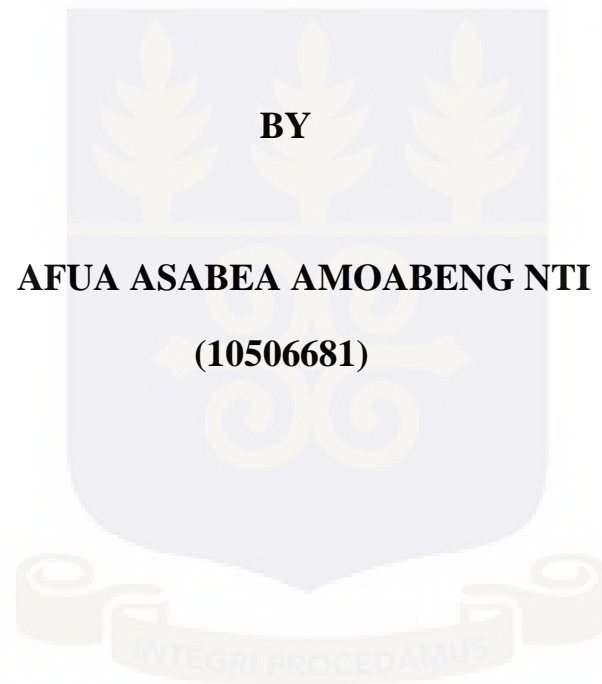


**SCHOOL OF PUBLIC HEALTH
SCHOOL OF GRADUATE STUDIES
UNIVERSITY OF GHANA**

EFFECTS OF E-WASTE ON RESPIRATORY FUNCTION AMONG E-WASTE WORKERS ENGAGED IN BURNING AT AGBOGBLOSHIE, ACCRA.



THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF SCIENCE IN OCCUPATIONAL MEDICINE DEGREE.

JULY, 2015

DECLARATION

I declare that except for the references to other people's investigations which have been duly acknowledged, this thesis is the result of my own research undertaken under the supervision of Dr. Reginald Quansah and Professor Julius Fobil. This work has not been submitted for the award of any other degree elsewhere.

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DEDICATION

I dedicate this thesis to my beautiful, intelligent and highly gifted ‘young ladies’ Awurama Sakaa Nti and Maame Afua Kusiwaa Nti. May God continually shed his light on your path and may you possess always the mind of Christ. Amen.



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ABSTRACT

Background: Open air burning of e-waste produces smoke, fumes and toxic gases such as furans, dioxins, brominated flame retardants and metallic dust which are deleterious to human health. High environmental and biological levels of heavy metals have previously been reported among e-waste workers at Agbogbloshie. However, no study has been conducted to investigate the effect of these contaminants on the respiratory function of the e-waste workers.

Objective: The primary objective of this study was to assess respiratory function (FEV₁, FVC, FEV₁/FVC) and the body burden of heavy metals such as cadmium, nickel, lead, manganese and chromium amongst e-waste burners at Agbogbloshie.

Methods: A cross sectional study was conducted from May to July 2015 among all 89 e-waste burners. Interviews, spirometry, blood and urine sampling were used to collect data from study participants. Analysis of the data for this report, however, was based on twenty- two e-waste burners whose data on heavy metals were available at the time of writing this report. Mean values and standard deviations of heavy metals and FVC, FEV₁ and FEV₁/FVC were computed. Proportions of respiratory symptoms were also computed. Multivariate linear regression analysis was used to assess the associations between heavy metals in blood and urine and indicators of respiratory functions.

Results: The mean age (SD) of e-waste burners was 22.4 years (7.02 years). Most (90.9%) of them were aware of the respiratory hazards associated with their work, however, over 72% of the workers used no personal respiratory protective equipment (PPE). The most commonly self-reported respiratory symptoms included easy tiredness (63.6%), and sore throat (50%). The remaining cited cold (36.4%), chest pains (45.5%), excessive phlegm (45.5%), and chronic

cough (37%). Mean concentrations (standard deviation) of the heavy metals ranged from bPb 119.16 $\mu\text{g/L}$ (47.10), bMn 9.61 $\mu\text{g/L}$ (3.23), uCd 0.39 $\mu\text{g/L}$ (0.02), uNi 8.77 $\mu\text{g/L}$ (1.26) and uCr 0.20 $\mu\text{g/L}$ (0.03). There was no significant association between the heavy metals and the measures of respiratory functions.

