryness under a gentle stream of nitrogen. The extract in the glass tube was reconstituted in 1ml of ethyl acetate and 2040 µL of 1% polyethylene glycol in ethyl acetate (v/v). The extract was transferred into 2ml GC vile to be analyzed.

2.4.4. Instrumental Analysis and Quantification

The samples were analyzed using an Agilent Technologies 7890B 7000C GC-MS/MS Triple Quard with autosampler 80 and Helium as the carrier gas. Injection temperature was 280 $^{\circ}$C, splitless mode and 2.0-µl injection volume. The ion source was EI mode, source temperature of 3000 $^{\circ}$C and MSD transfer line of 325 $^{\circ}$C. The column type HP-5ms (30 m x 0.25 mm x 0.25 um) was used with

column flow of 1.25 ml/min. The column temperature was first set as 70 °C and held for 2 mins ramped to 150 °C at 25 °C/min and then to 200 0C at 3 °C/min) and then finally to 280 0C and held at 3 mins. The solvent delay time was 4mins and a total time of 35 mins.

2.4.5Analytical Quality Controls

Quality assurance and quality control measures such as procedural blanks were analyzed alongside every batch of ten samples. This was to ascertain no contamination was emanating from solvents and glassware used during the sample preparation. In addition, a blank powdered milk baby formula was spiked with known concentrations of PCBs for recovery testing.

2.5 Data Analysis

Statistical data analysis was carried out using R software (version 3.4.0.).

2.6 Daily intake and health risk assessment

Daily intake (DI) of PCBs by infants was calculated on the assumption that average weight of a baby is 5kg and average milk consumption of a 5kg infant is 700g/day (Van Oostdam et al., 2005)

$$DI = \frac{C_{milk} \times 700\ g\ milk/day \times C_{lipid}/100}{5\ Kg\ body\ weight} \quad \text{Eq. 1}$$

Where,

C_{milk} is the concentrations of chemicals in milk (µg/g lipid wt.),

C_{lipid} is the ratio of lipid content in milk.

Health risk of infants' exposure to PCBs in mother's milk was calculated using hazard quotient (HQ), which is the ratio of the estimated daily intake of the compound through breastfeeding to the maximum acceptable dose for humans or reference dose (RFD). Hazard Quotient (HQ) greater than one (>1.0) suggests a potential risk. Hazard Quotients of PCBs were calculated using a reference dose (RF) values of 1 (Oostdam et al., 1999).

3.0. Result and Discussions

3.1. Anthropometric Characteristics of the Breast milk Donors

Detailed of the anthropometric characteristics of nursing mothers used in the study is shown in Table 1below.

Table 1. General demographic characteristics of the breast milk Donors.

	Agbogbloshie e-Waste site		Kwabenya	
	Mean	Range	Mean	Range
Age (Years)	27.6	18-41	28.6	19-35
Weight (Kg)	74.3	60.3-92	56	53-69
Height (m)	1.63	1.43-1.92	1.4o	1.90
BMI (Kg/m^2)	28.02	22.24-36.59	22.5	18-28
Diet	Mixed		Mixed	
Primiparae	47 Samples (44.76%)		8 (34.78%)	
Multiparae	58 Samples (55.24%)		15 (65.22%)	

One hundred and twenty-eight (128) individual human milk samples were in used in the study. Some of the women recruited in this research reside or work in and around Ghana's largest e-waste site Agbogbloshie which also is a very busy economic area in Accra. The other set of women are from areas without any industrial or economic activities. Nursing mothers from Agbogbloshie e-waste site were between the ages of eighteen and forty-one (18-41) and those from Kwabenya and its surrounding were also within the age range nineteen to thirty-five (19-35). Body mass index (BMI) range from 22.24 and 36.59 for donors from Agbogbloshie e-waste site and 18 to 28 for those from Kwabenya. 44.76% of donor mothers from Agbogbloshie e-waste site were first birth mothers and 55.24% multiple birth mothers and 34.78% and 65.22% for first birth and multiple mothers, respectively for donors from Kwabenya. The mean lipid content of their breast milk was 3.7% and was between the ranges of 2.2 to 5.1%.

3.2. Level of contamination

Seven individual PCB congeners were found in the breast milk samples. The levels (mean, standard deviation, median and range) of PCBs found in the breast milk from the 128 women residing and working in and around the e-waste site and Kwabenya and its neighborhood shown in Table 2 below.

Table 2. Concentrations (ng/g lipid wt.) of indicator PCBs in breast milk samples from Agbogbloshie e-waste site and Kwabenya

	Agbogbloshie e-Waste site n(105)				Kwabenya n(23)			
PCB	Mean±SD	Range	Median	Positive Samples	Mean±SD	Range	Median	Positive Samples
PCB18	1.056±0.976	<LOD -6.069	0.84636	104	<LOD	<LOD	NA	0
PCB28	1.303±1.247	0.002-5.750	0.79075	105	0.0291±0.139	<LOD -0.670	NA	1
PCB52	0.185±0.201	<LOD -1.031	0.11074	105	<LOD	<LOD	NA	0
PCB101	0.077±0.105	<LOD -0.532	0.04227	104	<LOD	<LOD	NA	0
PCB138	0.863±0.928	<LOD -4.424	0.61653	104	<LOD	<LOD	NA	0
PCB153	0.415±0.919	<LOD -5.428	0.10653	104	<LOD	<LOD	NA	0
PCB180	0.529±0.778	<LOD -5.969	0.36057	105	<LOD	<LOD	NA	0

Note: LOD= Limit of detection, NA=Not Applicable

Seven indicator PCBs (PCB 18, PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB180) were tested for in the milk samples. The sum of mean concentrations levels of PCBs in the milk samples from Agbogbloshie was 4.428 ng/g lipid wt. ranged from 0.002 ng/g lipid to 6.069 ng/g lipid wt. The total mean level of PCBs in milk from Kwabenya was 0.029 ng/g lipid and the ranges were between below limit of detection (<LOD) and 0.670 ng/g lipid wt. Only one PCB congener, PCB28 was recorded in the milk samples from Kwabenya and the surrounding environment, and its mean concentration was much lower (0.029 ng/g lipid wt.) as compared to themean concentration for PCB28 from Agbogbloshie (1.303ng/g lipid wt.). All the other PCB congeners were below detection limit in the milk samples of mothers from Kwabenya. In general, milk samples of mothers from Agbogbloshie e-waste site and surroundings were more contaminated with PCBs than milk samples from mothers at Kwabenya. The contamination of breast milk samples from mothers residing or working in and around Agbogbloshie e-waste site may be attributed to the e-waste dismantling and recycling activities in the vicinity. E-waste recycling activities are a major contributor of PCBs release to the environment (Gioia et al., 2014). The predominant PCB congeners in the breast milk samples from the e-waste site were PCB28, 18 and 138. PCB28

recorded the highest concentration (1.303ng/g lipid wt.), and PCB101 recorded the lowest concentration (0.077 ng/g lipid wt.). The mean concentration of PCB28 (1.303 ng/g lipid wt.) was higher than what was recorded by Asante et al. 2009 for PCB 28 (0.61ng/g lipid weight) in breast milk samples in Accra. However, from work done by Asante et al., 2009, mean concentration for PCB153 was higher (22ng/g lipid wt.) than what was recorded in this study (0.415ng/g lipid wt.). Total PCBs concentrations in breast milk is 3.637ng/g lipid wt. which is far lower than what was recorded in Accra in 2009 (62ng/g lipid wt.).This may be attributed to the reduction of PCBs in the Ghanaian environment over the years. PCB 28 was recorded in all the 105 milk samples from Agbogbloshie and one sample from Kwabenya. PCB congeners 18, 101, 138 and 153 were detected in 104 of the milk samples from Agbogbloshie whiles none of these congeners were detected in the milk samples from Kwabenya.

The mean concentrations of various PCBs in both first birth mothers (Primiparae) and mothers who have had one or more births (Multiparae) were compared, as shown in Table 3. There was no significant variation in the mean concentrations of the various PCB congeners in breast milk samples from both multiparae and primiparae mothers. This may be because the PCB contaminants are originating from the same source and the mothers are continually exposed (Someya et al., 2010; Tue et al., 2010).

Table 3. PCBs Concentrations (ng/g lipid wt.) in breast milk samples from primiparae and multiparae donors from Agbogbloshie e-waste site.

	Primiparae			Multiparae		
	Mean	Median	Range	Mean	Median	Range
PCB 18	1.066	0.814	0.055-4.371	1.047	0.881	<LOD-6.070
PCB 28	1.330	0.874	0.031-5.112	1.282	0.700	0.002-5.750
PCB52	0.164	0.098	0.001-0.666	0.202	0.117	0.002-1.031
101	0.071	0.427	0.002-0.390	0.082	0.038	<LOD-0.532
138	0.850	0.677	0.027-4.159	0.873	0.531	<LOD-4.420
153	0.312	0.093	0.007-4.349	0.495	0.109	<LOD-5.430
180	0.468	0.391	<LOD-2.360	0.577	0.345	0.001-5969

*Note: LOD- Limit of Detection

The mean concentrations of lower chlorinated PCB congeners (volatile and less lipophilic) for instance PCB18 and PCB28 were higher in both primiparae and multiparae donors as compared to the mean concentrations obtained for the other congeners. Lower chlorinated congeners are easily inhaled due to their volatile nature as compared to highly chlorinated congeners. The trend may be attributed to donors' proximity to the Agbogbloshie e-waste recycling site, where there is a probable release of PCBs in air and the smog emanating from the burning of the e-waste (Chan and Wong, 2013; Wong et al., 2007)

