

**SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA**

**LIVER AND IMMUNE FUNCTION AMONG E-WASTE RECYCLING
WORKERS AT THE AGBOGBLOSHIE E-WASTE PROCESSING SITE IN
ACCRA**

BY

KWAME YEBOAH

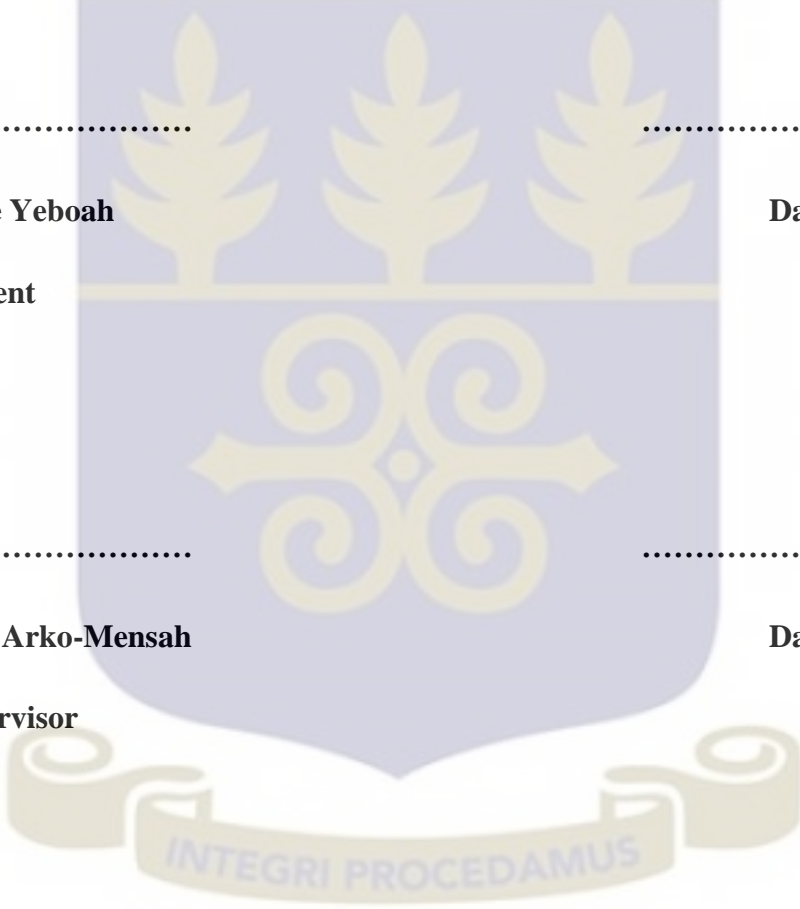
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**THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA,
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DEGREE**

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DECLARATION

I, Kwame Yeboah hereby declare that apart from references to other people's works which have been duly acknowledged, this dissertation is as a result of my own independent work and has not been submitted for the award of any degree in any institution.



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DEDICATION

I dedicate this dissertation to my wife – I am blessed and highly favoured to have you in my life.



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My foremost gratitude goes to the Almighty God for the wisdom, mercy, protection and divine guidance He bestowed upon me to get this far in my education. I wish to also express my sincere gratitude to Dr. John Arko-Mensah and Prof. Julius Fobil, my supervisors, for their advice and immense support during the course of this study.

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ABSTRACT

BACKGROUND: Electronic waste contains reusable components and precious metals, but recycling to retrieve these components could lead to the release of toxic substances into the environment, which could negatively impact on human health. Electronic waste recycling activities at Agbogbloshie are very informal, and workers use manual dismantling and open-air burning to retrieve valuable parts, which also exposes them to toxic substances such as heavy metals, organic toxins, dioxin, furans and fumes of metals. Although studies have been conducted at this dumpsite and shown increased levels of these contaminants in both environment media and urine of e-waste workers, none has looked at liver and immune function among e-waste workers.

OBJECTIVE: The aim of this study was to assess and compare immune status/liver function indices with heavy metals (Pb, As and Hg) levels among different categories of e-waste workers at the Agbogbloshie e-waste recycling/dumpsite.

METHODS: An analytical cross-sectional study was conducted at Agbogbloshie among e-waste workers. Levels of heavy metals in blood were analyzed using an atomic absorption Spectrophotometer and immune/haematological and liver function indices were analyzed in the laboratory of the University of Ghana hospital.

RESULTS: Overall, some e-waste workers had elevated levels of liver biochemical factors; AST, ALT and GGT (7.5%, 12.5% and 10.0% respectively). Dismantlers had the highest mean serum levels of AST, ALT and GGT (37.48 ± 14.45 [U/L], 31.69 ± 11.74 [U/L] and 46.39 ± 46.92 [U/L] respectively). The mean AST level difference among the groups was significant ($p=0.027$). Dismantlers had the highest prevalence of abnormalities in

three (Hb-40%, WBC-70% and granulocyte-100%) of the six immune/haematological parameters measured. Majority of the Sorters (9/10, 90%) had significantly higher abnormal monocytes levels as compared to the other groups ($p=0.001$). Most of the e-waste workers had abnormal granulocyte levels (82.5%) with all the Dismantlers recording abnormal levels. The total concentrations of heavy metals in blood were Pb ($10.92 \mu\text{g/L} \pm 8.88 \mu\text{g/L}$) and Hg ($43.74 \mu\text{g/L} \pm 72.05 \mu\text{g/L}$). Arsenic levels were below detection limit for all samples.

CONCLUSION: Finally, there was no statistically significant association found between blood levels of heavy metals (Hg and Pb) and liver biochemical function or immune/haematological parameters in this study even though occupational exposure to toxic/heavy metals especially Hg can lead to abnormal liver and immune function.

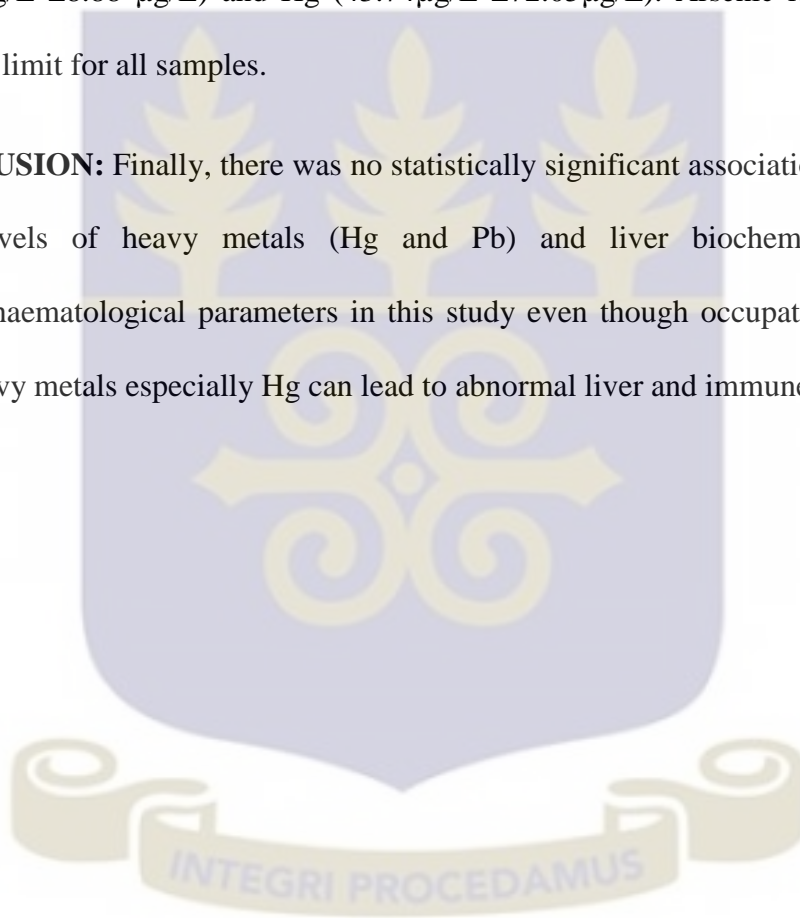


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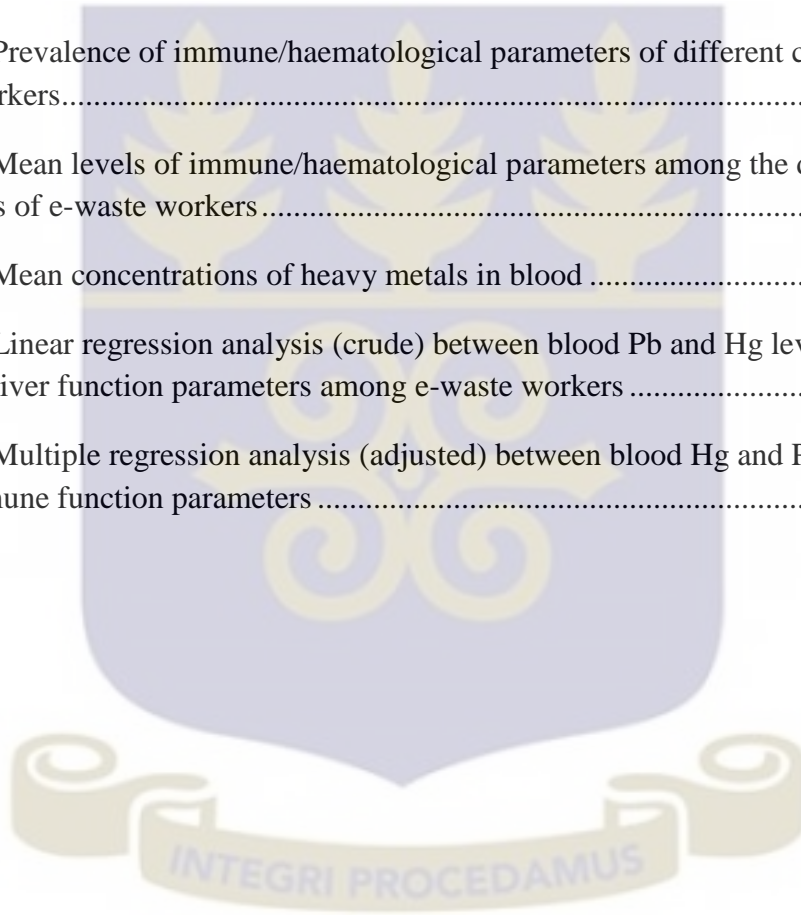
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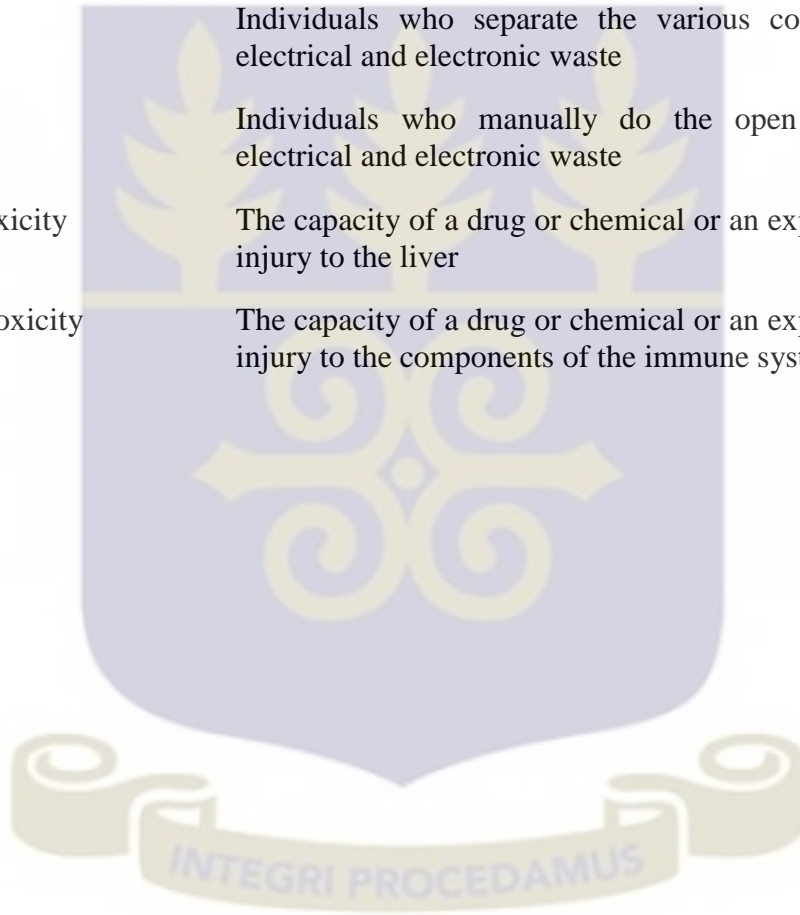


LIST OF ACRONYMS

Acronym	Meaning
ALAD	δ -aminolevulinic acid dehydratase
ALT	Alanine aminotransferase
As	Arsenic
AST	Aspartate aminotransferase
BFR	Brominated flame retardants
BLL	Blood lead levels
Cd	Cadmium
Cr	Chromium
EEE	Electrical and electronic equipment
E-waste	Waste electrical and electronic equipment/ electronic waste
Fe	Iron
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hg	Mercury
IgE	Immunoglobulin E
IgG	Immunoglobulin G
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PBB	Polybrominated biphenyls
PBDD/F	Polybrominated dibenzo-p-dioxins and dibenzofurans
PBDE	Polybrominated diphenyl ethers
PCB	Polychlorinated biphenyls
POP	Persistent organic pollutants
PVC	Polyvinyl Chloride
Sb	Antimony
Se	Selenium
UG	University of Ghana
WBC	White blood cells
WEEE	Waste electrical and electronic equipment

DEFINITION OF TERMS

Item	Operational Definitions
E-waste	End of life electrical and electronic equipment
Collectors	Individuals who go out to bring waste electrical and electronic equipments from homes and offices
Dismantlers	Individuals who manually disassemble the electrical and electronic waste
Sorters	Individuals who separate the various components of the electrical and electronic waste
Burners	Individuals who manually do the open burning of the electrical and electronic waste
Hepatotoxicity	The capacity of a drug or chemical or an exposure to produce injury to the liver
Immunotoxicity	The capacity of a drug or chemical or an exposure to produce injury to the components of the immune system



CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Waste electrical and electronic equipment, also referred to as electronic waste such as computers, TV-sets, fridges and cell phones are among the fastest growing waste streams in the world. Electronic waste (e-waste) contains more than 1000 different chemical substances, many of which are toxic and can potentially contaminate the environment (Itai et al., 2014).

Heavy metals: Lead (Pb), Mercury (Hg), Arsenic (As), Cadmium (Cd), Selenium (Se), Hexavalent chromium (Cr), Antimony (Sb), Aluminum (Al), Copper (Cu), Iron (Fe) and flame retardants which create toxic chemical intermediates such as dioxins and furans when burned are but a few examples of toxic substances in e-waste (Leung et al., 2011). In recent times, most developing countries, including those in West Africa such as Ghana and Nigeria have become major destinations for e-waste deposition and recycling. In these recipient countries, there are no standard protocols to safely recycle and dispose this hazardous e-waste. Also, the legislation dealing specifically with their flow and regulations for maintaining the environment and human health are not always effective (Amoyaw-Osei et al., 2011).

Except for used refrigerators, Ghana seems to have an unregulated and unrestricted import regime for second hand electrical and electronic equipment (EEE). Thus, e-waste enters the country under the guise of second hand EEE without detection. In 2009, the EEE imports into the country added up to 215,000 tons and per capita import of 9 kg due to increasing demand for the EEE in Ghana by the day (Amoyaw-Osei et al., 2011).

Unfortunately, no facility exists in Ghana for managing the disposal of e-waste in an environmentally sound manner, in spite of the existing large stocks and the ever-increasing high rate of generation of the waste. Most of this e-waste ends up in Agbogbloshie, a suburb in Accra which has been named the biggest e-waste dump-site in sub-Saharan Africa (Feldt et al., 2014).