Conclusions: This study showed elevated levels of lead in the blood and nickel in the urine of the e-waste burners. However, there was no significant association between lung function parameters and heavy metals (Pb, Cd, Mn, Cr, and Ni).

Key words: E-waste, respiratory function, heavy metals, lung function, e-waste burning, Agobloshie.



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LIST OF ABBREVIATIONS

ACGIH –American Association of Governmental Industrial Hygienists

bPb – Concentration of Lead in blood

bMn – Concentration of manganese in blood

Cd - Cadmium

COPD –Chronic Obstructive Pulmonary Disease

CRT - Cathode Ray Tube

Cr – Chromium

DNA – Deoxyribonucleic Acid

EEE –Electrical and Electronic Equipment

EPA – Environmental Protection Agency

ETS – Environmental Tobacco Smoke

E- Waste – Electrical/ electronic Waste

FEV1 – Forced Expiratory Volume in one second

FVC – Forced Vital Capacity

HSPGs – Heparin Sulfate Proteoglycans

IARC – International Agency for Radiation Commission

MAK values – Maximum concentration at the workplace values

MVV – maximum ventilation volume

NEAP – National Environmental Action Plan

Ni - Nickel

OS – Oxidative Stress

PAH – Polycyclic Aromatic Carbons

Pb - Lead

PBDE - Polybrominated Diphenyl Ethers

PBDD/F -Polybrominated Dibenzo- p- dioxins /Furans

PC – Personal Computer

PCB – Polychlorinated Biphenyls

PCDD/F -Polychlorinated Dibenzo-p-dioxins and Dibenzofuranes

POP - Persistent Organic Pollutants

PVC - Polyvinyl chloride

ROS – Reactive Oxygen Species

SHS – Second hand smoking

SOD – Superoxide Dismutase

TEs – Trace elements

TLVs – Threshold Limit Value

UNEP - United Nations Environmental Programme

uCr - Concentration of chromium in urine

uNi – Concentration of nickel in urine

uCd – Concentration of cadmium in urine





CHAPTER ONE INTRODUCTION

1.1 Background of study

Worldwide, the generation of electronic waste peaks at close to 20-50 million tons per annum (UNEP 2005; Hussain, & Mumtaz, 2014), of which 75-80% is shipped to countries in Asia and Africa (Caravanos et al., 2011; Asante et al., 2012). Agyei Mensah & Oteng-Ababio (2012) attributes the increased generation of e-waste to economic growth, the increased consumption and production of Electrical and Electronic Equipment (EEE) and increasing urbanization and globalization. Electronic waste (i.e. e-waste) contains several heavy metals and organic compounds. For example, a typical component of a Personal Computer (PC) consists of phosphor, brominated flame retardants, hexavalent chromium, lead, mercury, cadmium, barium and plastics made from poly vinyl chloride (PVC) (Appendix 1).

The Basel Action Network (2002) calculated that nearly 500 million computers contain 287 billion kilograms plastics, 716.7 million kilogram lead and 286,700 kilogram mercury and over 50 constituents from the periodic table such as cadmium, copper, nickel, lead, barium, hexavalent chromium and beryllium (Akormedi et al., 2013; Hussain and Mumtaz, 2014). E-waste, hence, has become both an emerging global environmental health challenge on one hand and a tremendous business opportunity on the other because of the content of useful metals and other products generated (Agyei Mensah & Oteng-Ababio., 2012; Asante et al., 2012; Alabi et al., 2012). It is estimated that about 60% of fractions recovered from electronic waste are precious metals and 30% are plastics generated from e-waste recycling (Oteng-Ababio, 2012). Most of these compounds or heavy metals however, are toxic, persistent, mobile and bio-accumulate in tissues and organs (Boghe, 2001) and could undergo bio-transformation into

carcinogenic, mutagenic or teratogenic metabolites (Boghe, 2001), they have been associated with several adverse health outcomes deleterious to human health (Hussain and Mumtaz, 2014; Alabi et al., 2012; Chen et al., 2011; Akormedi et al., 2013; Allsopp et al., 2006; Asante et al., 2012; CDC, 2009; Frazzoli et al., 2009; Wright et al., 2008).