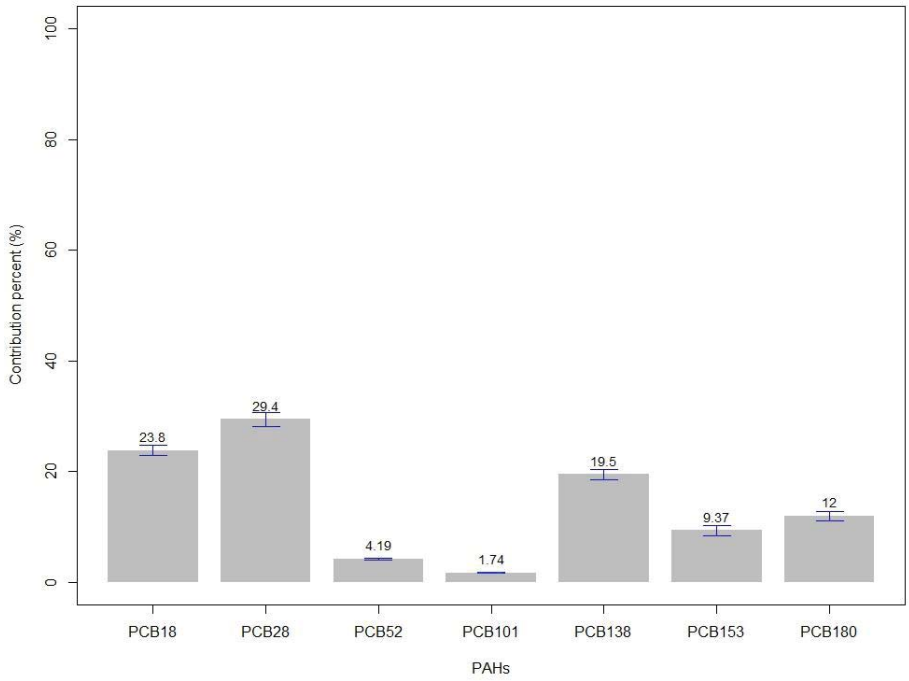


Fig. 2. Contribution (in percentages) of each PCB congener to the total PCBs

As shown in Fig. 2. the percentage contributions of PCB28 to the total PCBs in the milk samples was the greatest (29.5%) and PCB101 contributed the least (1.74%) as compared to the other congeners. The other congeners; PCB18, PCB138, PCB180, PCB52, PCB153 contributed 29.5%, 23.8%, 19.5% 11.9%4.18% and 9.35% respectively to the total PCBs in breast milk samples. The

first two congeners with higher contribution to total PCBs in the milk samples as compared to the other PCB congeners are both lower chlorinated PCB congeners. Lower-chlorinated PCB congeners consist of four or less chlorine substitutes on carbons of the biphenyl rings (Hu et al., 2010; Persoon et al., 2010; Wethington and Hornbuckle, 2005). The metabolism of PCBs is dependent on the number and position of the chlorine atoms (Mills et al., 1985; Schnellmann et al., 1985, Kato et al., 1980). Humans are easily exposed to lower chlorinated PCB especially through inhalation due to their low volatility (Bamford et al., 2000). The presence of these lower chlorinated PCBs in the milk samples may be recent exposure due to e-waste activities since old electric and electronic devices mainly contain congeners with lower chlorination (Takasuga et al., 2006). Old electric and electronic devices mainly contain congeners with low degrees of chlorination (Takasuga et al., 2006).

3.3 Daily intake and health risk assessment

The estimated daily intake (EDI) of PCBs in the milk samples was 0.02 µg/kg body wt./day with a range of <0.001- 0.03 µg/kg body wt./day. The ratio of the estimated daily intake to the Tolerable Daily Intake (TDI) of 1 µg/kg body wt./day established by health Canada for PCBs (Van Oostdam et al., 2005) is less than 1. This means that babies are under no risk. In addition, using a Tolerable risk value of 0.03 µg/kg body wt. /day by (ATSDR, 2000) there was no potential risk to babies but some were just at the threshold limit of 0.03 µg/kg body wt./day therefore the need for continuous monitoring of PCBs in humans. Comparing the estimated daily intake of PCBs in milk samples with work done in other parts of the world, the estimated daily intake in this work is lower as seen in Table 4.

Table 4. Mean Concentrations (ng/g lipid wt.) and estimated daily intake (µg/kg/day) of PCBs from different countries.

Country	Sampling year	Sample Size	PCBs (ng/g lipid wt.)	Daily Intake (µg/kg/day)	Reference
Ghana	2014-2016	128	3.638	0.02	This work
Ghana	2009	42	62	0.4	(Asante et al., 2012)
South Africa	2006	29	10	NA	(Darnerud et al., 2006)
Tunisia	2003-2005	237	196	0.83	(Ennaceur et al., 2008)
China	2003-2005	21	206	NA	(Zhao et al., 2007)
Republic Korea	2011	206	12.5	0.086	(Lee et al., 2013)
Turkey	2009	47	8.073	0.052	(Çok et al., 2012)
Russia	2003-2004	33	240	NA	(Tsydenova et al., 2007)
Spain	2005	9	125	NA	(Gómara et al., 2011)

4.0 Conclusion

A total of 128 human breast milk samples were collected from both primiparae and multiparae mothers from Agbogbloshie and Kwabenya in Accra Ghana and their surrounding areas. The breast milk samples were screened for seven indicator PCBs (PCB18, PCB28, PCB52, PCB101, PCB 138, PCB153 and PCB180). There were no significant variations between the arithmetic mean concentrations of PCBs in the human milk samples for primiparae and multiparae mothers. PCB28 and PCB180 were recorded in all the 105 individual milk samples from Agbogbloshie and 99% recorded PCB18, PCB52, PCB101, PCB138, and PCBs153. Only one individual sample out of 23 milk samples from Kwabenya tested positive to PCB 28, all other PCBs were below detection limit. PCBs28 had the highest contribution of 29.2% of the total PCBs in all the milk samples and PCB101 contributed the least of 1.745% of total PCBs. Using health Canada's guideline for maximum tolerable limit for total PCBs in a day (1 µg/kg body wt./day), this study found out that babies are at no risk. However, when considering minimal risk value of 0.03 µg/kg body wt. /day, it was found out from this study that some were at the threshold limit for potential risk. We therefore recommend continues monitoring of PCBs in humans among the Ghanaian populace.

Acknowledgement

We are grateful to the International Atomic Energy Agency for funding this study. We greatly appreciate all the nursing mothers who donated their milk samples for this research work.

We thank the Ghana health services and staff of the various health posts centres where milk was sampled for their support. We are also thankful to Ghana Atomic Energy Commission and Ghana Standard Authority. We also appreciate Aalborg University, Esbjerg campus.

References

Anim, A.K., Drage, D.S., Goonetilleke, A., Mueller, J.F., Ayoko, G.A., n.d. Distribution of PBDEs, HBCDs and PCBs in the Brisbane River estuary sediment. Mar. Pollut. Bull. doi:https://doi.org/10.1016/j.marpolbul.2017.05.002

Asante, K.A., Adu-Kumi, S., Nakahiro, K., Takahashi, S., Isobe, T., Sudaryanto, A., Devanathan, G., Clarke, E., Ansa-Asare, O.D., Dapaah-Siakwan, S., Tanabe, S., 2011. Human exposure to PCBs, PBDEs and HBCDs in Ghana: Temporal variation, sources of exposure and estimation of daily intakes by infants. Environ. Int. 37, 921–928. doi:10.1016/j.envint.2011.03.011

Asante, K.A., Agusa, T., Biney, C.A., Agyekum, W.A., Bello, M., Otsuka, M., Itai, T., Takahashi, S., Tanabe, S., 2012. Multi-trace element levels and arsenic speciation in urine of e-waste recycling workers from Agbogbloshie, Accra in Ghana. Sci. Total Environ. 424, 63–73. doi:https://doi.org/10.1016/j.scitotenv.2012.02.072

ATSDR, 2000. Toxicological Profile for Polychlorinated Biphenyls (PCBs). US Department of Health and Human Servicespp. 1‐948.

Bamford, H.A., Poster, D.L., Baker, J.E., 2000. Henry's Law Constants of Polychlorinated Biphenyl Congeners and Their Variation with Temperature. J. Chem. Eng. Data 45, 1069–1074. doi:10.1021/je0000266

Bordajandi, L.R., Abad, E., Gonzalez, M.J., 2008. Occurrence of PCBs, PCDD/Fs, PBDEs and DDTs in Spanish breast milk: enantiomeric fraction of chiral PCBs. Chemosphere 70, 567–575. doi:10.1016/j.chemosphere.2007.07.019

Brigden K, Labunska I, Santillo D, Johnston P. Chemical contamination at e-waste recycling and disposal sites in Accra and Korforidua, Ghana. Research Laboratories Technical Note; 10/2008

Cairns, T., Siegmund, E.G., 1981. PCBs. Regulatory history and analytical problems. Anal. Chem. 53, 1183A–1193A. doi:10.1021/ac00234a002

Chan, J.K.Y., Wong, M.H., 2013. A review of environmental fate, body burdens, and human health risk assessment of PCDD/Fs at two typical electronic waste recycling sites in China. Sci. Total Environ. 463–464, 1111–1123. doi:10.1016/j.scitotenv.2012.07.098

Çok, I., Mazmanci, B., Mazmanci, M.A., Turgut, C., Henkelmann, B., Schramm, K.-W., 2012. Analysis of human milk to assess exposure to PAHs, PCBs and organochlorine pesticides in the vicinity Mediterranean city Mersin, Turkey. Environ. Int. 40, 63–69. doi:https://doi.org/10.1016/j.envint.2011.11.012

Darnerud P.O, Aune M, Larsson L, Lignell S, Mutshatshi N, Agyei N, et al. Levels of POPs inhuman breast milk samples from Northern Province, South Africa; comparison to Swedish