Electronic-waste recyclers in Agbogbloshie use the informal and outmoded methods of recycling to salvage some valuable metals (i.e. manual dismantling and open-air burning). Much of this activity is carried out by young men, mostly using rudimentary tools and with no protective equipment. These unprotected workers at the site are exposed to mixtures of toxic substances through inhalation, ingestion and dermal routes (Robinson, 2009).

Exposure to toxicants such as organic toxins [Polybrominated diphenyl ethers (PBDEs), Polyvinyl Chloride (PVC), Polychlorinated biphenyls (PCBs) and Brominated flame retardants (BFRs)] as well as heavy metals found in e-waste can cause serious health effects to humans. Polycyclic aromatic hydrocarbons (PAH) emissions during recycling could pose a serious health threat to e-waste workers and persons living in the vicinity of e-waste recycling areas as PAH metabolites are recognized as carcinogenic (International Agency for Research on Cancer, 2012).

Polychlorinated biphenyls can cause reproductive and immune abnormalities (Harrison, 2014). PBDEs are hepatotoxic, embryotoxic and also cause thyroid abnormalities in humans (Darnerud, Eriksen, Jóhannesson, Larsen & Vileksela. 2001). BFRs have also been shown to damage endocrine system functions and may have an effect on the levels

of thyroid stimulating hormone and cancer risk (Tsydenova & Bengtsson, 2011). PVC produce dioxins, known to be teratogenic when burnt causing reproductive and developmental problems, immune system damage and interference with regulatory hormones (Kang, Lee, Joo, & Kim, 2005; Osuagwu & Ikerionwu, 2010).

Exposure to the heavy metals could have deleterious effect on organs such as lungs and kidneys, the nervous, digestive and immune systems and may also be fatal. Relatively low exposure of children and pregnant women to these heavy metals can cause serious, and in some cases, irreversible neurological damage and threaten foetal development (Material Safety Data Sheet, 2005; World Health Organisation [WHO], 2010).

A study on human monitoring survey by Asante et al. (2012) reported that e-waste workers in Agbogbloshie have high urinary levels of some metalloids, particularly Fe, Sb, As and Pb. All these metals have been associated with a variety of health issues including developmental neurotoxicity, immune function, cardiovascular disease, thyroid dysregulation, and impaired liver and renal functions (Clarkson, Vyas & Ballatori, 2007; Houston, 2011; Karagas et al., 2012).

This is the first study to describe the immune and liver function as well as their association to heavy metals (As, Pb and Hg) exposure among the e-waste workers at the Agbogbloshie recycling site in Ghana.

1.2 Statement of Problem

Agbogbloshie e-waste dump-site is reportedly the biggest in sub-Saharan Africa. Recyclers in this area use manual dismantling and open-air burning to collect small re-

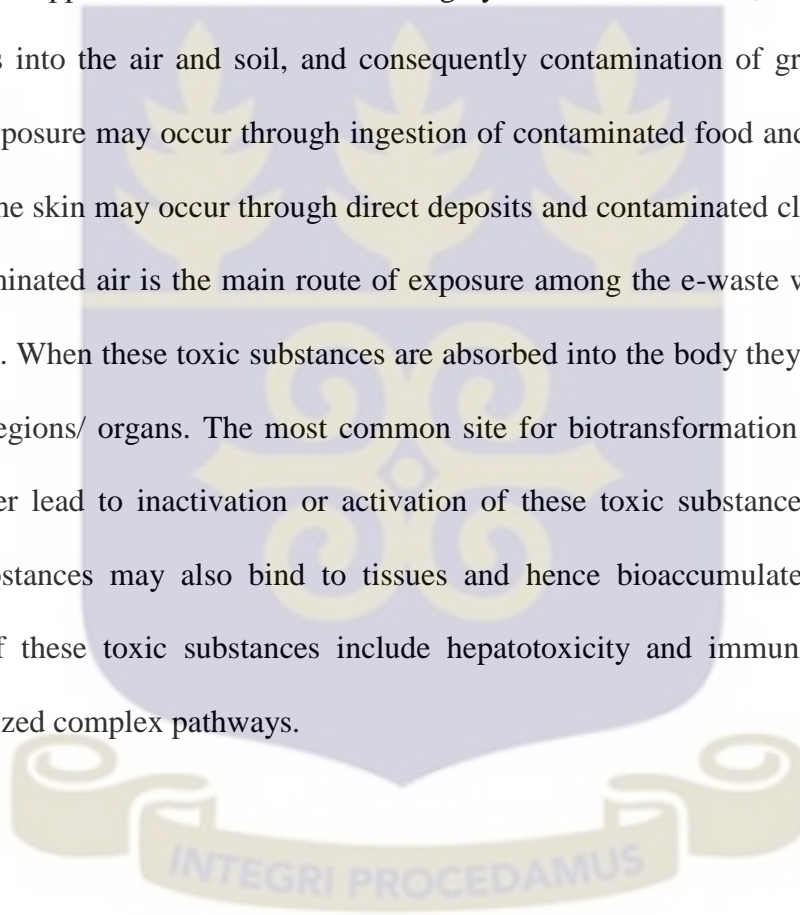
usable parts and oxidized copper which exposes them to several toxicants such as heavy metals in particulate matter, dioxin, furans and fumes of heavy metals (Akormedi, Asampong & Fobil, 2013). These pollutants can cause serious adverse health effects, including immunosuppression in both e-waste recyclers and the surrounding populations. In addition to the direct inhalation of smoke and fumes, toxicants from the burning process can contaminate food products sold in the nearby open market and rainwater harvested in the neighbourhood, making their consumption unsafe for humans.

Studies have also shown high urinary PAH concentration and high urinary levels of As and Hg (Asante et al., 2012; Feldt et al., 2014). For example, cadmium and chromium can lead to excessive inflammation and inappropriate activation of lymphoid cells (WHO, 2011). Lead is also a risk factor for chronic immune-related disease because it can precipitate atopy and autoimmune diseases. This will eventually reduce the host's defences against infectious agents and cancer. Arsenic is known to cause liver dysfunction (Islam et al., 2011). Polychlorinated biphenyls have been associated with immune suppression while Polybrominated diphenyl ethers with hepatic injury, embryotoxicity and thyroid dysfunction (Darnerud et al., 2001).

Although studies have been conducted at this dumpsite, none has looked at the liver and immune function of the e-waste workers. The immune response plays a critical role in the body's fight against diseases and the liver is the most common site for biotransformation of toxicants. This study assessed the liver biochemical function and selected immune indices among e-waste workers at the Agbogbloshie recycling site.

1.3 Conceptual framework

The manual/informal recycling of e-waste involves collection, sorting, dismantling and open air burning (Akormedi et al., 2013). During the dismantling process, electronic gadgets are forcefully pried open or crushed using manual tools such as hammer which releases particulates into the air and soil. Burning of e-waste to retrieve valuable metals, especially copper causes the release of highly toxic metal fumes, smoke and organic pollutants into the air and soil, and consequently contamination of ground and surface water. Exposure may occur through ingestion of contaminated food and water. Exposure through the skin may occur through direct deposits and contaminated clothing. Inhalation of contaminated air is the main route of exposure among the e-waste workers (Zheng et al., 2008). When these toxic substances are absorbed into the body they are distributed to various regions/ organs. The most common site for biotransformation is the liver. This may either lead to inactivation or activation of these toxic substances. Some of these toxic substances may also bind to tissues and hence bioaccumulate. Adverse health effects of these toxic substances include hepatotoxicity and immunotoxicity through hypothesized complex pathways.



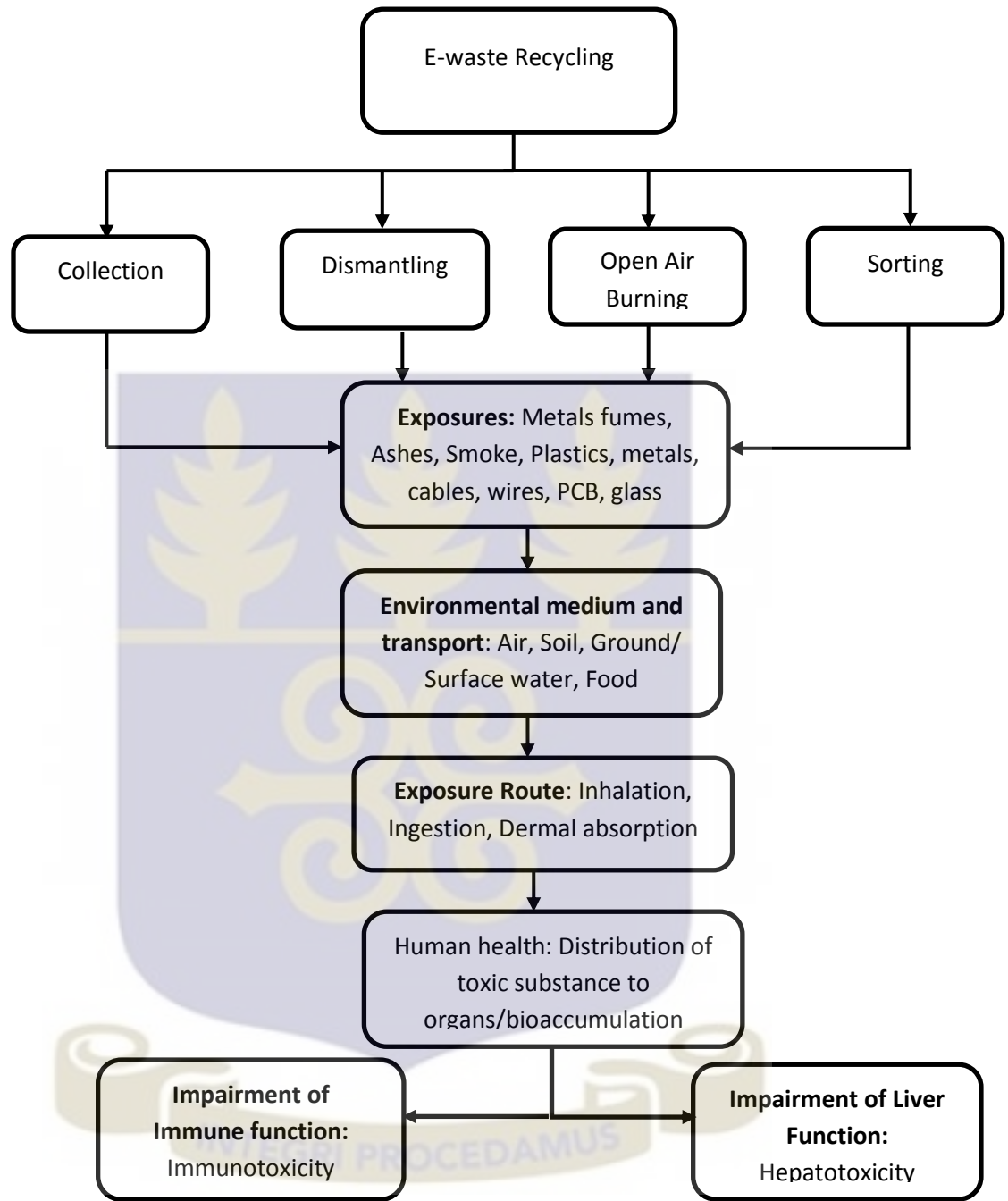


Figure 1: Conceptual framework

1.4 Justification

Importation of e-waste into Ghana has increased significantly over the past decade. Unfortunately, e-waste recycling remains unregulated and employs crude methods with little or no use of personal protective equipment (PPE). As more e-waste is recycled openly without use of PPE and proper control of toxic emissions, there is an increasing likelihood that more toxic chemicals will be released into the environment. Agbogbloshie is one of the heavily populated areas in Accra, and also has one of the biggest open markets where thousands of people traverse daily. This puts the health of e-waste recyclers as well as nearby communities at risk. This study determined whether there was an association between e-waste exposure through informal recycling activities and immune dysregulation and liver function in e-waste workers. This has helped to identify biochemical and/ or pathological damage that these exposures have on people and bring to the fore the need for adequate protection for the workers and the community. Data obtained from this study has also added to existing knowledge which is useful in developing appropriate policies to protect workers and communities from e-waste exposure in Ghana.

1.5 Objectives

1.5.1 General Objective

- To assess liver biochemical function and immune/haematological indices among e-waste workers at Agbogbloshie e-waste recycling site.

1.5.2 Specific Objectives

- To assess the general immune status by measuring levels of indices such as total and differential white blood cell count (WBC), haemoglobin (Hb) and Platelets in blood of e-waste workers.
- To carry out a liver function test by measuring levels of critical liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Gamma-glutamyl transpeptidase (GGT) among the e-waste workers.
- To measure the levels of Pb, As and Hg in e-waste workers.
- To determine whether there is an association between levels of Pb, As and Hg and immune/liver functions.
- To compare immune and liver function tests indices across the different groups of e-waste workers.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Burden of e-waste

Electronic waste (e-waste) has become an emerging environmental and human health problem in the world in this 21st century (Schmidt, 2006). According to the United Nations Environment Programme (UNEP), 20–50 million tons of e-waste is generated annually in the world (United Nations Environment Programme, 2005). It is estimated that more than 49 million tons of e-waste were generated worldwide in 2012 (Robinson, 2009).

The need to keep pace with advancement in technology, coupled with the inability of most people to afford new electronic products, has led to massive importation of second-hand electronic gadgets from developed countries to Ghana (Akormedi et al., 2013). Of the 215,000 tons of Electrical and Electronic Equipment (EEE) imported into the country in 2009, about 30% comprised of new products and 70% second hand. A significant portion of the second hand EEE (15%) was destined directly to informal recycling without being used (Amoyaw-Osei et al., 2011).

At Agboglobshie market in Accra, heaps of these EEE that are not usable are dumped continuously with no concern for the hazards that they may pose to the environment and the people living nearby (Amoyaw-Osei et al., 2011). Non-valuable fractions are informally dumped and periodically burnt, in order to reduce the waste volumes on the dump site (Akormedi et al., 2013). During these activities, high amounts of hazardous substances are released, with no thoughts given to the safety of the workers and the

protection of the environment (Feldt et al., 2014). This leads to significant negative impacts on soil, air and water as well as human health.

2.2 E-waste recycling

E-waste can be recycled through formal and informal ways. Informal e-waste recycling involves the dismantling of end-of-life electronics to retrieve valuable elements using primitive techniques, without exposure-minimizing technology or protective equipment, allowing the emission of dangerous chemicals to the surrounding environment (Orisakwe & Frazzoli, 2010; Wong, Duzgoren-Aydin, Aydin & Wong, 2007a). The informal recycling of e-waste is an emerging problem in West Africa due to the rapidly growing use of EEE in Africa, and particularly due to imports from industrialized countries (Amoyaw-Osei et al., 2011). The e-waste recycling activities generally involves scavenging for the waste EEE, dismantling scrap for useful components and open-air burning to recover precious components such as gold, copper, silver, aluminium, iron, and brass (Akormedi et al., 2013; Gabel, 2011; Wittsiepe et al., 2015).

These dangerous recycling practices have the potential to cause additional health risks (Ogunseitan, Schoenung, Saphores & Shapiro, 2009; Wong, Wu, Duzgoren-Aydin, Aydin & Wong, 2007b) and serious environmental pollution due to the release of various hazardous substances contained in e-waste, including heavy metals, polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and other toxic additives (Robinson, 2009). Many studies have reported that release of heavy metals and organic contaminants, such as brominated flame retardants (BFRs), polychlorinated dibenzo dioxins (PCDD) and furans (PCDF) into the environment are associated with the informal

e-waste burning (Atiemo, Ofosu, Aboh & Kuranchie-Mensah, 2012; Bridgen, Labunska, Santillo & Johnston, 2008; Caravanos, Clark, Fuller & Lambertson, 2011).

Environmental pollution from e-waste recycling sites and exposure of unprotected workers constitute an emerging problem in developing countries. Among the e-waste workers, the youngest people often perform the dirtiest jobs with the highest exposure risks (Wittsiepe et al., 2015). This is particularly alarming, since prenatal and early-life exposure to PCDD/F has been associated with negative effects on intrauterine and childhood growth, neurodevelopment, respiratory and immune function, in addition to carcinogenic potential (Burns et al., 2012; Gascon, Morales, Sunyer & Vrijheid, 2013; Kishi et al., 2013; Lee, Jacobs & Porta, 2007; Ten Tusscher et al., 2014).