Presently, in Ghana, it is estimated that there is an annual increment of about 20 thousand tons of e-waste dumped into the country (Huang et al., 2014; Asante et al., 2012; Agyei Mensah & Oteng- Ababio, 2012). This is due to the unrestricted entry and lax regulations that allow the free entry of EEE under the guise of second hand goods or donations (Asante et al., 2012; Agyei Mensah & Oteng- Ababio, 2012).

Agbogbloshie (see map in figure 2) is the hub of e-waste recycling in Ghana, the biggest in West Africa, (MacDougall, 2012; Akormedi et al., 2013; Feldt et al., 2014), and ranked among the top 10 most polluted places in the world (<http://www.blacksmithinstitute.org>). Here, e-waste recycling is conducted using informal processes. Informal e-waste recycling involve collection of e-waste, manual sorting and dismantling of gadget components using basic tools such as hammers, screwdrivers and cutters and the open air burning of cables to retrieve metals such as gold, copper and aluminum (Dwuma- Badu, 2014; Akormedi et al., 2013). The dismantling and grinding processes generate metallic dust and toxic gases that invariably contaminate nearby water and food sources (Brigden et al., 2008; Caravanos et al., 2011; Song and Li, 2014b). Open air burning of plastic materials encasing the metals using old lorry tyres produces thick billows of smoke and fumes and the release of toxic gases such as furans and dioxins, brominated flame retardants, metals and beryllium compounds into the ambient air. High environmental levels of aluminum, copper, iron, lead and zinc were reported recently at the Agbogbloshie e-waste site (Caravanos et al., 2011; Chan & Wong, 2013; Huang et al., 2014; Feldt et al., 2014). Charles

Amegah-Selorm in his article “Dealing with e-waste in Ghana”, wrote concerning the Agbogbloshie dump site,

“Thick smoke coming from burning these discarded gadgets on the dump sites also affects the respiratory tracts of human beings causing serious health complications. Inhabitants claim they suffer from nausea, headache and respiratory problems.”

The deleterious effects of e-waste on human health are many and the human body burden of the various toxic fractions and their metabolites are well documented in studies conducted elsewhere (Lars, 2003; CDC, 2009; Lau et al., 2014; Jiang et al., 2014; Song and Li, 2014a). Informal e-waste recycling has been associated with several adverse effects on mental and physical health, violence and criminal behaviour and adverse neonatal outcomes (Wright et al., 2008; Chen et al., 2011; Grant et al., 2013).

Zheng et al. (2012) conducted a study on school children living near an e-waste dump site in China where they examined the association between lung function in the children and exposure to three transition metals from improper e-waste recycling. They reported high levels of Ni and Mn in the blood of these school children between the ages of 8-13 years. The concentrations of blood manganese (bMn) and serum nickel (sNi) in the exposed group were significantly higher than those in the control group for all the age groups. Also, the forced vital capacity value (FVC) of the boys aged 8-9 years was significantly lower than that of the control and concluded that the accumulation of bMn and sNi may be risk factors for oxidative damage and decreased pulmonary function.

To the best of my knowledge, no study has enumerated the effects of e-waste on respiratory function among the adult human population. Only one study has reported on the issue of e-waste

on the lung function among Chinese children (Zheng et al. 2012). There is the need therefore for further studies, especially among the e-waste workers.