levels. Organohalogen Comp 2006;68:476–9.
Ennaceur, S., Gandoura, N., Driss, M.R., 2008. Distribution of polychlorinated biphenyls and organochlorine pesticides in human breast milk from various locations in Tunisia: Levels of contamination, influencing factors, and infant risk assessment. Environ. Res. 108, 86–93. doi:https://doi.org/10.1016/j.envres.2008.05.005
Erickson, M.D., Kaley, R.G. 2nd, 2011. Applications of polychlorinated biphenyls. Environ. Sci. Pollut. Res. Int. 18, 135–151. doi:10.1007/s11356-010-0392-1
Fitzgerald, E.F., Hwang, S.A., Bush, B., Cook, K., Worswick, P., 1998. Fish consumption and breast milk PCB concentrations among Mohawk women at Akwesasne. Am. J. Epidemiol. 148, 164–172.
Freels, S., Chary, L.K., Turyk, M., Piorkowski, J., Mallin, K., Dimos, J., Anderson, H., McCann, K., Burse, V., Persky, V., 2007. Congener profiles of occupational PCB exposure versus PCB exposure from fish consumption. Chemosphere 69, 435–443. doi:https://doi.org/10.1016/j.chemosphere.2007.04.087
Gioia, R., Akindele, A.J., Adebusoye, S.A., Asante, K.A., Tanabe, S., Buekens, A., Sasco, A.J., 2014. Polychlorinated biphenyls (PCBs) in Africa: a review of environmental levels. Environ. Sci. Pollut. Res. Int. 21, 6278–6289. doi:10.1007/s11356-013-1739-1
Gioia, R., Eckhardt, S., Breivik, K., Jaward, F.M., Prieto, A., Nizzetto, L., Jones, K.C., 2011. Evidence for Major Emissions of PCBs in the West African Region. Environ. Sci. Technol. 45, 1349–1355. doi:10.1021/es1025239
Gómara, B., Herrero, L., Pacepavicius, G., Ohta, S., Alaee, M., González, M.J., 2011. Occurrence of co-planar polybrominated/chlorinated biphenyls (PXBs), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk of women from Spain. Chemosphere 83, 799–805. doi:https://doi.org/10.1016/j.chemosphere.2011.02.080
Grossman, E., 2013. Nonlegacy PCBs: pigment manufacturing by-products get a second look. Environ. Health Perspect. doi:10.1289/ehp.121-a86
Hu, Z., Shi, Y., Niu, H., Cai, Y., Jiang, G., Wu, Y., 2010. Occurrence of synthetic musk fragrances in human blood from 11 cities in China. Environ. Toxicol. Chem. 29, 1877–1882. doi:10.1002/etc.258
Kato, S., Mckinney, J. D. and Matthews, H. B. (1980). Metabolism of symmetrical hexachlorobiphenyl isomers in the rat. Toxicol. Appl. Pharma. 53:389–398.
Klanova, J., Cupr, P., Holoubek, I., Boruvkova, J., Pribylova, P., Kares, R., Tomsej, T., Ocelka, T., 2009. Monitoring of persistent organic pollutants in Africa. Part 1: passive air sampling across the continent in 2008. J. Environ. Monit. 11, 1952–1963. doi:10.1039/b913415h
Lauby-Secretan, B., Loomis, D., Grosse, Y., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Baan, R., Mattock, H., Straif, K., 2013. Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls. Lancet. Oncol. doi:10.1016/S1470-2045(13)70104-9
Lee, S., Kim, S., Lee, H.-K., Lee, I.-S., Park, J., Kim, H.-J., Lee, J.J., Choi, G., Choi, S., Kim, S., Kim, S.Y., Choi, K., Kim, S., Moon, H.-B., 2013. Contamination of polychlorinated biphenyls and organochlorine pesticides in breast milk in Korea: Time-course variation, influencing factors, and exposure assessment. Chemosphere 93, 1578–1585. doi:https://doi.org/10.1016/j.chemosphere.2013.08.011
Luzardo, O.P., Ruiz-Suarez, N., Almeida-Gonzalez, M., Henriquez-Hernandez, L.A., Zumbado, M., Boada, L.D., 2013. Multi-residue method for the determination of 57 persistent organic pollutants in human milk and colostrum using a QuEChERS-based extraction procedure. Anal. Bioanal. Chem. 405, 9523–9536. doi:10.1007/s00216-013-7377-0
Mills, R. A., Millis, C. D., Dannan, C. A. Guengerish, E. P. and Anst, S. D. (1985). Studies on the structure activity relationship for the metabolism of PCB by rat liver microsome. Toxicol Appl

Pharmacol. 78:96 -104.
Persoon, C., Peters, T.M., Kumar, N., Hornbuckle, K.C., 2010. Spatial Distribution of Airborne Polychlorinated Biphenyls in Cleveland, Ohio and Chicago, Illinois. Environ. Sci. Technol. 44, 2797–2802. doi:10.1021/es901691s
Schnellmann, R. G. and Vickers, A. E. M. and Sipes, I. G. (1985). Metabolism and disposition of polychlorinated biphenyls. In: Hodgson EBJR, Philpot RM (editors). Reviews in Biochemical Toxicology. Vol 7. Armsterdam: Elsevier Press pp 247-282.
Someya, M., Ohtake, M., Kunisue, T., Subramanian, A., Takahashi, S., Chakraborty, P., Ramachandran, R., Tanabe, S., 2010. Persistent organic pollutants in breast milk of mothers residing around an open dumping site in Kolkata, India: specific dioxin-like PCB levels and fish as a potential source. Environ. Int. 36, 27–35. doi:10.1016/j.envint.2009.09.003
Stockholm Convention, 2015. Listings of POPs [Online]. Available: http://chm.pops.int/The Convention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx Accessed: October10, 2015.
Takasuga, T., Senthilkumar, K., Matsumura, T., Shiozaki, K. and Sakai, S. (2006). Isotope dilution analysis of polychlorinated biphenyls (PCBs) in transformer oil and global 234 commercial PCB formulations by high resolution gas chromatography–high resolution mass spectrometry. Chemosphere 62:469–484.
Tiernan TO, Taylor ML, Garret JH, Van Ness GF, Solchy G, Dais DA et al (1983) Chlorodibenzodioxins, chlorodibenzofurans, and relatedcompounds in the effluents from combustion processes.Chemosphere 12:595–606
Travis, C.C., Hattemer-Frey, H.A., Arms, A.D., 1988. Relationship between dietary intake of organic chemicals and their concentrations in human adipose tissue and breast milk. Arch. Environ. Contam. Toxicol. 17, 473–478. doi:10.1007/BF01055512
Tsydenova, O. V, Sudaryanto, A., Kajiwara, N., Kunisue, T., Batoev, V.B., Tanabe, S., 2007. Organohalogen compounds in human breast milk from Republic of Buryatia, Russia. Environ. Pollut. 146, 225–232. doi:https://doi.org/10.1016/j.envpol.2006.04.036
Tue, N.M., Sudaryanto, A., Minh, T.B., Isobe, T., Takahashi, S., Viet, P.H., Tanabe, S., 2010. Accumulation of polychlorinated biphenyls and brominated flame retardants in breast milk from women living in Vietnamese e-waste recycling sites. Sci. Total Environ. 408, 2155–2162. doi:10.1016/j.scitotenv.2010.01.012
Van Oostdam, J., Donaldson, S.G., Feeley, M., Arnold, D., Ayotte, P., Bondy, G., Chan, L., Dewaily, É., Furgal, C.M., Kuhnlein, H., Loring, E., Muckle, G., Myles, E., Receveur, O., Tracy, B., Gill, U., Kalhok, S., 2005. Human health implications of environmental contaminants in Arctic Canada: A review. Sci. Total Environ. 351–352, 165–246. doi:https://doi.org/10.1016/j.scitotenv.2005.03.034
UNEP (1999). UNEP chemicals guideline for the identification of PCBs and materials containing PCBs, First issue, Inter-organization program for the sound management of chemicals. pp. 562-563.
Wethington, D.M., Hornbuckle, K.C., 2005. Milwaukee, WI, as a Source of Atmospheric PCBs to Lake Michigan. Environ. Sci. Technol. 39, 57–63. doi:10.1021/es048902d
Wong, C.S.C., Duzgoren-Aydin, N.S., Aydin, A., Wong, M.H., 2007. Evidence of excessive releases of metals from primitive e-waste processing in Guiyu, China. Environ. Pollut. 148, 62–72. doi:10.1016/j.envpol.2006.11.006
Zhao, G., Xu, Y., Li, W., Han, G., Ling, B., 2007. PCBs and OCPs in human milk and selected foods from Luqiao and Pingqiao in Zhejiang, China. Sci. Total Environ. 378, 281–292. doi:10.1016/j.scitotenv.2007.03.008

PAPER III

Anita Osei Tutu, P.O. Yeboah, A.A. Golow, D. Denutsui and S. Blankson-Arthur

Assessment Organochlorine Pesticides Residues in the Breast Milk of Some Primiparae Mothers in La Community, Accra, Ghana

Research Journal of Environmental and Earth Sciences 3(2): 153-159, 2011

Research Journal of Environmental and Earth Sciences 3(2): 153-159, 2011
ISSN: 2041-0492

Received: November 26, 2010 Accepted: December 18, 2010 Published: March 05, 2011

Organochlorine Pesticides Residues in the Breast Milk of Some Primiparae Mothers in La Community, Accra, Ghana

Anita Osei Tutu, P.O. Yeboah, A.A. Golow, D. Denutsui and S. Blankson-Arthur
Department of Chemistry, Ghana Atomic Energy Commission NNRI,
P.O. Box LG 80 Legon-Accra, Ghana

Abstract: This study was conducted to determine the types and levels of Organochlorine pesticide residues in the breast milk of 21 primiparae mothers in La, a suburb of Accra an urban community in the Greater Accra region of Ghana. Liquid-liquid extraction procedure was employed and extract clean-up was done using silica gel solid phase extraction. Fourteen (14) different organochlorine pesticides residues namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were identified and quantified in the individual breast milk samples using a Gas Chromatograph (GC) with an Electron Capture detector. The GC recoveries of spiked samples were between 89 to 97%. P,p'- DDE recorded 100% incidence ratio. Also p,p'-DDT, delta-HCH, gamma-HCH, and endosulfan sulfate recorded incidence ratios of 76.79, 95.25, 80.95 and 85.71%, respectively for the breast milk samples. The concentrations of organochlorine pesticide residues in the human breast milk samples ranged from 1.839 to 99.05 μg/kg fats. With the exception of Endosulphan Sulphate whose mean concentration (99.052 μg/kg) was above the Australian Maximum Residue Limit (MRL) of 20 μg/kg for milk, the mean concentrations for all the other organochlorines detected were below their respective limits.