A study by Akormedi et al., (2013) to assess the working conditions of e-waste workers at Agbogboshie showed that the e-waste workers worked close to the polluted Korle lagoon and under harsh conditions. Dismantling of gadgets is done using tools such as hammers, screwdrivers and cutters. Burning of e-waste components took place at a small distance from where the dismantling, weighing, and selling of the e-waste products are done. The e-waste workers do not use any form of protective devices during the recycling process. Most of the e-waste workers at Agbogboshie are from the Northern part of the country and work for 10 -12 hours in a day and 6 days in a week (Akormedi et al., 2013; Siddarth et al., 2010).

Formal electronic waste recycling facilities use specifically designed equipment to safely remove salvageable materials from obsolete electronics while protecting workers from adverse health effects. However, these centres are very expensive to build and run and are

rare in less developed countries (Grant et al., 2013). Varying national safety standards can mean that workers at formal or semiformal recycling centres still risk exposure at low doses (Lundgren, 2012).

Sources of exposure to e-waste can be classified into three sectors: informal recycling, formal recycling, and exposure to hazardous e-waste compounds remaining in the environment (i.e. environmental exposure). The high levels of environmental, food and water contamination puts residents living within a specific distance of e-waste recycling areas also at risk of environmental exposure, although at lower levels than through occupational exposure (Lundgren, 2012).

Exposure routes can vary depending on the substance and recycling process. Generally, exposure to the hazardous components of e-waste is most likely to arise through inhalation (Grant et al., 2013).

2.2.1 Impact of e-waste recycling on the environment

Halogenated persistent organic pollutants (POPs) such as PCBs used as dielectric or flame-retardant plasticizers, BFRs like PBDEs and biphenyls (PBBs) have been found in the air, bottom ash, dust, soil, water and sediment samples from e-waste recycling sites worldwide, partly in tremendous high concentrations due to uncontrolled combustion and thermal processing of the e-waste (Brigden et al., 2008; Hosoda et al., 2014; Li, Yu, Sheng, Fu & Peng, 2007; Liu et al., 2008; Ni, Zeng, Tao & Zeng, 2010; Tue et al., 2010; Wang et al., 2013; Wong et al., 2007a; Xiao et al., 2014).

At Agbogbloshie, the recycling of this e-waste often takes place directly on unfortified ground which releases harmful substances directly into the soil. Insulating foam from dismantled refrigerators, primarily polyurethane, or old car tyres are the main fuels used for the fires contributing in itself to acute chemical hazards and longer-term contamination at the burning sites (Bridgen et al., 2008). Soil and ashes samples taken at burning sites in Agbogbloshie showed extremely high concentrations of copper, lead, tin, antimony and cadmium as compared to those typically seen in an uncontaminated soil (Bridgen et al., 2008).

Other studies also conducted at the dumpsite indicated high levels of lead, cadmium and chromium in the soil. Some of these contaminants were over 50 times higher than maximum exposure levels established by the World Health Organization (Gabel, 2011; Johnson et al, 2009; Bishop, 2011; Asante et al, 2011).

Incomplete combustion of chlorinated organic materials, including PVC coated wires, with the reaction catalysed by metals such as copper releases dioxins and furans (PCDD/F) to areas surrounding burning sites. This leads to contamination of surface soils and air (Bridgen et al. 2008).

During periods of heavy rainfall, much of the site becomes flooded and it is likely that surface dusts and soils, along with any chemical contaminant they may contain, are carried into the adjacent, lower-lying lagoons and the Odaw River which ultimately flows into the ocean. Sample of sediment collected from a shallow lagoon located near the disposal and open burning areas within the Agbogbloshie Market contained very high

metal concentrations and organic chemicals as to those in the more contaminated soil and ash samples (Bridgen et al. 2008).

2.2.2 Impact of e-waste recycling on human health

Developing countries like Ghana may be more vulnerable to long-term, transgenerational health risks from e-waste's chemicals, as they encounter problems associated with the control and management of long-term health risks. The most evident health-related issues are occupational and direct local exposure (Frazzoli, Orisakwe, Dragone & Mantovani, 2010). The modes of e-waste disposal, including simply dumping into landfills or burning in open space, expose the general population and generations to come to highly toxic e-waste related mixtures (EWMs) through inhalation, contact with soil and dust, and oral intake of contaminated locally-produced food and drinking water (Frazzoli et al., 2010).

Studies reviewed by Song & Li, (2014) showed that atmospheric pollution due to burning and dismantling activities seems to be the main cause of occupational exposure and contamination of neighbouring communities. The fine dust particles generated by the combustion is strongly associated with pulmonary and cardiovascular diseases, while the larger coarse dust particles which do not usually reach the lungs of humans, can irritate the eyes, nose and throat. The e-waste workers are also exposed to PBDEs and dioxins which is again a result of atmospheric emissions.

Studies conducted among residents around the e-waste sites showed elevated levels of PCBs and brominated flame retardants (BFRs) in breast milk (Asante et al., 2011), heavy metals in urine (Asante et al., 2012), and OH-metabolites of aromatic hydrocarbons in urine (Feldt et al., 2014).

Wittsiepe et al. (2015) found a clear impact of the e-waste exposure and exposure time on the internal PCDD/F i.e there was nearly a linear relationship between the time the e-waste workers had worked at the Agbogbloshie e-waste recycling site and their PCDD/F blood levels.

Differences in vulnerability/susceptibility in exposed individuals should be taken into account in assessing human data. Age and gender are major modulating factors, together with nutrition and lifestyles (e.g., smoking, alcohol consumption). Widespread genetic polymorphisms may also be of importance, as found in a study associating postmenopausal breast cancer risk with PCB exposure (Li et al., 2005). Thus, exposure levels that do not significantly increase health risks in the majority of population may do so in vulnerable/susceptible subgroups.

Evidence from experimental and human studies have shown that most of the toxins and chemicals released from the e-waste recycling activities affect human health severely. The PCDD/Fs can affect the reproductive, neurobehavioural and immune development, and also cause carcinogenicity (Larsen, 2006). The PBDEs can also affect reproductive and neurobehavioural development, and the thyroid function (Darnerud, 2008; Pacyniak et al., 2007). The PCBs exert a variety of toxicological effects, including carcinogenicity on multiple targets such as liver, thyroid, immune function, reproduction and neurobehavioral development (European Food Safety Authority, 2005). PAHs can cause carcinogenicity, mutagenicity and teratogenicity (International Agency for Research on Cancer, 2012).

Presence of Al in the human body can influence skeletal development and metabolism and also cause neurotoxicity and foetal toxicity (European Food Safety Authority, 2008). Arsenic can cause skin alterations, decreased nerve conduction, increased risk of diabetes and of cancer (skin and other tissues) and also liver damage (Gamble et al., 2006). Cadmium can cause kidney damage, renal toxicity, bone disease (osteomalacia and osteoporosis) and possibly reproductive damage and lung emphysema (European Food Safety Authority, 2004; European Commission, 2000). Copper and Fe can cause liver damage (European Food Safety Authority, 2004; European Commission, 2003). Chromium affects reproductive and endocrine functions and can also cause carcinogenicity (Banu, Samuel, Arosh, Burghardt & Aruldas, 2008; Quinteros, Poliandri, Machiavelli, Cabilla & Duvilanski, 2007). Lead and Hg can affect neurobehavioural development of children (especially methylmercury), cause anaemia, kidney damage and chronic neurotoxicity (European Food Safety Authority, 2003). Selenium can also cause hair loss, nail brittleness, cardiovascular, renal and neurological abnormalities (European Commission, 2000).

Exposure to PCBs in e-waste has also been associated with alterations in lymphocyte, monocyte and neutrophil counts (Glynn et al., 2008; Lyche et al., 2004; Leijds et al., 2009; Xu et al, 2015) and alteration of immune function (Li et al., 2013). Leijds et al., (2009) found a decrease in the number of polymorphic neutrophils in adolescents with higher PCBs.

In a study by Kyere, Greve & Atiemo (2016) to examine the spatial distribution and the extent of soil contamination by heavy metals resulting from the primitive, unconventional informal e-waste recycling in the Agbogbloshie e-waste processing site, the soil analysis

revealed that the concentrations of heavy metals measured were significantly higher than the Canadian Environmental Protection Agency and Dutch environmental standards. In an increasing order of Pb > Cd > Hg > Cu > Zn > Cr > Co > Ba > Ni contributed significantly to the overall degree of contamination. The overall degree of contamination was highest in the main working areas of burning and dismantling sites indicating the influence of recycling activities.

2.3 Heavy metals exposure and human health

Heavy metals by density are defined as those having a specific density of more than 5 g/cm³. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Asante et al., 2012). These metals have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO. Although several adverse health effects of heavy metals have been known for a long time, its exposure continues especially in less developed countries (Jarup, 2003).

Emissions of heavy metals to the environment occur via a wide range of processes and pathways, including to the air (e.g. during combustion, extraction and processing), to surface waters (via runoff and releases from storage and transport) and to the soil (and hence into ground waters and crops) [Jarup, 2003]. Atmospheric emissions tend to be of greatest concern in terms of human health, both because of the quantities involved and the widespread dispersion and potential for exposure that often ensues (Jarup, 2003).

2.3.1 Arsenic

Arsenic (As) is a naturally occurring metalloid that occurs in a variety of chemical forms and valence state. Occupational exposure to arsenic which is primarily through inhalation may occur in smelting of Copper, Gold or other non-ferrous metals. The liver is the primary target organ for the metabolism of arsenicals. The major metabolic pathway of inorganic arsenic (toxic arsenic) in humans is its methylation in the liver. The methylation of arsenic has been demonstrated by the presence of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in the urine and bile (Cui, Kobayashi, Hayakawa & Hirano, 2004; Li et al., 2008).

Inorganic arsenic is acutely toxic and intake of large quantities leads to gastrointestinal symptoms, severe disturbances of the cardiovascular and central nervous systems, and eventually death. This Arsenic toxicity (Arsenicosis) has also been reported to be associated with a variety of cancers, dermatitis, cardiovascular diseases, peripheral neuropathy, diabetes mellitus, renal failure and liver dysfunction (Chen et al., 2007; Meliker, Wahl, Cameron & Nriagu, 2007; Mumford et al., 2007; Tapio & Gosche, 2006; Vahidnia, Romijn, van der Voet & de Wolff, 2008; Wang et al., 2002).

Studies in India have also demonstrated the association of arsenic exposure with plasma cholinesterase and LDH activities which causes multi-organ dysfunction including liver intoxication. The arsenic exposure induces liver enlargement with concomitant elevation in levels of ALP, AST and ALT (Ali et al., 2010; Dalal, Nayak, Gangopadhyay & Mukherjee, 2009; Karim et al., 2010; Mazumder, 2005; Nabi, Rahman & Islam, 2005).

A study by Islam, et al., (2011) to demonstrate the effects of arsenic on serum hepatic enzymes showed an increased serum liver enzyme levels among those exposed to arsenic indicating liver toxicity. After adjustments were made for covariates (age, sex, BMI, smoking habit and skin lesions), the results explicitly still demonstrated that arsenic exposure was the main contributor to the increasing serum levels of hepatic enzyme activity. Other studies have also demonstrated that factors such as alcohol intake, age, sex, BMI and smoking habit may influence hepatic enzyme activity (Middelberg, Medland, Martin & Whitfield, 2007; Moranska, et al., 2004; Stranges et al., 2004).

Arsenic concentrations in blood, hair, nails and urine have been used as biomarkers of exposure. Arsenic accumulates in hair and nails, due to the element's affinity for the abundant sulfhydryl groups in keratin, thus As concentrations in these slow growing tissues are considered to be a good measure of past exposure (Hall et al., 2006). Since single doses of As are rapidly and extensively cleared from the blood via the kidney, blood As concentrations have been considered to reflect only recent exposure (Hall et al., 2006). However, with chronic and continuing exposure, steady-state concentrations in blood and urine are achieved thus either blood or urine can serve as biomarkers of past exposure (Morton & Dunnette, 1994). A study by Hall et al., (2006) showed that with long- term exposure, using blood to check As levels may better reflect an individual's total internal As burden due to inputs from recent exogenous exposure and tissue compartments. Arsenic concentration in blood of unexposed persons is mostly $<1\mu\text{g/L}$ thus a higher level may be harmful (Agency for Toxic Substances and Disease Registry [ATSDR], 2007).

2.3.2 Mercury

Mercury is a silvery heavy metal that is liquid at room temperature. Elemental mercury and soluble mercurial salts are efficiently absorbed after inhalation while alkyl mercury compounds are readily absorbed through all routes, including skin contact. Metallic mercury is an allergen, which may cause contact eczema. Exposure to even small amounts of mercury (Hg) can cause serious health problems in people (Lewis & Kosnett, 2014).

Methylmercury concentrates in erythrocytes; therefore, mercury levels in blood remain high in acute toxicity. When ingested by humans, methylmercury is easily absorbed and retained by the body; it has a half-life in blood of about 44 days, which makes blood tests useful measures of acute exposure (Ruedy, 2001).

A study by Tang, Cheng, Zhao, & Wang, (2015) identified high concentrations of total mercury in atmospheric, soil and dust samples and methyl mercury concentrations in rice and fish from an e-waste recycling area in Taizhou, China. This suggested that the e-waste recycling facility was a significant source of Hg. The inorganic Hg levels in hair samples of e-waste workers were also much higher than that in the reference samples. There were also strong positive correlations between hair inorganic Hg levels and time spent in industrial area, and between Methyl Hg levels and fish consumption frequency implying that workers were mainly exposed to Hg vapour through long-time inhalation of contaminated air and dust. Other population were mainly exposed to methyl Hg through high-frequency fish consumption.

According to Electronic Industries Alliance, Hg can be found in at least 26 categories of electronic devices, including electrical lighting, thermostats, LCDs from laptop computers, mobile phones and flat-panel TVs, and semiconductors. A report prepared by Silicon Valley Toxics Coalition also suggested that 22% of the world's annual consumption of Hg was used in electronics. This makes the e-waste recycling industry one of the major sources of environmental Hg contamination (Tang et al., 2015).

Elemental and methyl-mercury are toxic to the central and peripheral nervous system. Inhalation of mercury vapour can produce harmful effects on the nervous, digestive and immune systems, lungs and kidneys, and also cause cough, dyspnea and chemical pneumonitis with high concentration (WHO, 2007; Lewis & Kosnett, 2014). The inorganic salts of mercury are corrosive to the skin, eyes and gastrointestinal tract, and may induce kidney toxicity if ingested (WHO, 2007). Chronic overexposure to inorganic mercury is also associated with neurotoxicity while exposure to organic mercury compounds results in delayed insidious onset of progressive nervous system damage that could be fatal (Lewis & Kosnett, 2014). The most commonly accepted methods of assessing mercury exposure are to test urine or blood. Both tests usually measure levels of total mercury (elemental, inorganic and organic). Adverse health effects are expected when exposed persons have blood mercury concentration above 5ng/mL (New York State Department of Health, 2016). Biological exposure limit for workers exposed to mercury is 15µg/L (American Conference of Governmental Industrial Hygienists [ACGIH], 2012)

Mercury exposure on human's immune system appears to have unpredictable effect, with either an increase or decrease in immune activity depending on individual genetic

predisposition. Studies of workers exposed to elemental mercury vapour have failed to show consistent or marked changes in immune function parameters in large populations. For example, no effect on serum immunoglobulins (IgA, IgG, or IgM) and no increase in autoantibody titres were observed in a group of chloralkali workers exposed for an average of 13.5 years (Langworth, Elinder, Sundquist & Vesterberg, 1992). No increase in antglomerular basement membrane antibodies or IgE was seen in workers exposed for between 1.5 and 25 years (Cardenas et al., 1993).