1.2 Statement of the Problem

Informal recycling of e-waste involves manual dismantling of gadgets using basic tools with no protective equipment (Asante et al., 2012) and open burning of plastic casing to extract metals such as copper, gold and aluminum (Schmidt, 2006; Feldt et al., 2014). Many hazardous chemicals, including persistent organic pollutants (POP), lead, beryllium, cadmium, mercury, chromium and copper are released into the ambient air, water and soil (Weber et al., 2008; Atiemo et al., 2012; Zheng et al., 2012; Asante et al., 2013; Hosoda et al., 2014). Polyvinyl chloride, polybrominated Diphenyl ethers (PBDE), polybrominated dibenzo- p- dioxins /furans (PBDD/F), polychlorinated dibenzo-p-dioxins and dibenzofuranes (PCDD/F) and polyaromatic hydrocarbons (PAH), aluminum, copper, iron, lead, zinc and arsenic have been detected in urine or blood samples of e-waste workers at Agbogbloshie (Wittsiepe et al., 2015). Many other studies have associated informal e- waste scavenging to several adverse health outcomes such as a low intelligence quotient, violent behavior, poor academic achievement, criminal activity and many acute and chronic organ specific adverse effects (Allsopp et al., 2006; Cassius et al., 2008; Wright et al., 2008; Yapi et al., 2009; Frazzoli et al., 2009; CDC, 2009; Asare and Nani, 2010a; Chen et al., 2011; Asante et al., 2012a; Akormedi et al., 2013). Exposure to e-waste may lead to inflammation in the linings of the respiratory system due to sensitization or irritation leading to allergic reactions and consequently to reduce respiratory function, Asthma and other lung

diseases. (Fig 1) However, no studies have been conducted to investigate the effects of e-waste on the respiratory function especially among the adult population. This is the research question to be addressed in this study.

1.3 Conceptual Framework

As shown in figure 1 below, the manual processing of e-waste involves the collection, sorting, dismantling and open air burning or incineration to retrieve precious metals such as copper and gold (Akormedi et al., 2013). During the dismantling processes, the electronic gadgets are crushed using manual tools such as hammers, spanners and chisels (Akormedi et al., 2013). Heavy metal particulate dust is generated in the atmosphere and into the breathing zone of the workers. The burning of plastic castings to retrieve metals also release highly toxic dioxins, furans, PAH and POP and particulate metallic dust (e.g. Pb, Cd, particles) [Box 1]. Exposure to those pollutants may occur through ingestion of contaminated food and water. Also, dermal exposure may occur through contaminated clothing and direct deposition on the skin [Box 2] (Caravanos et al., 2013). Inhalation, however, is the main route of exposure among the workers (Zheng and Chen. 2013; Shen, Chen and Huang, 2009) [Box 2]. Depending on the size, the particles may be deposited into the nasal passage, pharynx, bronchial passage or the alveolar. Inhalation into the respiratory tract induces a hypersensitivity response leading to inflammation of the respiratory lining and giving rise to acute respiratory symptoms such as cough, cold, rhinitis, rhinorrhea, sore throat, chest pains, difficulty in breathing and wheezing with associated reduced lung function [Box 3] (Caravanos et al., 2013; Lars, 2003). Prolonged and chronic exposure may lead to asthma, bronchitis, lung cancer and Chronic Obstructive Pulmonary Disease (COPD) (Fig. 1.1).