Key words: Breast milk, gas chromatograph, Ghana, maximum residue limit, organochlorine pesticide residues, primiparae

INTRODUCTION

The use of pesticides became very relevant in an attempt to control and eradicate crop pest and also to produce quality and bumper harvest to feed the ever growing population. Over the years, human population has been on the increase thus the territory of these pests became larger and larger hence the need to look for stronger and more effective alternatives to meet food security and to survive from disease vector organisms (Hodgson, 2003). It is estimated that as much as 45% of the world's crop is destroyed by plant pest and disease (Bhanti and Taneja, 2007). In Ghana, the merits of these pesticides cannot be disputed as they were massively used in the agriculture and public health sectors to curb crop pest and for disease control (Clarke *et al*., 1997). Organochlorine pesticides were extensively used by most Ghanaian farmers due to their low cost, high efficacy and its wide range suitability for plants (Osafo and Frempong, 1998). These pesticides were greatly used in most farming communities in the Western, Ashanti and Brong Ahafo regions of Ghana (Amoah *et al*., 2006) in vegetable production, cocoa farms, and mixed crop farms (Gerken *et al*., 2001, Ntow *et al*., 2006). Organochlorine pesticides such as DDT, Lindane and endosulfan were also employed to control ectoparasites of farm animals and pets in Ghana (Ntow *et al*., 2006). Pesticides have also been used to control black flies along the banks of the Tano and Pra Rivers (Ntow, 2001). Unfortunately, pesticides usage has been abused since most pesticide users are ignorant or have little knowledge about these chemicals. Some farmers are of the view that the more or as often as they apply pesticides the greater their chances of higher yield and also destroying crop pest (Ntow *et al*., 2006). They have no idea of the half lives of these chemicals nor the dangers they pose when misused. The environment is contaminated with pesticides because of their massive use in both the agriculture and public health sectors. The deleterious effects of these organochlorine pesticides on wildlife primarily led to their ban from routine use in the US and many other countries in 1970s and 1980s (Carson, 1962; Dunlap, 1981). With the exception of Endosulphan which was considered for restricted use in 2008, Ghana banned the use of most organochlorine pesticides since 1985 and Lindane in 2006, the persistent, long range transport, lipophilic and bioaccumulative nature has resulted in residual amount in the environment. There is evidence of organochlorine

Corresponding Author: Anita Osei Tutu, Department of Chemistry, Ghana Atomic Energy Commission NNRI, P.O. Box LG 80 Legon-Accra, Ghana

pesticide residues in sediments, water and biota, crops, meat and human fluids (Osafo and Frimpong, 1998; Ntow, 2001; Kalantari and Ebodi, 2006; Khalid *et al*., 2007; Darko and Acquaah, 2007). Increase accumulation of these chemicals in the food chain may pose serious health hazards in the general populace (Jayashree and Vasudevan, 2007). For example, exposure to organochlorine compounds has been reported to affect thyroid function in preschool children (Natural Health News, 2008). Low sperm count in males, birth defects, increased in testicular cancer and other reproductive and development effects (Weltman and Norback, 1983) have also been reported as a result of organochlorine contamination. In spite of the massive use of organochlorine pesticides in Ghana, there is paucity of information on their environmental levels. Consequently, there is much concern over the environmental quality of Ghana and the health of its inhabitants. Very little work has been done to measure body burden of organochlorines in Ghana especially on human breast milk. Samples of body fluids such as breast milk have been shown as an adequate indicator of body burden of organochlorine pesticide residues (Ntow, 2001). The breast, a lipid-rich tissue acts as a depot or reservoir of lipophilic pesticide by virtue of physiochemical interactions of the cellular component with the pesticide (Mussalo-Rauhamaa, 1991). Ntow (2001) worked on organochlorine pesticide residues in human breast milk of some women in Akomadan, a farming community in the Ashanti region of Ghana and recorded 40 μg/kg fats of Hexachloro Cyclo Benzene (HCB) and 490 μg/kg fats of p,p'-DDE . The current study however, is limited to only first birth mothers. Such mothers have never breast fed any children to release body burden of organochlorine through breast feeding and are more likely to have most of the organochlorine pesticide they have accumulated in their life time. This will give an idea on the levels of organochlorine pesticide residues in first birth mothers as compared to mothers of subsequent births.

MATERIALS AND METHODS

The study was conducted in the Ghana Standards Board Pesticides Residue Laboratory within August 2008 to June 2009. Ethical clearance was sought from the Ministry of Health before selecting potential donors for the study.

Chemicals and reagents: The reagents used for the analysis were analytical grade petroleum ether 40-60ºC (Scientific and Chemical Supplies Ltd); N-Hexane, 95% HPL grade (Sigma-Aldrich), acetone (Scientific and Chemical Supplies Ltd.), diethyl ether AnalaR (BDH Chemical Ltd.), concentrated sulphuric acid, >95-97% (Fluka), ethylacetate pestanal (Riedel Haen), individual pesticide reference standards (>95.0% purity) from Dr. Ehrenstofer GmbH, Germany and stored in a freezer at -20ºC to minimize degradation. Solid Phase Extraction (SPE) cartridges (strata sI-1 Silica) (55 μm, 70 A) of density, 500 mg/6 mL.

Glassware: Twenty one (21) Schott Duran 100ml glass bottles with protective caps used for sampling were imported from Germany.

Equipment: Centrifuge Cri multifunction (Thermo Electron Industries SAS, France), T 25 Basic Ultra turax macerator (IKA® Werke Germany), Metler Toledo PG 10035 weighing balance, rotary evaporator Buchi RE-200 equipped with Buchi B740 re-circulating water chiller and Buchi V700 vacuum pump (BÜchi Labortechnic AG Postfach Switzerland). A gas chromatograph, Varian CP-3800 (Varian Association Inc. USA) equipped with ^{63}Ni Electron Capture Detector (ECD), CTC Analytic Combi PAL autosampler, split-splitless injector, Programmed Pneumatic Control (PPC) and a computer running star workstation data processor. For separation, a 5% diphenyl 95% dimethyl siloxane capillary column (30m × 0.25(lid) × 0.25 μm, thickness plus 10m guard column.

Study area: The city Accra was selected for this study. Labadi General Hospital was chosen as the main site for the collection of the human milk samples. The study area is shown below in Fig. 1.

Cleaning of glassware: All glasswares used for this study were rigorously scrubbed with a brush in hot water and detergent. The glasswares and the sampling bottles were rinsed five times with tap water and twice with distilled water. They were further rinsed with acetone followed by hexane. They were placed over night in an oven at 300ºC. The glasswares were then sealed tightly with aluminum foil to prevent contamination and were then stored in a dust free cabinet when not in use.

Sample collection: A total of twenty one (21) nursing mothers satisfied the criteria and were selected for the study. Mothers who expressed their willingness to partake in the study were made to fill a questionnaire which was basically about their personal information and diet. Selected mothers were made to sign a Prior Informed Consent Form before samples were taken. After the signing of the Consent Form, mothers were given the already cleaned glass jar with a very tight and well protected lid labelled with their individual identification code, A1 to A21 for the human breast milk samples. The samples were collected manually and directly into the individual glass jars. Babies whose mothers donated some of their milk samples were giving a baby Tee shirt donated by the World Health Organization as a sign of appreciation to the mothers.

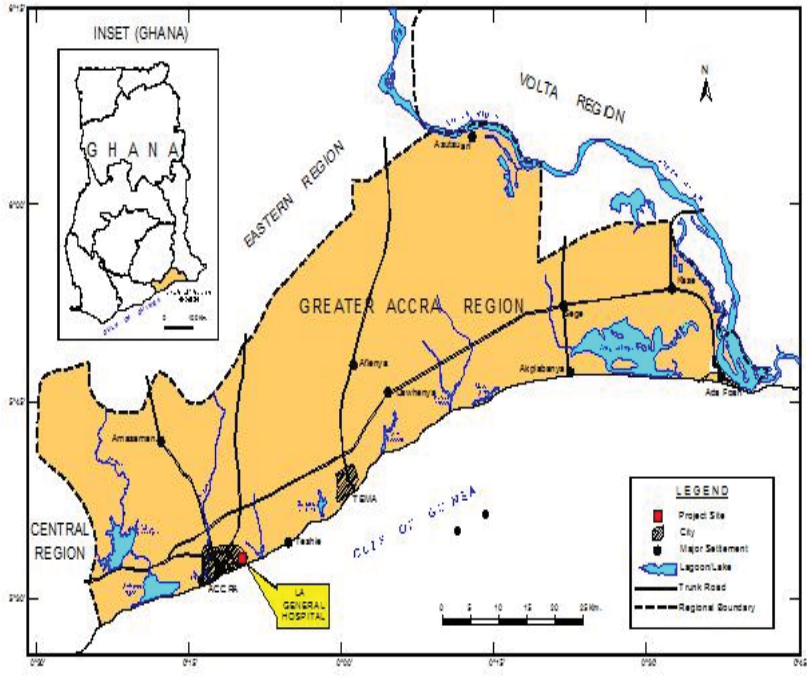


Fig. 1: A map showing the sampling area

The samples were stored in an ice chest with dried ice at -4ºC. The samples were later transported to the Ghana Standard Board Pesticide Residues Laboratory and stored at -20ºC in a freezer prior to analyses. This is the recommended temperature at which all microbial actions in biological samples are ceased (Kiriluk *et al*., 1996).

Sample preparation and extraction of the human breast milk samples: The extraction procedure carried out was that described by Weisenberg *et al. (*1985) and cited by Ntow (2001) with slight modifications. The human breast milk samples frozen at -20ºC were allowed to thaw and then stirred thoroughly. 10 mL of the milk samples were then pipetted and homogenised with 40 mL of 1:1 petroleum ether/acetone mixture by macerating the mixture with the aid of an Ultra-Turrax T 25 basic at a speed of 9,500 rpm for 2 min to enhance extraction. The homogenate were then centrifuged at 2500 rpm for 2 min. After centrifuging the organic layer was collected into an already weighed round bottom flask. The milk phase was re-extracted twice with two separate aliquots of 30 mL petroleum ether. The combined organic phase collected was evaporated to dryness by the rotary evaporator with water bath at 40ºC. The dried organic phase was weighed and dissolved in 5 mL hexane and then subjected to clean-up procedure below. The same extraction procedure was followed for the other individual milk samples. Spiked samples were treated in a similar manner Sample extracts clean-up: The silica solid phase extraction column (500 mg / 6 mL) cartridges were conditioned with 10 mL petroleum ether. The organic layer dissolved in 5 mL hexane was cleaned up by shaking for 1min in 2 mL concentrated sulphuric acid. The sample extracts were loaded onto the columns and eluted with 1: 9 diethyl ether/petroleum ether mixture. Spiked samples were treated similarly.

The cleaned extracts were concentrated to dryness by rotary evaporation. The dried residues were for each sample were dissolved in 1 mL ethyl acetate and then picked into a 2 mL vial for analysis by the gas chromatograph.