Slight decreases in IgA and IgG were observed in workers after more than 20 years of exposure to metallic mercury vapours when compared to unexposed controls (Moszczynski, Lisiewicz, Bartus & Bem, 1990). No significant differences in the concentrations of immunoglobulins or complement components were found in a study of 76 chloralkali workers previously exposed to mercury vapour for an average of 7.9 years (range, 1.1–36.2 years) [Ellingsen, Gaarder & Kjuus, 1994]. No increase in the prevalence of autoantibodies was also observed between the formerly exposed worker group and a control group of 53 age-matched referents. The average time elapsed since the cessation of occupational exposure was 12.3 years (range, 1–35 years).

2.3.3 Lead

Lead is a soft malleable, greyish divalent metal which occurs naturally in the earth crust as inorganic or organic compound. Lead has been persistently used by man for various purposes because of its peculiar chemical properties coupled with its poor ability to conduct heat and electricity (Onyeneke & Omokaro, 2016). It is used primarily in storage battery and also in plastics and rubber materials (pipes and cable sheathing). Lead is

absorbed by the gastrointestinal tract via food, beverages, soil and dust. Dietary factors, nutritional status, chemical form of the metal and patterns of food intake affect lead absorption. In humans, lead causes a wide range of biological effects depending upon the level and duration of exposure. It affects several organs and organ systems including nervous, renal, reproductive, hematological and immune system (Dongre et al., 2011).

Lead poisoning is a global health problem but it is unrecognized as such in a number of African countries. Lead poisoning indicated by elevated blood lead levels has been observed in the general population in some parts of Nigeria (Onyeneke & Omokaro, 2016). This study sought to ascertain if some occupational exposure to lead in Nigeria results in elevated blood lead levels and impairment of liver function and found that occupationally exposed subjects had significantly elevated blood lead levels (BLL) compared to controls (subjects: $69.79 \pm 62.30\mu\text{g/dl}$, control: $1.98 \pm 3.85\mu\text{g/dl}$). The occupationally exposed subjects had significantly higher prevalence of lead toxicity, (57%), compared to the controls (0%; $P= 0.05$) which concluded that occupational exposure to lead in Nigeria was associated with significant elevation of BLL, increased prevalence of lead toxicity and liver dysfunction (Onyeneke & Omokaro, 2016).

In adults, occupational exposure to lead is the most common cause of lead poisoning. Inhalation of lead fumes during burning operations is the most common source of acute exposure with approximately 50% of inhaled lead oxide fumes absorbed in the lungs (Jarup, 2003).

BLL are an indication of a recent exposure (Lewis & Kosnett, 2014). BLL is a test that measures the amount of lead in the blood. Small amounts of lead in adults are not thought

to be harmful i.e. $<10\mu\text{g/dL}$ of lead in the blood while adults who have been exposed to lead at work should have BLL below $30\mu\text{g}/100\text{mL}$ (ACGIH, 2012). In less serious cases, the most obvious sign of lead poisoning is disturbance of haemoglobin synthesis, and long-term lead exposure may lead to anaemia.

Lead is known to alter the haematological system. The lead induced anaemia is microcytic, hypochromic one and results primarily from both the inhibition of heme synthesis and shortening of the erythrocyte lifespan. Lead interferes with heme synthesis by altering the activities of δ -aminolevulinic acid dehydratase (ALAD) and ferrochelatase. Studies of children in India and China also have reported significant decreases in ALAD activity associated with $\text{BLL} \geq 10 \mu\text{g/dL}$ (Ahamed, Verma, Kumar & Siddiqui, 2005; Jin et al., 2006).

A study of workers in the United Arab Emirates reported that a group of 100 workers with a mean BLL of $78 \mu\text{g/dL}$ had significantly higher concentrations of amino acids in serum than 100 controls whose mean BLL was $20 \mu\text{g/dL}$ (Al-Neamy et al., 2001). Tests for liver function that included serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities found small ($\leq 10\%$) but statistically significant increases in alkaline phosphatase and lactate dehydrogenase activities in the serum of the workers.

Another study found no alterations in serum IgA and IgM levels among 25 workers with a mean BLL of $74.8 \mu\text{g/dL}$ (range, $38\text{--}100 \mu\text{g/dL}$) compared to $16.7 \mu\text{g/dL}$ (range, $11\text{--}30 \mu\text{g/dL}$) among 25 controls; however, IgG level was significantly reduced among the workers (Basaran & Undeger, 2000). Also, a study of 606 Korean workers found that

mean serum IgE levels were positively related to BLL in the range of <10 – ≥ 30 $\mu\text{g/dL}$ (Heo, Lee, Ahn, & Lawrence, 2004). Changes in T-cell subpopulations also have been reported. Basaran & Ündeger (2000) described a significant decrease in the number of CD4^+ cells and C3 and C4 complement levels in workers with a mean BLL of 74.8 $\mu\text{g/dL}$.

Lutz et al., (1999) conducted a survey of the immune system's function in a cohort of 279 children aged 9 months to 6 years, with BLL ranging from 1 to 45 $\mu\text{g/dL}$. Exposure was due primarily to lead-based paint. Of the comprehensive number of parameters of cellular and humoral immunity evaluated, only the serum IgE levels showed a statistically significant relationship with BLL, as BLL increased so did IgE levels. Variables controlled for in this study included age, race, gender, nutrition, and socioeconomic level.

2.4 Liver biochemical function

The liver filters and processes blood as it circulates through the body. It metabolizes nutrients and drugs, makes blood clotting proteins, and performs many other vital functions. The liver may serve as a target organ and also play a central role in the detoxification and elimination of many occupational and environmental chemicals. This makes it especially vulnerable to chemical injury (Harrison, 2014).

The role of the liver as a primary defence against xenobiotics is facilitated by its cellular enzyme system that drives these chemical reactions. When liver cells (hepatocytes) are damaged or destroyed, the enzymes in the hepatocytes leak into the blood where they are

routinely measured to assess liver function (Harrison, 2014). Liver function tests check the blood for two main liver enzymes:

- Aspartate aminotransferase (AST): This is an enzyme that helps to process proteins. It is also found in muscle tissues besides the liver.
- Alanine aminotransferase (ALT): This enzyme is almost exclusively found in the liver.

Normal laboratory reference range for serum AST and ALT enzyme level is 0.1-40.0U/I (University of Ghana hospital laboratory, 2016). Other reference ranges have also been reported from studies among healthy Ghanaian males for AST and ALT. These were 17.0-60.0U/I (Dosoo et al., 2012); 15.5-46.5U/I (Koram et al., 2007) for AST and 8.0-54.0U/I (Dosoo et al., 2012); 9.5-39.2U/I (Koram et al., 2007) for ALT. During hepatic injury, serum levels of these enzymes usually rises.

The ratio of AST to ALT sometimes can help determine whether the liver or another organ has been damaged. If ALT and AST are found together in elevated amounts in the blood, liver damage is most likely present. In most types of liver disease, the ALT level is higher than AST and the AST/ALT ratio will be low (less than 1). There are a few exceptions; the AST/ALT ratio is usually increased in alcoholic hepatitis, cirrhosis, and in the first day or two of acute hepatitis or injury from bile duct obstruction. With heart or muscle injury, AST is often much higher than ALT (often 3-5 times as high) and levels tend to stay higher than ALT for longer than with liver injury (Ratini, 2014).

Another key function of the liver is the production of bile which helps digest fat. Bile flows through the liver in a system of biliary ducts, and is eventually stored in the

gallbladder under the liver. When bile flow is slow or blocked, blood levels of certain liver enzymes (Alkaline phosphatase, 5' nucleotidase, Gamma-glutamyl transpeptidase [GGT]) rises. Liver tests may check for any or all of these enzymes in the blood. If alkaline phosphatase and/or 5' nucleotidase and GGT are elevated, a problem with bile flow is most likely present. Bile flow problems can be due to a problem in the liver, the gallbladder, or the tubes connecting them (Ratini, 2014). Normal laboratory reference range for serum GGT enzyme level is 0.1-55.0U/I (University of Ghana hospital laboratory, 2016) while a population based study among Ghanaian males quoted 9.0-71.0U/I (Koram et al., 2007).

2.5 Immune/haematological indices

The immune system serves to protect the host from invasion by foreign antigens. It does this through distinguishing itself from non-self antigens. A normal immune response relies on a careful coordination of specialised cells, organs and biological factors necessary for the recognition and subsequent elimination of foreign antigens.

Effector cells that participate in immune defence and hypersensitivity reactions include mast cells, basophils, polymorphonuclear neutrophils, eosinophils, macrophages, monocytes, platelets and lymphocytes (Kishiyama, 2014). The neutrophils, lymphocytes, eosinophils, monocytes and basophils together constitute the white blood cells (WBC). These WBCs are formed via differentiation from pluripotent hematopoietic stem cells of the bone marrow (Yuksel, Verit, Sahin, Urkmez & Uruc, 2016).

The normal total WBC count for healthy adults range between $5.00-10.00 \times 10^3/\mu\text{L}$ (University of Ghana Hospital Laboratory, 2016). Leucocytosis is WBC count above upper limit of $10.00 \times 10^3/\mu\text{L}$ and leukopenia represents WBC count below the lower limit of $5.00 \times 10^3/\mu\text{L}$. Leucocytosis is most commonly due to an increase in the absolute number of mature neutrophils (neutrophilia), it can also reflect a marked increase in the absolute numbers of any of the WBC. Neutrophilic leukocytosis is commonly seen in infection, stress, smoking, pregnancy and following exercise (Coates, 2016).

Common myeloid progenitor cell first differentiates into granulocyte/macrophage cells, and then through a series of differentiation phases i.e. induce formation of dendritic cell, granulocytes (neutrophils, eosinophils, basophils and mast cells), monocyte/macrophages (Yuksel et al., 2016). Neutrophils are granulocytes that play an important role in destroying foreign antigens by phagocytosis or local release of lysosomal enzymes for larger particles. These cells generate a number of antimicrobial factors that play a part in hypersensitivity reactions. Eosinophils play both proactive and modulating role in inflammation. Monocytes /macrophages are involved in ingesting, processing and presenting antigens to lymphocytes. Mast cells and basophils are involved in immediate hypersensitivity and have cell surface receptors for Immunoglobulin E (IgE) [Kishiyama, 2014].

Lymphocytes and natural killer (NK) cells differentiate from common lymphoid progenitor cells (Yuksel et al., 2016). The lymphocytes are divided into B cells involved in humoral immunity and T cells (thymus derived lymphocytes) involved in cellular immune response. These cells are responsible for the initial specific recognition of

antigens. The helper-inducer (CD4) subpopulation of T cells helps to regulate immune response and also amplify B cells production of immunoglobulin. Natural killer (NK) cells are non B, non T lymphocytes that can bind Immunoglobulin G (IgG) and are involved in antibody dependent cell mediated cytotoxicity. They can also non-specifically destroy virally infected and tumour cells (Kishiyama, 2014).

Platelets are irregularly shaped cytoplasmic fragments of a megakaryocyte found in the peripheral blood, where it functions in clotting. It has no definite nucleus and is about one-third to one-half the size of an erythrocyte. Platelets survive in the circulation for 8 to 10 days, after which they are removed from the circulation by cells of the monocyte-macrophage system (Landaw, & George, 2014). Normal range for platelets is 150-400 $\times 10^3/\mu\text{L}$ (University of Ghana Hospital Laboratory, 2016).

The full blood count (FBC) with differential provides basic but valuable information about the haematological and immune system. The parameters measured include haemoglobin concentration and red cells indices; platelet count; and the total and differential WBC counts. These parameters may be used as a measure of immunotoxicity (ATSDR, 1994). According to ATSDR (1994), immune tests are divided into three levels namely Basic tests (level 1), Focused tests (level 2) and Research tests (level 3). The Basic tests are easily performed and are also relevant to the detection of immune deficiency, hypersensitivity and autoimmunity. They include serum levels of antinuclear antibodies; C reactive protein; immunoglobulins (IgG, IgM and IgA); total proteins; total white blood cell count; total lymphocytes count; total eosinophil count; and CD4/CD8 lymphocyte counts. Focused tests are for follow up of abnormal basic test results.

Another important parameter measured in the full blood count is the haemoglobin (Hb) concentration. Haemoglobin (Hb) is the respiratory protein found in red blood cells (RBC) with normal range for adult males as 13.0-17.4g/dl and adult females 12-15g/dl (University of Ghana Hospital Laboratory, 2016). Erythropoiesis is the formation of RBCs which takes place in the bone marrow of adults under the influence of the stromal framework, cytokines and erythropoietin (an endocrine hormone produced by the kidney). The mature RBC circulates in blood for approximately 110 to 120 days after which it is removed by macrophages. Under normal conditions, the rate of production equals the rate of loss (Schrier, 2014).

Anaemia is Hb below the lower limit (adult males-13.0g/dl and adult females-12.0g/dl) [WHO, 2011]. Occupational related anaemia caused by exposure to industrial pollutants include Aplastic anaemia following exposure to benzene, pesticides, arsenic, cadmium and copper; Megaloblastic anaemia following exposure to arsenic, chlordane, benzene and nitrous oxide and; Haemolytic anaemia following exposure to arsenic, methyl chloride, naphthalene, lead, cadmium and mercury (Wiwanitkit, 2007).

Minor defects in haematological and immune response has the potential of reducing an individual's resistance against infections and underlying neoplastic changes, increasing susceptibility to clinical disease (Lyche et al., 2004).

CHAPTER THREE

3.0 METHODS

3.1 Study Area

The study was conducted at Agbogbloshie e-waste recycling site situated on the bank of the Odaw River and in the upper reaches of the Korle-Lagoon, adjacent to the Agbogbloshie food market. It is one of the largest e-waste dumps in sub-Saharan Africa, processing millions of tons of e-waste each year. Most old/waste electrical and electronic equipment from households and offices end up there every month as a final resting place, where they are broken apart to salvage copper and other metallic components that can be sold (Akormedi et al., 2013). Thick plumes of smoke and irritating fumes from the burning of e-waste materials including old car tyres at the site to retrieve valuable metals engulf the air continuously, contaminating the atmosphere and compromising the health of the many people who live, work or visit Agbogbloshie. About 40,000 people live in this area under the most deplorable environmental conditions and largely represent a migrant population from Northern parts of Ghana (Wittsiepe et al., 2015). A densely populated slum housing where the e-waste workers, their families and other residents live is located south of the recycling area and the Agbogbloshie food market and other businesses including pharmaceutical companies, breweries, manufacturing companies and small self-employed traders located eastwards.

3.2 Study design

An analytical cross-sectional study was conducted among the e-waste workers over the period of 8th – 17th June, 2016 (data collection period). Demographical data was collected

from study participants using a semi-structured questionnaire with short interviews. This was followed by collection of blood samples via venepuncture. Blood samples were sent to the laboratory for liver biochemical function, haematological/ immunological indices and heavy metals levels analysis. Ethical approval for the study was granted by the Ghana Health Service Ethics Review Committee (GHS-ERC: 79/12/15).

3.3 Study variables

Dependent variables: Immune function and liver biochemical function

- Liver biochemical function: AST, ALT, GGT,
- Immune function: Haemoglobin, White blood cell with differentials (lymphocyte, neutrophils, eosinophils, basophils (granulocytes) and monocytes); Platelets.

(markers selected from ATSDR recommended bio-makers for immunotoxicology [basic tests]).

Independent variables: Blood levels of lead, mercury & arsenic; category of recycling activity, exposure duration and use of personal protective equipment (PPE).

Confounders to be assessed include age, sex, smoking habits, alcohol use and hepatitis B status (assessed through laboratory test for HBsAg from sample taken).

3.4 Study Population

Inclusion criteria: Adult (18 years or older), occupation in the e-waste recycling process or on the recycling site for 6 months or more and also not visibly ill.

Exclusion criteria: Not willing or able to understand or comply with study procedures

3.5 Sample size

Due to the high cost of sample analysis (heavy metals analysis and immunological/liver function parameter), 40 e-waste workers were selected for the study. This number was evenly distributed among the four categories of e-waste workers (collectors, sorters, dismantlers and burners). Other studies conducted among these e-waste workers had sample size ranging from 29 to 75 (Asante et al., 2012; Feldt et al., 2014; Wittsiepe et al., 2015). Thus, the whole population was targeted for this study.