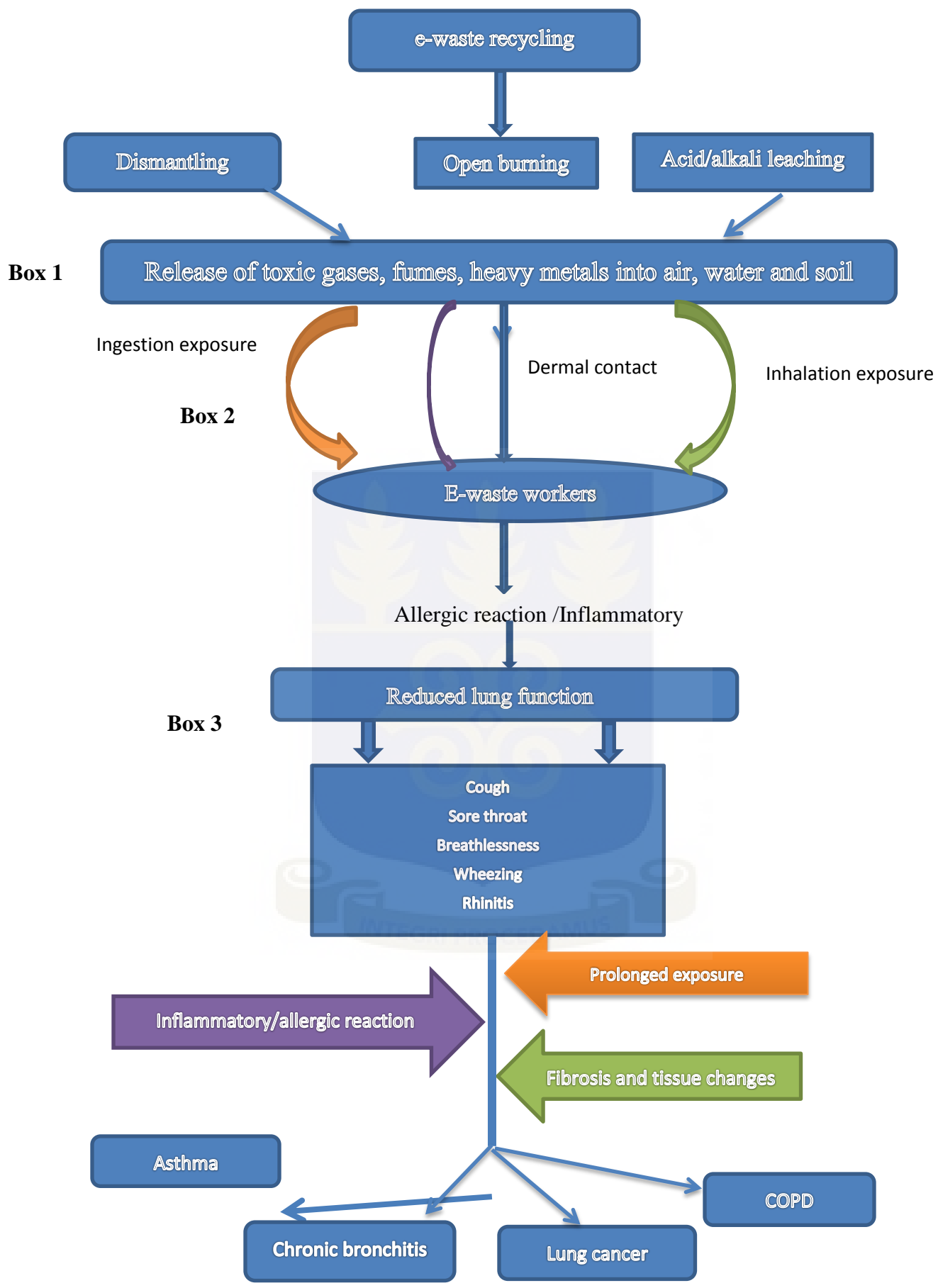


Figure 1.1: Pathway linking e-waste exposure to reduced lung function and respiratory diseases

1.4 Justification

E-waste scavenging is a critical source of financial support for many individuals and families. About 121,800- 201,600 people (0.4-1.72% of the urban population) are employed as collectors, recyclers in Ghana. Furthermore, the sector is said to contribute 105-268 million US dollars to the Ghanaian economy (Amoyaw-Osei et al., 2011). Many other second hand electronic repairers depend on the recycling processes to obtain parts for their repair businesses. The metallic components such as aluminum, gold and copper serve as raw materials for other industries. E-Waste recycling has huge economic potential and is a source of employment for the many teeming unemployed youth in Ghana (Amoyaw-Osei et al, 2011). There are various toxic chemicals and fumes released into the environment largely as a result of the informal recycling of e-waste and thereby polluting the air, water and food (Song and Li, 2014b). Such chemical emissions and fumes releases are of global public health importance for which policy intervention is urgently required to curtail the harmful effects to human health and the environment (Chan & Wong, 2013). The cumulative lifetime exposure to these hazardous chemicals to the workers and the community could lead to high cost in health care bills and environmental cost to both fauna and flora (Amoyaw-Osei et al, 2011). Whilst the Government could help these workers with new environmentally friendly technologies and trainings, there is the need to quantify the burden of diseases due to e-waste activities and to monitor environmental pollutants. Such information may be important for long term strategic measures to minimize exposure and improve the health of the teeming youth in this industry.

1.5 Study Objectives

1.5.1 General Objective

The general objective is to assess respiratory function (FEV₁, FVC FEV₁/FVC) and the body burden of heavy metals such as cadmium, nickel, lead, manganese and chromium amongst e-waste burners at Agbogbloshie.