Analysis of milk extract for organochlorine pesticide residues: The sample extracts as well as spiked extracts were analyzed by a Varian gas chromatograph CP-3800 equipped with ^{63}Ni electron capture detector which is very sensitive for detecting halogens. The GC conditions used for the analysis included a capillary column coated with

Table 1: Organochlorine pesticide residue (μg/kg) in human breast milk samples from Accra

	Milk fat		*Whole milk		
Organochlorine pesticide	Mean	SD	Mean	SD	**Incidence ratio (%)
Gamma-HCH	4.207	0.608	0.298	0.068	80.95
Delta-HCH	13.855	2.003	0.686	0.071	95.24
Heptachlor	11.791	1.223	0.514	0.033	76.19
Aldrin	2.962	0.210	0.156	0.017	85.71
Gamma-chlordane	1.839	0.182	0.101	0.007	33.33
Alpha-endosulfan	4.704	0.477	0.211	0.015	80.95
p,p'- DDE	23.367	3.233	1.124	0.117	100
Dieldrin	2.407	0.316	0.115	0.010	71.43
p,p'- DDT	3.085	0.398	0.371	0.029	76.19
Endrin	7.669	1.004	0.125	0.015	80.95
Endrin aldehyde	7.769	2.735	0.224	0.040	42.86
Endosulfan sulfate	99.052	10.693	4.907	0.503	85.71
Endrin ketone	63.846	33.097	0.153	0.024	42.86
Methoxychlor	20.116	4.149	0.716	0.115	42.86

SD = Standard Deviation; **: Incidence ratio = Number of samples that tested positive; *: => Whole milk is the total composition of milk expressed for analysis of which fat is a part

RB-5 (30×0.25 mm, 0.25 μm film thickness), a carrier gas at a flow rate of 1.0 mL/min and a make-up gas of Nitrogen also at a flow rate of 29 mL/min. The temperature of injector operating in splitless mode was held at 225ºC, oven temperature was set at 225ºC and electron capture detector was also set at 300ºC. The column oven temperature was programmed as follows; 60ºC for 2 min, 180ºC/min up to 300ºC held for 31.80 min. The injection volume of the Gas Chromatograph (GC) was 1.0 μL. The residues detected by the GC analysis were confirmed by the analysis of the extract on two other columns of different polarities. The first column was coated with ZB-1 (methyl polysiloxane) connected to ECD and the second column was coated with ZB-17 (58% phenyl, methyl polysiloxane) and ECD was also used as detector.

Quantification: The quantities of residues in the samples were determined using an external standard method. An organochlorine standard mixture with known concentrations was run and the detector response for each compound was determined. The areas of the corresponding peak in the samples were compared with that of the known standards.

Recovery test: One sample in each batch of analysis was spiked with 0.1 mL/kg of a mixed standard. The spiked samples were extracted and analyzed under the same conditions as the samples. The percentage recovery was calculated as:

$$\text{Recovery (\%)} = \frac{\text{(Amount of analyte received)}}{\text{(Amount of analyte spiked)}} \times 100$$

The recovery for the different organochlorine pesticides in the milk samples were between 89 to 97%.

RESULTS AND DISCUSSION

Table 1 presents the mean concentrations of organochlorine pesticide residues analysed in the fat and whole milk of the samples. It is evident that the concentrations of the organochlorine pesticide residues in the milk fat were higher than respective concentrations in the whole milk. This is due to the fact that organochlorines are lipophilic and thus accumulate more in the fatty medium. Fourteen organochlorine pesticides namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and Methoxychlor were detected in the 21 samples analysed.

Figure 2 shows the percentage incidence of the various organochlorine pesticide residues that were analysed in the samples. All the samples tested positive to p,p'-DDE thus 100% incidence ratio and 76.19% tested positive to p,p'-DDT. Although DDT has long been banned in Ghana; in 1985, its residues can still be detected in the environment after a decade and halve of its ban. This is due to the high persistent nature. About 95.24 and 80.95% of the breast milk sampled tested positive to Delta-HCH and Gamma-HCH, respectively. Alpha-Endosulphan was recorded in 80.95% of the samples from Ada and 85.71% recorded Endosulphan-Sulphate.

From Table 1, the concentrations of p,p'-DDT and p,p'-DDE in the milk fat were 3.085 and 23.367 μg/kg respectively in the human breast milk samples. The presence of both DDT and its metabolite DDE in the human milk fat samples even though they have been banned since 1985 (EPA Ghana, 2008), may be due to the persistence and long range transport nature of DDT and its metabolite DDE (Ritter *et al*., 1995) and also their ability to bioaccumulate and biomagnify in the food chain (Travis and Arms, 1988). The body burden of DDT and DDE might be through food since residual levels of both

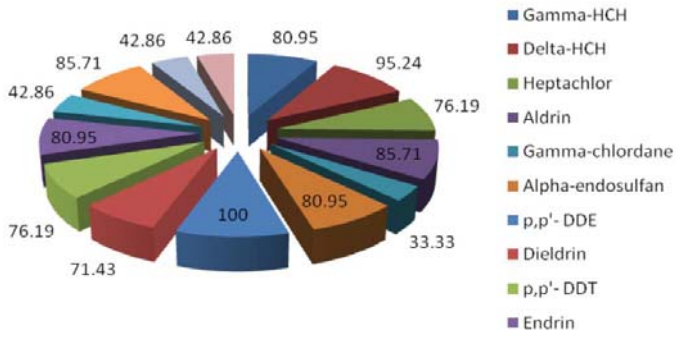


Fig. 2: incidence ratio of organochlorine pesticides in samples from Accra

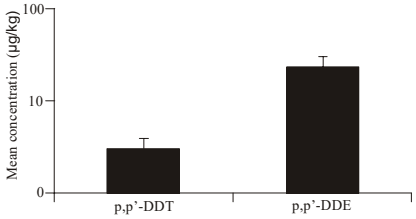


Fig. 3: Mean concentration of DDT and DDE in milk fat samples from Accra. The error bars represent standard deviation

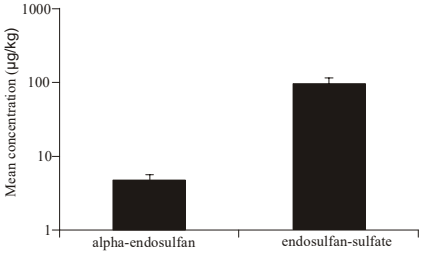


Fig. 4: Mean concentration of endosulfan and endosulfan sulfate in human milk (fat) samples from Accra. The error bars represent standard deviation

DDT and DDE have been detected in crops and the tissues of animals (Ntow, 2001).

Figure 3 compares the mean concentrations of p,p'-DDT and p,p'-DDE in the samples. The mean concentration of p,p'-DDT is lower than that of its metabolite p,p'-DDE. This may be that most of the DDT massively used in the past is in the metabolite state and fresh input of DDT in the environment is minimal.

The mean concentrations of alpha-endosulfan and endosulfan-sulphate are shown in Fig. 4. Endosulfan

Table 2: Comparison of organochlorine pesticides residue in human milk (fat) samples from selected nursing mothers in Accra with Australia maximum residue limits (µg/kg)

Name of Pesticides	Mean (fat)	Australian MRL(fat)
Gamma HCH	4.207	200
Delta-HCH	13.855	200
Heptachlor	11.791	150
Aldrin	2.962	150
Dieldrin	2.407	150
Gamma-chlordane	1.839	50
Alpha-Endosulphan	4.704	20
Endosulfan Sulphate	99.052	20
p,p'-DDT	3.085	1250
p,p'-DDE	23.367	1250
Endrin	7.669	-
Endrin aldehyde	7.769	-
Endrin Ketone	63.846	-
Methoxychlor	20.116	-

Sulphate, a metabolite of alpha-endosulfan recorded the highest mean concentration of 99.052 µg/kg (fat) and alpha endosulfan also recorded a mean concentration of 4.704 µg/kg (fat).

Endosulfan, just like other organochlorine pesticides are known to persist in the environment even years after their use. Darko and Acquaah (2007) detected endosulfan- sulphate mean concentration of 21.35 µg/kg in meat. Ntow (2001) recorded 30.8 µg/kg mean concentration of Endosulfan sulphate residues in water (Ntow, 2001). Alpha-endosulfan residues have also been recorded in crops and in fish (Ntow, 2001; Osafo and Frimpong, 1998). An appreciable concentration of alpha-endosulfan was measured in the breast milk samples and this might be due to the fact that it was recently considered for restrictive use in Ghana, precisely 2008. The relatively higher level of endosulfan-sulphate compared to alpha-endosulfan may also be due to the fact that previous inputs of alpha-endosulfan has metabolized to endosulfan- sulphate or there is minimal inputs of alpha-endosulfan at present.

Table 2 gives the mean concentrations in µg/kg of the various organochlorine pesticides residues detected in the human breast milk sample analyzed compared to that of the Australia maximum residue limit.

It is clear from Table 2 that with the exception of endosulfan Sulphate whose mean concentration, 99.052 µg/kg was found to be higher, the mean concentrations of the organochlorine pesticides detected were lower than that of the Australian Maximum Residue Limit.

CONCLUSION

The results obtained from this research work revealed that there are still residues of some organochlorine pesticides in the environment. Fourteen different organochlorine pesticides namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-aldehyde, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were detected. Seven out these fourteen organochlorine pesticide residues; aldrin, chlordane, DDT, dieldrin, Endrin, Lindane and heptachlor are on the list of banned pesticides by the Environmental Protection Agency of Ghana. The mean concentrations of the organochlorine pesticides detected were between the ranges of 1.839 -99.052 µg/kg (fat). P, p'- DDE recorded 100% incidence ratio. Endosulfan Sulphate recorded the highest mean concentration of 99.052 µg/kg (fat) which was about five times greater than the Australian Maximum Residue Limit (MRL) value for milk fat.

ACKNOWLEDGMENT

We are grateful to WHO for providing the sampling bottles, also to the Ghana Standard Board Pesticide Residue Laboratory and The National Nuclear Research Institute of Ghana Atomic Energy Commission, The Ghana Health Services for the provision of ethical clearance for the study and finally to all the nursing mothers who donated their milk.

REFERENCES

Amoah, P., P. Drechsel, R.C. Abaidoo and W.J. Ntow, 2006. Pesticide and pathogen contamination of vegetables in Ghana's urban markets. Arch. Environ. Contam. Toxicol., 50: 1- 6.

Bhanti, M. and A. Taneja, 2007. Contamination of vegetables of difference seasons with organophosphorous pesticides and related health risk assessment in Northern India. Chemosphere, 69(1): 63-68.

Carson, R., 1962. Silent Spring. Houghton Mifflin, pp: 378.

Clarke, E.E.K., L.S. Levy, A. Spurgeon and I.A. Calvert, 1997. The problems associated with pesticide use by irrigation workers in Ghana. Occup. Med., 47(5): 301-308.

Darko, G. and S.O. Acquaah, 2007. Levels of organochlorine pesticides residues in meat. Int. J. Environ. Sci. Tech., 4(4): 521-524.

Environmental Protection Agency-Ghana, 2008. Register of pesticides as at 31st December 2008 under Part 11 of the environmental protection agency act, 1994 (Act 490).

Gerken, A., J.V. Suglo and M. Braun, 2001. Pesticide Policy in Ghana. MoFA/PPRSD, ICP Project, Pesticide Policy Project/ GTZ. Accra, Ghana.

Dunlap, T.R., 1981. DDT: Scientists, Citizens, and Public Policy. Princeton University Press, Princeton, NJ.

Hodgson, A., 2003. The high cost of pesticide poisoning in northern Ghana. Pestic. News, 62(3): 4-8.