3.6 Recruitment/ Sampling Method

Generally, approaching and recruiting people for studies involving invasive procedures tend to be difficult hence require a lot of trust. This was not different among the e-waste workers. In addition, the superstitious beliefs associated with blood samples also hindered their acceptance to participate in the study. After an initial engagement some weeks earlier with community leaders and some e-waste workers, recruitment of study participants was done. Volunteers were selected among them who played the role of local guides and translators. The data collection period coincided with the Ramadan fasting, and since majority of e-waste workers are Muslims, blood collection was done only in the evening after they broke their fast.

3.7 Data collection

3.7.1 Questionnaire Administration

A short interview with the aid of translators was conducted to obtain basic demographic information such as participants' contact information, age, sex, ethnicity, highest educational level, type of recycling activity worker is engaged in, exposure duration, use of personal protective equipment (PPE), smoking habits, alcohol use and current medications being taken. Written consent was obtained from each participant before the questionnaire was administered. Questionnaire administration and biological specimen collection were done in a small shed at the site that was provided by the community elders.

3.7.2 Biological Specimens Collection

Venous blood was collected from the antecubital fossa of participant's arm of choice or their non-dominant hand into 2 separate tubes using standard laboratory procedures by a phlebotomist from University of Ghana Hospital. After explaining the procedure and risks to the participant and obtaining their consent for the blood draw, participants' arm was placed in an extended position while he was comfortably seated. An appropriate vein was located (commonly used veins included the cubital, basilic of cephalic vein) and a tourniquet applied 3-4 inches above the collection site. The site was cleaned with a cotton swab containing 70% isopropyl alcohol. With the appropriate needle (18 gauge) attached to the hub and the skin held tightly, the needle was inserted into the vein followed by attaching the vacutainer tubes to draw blood while the hub is held securely. The tourniquet was removed immediately blood began to flow.

Four (4) ml of blood was drawn into each of the 2 vacutainer tubes and immediately labelled with participants' identity code (same as code on questionnaire). The first tube containing ethylenediamine tetra-acetic acid (EDTA) K3 was used for heavy metal analysis (Pb, Hg and As) and haematological tests (Full blood count (FBC): Hb, total WBC and differential, platelet count). The second tube containing a serum separator was used to run the liver biochemical function test (Liver function test (LFT): AST, ALT, GGT) and hepatitis B surface antigen test (HBsAg). After collection, the samples were kept in coolers on ice pack and transported to the University of Ghana Hospital Laboratory after field work for analysis. The FBC, LFT and HBsAg were run the same day the samples were collected. Samples for blood Pb, Hg and As levels were stored overnight at the University of Ghana Hospital Laboratory and transported to Ecolab the following day for heavy metal analysis.

3.8 Specimen Analysis

3.8.1 General Laboratory practices

Calibrated analytical balances and pipettes were used as well as standard reference materials. All analysers were calibrated to conform with International Standard Organisation (ISO) specifications. Instruments for analysis of heavy metal were washed with 1M nitric acid and demineralised water prior to use in the analysis

3.8.2 Immune indices and liver biochemical function test

The liver biochemical function test (AST, ALT and GGT) and full blood count (Hb, platelet count, WBC with differentials) were done at the University of Ghana Hospital Laboratory. The AST, ALT and GGT were run using a Selectra Pro S biochemical analyser (Puteaux, France). This is an automated biochemical analyser and runs up to 120 photometric tests per hour. Whole blood samples collected in a serum separator tubes (yellow top) were centrifuged at 1000g for 10 minutes to obtain serum. After calibration/programming, the analyser picked a fixed volume serum from the sample tube for analysis, eliminating all forms of contact with the sample whilst in operation and results were then displayed on the monitor and printed.

An Abott Cell-Dyn Emerald haematology analyser (Illinois, U.S.A) was used for the FBC with differential count test. This model was a 3-part differential analyser and hence gave granulocyte, lymphocyte and monocyte counts. Whole blood sample in EDTA tubes were used. The whole blood was uniformly and gently mixed by gently inverting each tube up-and-down 8-10 times, the cap removed and the probe of the analyser fully immersed in the blood sample. An aspirator button was then pushed and the required aliquot of whole blood was aspirated for analysis with an auto-printing of results.

Hepatitis B screening test (HBsAg) was performed with a Core technology hepatitis B test kit (Beijing, China). This had an accuracy of 99% and sensitivity of 2ng/ml. Following the manufacturers' instructions, drops of whole blood was placed on the sample well of the cassette using a 25 μ l pipette after which the buffer was added. Results were available in 10-15 minutes.

3.8.3 Blood sample analysis for Heavy metals (As, Pb and Hg)

Measurements of blood heavy metals levels (Hg, As and Pb) was performed using a Perkin-Elmer PinAAcle 900t atomic absorption spectrophotometer (Waltham, Massachusetts-USA) coupled to a flow injection analysis system at the Ecological Laboratory of the University of Ghana. Default parameters found in the software were used for all three elements: As, Hg, and Pb. Prior to the measurements, the blood samples were digested in nitric acid. The instrument was calibrated with standard stock solutions. The acidified sample, blank, or standard were placed into the instrument. A flow of Argon was added which allowed the gaseous phase containing the analyte vapour to enter the quartz cell on the Atomic Absorption Spectrophotometer for analysis. Values detected for arsenic, cadmium and lead were recorded accordingly.

3.9 External Quality Control

To ensure quality and consistent results, Research assistants received training in questionnaire administration; Sample containers codes were always cross-checked with participant's code on the questionnaire before the blood sample was taken; Skin site for the blood collection was well cleaned with alcohol prep pads before blood samples were taken; All equipment including gloves, needles and sample tubes were kept in an air tight container and only removed when it was about to be used; One pair of non-sterile gloves was used per patient; Needles and sharps were disposed of into appropriate sharp bins immediately after use; the first sample of blood collected for every participant was used for blood heavy metal levels; and also Pre- screened heavy metal free sample collection tubes were used.

3.10 Data Processing and Analysis

The pre-coded questionnaires were serialised at the time of entry and entered in SPSS version 21.0 (IBM, Armonk, USA) after it had been checked for completeness. It was then exported to STATA version 13.1 (STATA Corp LP, Texas, USA) for statistical analysis. Missing data and errors were checked by using the *codebook* command. The data was explored by running frequencies for all the variables.

We computed means, standard deviations and confidence intervals for all the continuous variables while for categorical variables, we computed proportions. Liver and immune function statistics were compared across the different subgroups of e-waste workers using chi-square test for the difference in proportions, one-way ANOVA for the difference in means, as well as comparing their means with the reference normal ranges provided by the University of Ghana Hospital Laboratory.

The distributions of serum Pb and Hg levels, liver biochemical function and immune/haematological parameters were then assessed by the normality test. The data that did not fit normal distribution were transformed, and the statistics were performed on the transformed data.

Simple linear regression was conducted to evaluate the relationship between all potential exposure variables and the liver/immune function parameters. This exploratory analysis served as the primary mechanism for determining which exposure variables seemed important and which were not, and was used to screen out insignificant exposure variables. A 95% confidence interval (CI) was used to test the significance levels. A

general linear regression was also used to investigate the association between immune/haematological and liver function, and the heavy metal (Pb and Hg) levels.

Multiple linear regression analysis was used to determine the independent association between the levels of Pb and Hg with liver biochemical function and immune/haematological parameters measured adjusting for other potential confounders.

P-value <0.05 was used to determine statistical significance of all tests.

3.11 Return of laboratory results to participants/ Follow-up

Results obtained from the blood samples collected from participants were discussed with them after the analysis had been completed. This was by an initial phone conversation through the contact numbers they had provided. The principal investigator (PI) also sent copies of the laboratory results to them at their workplace. Participants with abnormal laboratory results had the implications further explained to them by the PI who is also a medical doctor. These participants were then assisted with a referral letter to see the appropriate physician/ specialist for further medical care.

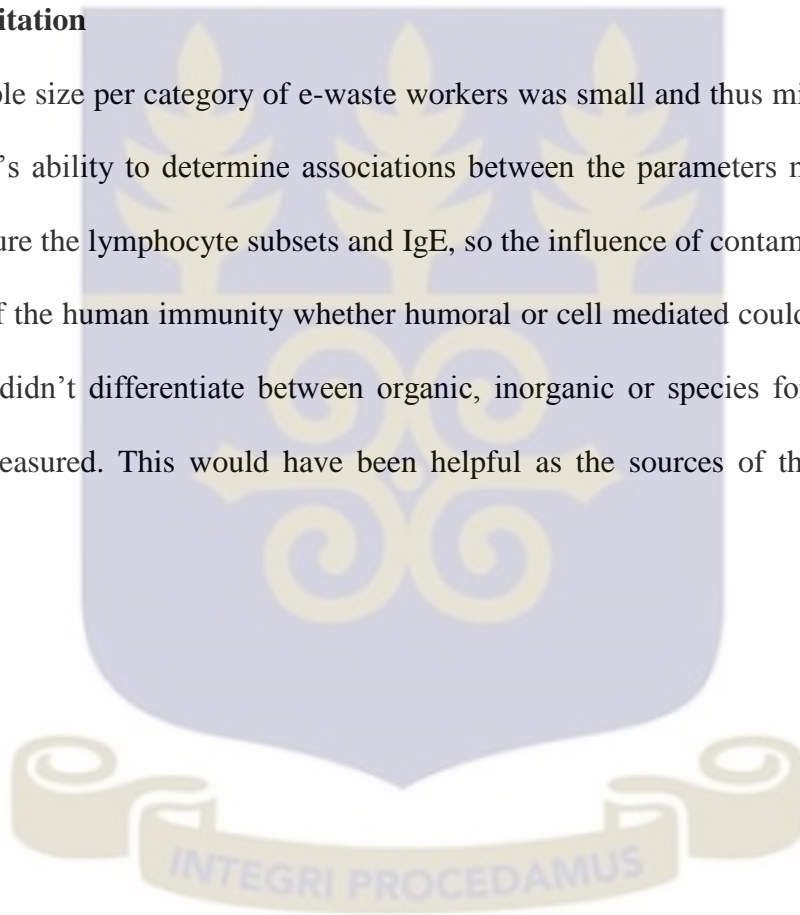
3.12 Ethical consideration/issues

Ethical clearance was obtained from the Ghana Health Service Ethical Review Board. Permission was also sought from the e-waste informal Association leaders, as well as the community leaders at where the study was conducted. The study objectives, procedures and possible risks/ benefits associated with participating in the study was carefully explained in English and the local language to the e-waste workers before they were

recruited. Persons eligible for the study were recruited after written informed consent had been obtained from them. Participants were informed that they had the right to stop at any point in the study. Compensation was given at the time of data/specimen collection. All responses obtained were kept confidential.

3.13 Limitation

The sample size per category of e-waste workers was small and thus might have affected the study's ability to determine associations between the parameters measured. We did not measure the lymphocyte subsets and IgE, so the influence of contaminants on specific aspects of the human immunity whether humoral or cell mediated could not be analysed. We also didn't differentiate between organic, inorganic or species forms of the heavy metals measured. This would have been helpful as the sources of these contaminants vary.



CHAPTER FOUR

4.0 RESULTS

4.1 Demographic characteristics of e-waste workers

All forty (40) e-waste workers studied were males comprising 10 burners, 10 collectors, 10 sorters and 10 dismantlers. The demographic characteristics of the study population are presented in Table 1. In all, 32 (80%) of the e-waste workers studied were below the age of 30 years and 13 (32.5%) between ages 20-24 years. The mean age of these e-waste workers was 24.7 ± 1.2 years with burners and dismantlers recording the lowest (19.9 ± 2.1 and 23.4 ± 6.8 respectively), and collectors the highest (30.1 ± 7.6). The difference between the mean age among the various categories of e-waste workers (Burners, Dismantlers, Collectors and Sorters) was statistically significant ($p=0.013$) as shown in Table 1.

About half (50%) of the study population had no formal education, with only a few 4 (10%) educated beyond the Junior Secondary level. Collectors were the most uneducated (80%), while half (50%) of all sorters were without formal education. The difference in educational level among the categories of e-waste workers was statistically significant ($p=0.037$) [Table 1]. With regard to ethnicity, majority 35 (87.5%) of the study population were Dagombas, 4 (10%) were Frafras, with one (1) person being a non-Ghanaian, an Igbo from Nigeria. Most of the e-waste workers 35 (87.6%) had worked for less than 10 years.

Almost all the workers studied work for at least 12 hours a day, and 6 days in a week. Among the different categories of e-waste workers, majority, (6/10, 60%) of the collectors worked the longest hours, more than 12 hours in a day as shown in Table 1. Also, most of the e-waste workers studied 36 (90%) do not wear any personal protective

equipment (PPE) during recycling activities, and even for those who use PPE, only hand, foot and body protective equipment are sometimes used, which did not differ among the groups (Table 1). Finally, only few (22.5%) of the study participants smoke cigarette, or take alcohol (22.5%), or take any recreational drugs (15.0%) as indicated in Table 1. Marijuana was the recreational drug being taken by the e-waste workers.

Table 1: Demographic characteristics of study population

Characteristics	Frequency (%)					P-value
	Total (N=40)	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	
Age (mean±SD)	24.7±7.4	19.9±2.1	23.4±6.8	30.1±7.6	25.3±8.2	0.013*
Highest educational level						
No formal education	20(50.0)	3(30.0)	4(40.0)	8(80.0)	5(50.0)	0.037*
Primary	9(22.5)	6(60.0)	1(10.0)	0	2(20.0)	
Middle/JHS	7(17.5)	1(10.0)	4(40.0)	1(10.0)	1(10.0)	
SSS/SHS	4(10.0)	0	1(10.0)	1(10.0)	2(20.0)	
Ethnicity						
Dagomba	35(87.5)	7(70.0)	9(90.0)	10(100)	9(90.0)	0.145
Frafra	4(10.0)	3(30.0)	1(10.0)	0	0	
Igbo (Nigerian)	1(2.5)	0	0	0	1(10.0)	
Cigarette smoking						
No	31(77.5)	7(70.0)	8(80.0)	8(80.0)	8(80.0)	0.934
Yes	9(22.5)	3(30.0)	2(20.0)	2(20.0)	2(20.0)	
Alcohol intake						
No	31(77.5)	4(40.0)	8(80.0)	10(100)	9(90.0)	0.008*
Yes	9(22.5)	6(60.0)	2(20.0)	0	1(10.0)	
Recreational drugs use						
No	34(85.0)	6(60.0)	8(80.0)	10(100)	10(100)	0.035*
Yes	6(15.0)	4(40.0)	2(20.0)	0	0	
No. of hours worked/day						
1-12	33(82.5)	10(100)	9(90.0)	4(40.0)	10(100)	0.001*
13-24	7(17.5)	0	1(10.0)	6(60.0)	0	
No. of days worked/week						
6	37(92.5)	10(100)	8(80.0)	9(90.0)	10(100)	0.265
7	3(7.5)	0	2(20.0)	1(10.0)	0	
PPE use						
No	36(90.0)	10(100)	9(90.0)	9(90.0)	8(80.0)	0.528
Yes	4(10.0)	0	1(10.0)	1(10.0)	2(20.0)	

*Statistically significant (p<0.05)

4.2. Health Status of e-waste workers

The health status of e-waste workers studied is presented in Table 2 below. Hepatitis B viral infection which attacks the liver and can cause both acute and chronic disease, had a prevalence of 10% among the e-waste workers studied, which was not statistically significant ($p=1.000$) as indicated in Table 2. The difference among their knowledge of any illness was statistically significant ($p=0.028$) [Table 2].