1.5.2 Specific Objectives

- i. To find out the level of awareness of potential respiratory hazards associated with e-waste burning from the workers.
- ii. To determine a mean concentration of heavy metals in blood (Pb, Mn) and urine (Cd, Ni, Cr) of e-waste burners.
- iii. To determine the association between respiratory symptoms, lung function and concentration of heavy metals in blood and urine of e-waste burners.

1.6 Research Questions

- i. Is there an awareness of possible respiratory hazards associated with informal e-waste recycling among e-waste burners at Agbogbloshie?
- ii. Is there any difference in mean urinary nickel, chromium and cadmium and blood lead, manganese levels among e-waste burners at Agbogbloshie?
- iii. Is there any decline in respiratory function among e-waste workers engaged in e-waste burning?

- iv. Is there any significant association between lung function (FEV1, FVC, FEV1 /FVC), respiratory symptoms and blood, or urinary levels of heavy metals among e-waste burners?



CHAPTER TWO

LITERATURE REVIEW

2.1 Scope of the Review

The focus of this review was on the effect of mixtures of heavy metal contamination on respiratory function during informal e-waste processing. The health effects of mixtures of heavy metal contamination occur at the informal e-waste recycling site, in fact, more than 50 transition metals are known to be part of electronic gadgets (Ababio, 2012). The literature review will give a brief introduction to the legislations on e-waste both internationally and nationally (section 2.2), the importance of e-waste (section 2.3), the general health effects of improper e-waste processing (section 2.4), and the health effects of heavy metals on respiratory function with a focus specifically on Cr, Pb, Cd, Mn and Ni (section 2.5). Section 2.6 will discuss lung function and airflow limitation due to respiratory insults from informal e-waste recycling exposure. This will be followed by a short summary of the chapter.

2.2 Legislation on E-waste

The Basel and Bamako Conventions were promulgated following the toxic deposits of hazardous waste in Africa and other developing countries by the industrialized countries to control the trans-boundary movements of hazardous wastes and its disposal (Idaho, 2012). Ghana is signatory to these conventions. The 1992 Constitution of Ghana provides the broad basis for the protection of the environment in general. The Economic Development - Article 36 (9) and (10) mandates the state to take appropriate measures needed to protect and safeguard the national environment for posterity; and to seek co-operation with other states and bodies for the purposes of protecting the wider international environment for mankind and to safeguard the health, safety and welfare of all persons in employment, and establish the basis for the full deployment of the

creative potential of all Ghanaians (Constitution of Ghana, 1992). The National Environmental Action Plan (NEAP), which incorporates the Environmental Policy of Ghana, was published in 1991. The aim of the environmental policy is to improve the surroundings, living conditions and the quality of life both of the present and future generations. The policy requires the State to take appropriate measures to control pollution and the importation and use of potentially toxic substances (which include EEE). The Draft Bill on the Control and Management of e-waste, (2012) covers the control and management of hazardous waste in general. The Part Two of the Bill addresses E-Waste. The Bill is yet to be considered by Ghana's Parliament. When the bill becomes a law, a manufacturer or importer of electronic equipment will be required to register with the Environmental Protection Agency and pay electronic waste levy in respect of electronic equipment that is imported into the country or manufactured in the country (Parliament of Ghana website).

2.3 Importance of Recycling E-Waste

E-waste contains toxic heavy metals and hazardous chemicals, materials of strategic value such as indium and palladium and precious metals such as gold, copper and silver which can be recovered and recycled, thereby serving as a valuable source of secondary raw materials for other industries (SBC 2011; Akormedi et al., 2013). These materials could be used to make new products. This exercise would help to preserve natural resources, preserve energy, and reduce contamination and greenhouse gas emissions by extracting fewer raw materials from the ground. E-waste recycling is a source of financial reward for both the workers and the country as a whole. About 121,800-201,600 representing between 0.4- 1.72% of the urban population benefit directly or indirectly from e-waste activities. An estimated 105- 268 million US dollars accrue to the national economy annually (Amoyaw-Osei et al., 2011).