Jayashree, R. and N .Vasudevan, 2007. Effect of tween 80 added to the soil on the degradation of endosulfan by *Pseudomonas aeruginosa*. Int. J. Environ. Sci. Tech., 4(2): 203-210.

Kalantari, M.R. and A.G. Ebodi, 2006. Study and measurement of some persistent organochlorine residues in sediments from the two great rivers (Tojan and Neka) of Mazanderran Province (Iran). J. Appl. Sci., 6(5): 1028-1032.

Khalid, I.S., A.E. Mohammed and A. Morshedy, 2007. Organochlorine pesticide residues in camel, cattle and sheep carcasses slaughtered in Sharkia Province, Egypt. Food Chem., 108: 154- 164.

Kiriluk, R.M., W.H. Hyatt, M.J. Keir and D.M. Whittle, 1996. Fluctuations in levels of total PCB, organochlorine residue, lipid and moisture in whole lake trout homogenate samples withinfour years of frozen storage. Fisheries and Oceans Canada, Ottawa, ON, pp: 32.

Mussalo-Rauhamaa, H., 1991. Partitioning and levels of neutral organochlorine compounds in human serum, blood cells and adipose and liver tissue. Sci. Tut. Environ., 103: 159-175.

Ntow, W.J., H.J. Gijzen and P. Drechsel, 2006. Farmer perceptions and pesticide use practices in vegetable production in Ghana. Pest Manage. Sci., 62(4): 356-365.

Ntow, W.J., 2001. Organochlorine pesticide in sediment crops and human fluids in a farming community in Ghana. Arch. Environ. Contam. Toxical., 40: 557-563.

Osafo A.S. and E. Frempong, 1998. Lindane and endosulfan residues in water and fish in the Ashanti region of Ghana. J. Ghana Sci. Assoc., 1(1): 135-140

Ritter, L., K.R. Solomon, J. Forget, M. Stemeroff and C.O. Leary, 1995. An Assessment Report on Persistent Organic Pollutants, May.

Travis C.C. and A.D. Arms, 1988. Bioconcentration of organics in beef, milk and vegetation. Environ. Sci. Technol., 22: 271-274 .

Weisenberg, E., I. Arad, F. Graver and Z. Sahm, 1985. Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch. Environ. Contam. Toxicol., 14: 517-521.

Weltman, R.H. and D.H. Norback, 1983. Lack of hepatocarcinogenic activity after 2,3,6,2_,3_,6_-hexachlorobiphenyl (HCB) exposure in Sprague-Dawley rats: a sequential ultra-structural study. Toxicologist, 3: 101 (abstract 401).

PAPER IV

Anita Osei Tutu, Philip Owiredu Yeboah, A. A. Golow, Samuel Adu- Kumi, Edith. Clarke and Paul Osei-Fosu

Levels of Organochlorine pesticide residues found in the breast milk of some first-birth mothers from a rural community (Ada) in Ghana

Elixir Pollution 54 (2013) 12668-12672

Available online at www.elixirpublishers.com (Elixir International Journal)

Pollution

Elixir Pollution 54 (2013) 12668-12672

ISSN: 2229-712X

Levels of Organochlorine pesticide residues found in the breast milk of some first-birth mothers from a rural community (Ada) in Ghana

Anita Osei Tutu[1], Philip Owiredu Yeboah[2], A. A. Golow[2], Samuel Adu- Kumi[3], Edith. Clarke[4] and Paul Osei- Fosu[5]

[1]NCERC, NNRI, GAEC, P.O.Box LG 80, Legon – Accra, Ghana.

[2]Graduate School of Nuclear and Allied Sciences, University of Ghana, P.O.Box AE 1 Atomic Kwabenya – Accra, Ghana.

[3]CCMC-Environmental Protection Agency, P.O.Box MB 326, Accra – Ghana

[4]Occupational Health, Ministry of Health, P.O.Box M 44, Accra – Ghana.

[5]PML-Ghana Standards Board, P.O.Box M 245, Accra – Ghana.

ARTICLE INFO

Article history:

Received: 14 September 2012;

Received in revised form:

15 January 2013;

Accepted: 24 January 2013;

Keywords

Breast milk,

Organochlorine pesticide residues,

Gas Chromatograph,

First-Birth Mothers ,

Ghana, Maximum Residue Limit.

ABSTRACT

The aim of this study was to determine the types and levels of organochlorine pesticide residues in the breast milk of some first birth mothers in Ada, a rural community in the greater Accra region of Ghana. Liquid-liquid extraction procedure was employed and extract clean-up was done using silica gel solid phase extraction. Thirteen different organochlorine pesticides residues namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, endrin, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were identified and quantified in the individual breast milk samples using a Gas Chromatograph (GC) with an Electron Capture detector. The GC recoveries of spiked samples were between 89 to 97%. Gamma-HCH recorded the highest incident ratio of 95.2% and p, p'-DDE, endosulphan sulphate, delta-HCH and dieldrin also recorded incidence ratios of 90.5%, 81.0%, 66.7% and 57.1% respectively in the twenty-one individual human breast milk samples. The mean concentrations of organochlorine pesticide residues in the human breast milk samples ranged from 0.682 to 63.803 µg/kg fats. Endosulphan-sulphate recorded the highest concentration of 63.803 µg/kg fats which is about three times greater than the Australian Maximum Residue Limit (MRL) of 20 µg/kg for milk. The mean concentrations for all the other organochlorines detected were below their respective maximum residue limits.

Introduction

Organochlorine pesticides are a class of chemicals that came into widespread use in the late1940s. Until the early 1980s, many chlorinated insecticides, mainly aldrin, dieldrin, DDT, and lindane had been used in controlling pests of crops, vectors of some diseases and other aspects of public health in Ghana (UNEP, 2002). Some of these pesticides are still widely used by farmers because of their effectiveness and their broad-spectrum activity (Amoah *et al.,* 2006). Despite being banned in industrialized countries since the 1970s, or subjected to restrictions in use in many others, they persist to this day in the environment. Apart from occupationally

exposed individuals, most exposure to these chemicals occurs via dietary intake (DeVoto *et al.,* 1998; Ahlborg *et al*., 1995), especially food of animal origin, but also through water, ambient and indoor air, dust and soil (Covaci *et al*., 2002; Dua *et al.*, 2001; Manirakiza *et al*., 2002). These lipophilic compounds accumulate and even biomagnify their concentrations along the food chain, especially in fatty foods (Manirakiza *et al*., 2002). Some published reports (Ahlborg *et al*., 1995; DeVoto *et al*., 1998; Hanaoka *et al*., 2002; Manirakiza *et al*., 2002) suggest that serum levels of organochlorines are related to the consumption of various foods. In Ghana, analytical investigations of a number of organochlorine pesticides in human organs, body fluids and other reported incidents suggest that some of these chemicals are still in use illegally despite their ban or considered strictly under restrictive use. (Ghana NIP, 2007). Organochlorine pesticides are very harmful to both humans and the environment at large (Hunter *et al*., 1997). Adverse health effects including reproductive failures, tumor induction, endocrine disruption and cancers can occur once living organisms are exposed to organochlorine pesticides (Makris and Rowe, 1998). These chemicals pose a serious risk to health, especially for infants in whom enzymatic and metabolic systems are not fully active (Garry, 2004). Information from research indicates that exposure to organochlorine compounds affect thyroid function in preschool children (Natural Health News, 2008). Low sperm count in males, birth defects, increase in testicular cancer and other reproductive and development effects (Weltman, 1983) have also been reported as a result of organochlorine contamination. With regard to exposure to organochlorine pesticides in early pregnancy, several epidemiological studies suggest that maternal employment in agriculture may be a risk factor for birth defects (Nurminen, 1995; Weidner *et al*., 1998; Engel *et al*., 2000). In spite of the massive use of organochlorine pesticides in Ghana, there is paucity of information on their environmental levels and their levels in humans. Consequently, there is much concern over the environmental quality of Ghana and the health of its inhabitants. Very little work has been done to measure body burden of organochlorines in Ghana especially on human breast milk. Samples of body fluids such as breast milk have been shown as an adequate indicator of body burden of organochlorine pesticide residues (Ntow *et al.,* 2001). The breast, a lipid-rich tissue acts as a depot or reservoir of lipophilic

Tele:

E-mail addresses: annieoseitutu@yahoo.com

pesticide by virtue of physiochemical interactions of the cellular component with the pesticide (Mussalo-Rauhamaa, 1991). . The current study however, is limited to only first birth mothers in a rural community. Such mothers have never breast fed any children to release their body burden of organochlorine through breast feeding and are more likely to have most of the organochlorine pesticide they have accumulated in their life time. This study will give vital information on the levels of these organochlorine pesticide residues in first birth mothers as compared to mothers of subsequent births.

Materials And Methods

The study was conducted in the Ghana Standards Board Pesticides Residue Laboratory within August 2008 to June 2009. Ethical clearance was sought from the Ministry of Health before selecting potential donors for the study.

Chemicals and reagents*:* The reagents used for the analysis were analytical grade petroleum ether 40-60^{0}C (Scientific and Chemical Supplies Ltd); N-Hexane, 95% HPL grade (Sigma-Aldrich), acetone (Scientific and Chemical Supplies Ltd), diethyl ether AnalaR (BDH Chemical Ltd), concentrated sulphuric acid, >95-97% (Fluka), ethylacetate pestanal (Riedel Haen), individual pesticide reference standards (> 95.0% purity) from Dr. Ehrenstofer GmbH, Germany and stored in a freezer at -20^{0}c to minimize degradation. Solid phase extraction (SPE) cartridges (strata sI-1 Silica) (55um, 70 A) of density, 500mg/6ml.

Glassware*:* Twenty one (21) Schott Duran 100ml glass bottles with protective caps used for sampling were imported from Germany.

Equipment*:* Centrifuge Cri multifunction (Thermo Electron Industries SAS, France), T 25 Basic Ultra turax macerator (IKA® Werke Germany), Metler Toledo PG 10035 weighing balance, rotary evaporator Buchi RE-200 equipped with Buchi B740 re-circulating water chiller and Buchi V700 vacuum pump (BÜchi Labortechnic AG Postfach Switzerland). A gas chromatograph, Varian CP-3800 (Varian Association Inc. USA) equipped with ^{63}Ni electron capture detector (ECD), CTC Analytic Combi PAL autosampler, split-splitless injector, programmed pneumatic control (PPC) and a computer running star workstation data processor. For separation, a 5% diphenyl 95% dimethyl siloxane capillary column (30m × 0.25(lid) × 0.25µm, thickness plus 10m guard column.