Table 2: Health status of study population

Characteristics	Frequency (%)					P-value
	Total	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	
Knowledge of any illness (N=40)						
No	30(75.0)	4(40.0)	8(80.0)	9(90.0)	9(90.0)	0.028*
Yes	10(25.0)	6(60.0)	2(20.0)	1(10.0)	1(10.0)	
Type of Illness (N=10)						
Body aches	6(60.0)	4(40.0)	1(10.0)	0	1(10.0)	
Chest pains	2(20.0)	1(10.0)	0	1(10.0)	0	0.179
Fever	1(10.0)	1(10.0)	0	0	0	
Heart burns	1(10.0)	0	1(10.0)	0	0	
Hepatitis B surface antigen (N=40)						
Non-reactive	36(90.0)	9(90.0)	9(90.0)	9(90.0)	9(90.0)	1.000
Reactive	4(10.0)	1(10.0)	1(10.0)	1(10.0)	1(10.0)	

*Statistically significant ($p<0.05$)

4.3 Liver biochemical function test

Overall, prevalence of elevated serum levels of active liver biochemical enzymes ALT, AST and GGT measured among the study population were 7.5%, 12.5% and 10.0% respectively as shown in Table 3. These results were compared to the laboratory

reference range (Reference range: AST & ALT: 0.1U/L -40.0U/L, GGT: 0.1U/L-55.0U/L) from the University of Ghana hospital. Burners had the highest elevated prevalence levels of biochemical enzymes ALT, AST and GGT; 30%, 20% and 20% respectively. Values obtained for sorters were all within the reference ranges. A chi-squared test for differences in elevated liver enzymes levels among the different categories of e-waste workers (Burners, Dismantlers, Collectors and Sorters) was statistically significant for AST and ALT ($p=0.04$, $p=0.04$) but not for GGT ($p=0.14$) as shown in Table 3.

Table 3: Prevalence of elevated levels of liver biochemical enzymes among different categories of e-waste workers

Enzyme	Total (N=40)	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	P-value (<0.05)
Prevalence of AST elevation (>40U/L)	3(7.5%)	2(20.0%)	1(10.0%)	0	0	0.04*
Prevalence of ALT elevation (>40U/L)	5(12.5%)	3(30.0%)	1(10.0%)	1(10.0%)	0	0.04*
Prevalence of GGT elevation (>55U/L)	4(10.0%)	2(20.0%)	1(10.0%)	1(10.0%)	0	0.14

*Statistically significant at $p<0.05$

The overall mean AST and GGT levels ($31.83\text{U/L} \pm 10.63\text{U/L}$, $31.16\text{U/L} \pm 26.41\text{U/L}$) were higher compared to ALT levels ($25.99\text{U/L} \pm 10.13\text{U/L}$) [Table 4]. Among the category of workers, burners and dismantlers recorded the highest mean levels of all the liver biochemical enzymes measured as shown in Table 4. A one-way ANOVA revealed that the differences between mean levels of AST among the various categories was statistically significant ($p=0.027$). However, there was no statistically significant

difference between ALT and GGT levels among the different categories of e-waste workers ($p=0.067$, $p=0.420$) as shown in Table 4.

Table 4: Mean levels of liver biochemical enzymes of different categories of e-waste workers

Enzyme	Total (N=40)	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	P-value (<0.05)
AST (U/L)	31.83±10.63	37.48±14.45	35.27±12.17	26.43±4.83	28.14±3.58	0.027*
ALT (U/L)	25.99±10.13	31.69±11.74	28.62±9.36	21.94±9.58	21.70±6.70	0.067
GGT (U/L)	31.16±26.41	46.39±46.92	27.45±13.57	27.56±15.07	23.25±7.33	0.420

Values represent mean \pm standard deviation (S.D)

*Statistically significant at $p<0.05$

4.4 Immune/ Haematological parameters

The immune/haematological parameters were classified as abnormal (low or high) or normal in reference to standard reference values provided by the University of Ghana hospital laboratory (Haemoglobin level (g/dL):13.00 – 17.00; Platelets ($\times 10^3/\mu\text{L}$):150 – 400; Granulocytes ($\times 10^3/\mu\text{L}$):2.50 – 7.50; Lymphocytes ($\times 10^3/\mu\text{L}$):1.30– 4.00; Monocytes ($\times 10^3/\mu\text{L}$): 0.15 – 0.70; White blood cell ($\times 10^3/\mu\text{L}$):5.00 – 10.00). Most of the e-waste workers had normal levels of haemoglobin (Hb), platelet, white blood cell (WBC) and lymphocytes counts (75.0%, 87.5%, 52.5% and 90.0% respectively) [Table 5]. Among the four categories of e-waste workers studied, there was no significant difference in immune/haematological parameters except for monocytes ($p=0.001$) [Table 5]. Most of the Sorters 9 (90%) had abnormal monocytes levels as compared to the other groups. Majority of the e-waste workers had abnormal granulocyte levels (82.5%) with all the Dismantlers recording abnormal levels (Table 5).

Of the six parameters measured for immune/haematological function, dismantlers presented the highest prevalence of abnormalities in Hb (40%), WBC (70%) and granulocyte (100%) as shown in Table 5. Burners had the highest prevalence of abnormal lymphocytes counts (20%), Collectors in platelets counts (20%) and Sorters in monocytes counts (90%) [Table 5].

Table 5: Prevalence of immune/haematological parameters of different categories of e-waste workers

Parameters	Total (N=40)	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	P-value (<0.05)
Haemoglobin level (g/dL)						
Anaemic	10(25.0%)	3(30.0%)	4(40.0%)	1(10.0%)	2(20.0%)	0.325
Normal	30(75.0%)	7(70.0%)	6(60.0%)	9(90.0%)	8(80.0%)	
Platelets ($\times 10^3/\mu\text{L}$)						
Abnormal	5(12.5%)	1(10.0%)	1(10.0%)	2(20.0%)	1(10.0%)	0.798
Normal	35(87.5%)	9(90.0%)	9(90.0%)	8(80.0%)	9(90.0%)	
White blood cell ($\times 10^3/\mu\text{L}$)						
Abnormal	19(47.5%)	4(40.0%)	7(70.0%)	4(40.0%)	4(40.0%)	0.439
Normal	21(52.5%)	6(60.0%)	3(30.0%)	6(60.0%)	6(60.0%)	
Granulocytes ($\times 10^3/\mu\text{L}$)						
Abnormal	33(82.5%)	6(60.0%)	10(100%)	8(80.0%)	9(90.0%)	0.109
Normal	7(17.5%)	4(40.0%)	0	2(20.0%)	1(10.0%)	
Lymphocytes ($\times 10^3/\mu\text{L}$)						
Abnormal	4(10.0%)	2(20.0%)	1(10.0%)	1(10.0%)	0	0.528
Normal	36(90.0%)	8(80.0%)	9(90.0%)	9(90.0%)	10(100%)	
Monocytes ($\times 10^3/\mu\text{L}$)						
Abnormal	23(57.5%)	4(40.0%)	4(40.0%)	6(60.0%)	9(90.0%)	0.001*
Normal	17(42.5%)	6(60.0%)	6(60.0%)	4(40.0%)	1(10.0%)	

*Statistically significant at $p < 0.05$

Table 6 shows the mean immune/haematological parameters measured among different categories of e-waste workers recruited for this study. Overall, the total mean granulocyte level of $1.93 \times 10^3/\mu\text{L} \pm 0.86 \times 10^3/\mu\text{L}$ was lower than the reference range ($2.50 \times 10^3/\mu\text{L}$ –

$7.50 \times 10^3/\mu\text{L}$), while Hb ($13.55\text{g/dL} \pm 1.45\text{g/dL}$) and WBC ($5.12 \times 10^3/\mu\text{L} \pm 1.42 \times 10^3/\mu\text{L}$) levels were also closer to the lower limits (13.0g/dL and $5 \times 10^3/\mu\text{L}$ respectively) as shown in Table 6.

Among the groups, dismantlers had the lowest mean haemoglobin level ($12.71\text{g/dL} \pm 2.07\text{g/dL}$) which was also lower than the reference lower limit of 13.00g/dL indicating that they were anaemic. Sorters had the lowest mean platelet count ($194.20 \times 10^3/\mu\text{L} \pm 50.53 \times 10^3/\mu\text{L}$) and burners had the lowest mean lymphocyte count ($2.18 \times 10^3/\mu\text{L} \pm 0.82 \times 10^3/\mu\text{L}$) though all values were within the normal reference range [Platelets ($\times 10^3/\mu\text{L}$): $150 - 400$; Lymphocytes ($\times 10^3/\mu\text{L}$): $1.30 - 4.00$]. Dismantlers had the lowest mean WBC count ($4.63 \times 10^3/\mu\text{L} \pm 1.43 \times 10^3/\mu\text{L}$) and both dismantlers and burners had WBC counts below the reference lower limit $5.00 \times 10^3/\mu\text{L}$. All groups recorded mean levels of granulocytes (burners: $2.29 \times 10^3/\mu\text{L} \pm 1.22 \times 10^3/\mu\text{L}$, dismantlers: $1.82 \times 10^3/\mu\text{L} \pm 0.48 \times 10^3/\mu\text{L}$, collectors: $1.83 \times 10^3/\mu\text{L} \pm 0.73 \times 10^3/\mu\text{L}$, sorters: $1.77 \times 10^3/\mu\text{L} \pm 0.89 \times 10^3/\mu\text{L}$) below the reference lower limit of $2.5 \times 10^3/\mu\text{L}$ with sorters recording the least value. Also, Collectors and Sorters recorded higher values of monocytes levels ($0.79 \times 10^3/\mu\text{L} \pm 0.36 \times 10^3/\mu\text{L}$ and $0.88 \times 10^3/\mu\text{L} \pm 0.25 \times 10^3/\mu\text{L}$ respectively) above the reference upper limit of $0.70 \times 10^3/\mu\text{L}$ with sorters recording the highest value. A one-way ANOVA revealed that these differences in mean levels of immune/haematological parameter among the various groups (burners, dismantlers, collectors and sorters) were not statistically significant as shown in Table 6.

Table 6: Mean levels of immune/haematological parameters among the different categories of e-waste workers

Parameters	Total (N=40)	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	P-value (<0.05)
Haemoglobin level (g/dL)	13.55±1.45	13.41±1.41	12.71±2.07	14.13±0.60	13.95±1.05	0.118
Platelets ($\times 10^3/\mu\text{L}$)	236.48±77.62	230.80±59.10	270.70±92.45	250.20±89.35	194.20±50.53	0.145
White blood cell ($\times 10^3/\mu\text{L}$)	5.12±1.42	4.96±1.75	4.63±1.43	5.41±1.35	5.46±1.13	0.533
Granulocytes ($\times 10^3/\mu\text{L}$)	1.93±0.86	2.29±1.22	1.82±0.48	1.83±0.73	1.77±0.89	0.058
Lymphocytes ($\times 10^3/\mu\text{L}$)	2.47±0.77	2.18±0.82	2.21±0.83	2.69±0.88	2.80±0.37	0.635
Monocytes ($\times 10^3/\mu\text{L}$)	0.62±0.43	0.23±0.15	0.60±0.55	0.79±0.36	0.88±0.25	0.181

Values represent mean \pm standard deviation (S.D)

*Reference range:- Haemoglobin level (g/dL):13.00 – 17.00; Platelets ($\times 10^3/\mu\text{L}$):150 – 400; Granulocytes ($\times 10^3/\mu\text{L}$): 2.50 – 7.50; Lymphocytes ($\times 10^3/\mu\text{L}$): 1.30– 4.00; Monocytes ($\times 10^3/\mu\text{L}$): 0.15 – 0.70; White blood cell ($\times 10^3/\mu\text{L}$):5.00 – 10.00.

4.5 Blood concentrations of heavy metals (Pb, As and Hg) in e-waste workers

Overall, the total concentrations of heavy metals in blood were Pb (10.92 $\mu\text{g/L} \pm 8.88 \mu\text{g/L}$) and Hg (43.74 $\mu\text{g/L} \pm 72.05 \mu\text{g/L}$). Arsenic levels were below detection limit for all samples. The minimum blood Pb level was 2.80 $\mu\text{g/L}$ while the maximum level was 54.02 $\mu\text{g/L}$, minimum blood Hg level was 0 $\mu\text{g/L}$ while the maximum level was 321.70 $\mu\text{g/L}$.

Among the groups, dismantlers had the highest mean blood levels of Hg ($66.28\mu\text{g/L} \pm 109.62 \mu\text{g/L}$), with burners having the lowest ($7.52 \mu\text{g/L} \pm 6.86 \mu\text{g/L}$) as shown in Table 7. On the other hand, burners had the highest mean blood Pb levels ($14.02 \mu\text{g/L} \pm 14.48 \mu\text{g/L}$) with collectors having the lowest ($8.47 \mu\text{g/L} \pm 6.26 \mu\text{g/L}$) as shown in Table 7. A one-way ANOVA revealed that only differences in mean blood Hg levels among the groups were statistically significant ($p=0.003$) [Table 7].

Table 7: Mean concentrations of heavy metals in blood

Heavy Metals	Burners (N=10)	Dismantlers (N=10)	Collectors (N=10)	Sorters (N=10)	P-value (<0.05)
Pb ($\mu\text{g/L}$)	14.02 ± 14.48	12.26 ± 6.58	8.47 ± 6.26	8.95 ± 5.09	0.252
As ($\mu\text{g/L}$)	0	0	0	0	
Hg ($\mu\text{g/L}$)	7.52 ± 6.86	66.28 ± 109.62	43.51 ± 62.20	57.65 ± 65.75	0.003*

*Statistically significant at $p < 0.05$

4.6 Association between heavy metals (Pb & Hg) and immune/liver function parameters

Linear regression was used to evaluate the relationship between levels of heavy metals (Pb and Hg) and liver biochemical function and immune/haematological indices. As shown in Table 8, there was a negative relationship between blood Pb and Hg with levels of ALT ($\beta = -2.212$, $\beta = -0.205$ respectively), and no relationship with AST ($\beta = 0.002$, $\beta = 0.000$). GGT showed a negative relationship with Pb ($\beta = -0.040$) and no relationship with Hg ($\beta = 0.000$). However, there was no statistical significance between the different associations, and also, the levels of reduction or increment in the presence of Pb and Hg was low (Table 8).

With regard to immune/haematological parameters, blood Pb level showed a negative relationship with monocytes, but positive relationship with granulocytes and lymphocytes. This means that with a one unit increase in Pb level, we would expect 27 unit increase in lymphocytes levels and an 18 unit reduction in monocytes level as shown in Table 8. The reduction in monocytes was not statistically significant ($p=0.277$). Hg levels also showed negative relationship with granulocyte and monocytes, but positive relationship with lymphocytes (Table 8). Only blood Pb had a statistically significant association with blood lymphocytes counts ($p=0.049$) as shown in Table 8. Additionally, blood Pb level showed a high positive relationship with WBC ($\beta=7.467$) but blood Hg level showed a very low negative relationship with WBC ($\beta=-0.030$). Blood levels of Pb and Hg were both negatively associated with platelets ($\beta=-0.569$, $\beta=-0.066$ respectively), while levels of blood Pb and Hg were positively and negatively associated with the haemoglobin levels ($\beta=4.059$, $\beta=-0.152$ respectively) [Table 8]. The increase in Hb and WBC in association with Pb was not statistically significant ($p=0.479$, $p=0.180$ respectively).

Table 8: Linear regression analysis (crude) between blood Pb and Hg levels and immune/liver function parameters among e-waste workers

Parameters	Pb		Hg	
	β	P-value(<0.05)	β	P-value(<0.05)
AST (U/L)	0.002	0.287	0.000	0.670
ALT (U/L)	-2.212	0.151	-0.205	0.206
GGT (U/L)	-0.040	0.253	0.000	0.332
Haemoglobin level (g/dL)	4.059	0.479	-0.152	0.301
Platelets ($\times 10^3/\mu\text{L}$)	-0.569	0.650	-0.066	0.687
White blood cell ($\times 10^3/\mu\text{L}$)	7.467	0.180	-0.030	0.830
Granulocytes ($\times 10^3/\mu\text{L}$)	0.308	0.857	-0.081	0.401

Lymphocytes ($\times 10^3/\mu\text{L}$)	27.583	0.049*	0.050	0.759
Monocytes ($\times 10^3/\mu\text{L}$)	-18.621	0.277	-0.152	0.348

*Statistically significant at $p < 0.05$

Multiple linear regression analysis which adjusted for the effects of all the possible risk factors (type of recycling activity, hours worked in a day, alcohol consumption, recreational drugs intake, presence of illness, smoking habits and use of personal protective equipment during working) which were statistically significant after univariate analysis was used to determine the independent effect of Pb and Hg on the liver/immune function. Only Hg was found to be independently associated with blood Hb ($p=0.049$) as shown in Table 9. Effect of Hepatitis B on liver biochemical function tests was adjusted for, although it was not statistically significant after univariate analysis. The results of this model showed that the presence of Pb and Hg in the blood of the e-waste workers examined had no statistically significant association with the liver and immune function as shown in Table 9.