Study area:

A rural community (Ada) was considered for this study. Ada is a rural community in the Greater Accra region of Ghana. Ada Foah Health Centre was selected for the collection of the human milk sample. Ada is farming and fishing community. Most of the farmers are engaged in vegetable production along the Volta River. The study area is shown in figure 1.

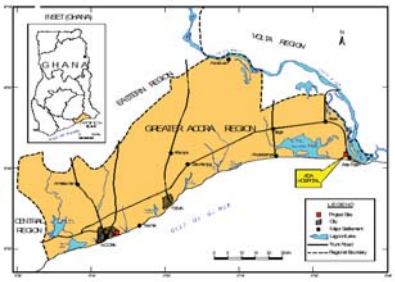


Figure 1.0: Map showing the sampling sites

Cleaning of Glassware: All glasswares used for this study were rigorously scrubbed with a brush in hot water and detergent. The glasswares and the sampling bottles were rinsed five times with tap water and twice with distilled water. They were further rinsed with acetone followed by hexane. They were placed over night in an oven at 300^{0}c. The glasswares were then sealed tightly with aluminum foil to prevent contamination and were then stored in a dust free cabinet when not in use.

Sample Collection: A total of twenty one (21) nursing mothers satisfied the criteria and were selected for the study. Mothers who expressed their willingness to partake in the study were made to fill a questionnaire which was basically about their personal information and diet. Selected mothers were made to sign a Prior Informed Consent Form before samples were taken. After the signing of the Consent Form, mothers were given the already cleaned glass jar with a very tight and well protected lid labelled with their individual identification code, A22 to A42 for the human breast milk samples. The samples were collected manually and directly into the individual glass jars. Babies whose mothers donated some of their milk samples were giving a baby Tee shirt donated by the World Health Organization as a sign of appreciation to the mothers.

The samples were stored in an ice chest with dried ice at -4^{0}C. The samples were later transported to the Ghana Standard Board Pesticide Residues Laboratory and stored at -20^{0}C in a freezer prior to analyses. This is the recommended temperature at which all microbial actions in biological samples are ceased (Kiriluk *et al.,* 1996).

Sample preparation and extraction of the human breast milk samples: The extraction procedure carried out was that described by Weisenberg *et al. (*1985) and cited by Ntow (2001) with slight modifications. The human breast milk samples frozen at -20^{0}c were allowed to thaw and then stirred thoroughly. 10 ml of the milk samples were then pipetted and homogenised with 40ml of 1:1 petroleum ether / acetone mixture by macerating the mixture with the aid of an Ultra-Turrax T 25 basic at a speed of 9,500 rpm for 2 min to enhance extraction. The homogenate were then centrifuged at 2500 rpm for 2 min. After centrifuging the organic layer was collected into an already weighed round bottom flask. The milk phase was re-extracted twice with two separate aliquots of 30 ml petroleum ether. The combined organic phase collected was evaporated to dryness by the rotary evaporator with water bath at 40^{0}c. The dried organic phase was weighed and dissolved in 5 ml hexane and then subjected to clean-up procedure below. The same extraction procedure was followed for the other individual milk samples. Spiked samples were treated in a similar manner

Sample extracts clean-up: The silica solid phase extraction column (500mg/6ml) cartridges were conditioned with 10 ml petroleum ether. The organic layer dissolved in 5ml hexane was cleaned up by shaking for 1min in 2 ml concentrated sulphuric acid. The sample extracts were loaded onto the columns and eluted with 1: 9 diethyl ether / petroleum ether mixture. Spiked samples were treated similarly.

The cleaned extracts were concentrated to dryness by rotary evaporation. The dried residues were for each sample were dissolved in 1ml ethyl acetate and then picked into a 2 ml vial for analysis by the gas chromatograph.

Analysis of Milk Extract for Organochlorine Pesticide Residues: The sample extracts as well as spiked extracts were analyzed by a Varian gas chromatograph CP-3800 equipped with ^{63}Ni electron capture detector which is very sensitive for detecting halogens. The GC conditions used for the analysis included a capillary column coated with RB-5 (30×0.25mm,

0.25µm film thickness), a carrier gas at a flow rate of 1.0 ml/min and a make-up gas of Nitrogen also at a flow rate of 29 mL/min. The temperature of injector operating in splitless mode was held at 225°C, oven temperature was set at 225°C and electron capture detector was also set at 300°C. The column oven temperature was programmed as follows; 60°C for 2min, 180°C/min up to 300°C held for 31.80 min. The injection volume of the Gas Chromatograph (GC) was 1.0µL. The residues detected by the GC analysis were confirmed by the analysis of the extract on two other columns of different polarities. The first column was coated with ZB-1 (methyl polysiloxane) connected to ECD and the second column was coated with ZB-17 (58% phenyl, methyl polysiloxane) and ECD was also used as detector.

Quantification: The quantities of residues in the samples were determined using an external standard method. An organochlorine standard mixture with known concentrations was run and the detector response for each compound was determined. The areas of the corresponding peak in the samples were compared with that of the known standards.

Recovery Test: One sample in each batch of analysis was spiked with 0.1ml/kg of a mixed standard. The spiked samples were extracted and analyzed under the same conditions as the samples. The percentage recovery was calculated as:

$$\% \text{ of Recovery} = \frac{\text{(Amount of analyte received)}}{\text{(Amount of analyte spiked)}} * 100$$

The recovery for the different organochlorine pesticides in the milk samples were between 89 to 97%.

Results And Discussion

Based on the analysis carried out, thirteen different types of organochlorine pesticides were detected in the twenty-one individual human breast milk sample analysed. They include p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-ketone, alpha-Endosulphan, Endosulphan-sulphate, gamma-chlordane, dieldrin, and Methoxychlor. The mean concentrations of the various analyte were recorded in µg/kg fat.

Figure 2 gives detailed description of incidence ratios of the various organochlorine pesticides in the human breast milk samples.

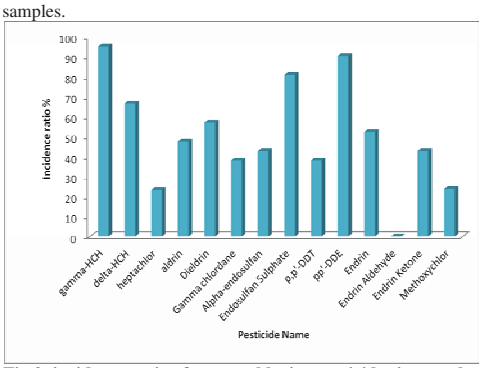


Fig 2: incidence ratio of organochlorine pesticides in samples from Ada

Gamma-HCH recorded the highest incidence ratio of 95.2%. This indicates that majority of the first birth mothers selected for the study has gamma-HCH in their breast milk. About 90.5% of the breast milk samples tested positive to p, p'-DDE. Endosulphan Sulphate recorded an incidence ratio of 81%. Delta-HCH, Dieldrin, and Endrin recorded incidence ratios of 66.7%, 57.1, and 52.4% respectively. The incidence ratios of the other organochlorine pesticides detected in the milk samples were below 50% and their individual incidence ratios can be seen from figure 2.

Table 1 shows the mean concentrations in µg/kg of the various organochlorine pesticide residues in the human breast milk samples.

Endosulphan Sulphate recorded the highest mean concentration of 63.803 µg/kg fat. Endosulphan sulphate is the main degradation product of Endosulphan; it is equally toxic as the parent compound and perhaps even more persistent (U.S. EPA, 2002). Endosulphan, marketed as thiodan, was widely used in cotton growing areas, on vegetable farms, and on coffee plantations (Gerken, *et al.,* 2001). Endosulphan was also employed to control ectoparasites of farm animals and pets in Ghana (Ntow *et al.,* 2006). The massive use of Endosulphan, its persistent nature, long range transport as well as its ability to bioaccumulate has resulted in it getting into the food chain (U.S. EPA, 2002).

The use of Endosulphan was only recently considered for restricted use in Ghana in 2008. This might have resulted in its residues still being recorded in the human milk fat samples analysed. From figure 3, Alpha Endosulphan which is a major component of the technical Endosulphan, recorded a lower mean concentration as compared to the metabolite Endosulphan sulphate, thus 63.803 µg/kg for Endosulphan sulphate as against 2.588 µg/kg for alpha Endosulphan. This means that most of the Endosulphan used in the past is in the metabolite state. Exposure in humans might have resulted through contaminated food or through direct contact during its application in agriculture and household use.

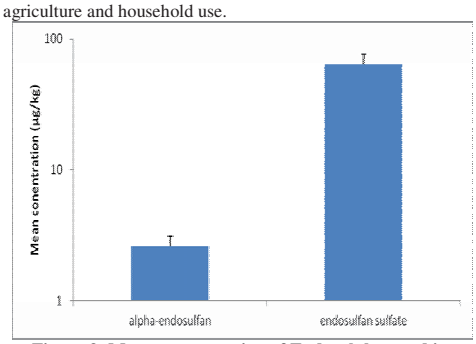


Figure 3: Mean concentration of Endosulphan and its metabolite in human milk fat samples from Ada. The error bars represent standard deviation.

The mean concentration for p, p,-DDE recorded in the human milk samples from Ada is 24.165µg/kg fat and that of p, p'-DDT is 6.339µg/kg fat. Before the ban of DDT in most parts of the world, DDT was used in Ghana in the agriculture sector to control crop pest and in public health for disease vector control. It was also used to control ecto-parasites on household animals (Ntow *et al.,* 2001; Ntow *et al.,* 2006). Even though DDT is under restrictive use in Ghana, it is no more imported or used, not even in the public health sector, this is to prevent misapplication. p, p'-DDE is the main metabolite of DDT, and it is more persistent in the environment than the parent DDT. DDE levels in the breast milk may reflect previous exposure to DDT which has degraded to DDE or exposure to DDE itself through food or other means.

Table 1: Organochlorine pesticide residue (µg/kg) in human breast milk samples from Ada.

Name of Organochlorine Pesticide	Mean (fat)	SD	*Mean (whole milk)	SD	**Incidence ratio (%)
gamma-HCH	5.438	1.573	0.372	0.099	95.2
delta-HCH	6.728	3.489	0.206	0.029	66.7
heptachlor	0.682	0.148	0.054	0.021	23.3
aldrin	2.387	0.605	0.172	0.035	47.6
Dieldrin	2.222	0.542	0.18	0.047	57.1
Gamma chlordane	1.304	0.372	0.101	0.031	38.1
Alpha-Endosulphan	2.588	0.704	0.18	0.046	42.9
Endosulphan sulphate	63.803	11.167	4.241	0.635	81
p,p'-DDT	6.339	1.987	0.283	0.115	38.1
p,p'-DDE	24.165	7.597	1.618	0.623	90.5
Endrin	3.468	1.287	0.474	0.159	52.4
Endrin Aldehyde	ND	-	ND	-	-
Endrin Ketone	1.441	0.348	0.127	0.031	42.9
Methoxychlor	4.896	0.703	0.424	0.06	23.8

SD = Standard Deviation
ND = Not Detected
**Incidence ratio = Number of samples that tested positive
* => Whole milk is the total composition of milk expressed for analysis of which fat is a part.

Table 2: Comparison of organochlorine pesticides residue in human milk (fat) samples from selected nursing mothers in Ada with Australia maximum residue limits (µg/kg)

Name of pesticides	Ada Mean (fat)		Australian MRL (fat)
Gamma HCH	5.438		200
Delta-HCH	6.728		200
Heptachlor	0.682		150
Aldrin	2.387		150
Dieldrin	2.222		150
Gamma-chlordane	1.304		50
Alpha-Endosulphan	2.588		20
Endosulphan Sulphate	63.803		20
p,p'-DDT	6.339		1250
p,p'-DDE	24.165		1250
Endrin	3.468		-
Endrin aldehyde	ND		-
Endrin Ketone	1.441		-
Methoxychlor	4.896		-

ND = Not Detected

Although DDE is more toxic and persistent in the environment than the DDT, the results give a positive signal that fresh input of DDT has minimized if not halted. This also means that the ban on DDT has been effective in Ghana.

The mean concentration for aldrin is 2.387 µg/kg fat and that of dieldrin is 2.222 µg/kg fat. Dieldrin is a metabolite of aldrin and from the result from Ada, the mean concentration for dieldrin is slightly lower than the mean concentration for aldrin. This means there is more Aldrin in the environment than the metabolite Dieldrin (Figure 5).

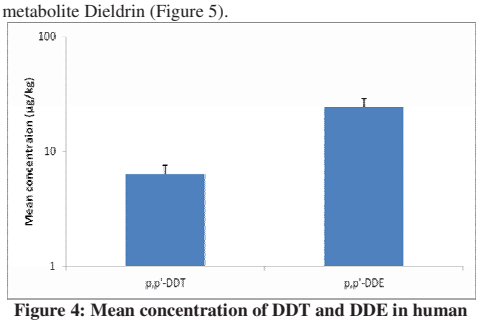


Figure 4: Mean concentration of DDT and DDE in human milk fat samples from Ada. The error bars represent standard deviation.

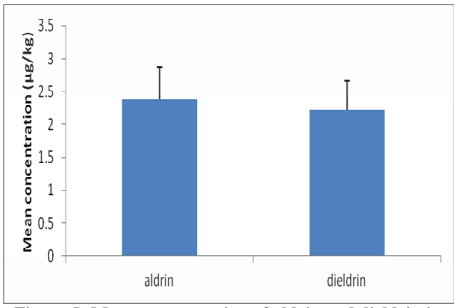


Figure 5: Mean concentration of aldrin and dieldrin in human milk fat samples from Ada. The error bars represent standard deviation.

Even though aldrin and dieldrin are the most widely banned and restricted class of pesticide in the world (Siedenburg, 1991) its persistent nature allows levels of its residues to be detected in the environment. Dieldrin has been detected in more than 99% of breast milk sample tested in most countries (WHO, 1989).

Table 2 gives the mean concentrations in µg/kg fat of the various organochlorine pesticides residues detected in the human breast milk sample analyzed compared to that of the Australia maximum residue limit. The mean concentration of

Endosulphan sulphate was about three times greater than the recommended Australia Maximum Residue Limit for milk. The mean concentration recorded in the milk sample was found to be 63.803 µg/kg as against the recommended MRL value of 20 µg/kg. The mean concentrations for all the other organochlorine pesticides detected were below their recommended MRLs for milk. This is clearly shown in the table 2.

Conclusion

The results obtained from this research work revealed that there are still residues of some organochlorine pesticides in the environment. Thirteen different organochlorine pesticides namely p,p'-DDT, p,p'-DDE, gamma-HCH, delta-HCH, heptachlor, aldrin, Endrin, endrin-ketone, alpha-endosulphan, endosulphan-sulphate, gamma-chlordane, dieldrin, and methoxychlor were detected. Seven out these fourteen organochlorine pesticide residues; aldrin, chlordane, DDT, dieldrin, Endrin, Lindane and heptachlor are on the list of banned pesticides by the Environmental Protection Agency of Ghana. The mean concentrations of the organochlorine pesticides detected were between the ranges of 0.682 -62.803 µg/kg (fat). Gamma –HCH recorded the highest incidence ratio of 95.2%. Endosulphan Sulphate recorded the highest mean concentration of 63.803µg/kg (fat); which was about five times greater than the Australian Maximum Residue Limit (MRL) value for milk fat.

Acknowledgement

We are grateful to WHO for providing the sampling bottles, also to the Ghana Standard Board Pesticide Residue Laboratory and The National Nuclear Research Institute of Ghana Atomic Energy Commission, The Ghana Health Services for the provision of ethical clearance for the study and finally to all the nursing mothers who donated their milk.

References

Ahlborg U.G., L. Lipworth, L. Titus-Ernstoff, C.C. Hsieh, A. Hanberg, J. Baron, D.

Trichopoulos. H.O. Adami. Organochlorine compounds in relation to breast cancer, endometrial cancer,and endometriosis: an assessment for the biological and epidemiological evidence. Crit Rev.Toxicol 1995; 25(6):463 –531.

Amoah, P., P. Drechsel, R.C. Abaidoo, W.J .Ntow, (2006). Pesticide and pathogen contamination of vegetables in Ghana's urban markets. *Arch. Environ. Contam. Toxicol.,* **50**:1- 6.

Covaci A., P. Manirakiza, P. Schepens. Persistent organochlorine pollutants in soils from Belgium, Italy, Greece, and Romania. Bull Environ Contam Toxicol 2002; 68:97 –103.

DeVoto E., L. Kohlmeier, W. Heeschen. Some dietary predictors of plasma organochlorine

concentrations in an elderly German population. Arch Environ Health 1998;53(2):147 – 155.

Dua V.K., R. Kumari, V.P. Sharma, S.K. Subbarao. Organochlorine residues in human blood From Nainital (U.P.), India. Bull Environ Contam Toxicol 2001; 67:42 –45.

Engel, L.S., O.Meera, E.S Schwartz, S.M., 2000. Maternal occupation in agriculture and risk of defects in Washington state, 1980–1993. Scand. J. Work Environ. Health 26, 193–198

Garry, V.F., 2004. Pesticides and children. Toxicol. Appl. Pharmacol. 198, 152–163.

Gerken, A., J.V. Suglo, M. Braun, 2001. Pesticide policy in Ghana. MoFA/PPRSD, ICP Project,

Pesticide Policy Project/ GTZ. Accra, Ghana.

Ghana National Implementation Plan, 2007. <http://www.pops.int/>

Hanaoka T., Y. Takahashi, M. Kobayashi, S. Sasaki, M. Usuda, S, Okubo, M. Hayashi, S.Tsugane Residuals of beta-hexachlorocyclohexane, dichlorodiphenyltrichloroethane, and hexachlorobenzene in serum, and relations with consumption of dietary components in rural residents in Japan. Sci Total Environ 2002; 286:119 –127.

Hunter, D.J., Hankinson, S.E., Laden, F., Colditz, G.A., Manson, J.E., Willet, W.C., Speizer, F.E., Wolf, M.S., 1997. Plasma organochlorine levels and the risk of breast cancer. N. Engl. J. Med. 337 (18), 1253–1258

Kiriluk, R. M., W.H. Hyatt, M.J. Keir, D.M. Whittle, 1996. Fluctuations in levels of total PCB,organochlorine residue, lipid and moisture in whole lake trout homogenate samples within four years of frozen storage. Fisheries and Oceans Canada, Ottawa, ON, pp 32.

Makris, S.L., J.N. Rowe,., 1998. Implementation of the Food Quality Protection Act (FQPA) as it relates to enhanced sensitivity of children. Teratology 57, 246.

Manirakiza, P., A. Covaci, L. Nizigiymana, G. Ntakimazi, P. Schepens,., 2002.Environ. Pollut. 117, 447–455.

Mussalo-Rauhamaa, H., 1991. Partitioning and levels of neutral organochlorine compounds in human serum,blood cells and adipose and liver tissue. Sci. Tut.Environ., 103: 159-175.

Natural Health News, Organochlorine exposure alters thyroid function . Tuesday, July 22nd 2008.

Ntow, W.J., H.J. Gijzen and P. Drechsel, 2006. Farmer perceptions and pesticide use practices in vegetable production in Ghana. Pest Manage. Sci., 62(4):356-365.

Ntow, W.J., 2001. Organochlorine pesticide in sediment crops and human fluids in a farming community in Ghana. Arch. Environ. Contam. Toxical., 40:557-563

Nurminen, T., 1995. Maternal pesticide exposure and pregnancy outcome. J. Occup.Environ. Med. 37, 935–940.

Siedenburg, K. "Demise of the Drins," *Global Pesticide Campaigner* ; (January 1991).

United Nations Environment Programme, 2002. <http://www.unep.org>.

U.S. Environmental Protection Agency, Office of Pesticide Programs, *Endosulfan Reregistration Eligibility Decision*, at 14-15 (2002).

Weisenberg, E., I. Arad, F. Graver and Z. Sahm, 1985. Polychlorinated biphenyls and organochlorine insecticides in human milk in Israel. Arch. Environ. Contam. Toxicol.,14: 517-521.

Weidner I.S, H. Møller, T.K. Jensen, Skakkebæk NE. Cryptorchidism and hypospadias in sons of gardeners and farmers. Environ Health Perspect. 1998;106:793–796. [PubMed]

Weltman, R.H. and D.H. Norback, 1983. Lack of hepatocarcinogenic activity after 2,3,6,2_,3_,6_ hexachlorobiphenyl (HCB) exposure in Sprague- Dawley rats: a sequential ultra-structural study. Toxicologist, 3: 101 (abstract 401).

WHO, Aldrin and Dieldrin, World Health Organization: Geneva (1989).

PAPER V

Anita Asamoah, Erik Gydesen Søgaard and David Kofi Essumang

Assessment of PCBs and PAHs in soil samples from Agbogbloshie e-waste site, Accra Ghana

Preparatory Stage

ISSN (online): 2446-1636
ISBN (online): 978-87-7112-980-9

AALBORG UNIVERSITY PRESS