Lymphocytes levels on the other hand, had a statistically significant high positive association ($\beta=27.583$; $p=0.049$) with Pb after univariate analysis. After adjusting for other factors, this association was no longer statistically significant ($p=0.098$) [Table 9].

The association of almost all the immune/haematological parameters and Pb in both univariate and multivariate analysis showed high positive or negative increase with a one unit increase in Pb levels but not statistically significant as shown in Tables 8 and 9.

Table 9: Multiple regression analysis (adjusted) between blood Hg and Pb levels and liver/immune function parameters

Parameters	Pb		Hg	
	β	P-value (<0.05)	β	P-value (<0.05)
AST (U/L)	0.0002	0.884	0.000	0.305
ALT (U/L)	-0.650	0.685	-0.003	0.113
GGT (U/L)	-0.060	0.092	0.000	0.538
Haemoglobin level (g/dL)	2.021	0.701	-0.012	0.049*
Platelets ($\times 10^3/\mu\text{L}$)	-1.097	0.358	-0.001	0.668
White blood cell ($\times 10^3/\mu\text{L}$)	3.110	0.560	0.001	0.864
Granulocytes ($\times 10^3/\mu\text{L}$)	0.113	0.949	-0.002	0.417
Lymphocytes ($\times 10^3/\mu\text{L}$)	21.810	0.098	-0.001	0.950
Monocytes ($\times 10^3/\mu\text{L}$)	-7.957	0.614	-0.002	0.893

*Statistically significant ($p < 0.05$)

CHAPTER FIVE

5.0 DISCUSSIONS

Electronic waste (e-waste) contains toxic substances, including heavy metals and organic substances, and the use of inappropriate recycling methods could lead to release of these pollutants into the environment. Direct exposure of persons involved in e-waste recycling to these toxic substances can impact negatively on their health. In this study, levels of heavy metals (Pb, As and Hg) as well as indices of immune status and liver function were assessed in different categories of e-waste workers: Collectors, Sorters, Dismantlers and Burners at Agbogbloshie, a well-documented e-waste dumpsite.

Our demographic results were consistent with that of Akormedi et al. (2013) and Siddarth et al. (2010) who reported that in developing countries, e-waste recycling is mostly carried out by the poor and marginalized. The Northern Region of Ghana, from where almost all the e-waste workers migrated from is one of the poorest regions in Ghana. The poor economic conditions, compounded by past ethnic conflicts and chieftaincy disputes compelled many young men to migrate to the Capital, Accra in search of greener pastures.

About half (50%) of the e-waste workers sampled had no formal education, with collectors having the highest number of persons with formal education (80%). Suffice it to say that e-waste recycling at Agbogbloshie is informal and does not require that one has higher education. Many of the e-waste workers, who had knowledge of existing illness, complained of back and chest pains which probably can be attributed to the

rigorous and physical nature of e-waste collection and recycling activities: lifting, transportation of heavy appliances and dismantling.

Although Ghana is signatory to all the conventions of the International Labour Organization (ILO) that deal with the issue of working hours, overtime, overtime compensation and rest periods, their implementation in informal sectors especially in the e-waste recycling industry is non-existent due to its unregulated and informal nature (Siddarth et al., 2010). Thus, most e-waste workers do not have any fixed working time in terms of hours per day or per week. Majority of the recyclers in the Agbogbloshie scrap yard were found to work for 12 hours per day, and 6 days in a week. They also do not wear any personal protective equipment during the recycling activities which were consistent with previous findings (Akormedi et al., 2013; Siddarth et al., 2010).

Lead (Pb) was detected in the blood of all categories of e-waste workers examined, whereas As was below detection level of the instrument. Detectable levels of Pb were also measured in the urine of e-waste workers assessed at the current study site, Agbogbloshie by Asante et al. (2012). Overall, Hg had the highest mean concentration in blood ($43.74\mu\text{g/L} \pm 72.05\mu\text{g/L}$). Among the different categories of e-waste workers, burners recorded the lowest mean blood Hg but the highest mean blood Pb levels. The difference between the mean blood levels of Hg among the various categories of e-waste workers was also significant with dismantlers having the highest. The nature of recycling activity significantly impacts on the kind of contaminants e-waste workers are usually exposed to. For example high levels of Pb and Hg have been reported in environmental journals at the e-waste site by several authors (Asante et al., 2011; Bishop, 2011; Gabel, 2011; Johnson et al., 2009; Kyere et al., 2016).

In this study, dismantlers and burners recorded the highest blood Hg and Pb levels respectively. Consistent with this study, Kyere et al. (2016) recently reported that the highest degree of contamination was at working areas of burners and dismantlers. This is not surprising because among all categories of e-waste activities, dismantling and burning exposes workers and the environment to direct release of toxic chemicals.

The mean blood Hg level was approximately 3 times higher than the acceptable biological exposure limit established by the American Conference of Governmental Industrial Hygienists (ACGIH) for exposed individuals (ACGIH, 2012), indicating high Hg exposure among e-waste workers.

Previously, high levels of As was detected in the urine of e-waste workers, as well as in soil and ash at the same study site (Asante et al., 2011; Asante et al., 2012; Itai et al., 2014). Unlike the current study where blood was used, the studies cited used either environmental media or urine, which may explain the presence of As. Also, single doses of As are rapidly and extensively cleared from the blood via the kidney (Hall et al., 2006) thus blood concentrations are exceedingly low and are not detectable by conventional atomic absorption spectrophotometric techniques (Hall et al., 2006). The use of the Perkin-Elmer PinAAcle 900t atomic absorption spectrophotometer to analyse the blood serum for As by this study may have contributed to the non-detection of As in the blood. A study by Hall et al. (2006) which used a Perkin-Elmer Elan DRCII ICP-MS equipped with an AS 93+ autosampler to analyse the blood samples found blood As levels of 137.3 μ g/L in the blood of well users in Araihaazar, Bangladesh.

ALT, AST and GGT liver biochemical factors or indices measured showed that 7.5%, 12.5% and 10% respectively of the e-waste workers had levels above acceptable limits as defined by the Legon hospital laboratory reference range. High levels of toxic metals, PCBs and PBDEs have been measured in the soil, air and dust at Agbogbloshie (Atiemo et al., 2012; Bridgen, et al, 2008). Studies conducted among residents around the e-waste sites showed elevated levels of PCBs and brominated flame retardants (BFRs) in breast milk (Asante et al., 2011), heavy metals in urine (Asante et al., 2012), polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs), biphenyls (PCBs) in blood (Wittsiepe, 2015) and OH-metabolites of aromatic hydrocarbons in urine (Feldt et al., 2014). These toxins and heavy metals have been shown severally to be associated with liver malfunction (European Food Safety Authority, 2004; 2005; Gamble et al., 2006; Larsen, 2006; Xu et al., 2015). This might have contributed to the elevated levels of the liver biochemical enzymes found.

Prevalence of elevated levels of AST, ALT and GGT were highest among burners and dismantlers, who again recorded the highest mean serum levels. Dismantling and burning activities may increase exposure to higher levels of hepatotoxic pollutants as compared to collecting and sorting activities thus the difference in prevalence of liver biochemical levels among the groups. There was no statistically significant association between blood Pb or Hg levels and the liver biochemical factors measured. This is in contrast with previous studies that found associations between Pb and Hg and liver biochemical factors among mechanics, panel beaters, petrol attendants, battery chargers and motorcycle riders (Harrison, 2014; Lewis & Kosnett, 2014; Onyeneke & Omokaro, 2016). Electronic waste recyclers are exposed to multiple hepatotoxic contaminants such as heavy metals, PCBs,

PAHs, PDBEs, BFRs as well as biological hazards including hepatitis viruses and malaria parasites all of which can negatively impact on the integrity and function of the liver.

To assess the immune status of the study population, haematological parameters such as leukocytes, haemoglobin, platelets were measured. Overall, the mean granulocyte, haemoglobin and platelets counts were 1.93 ± 0.86 ($\times 10^3/\mu\text{L}$), 13.55 ± 1.45 (g/dL) and 236.48 ± 77.62 ($\times 10^3/\mu\text{L}$) respectively. Majority (82.5%) of the study population had granulocytes count below the lower limit of the reference value from the University of Ghana Hospital laboratory. Also, majority of the study population had mean WBC and Hb counts which were closer to the lower limit of the reference ranges. On the other hand, the mean monocyte count ($0.62 \pm 0.43[\times 10^3/\mu\text{L}]$) was closer to the upper reference value ($0.7 \times 10^3/\mu\text{L}$) limit with 45.0% of e-waste workers recording abnormal high values. This is in contrast to a study by Xu et al. (2015) which reported higher granulocytes and haemoglobin levels, but lower lymphocytes and monocytes counts among persons living near an e-waste dumpsite (e-waste exposed) compared to unexposed individuals. Several factors other than exposure to e-waste: such as race/genetic background, nutrition and lifestyle can impact on levels of immune/haematologic parameters, and this may explain the differences between the present study and that of Xu and co-workers (Xu et al., 2015). The negative impact of biological agents and chemicals on immune parameters has also been reported by several studies (Karagas et al., 2012; Lewis & Kosnett, 2014; World Health Organisation, 2007; Xu et al., 2015).

Of the six parameters measured for immune/haematological function, dismantlers presented the highest prevalence of abnormalities in haemoglobin, white blood cells and granulocyte levels. Also, the mean Hb level was below the reference lower limit. All the

dismantlers recorded abnormal granulocytes levels. This may suggest that dismantling activities increases exposure to toxic substances that affects the immune/haematological function as compared to the other groups of e-waste workers.

There was no statistically significant association between the levels of haemoglobin, platelets, white blood cells, granulocytes and monocytes with Pb and Hg. Thus, the results were similar to other studies where workers exposed to elemental mercury vapour failed to show consistent and/or marked changes in immune function parameters (Langworth et al., 1992).

Similar to findings by Li et al. (2013), lymphocytes counts were significantly associated with blood Pb levels after univariate analysis. The association of almost all the immune/haematological parameters and Pb showed higher or lower unit increase with a one unit increase in Pb levels. This is consistent with a study by Islam et al. (2011) where they found Pb to be a risk factor for chronic immune-related disease because it can precipitate atopy and autoimmune diseases.



CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

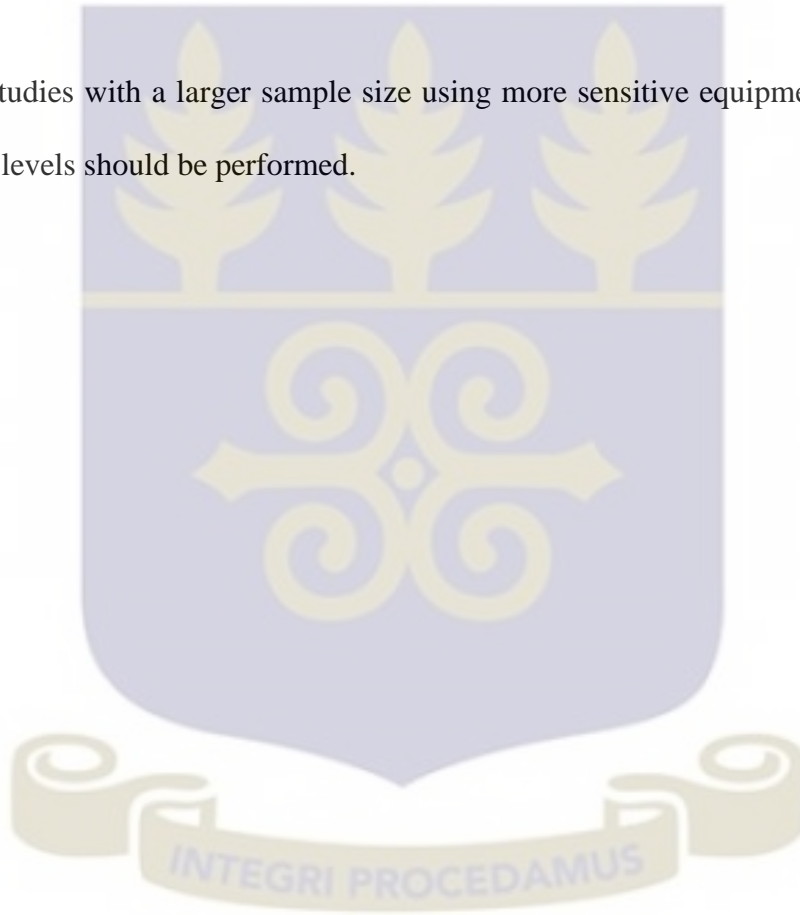
Occupational exposure to toxic substances including heavy metals can cause abnormal liver and immune/haematological function. This study has shown that e-waste workers who are involved in burning and dismantling activities have higher prevalence of abnormal liver biochemical function. The mean levels of AST, ALT and GGT for the various groups were normal. Immune/haematological function levels varied among burners, dismantlers, sorters and collectors, with dismantlers having the most abnormal levels of immune/haematological parameters. Though Pb was found in the blood of most e-waste workers, the levels were within normal range. Arsenic was not detected in any of the e-waste workers sampled. Even though occupational exposure to toxic/heavy metals especially Hg can lead to abnormal liver and immune function, there was no statistically significant association found between Hg and Pb and liver biochemical function or immune/haematological parameters in this study.

6.2 Recommendations

Improved methods of e-waste recycling should be encouraged to minimize the e-waste workers' exposure to health hazards and also regular and early biomonitoring to identify exposure to health hazards and their adverse effects should be instituted in the country. Workers' use of personal protective equipment should be enforced and supervised by the Ghana Labour Authority to ensure stronger adherence to the safety protocols, and thus

minimise the levels of exposure to these physical hazards. The Environmental Protection Agency (EPA) should incorporate the informal e-waste recycling activities into the national waste management program. The EPA can also assist the informal e-waste recycling sector with the construction of cost effective incinerators for safe burning of e-waste materials which can protect the e-workers and the general public from the harmful health effect of e-waste.

Finally, studies with a larger sample size using more sensitive equipment, especially for blood As levels should be performed.



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8.0 APPENDICES

8.1 Informed Consent Form

Title of study: Liver and immune function among e-waste recycling workers at the Agbogbloshie e-waste processing site in Accra.

Introduction

I am a master's student of the School of Public Health, University of Ghana, legon. As part of the academic programme, we carry out research work. My work is on the health effects of coming into contact with electronic waste (e-waste). E-waste recyclers in Agbogbloshie use the manual dismantling and open-air burning to retrieve some valuable metals which render them and the surrounding community to mixtures of poisonous substances such as fumes of lead, mercury and arsenic through breathing, by mouth and through the skin. These chemicals are known to have severe negative health effects on all organs in human body.

It is hoped that the findings of this study will help identify the association between coming into contact with e-waste and the liver/ body defense mechanism (immune) of the Agbogbloshie e-waste recyclers.

Purpose of the study

The study aims at assessing the health status and function of the liver and the body's defense mechanisms among e-waste workers at Agbogbloshie recycling site.

Eligibility criteria

Anyone who is 18 years or older and has been involved in the e-waste recycling process for not less than 6 months can take part in the study. Persons with severe anaemia or very

sick and those who are not willing or able to understand or comply with study procedures will not be allowed to be part of the study.

Study Procedure

A short interview will be conducted to obtain the contact information, age, sex, highest educational level, ethnicity, type of recycling activity one is engaged in, hours of work per week, use of protective clothing, location and distance of your work area from burning site, smoking habits, alcohol use and current medications that you are taking.

Blood will be collected from a vein in the arm of e-waste workers by the principal investigator (a medical doctor assisted by a laboratory technologist). Maximum of 15mls blood will be collected into 3 different tubes from an arm of your choice for analysis at the laboratory.

Risks and Benefits

This research will increase our understanding regarding the possibility of harm to the liver and/ or the bodies defense system when you come into contact with e-waste and also find the association between specific heavy metal (Pb, Hg and As) and liver/ bodies defense system. A better understanding of these relationships may provide information to put in place measures to protect the workers. Furthermore, this research may provide evidence to support the need for infrastructure in Ghana for the safe disposal of e-waste and also provide critical environmental and occupational health research practical for the principal investigator, Dr. Kwame Yeboah at the School of Public Health, University of Ghana. The laboratory tests will be done for free.

Possible risks/ complications of blood collection include bruising or swelling at the puncture site, accidental blood spillage, damage to underlying tissue and infection at the

puncture site. The risks of any serious complication occurring is very low. These risks will be avoided by ensuring strict hygienic practices by cleaning skin with alcohol swabs before blood is taken; using single-use disposable needles; using experienced persons for taking blood samples; removing tourniquet immediately after entering the vein; using clean dressing/plaster to cover the puncture site when needle is removed from vein; ensuring puncture site is away from vital/ important tissue; and also ensuring great care to prevent accidental spillage.

Freedom to participate/ Voluntary withdrawal

E-waste worker opinions and experiences are important to us, so we want you to be honest and truthful in answering our questions. Your participation is completely voluntary and you may refuse to participate at any time. You may ask me to stop the interview or blood sample collection at any point or you may also decline to answer any question if it makes you uncomfortable.

Privacy and Confidentiality

To ensure confidentiality and privacy we will not mark any of the samples with study e-waste worker's names; rather we will code numbers to the samples and keep an encrypted file that coordinates numbers to names on a secure laptop.

Protection of subjects' privacy

E-waste workers do not have to answer any questions that they feel are an invasion of their privacy. Also, subjects do not have to participate in any particular aspects of the study that they find invasive. Results from their immune and liver function test will be communicated directly to them through the contact details they will provide.

Additionally, client with abnormal test results will be assisted with a referral letter to the appropriate facility.

Provision to prematurely end a particular subject's participation in the study

E-waste workers can opt to be interviewed in a location of their choice to increase privacy. In the case of an adverse event or situation of distress, a subject's participation in the study will be concluded.

Compensation for e-waste workers

Compensation will be given at the time of data/specimen collection. Compensation is not payment for participating in this study but serves as a token of appreciation for e-waste workers' time as these workers will in a normal working day earn more than the compensation reward. A milo drink will be provided for all e-waste workers who agree to participate in the study. Additionally, a payment of GH¢ 10 will be given to study e-waste workers who complete all aspects of the study (questionnaire and blood draw).

Data storage and protection

All research records, data and blood (specimens) will be protected against inappropriate use or disclosure, or malicious or accidental loss or destruction. Data will be locked with restricted access on a secure laptop. There will be safe disposition/destruction of data or devices, as appropriate (e.g., shredding paper documents, secure erasure of electronic media) at the conclusion of the study.

The data and/or any specimens will be destroyed at the conclusion of this study.

Specimens of blood will be stored for a maximum of 2 weeks during the analysis period to enable us confirm inconsistent or abnormal results after which they will be destroyed

as well as the identifiers on their storage containers. Study survey forms (hard copy) will be destroyed at the conclusion of the study.

Declaration of conflict of interest

I Kwame Yeboah (Principal Investigator), declare that, to the best of my knowledge, there is no actual, perceived or potential conflict of interest that will or may arise as a result of my involvement with this study.

Who to contact

In cases of any questions regarding the research, you can contact:

- GHS/ Ethical Review Committee administrator, Hannah Frimpong (mobile: 0507041223)
- School of Public health, University of Ghana, Legon.

or

- Dr. Kwame Yeboah

Mobile number: 0244661187/ 0202640478

Email: kwameyeboah@outlook.com

Before taking Consent

Do you have any questions you wish to ask about the study? Yes No

(If yes, please, indicate the questions below).....

.....

Statement of consent

I, declare that the purpose, procedures to be followed, risks and benefits of the study have been read/ had been explained and every question(s) have been answered to my satisfaction. I hereby give consent to participate in this study.

Signature/Thumbprint of e-waste worker.....

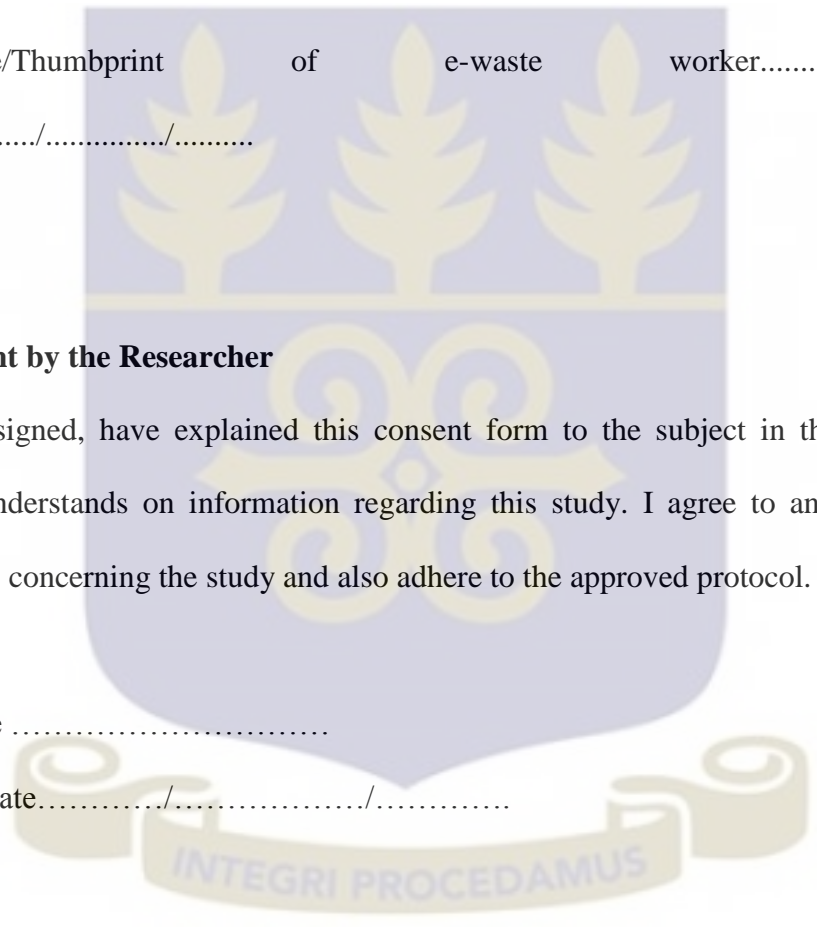
Date...../...../.....

Statement by the Researcher

I, undersigned, have explained this consent form to the subject in the language that he/she understands on information regarding this study. I agree to answer any future questions concerning the study and also adhere to the approved protocol.

Signature

Date...../...../.....



8.2 Questionnaire

My name isfrom the School of Public Health, University of Ghana. We are asking for your help in carrying out an important scientific study on the effect of electronic waste exposure on your immune/liver function.

This study will give you information about your health in relation to the job you are currently doing. Your participation is very important to the success of the study. All information that you give us will be treated with care and will not be released to anyone but researchers conducting the study. Confidential information will be stored in locked files accessible only to study staff.

We would administer a questionnaire and also take some blood and urine sample from you. Do feel free to skip any question in the form or stop at any point of the interview/procedure.

Please do you have any questions about the study?

Thank you for agreeing to participate in this important research project.

A: General Information

E-waste workers code:

Name of e-waste worker:.....

Contact information:.....

Name of interviewer:.....

Date of interview:

Place of interview:

B: Personal Information

1. Date of birth:/...../..... (DD/MM/YY) Age:.....

2. Sex Male Female

3. Ethnicity:

4. Highest level of education. No formal education Primary JHS SHS

C: Occupational Information

5. Which recycling activity do you undertake?

Burning Dismantling Collector Sorting

6. How long have you worked in this job?

7. How many hours do you work per day?

7b. How many days do you work per week?.....

8a. Do you use/wear any personal protective equipment (PPE) at work?

Yes No

8b. If yes, Tick (✓) what applies

Face / Eye protection e.g. safety glasses, face shield	
Hand protection e.g. safety gloves	
Body protection e.g. overall coat, aprons	
Foot protection e.g. safety boots	
Inhalation protection e.g. nose masks	
Head protection e.g. Helmets, hard hats	
Hearing protection e.g. ear plugs	

Other, please specify.....

D: Health in relation to your work

9a. Do you have any illness you know about? Yes No

9b. If Yes, please specify the illness.

9c. Was it diagnosed by a doctor? Yes No

E: Habits/ lifestyle

10a. Do you smoke? Yes No In the past

10b. If yes, how many sticks do you smoke per day?

10c. How long have you being smoking?

11a. Do you take alcohol? Yes No In the past

11b. How long have you being taking alcohol?

11c. Which type of alcohol do you usually take? Please tick (✓) as appropriate.

Spirits	
Bitters	
Beers	

Other, please specify.....

11d. How much alcohol (tots/ bottles) do you take per day?

12a. Have you used any recreational drugs in the last 3 months? Yes No

12b. If yes, please specify the type.....

Thank you for your time. Your participation is very much appreciated.

8.3 Tables from demographic characteristics and linear regression analysis

Table A: Demographic characteristics of study population

Characteristics	Frequency	Percentage (%)
Age (N=40)		
<20	11	27.5
20-24	13	32.5
25-29	8	20.0
>29	8	20.0
Type of PPE used (N=4)		
Hand protection (safety gloves)	2	50.0
Body Protection (overall coat, aprons)	1	25.0
Foot protection (safety boots)	1	25.0
Number of sticks smoked in a day (N=9)		
1-5	6	66.7
>5	3	33.3
Duration of Smoking (years) [N=9]		
1-5	7	77.8
>5	2	22.2
Duration of Alcohol intake (years) [N=9]		
1-5	7	77.8
>5	2	22.2
Type of Alcohol intake (N=9)		
Bitters	3	33.3
Beer	6	66.7
Alcohol intake per day (bottles) [N=9]		
1	3	33.3
2	5	55.6
3	1	11.1

Table B: Linear regression analysis to evaluate the association between immune/haematological parameters and other risk factors among e-waste workers

Variables	β (P-value)		
	Haemoglobin	Platelets	White blood cells
Age	0.232 (0.149)	0.044 (0.785)	-0.122 (0.452)
Highest educational level	0.069 (0.674)	0.021 (0.899)	0.016 (0.922)
Type of recycling activity	0.237 (0.141)	-0.227 (0.160)	0.181 (0.264)
No. of hours worked/day	0.133 (0.412)	-0.096 (0.558)	0.123 (0.451)
No. of days worked/week	0.162 (0.317)	0.241 (0.135)	-0.096 (0.555)
PPE use	0.233 (0.148)	-0.051 (0.754)	0.349 (0.027)*
Type of PPE used	-0.227 (0.159)	0.097 (0.551)	-0.342 (0.031)*
Presence of illness	-0.443 (0.004)*	0.203 (0.209)	0.438 (0.004)*
Smoking habit	-0.056 (0.730)	-0.236 (0.143)	-0.111 (0.495)
Number of sticks smoked	-0.361 (0.340)	0.055 (0.889)	-0.003 (0.994)
Alcohol intake	-0.165 (0.309)	-0.090 (0.579)	-0.324 (0.041)*
Recreational drug intake	-0.239 (0.137)	-0.107 (0.512)	-0.111 (0.495)
Hepatitis B surface antigen	0.105 (0.520)	-0.188 (0.246)	-0.082 (0.614)

*Statistically significant at $p < 0.05$



Table C: Linear regression analysis to evaluate the association between immune/haematological parameters and other risk factors among e-waste workers

Variables	β (P-value)		
	Monocytes	Granulocytes	Lymphocytes
Age	-0.215 (0.182)	-0.150 (0.357)	-0.132 (0.415)
Highest educational level	-0.031 (0.851)	-0.110 (0.498)	0.135 (0.408)
Type of recycling activity	-0.401 (0.010)*	-0.204 (0.207)	0.323 (0.041)*
No. of hours worked/day	-0.074 (0.649)	0.236 (0.142)	0.042 (0.798)
No. of days worked/week	-0.096 (0.555)	0.022 (0.894)	0.041 (0.804)
PPE use	-0.120 (0.462)	0.312 (0.050)	0.119 (0.467)
Type of PPE used	0.118 (0.468)	-0.309 (0.053)	-0.125 (0.444)
Presence of illness	0.438 (0.005)*	-0.046 (0.781)	-0.381 (0.015)*
Smoking habit	0.176 (0.277)	-0.085 (0.603)	-0.126 (0.440)
Number of sticks smoked	-0.072 (0.855)	0.414 (0.268)	-0.149 (0.702)
Alcohol intake	-0.063 (0.698)	-0.079 (0.627)	-0.329 (0.038)*
Recreational drug intake	0.193 (0.234)	0.115 (0.482)	-0.228 (0.156)
Hepatitis B surface antigen	-0.077 (0.639)	0.043 (0.794)	-0.147 (0.364)

*Statistically significant at $p < 0.05$



Table D: Linear regression analysis to evaluate the association between liver biochemical parameters and other risk factors among e-waste workers

Variables	β (P-value)		
	AST	ALT	GGT
Age	0.191 (0.238)	-0.009 (0.956)	-0.175 (0.280)
Highest educational level	0.241 (0.135)	-0.152 (0.349)	-0.089 (0.587)
Type of recycling activity	0.344 (0.030)*	-0.392 (0.012)*	0.267 (0.096)
No. of hours worked/day	0.280 (0.081)	-0.168 (0.301)	-0.200 (0.216)
No. of days worked/week	0.303 (0.057)	-0.224 (0.165)	0.001 (0.994)
PPE use	0.207 (0.200)	-0.265 (0.099)	0.276 (0.085)
Type of PPE used	-0.172 (0.288)	0.263 (0.101)	-0.313 (0.049)*
Presence of illness	0.025 (0.878)	0.156 (0.332)	-0.201 (0.215)
Smoking habit	-0.113 (0.494)	0.246 (0.126)	-0.108 (0.507)
Number of sticks smoked	-0.483 (0.187)	0.150 (0.700)	-0.021 (0.958)
Alcohol intake	-0.447 (0.004)*	0.398 (0.011)*	-0.232 (0.150)
Recreational drug intake	-0.217 (0.179)	0.324 (0.041)*	-0.322 (0.043)*
Hepatitis B surface antigen	-0.211 (0.191)	0.084 (0.608)	-0.246 (0.126)

*Statistically significant at $p < 0.05$



8.4 Ethical clearance from Ghana Health Service

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

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

*My Ref. GHS/RDD/ERC/Admin/App
Your Ref. No.*



Research & Development Division
Ghana Health Service
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Email: Hannah.Frimpong@ghsmail.org

11th March, 2016

Kwame Yeboah
University of Ghana
School of Public Health
Legon, Accra

ETHICS APPROVAL - ID NO: GHS-ERC: 79/12/15

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

“Liver and Immune Function among E-Waste Recycling Workers at the Agbogloshie E-Waste Processing Site in Accra”

This approval requires that you submit yearly review of the protocol to the Committee and a final full review to the Ethics Review Committee (ERC) on completion of the study. 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 three days verbally and seven 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 note that this approval is given for a period of 12 months, beginning 11th March, 2016 to 10th March, 2017. However, you are required to request for renewal of your study if it lasts for more than 12 months.

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

SIGNED.....


PROFESSOR MOSES AIKINS
(GHS-ERC VICE-CHAIRPERSON)

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