2.4: Health Effects of Improper E-Waste Recycling

Electronic products contain toxic chemicals which are detrimental to human health and the environment (Ha et al., 2009; Noel-Brune et al., 2013; Huang et al., 2014). Chemicals such as lead, cadmium, mercury, polyvinyl chloride (PVC), brominated flame retardants (BFRs), chromium, among others, are integral parts of components of Electrical and Electronic Equipment (Herat & Agamuthu, 2012; Santhanam, Melvin & Ramalingam, 2014). For example televisions, video and computer monitors use cathode ray tubes (CRTs), which have significant amounts of lead and the long term exposure to these substances can damage the nervous system, the kidney, the respiratory system, bones and the endocrine system (Liu et al., 2009; Wu et al., 2010) and the endocrine systems. Other studies have associated e-waste to adverse pregnancy outcomes such as low birth weight and learning difficulties in children (Chen et al., 2011). Likewise, more or less of the toxicants such as lead and PCBs are either cytotoxic, genotoxic, carcinogenic, mutagenic or teratogenic (Wang et al., 2011; Ni et al., 2013). Improper recycling of e-waste has long lasting repercussions on the environment; contaminating the top soil, water and air (Wang et al., 2011; Asante et al., 2012; Feldt et al., 2014). Often, these hazards arise due to the improper recycling and disposal processes employed and are a constant threat to communities close to the dumping sites (Grant et al., 2010; Yang et al., 2013). Among children living close to e-waste sites in China and Nigeria, high levels of blood lead were reported (Njoroge, 2007; Chen et al., 2011). Similar surveys have been attempted at various e-waste sites (Faller et al., 2001; Song and Li, 2014; Feldt et al., 2014). There are several mixtures of heavy metals contamination from e-waste recycling, thus the resulting health effects may be additive.

2.5 Respiratory Health Effects of Heavy Metal Exposure

2.5.1 Lead

The general population is exposed to lead from air and food in roughly equal proportions (Lars, 2003). A major source of lead emissions into ambient air originate from leaded petrol and more recently e-waste processing. According to the US-EPA (2008), an old CRT television contains 1.5-2.0 kg of lead. Lead is used for soldering on printed circuit boards. In adults, nearly 50% of inhaled lead is taken up in the lungs, whereas similar percentages of absorption occur in the gastrointestinal tract (GIT) of children (Lars, 2003). Also, the Blood–Brain Barrier (BBB) in children is under-developed and easily permeable to lead. The high GIT absorption and the permeability of the BBB make children highly susceptible to brain damage (Schümann, 1990; Lars, 2003). Penetration through the skin and brain may occur in adults resulting in lead encephalopathy from acute poisoning (Lars, 2003). Lead binds to erythrocytes and are transported into the bone where it bio-accumulates with a half-life of 20-30 years (Lars, 2003). Lead is eliminated slowly in the urine (Lars, 2003). It is one of the most studied developmental neurotoxicant and a major toxicant in e-waste processing (Barbosa et al., 2005; Chen et al., 2011).

Zheng et al., (2012) and Ha et al., (2009) observed high mean levels of lead in the blood of children living close to an e-waste site compared to those children in a central site. Lead has been associated with impaired cognitive function, behavioral disturbances, attention deficits, hyperactivity and behavioral problems in children (Chen et al., 2011). It also causes blood dyscrasias, and kidney damage (Shih et al., 2007). Lead compounds suppress the activity of

superoxide dismutase and total antioxidant capacity and increase serum malonaldehyde in blood and lung tissue with accompanying inflammatory response in the lung tissue (Li et al., 2013).

2.5.2 Manganese and Nickel

Nickel occurs naturally in the earth's crust and natural exposure may occur through volcanic eruptions and wind blowouts. Anthropogenic exposure occurs through industrial activities such as nickel mining, smelting, waste incineration and cadmium-nickel battery manufacturing (ATSDR, 2008).

Occupational exposure to nickel may occur by inhalation or dermal contact of aerosols, dusts, fumes or mists containing nickel (Angerer & Lehnert, 1990). Dermal contact may also occur with nickel solutions, such as those used in electroplating, nickel salts, and nickel metal or alloys. Also, nickel-containing dust may also be ingested in the face of poor work practices or poor personal hygiene (Angerer & Lehnert, 1990). Water soluble nickel compounds have greater affinity for the lungs and easily pass into the lungs through the blood and subsequently dissolved into the bloodstream (Angerer & Lehnert, 1990).

Common health effects of nickel exposure include allergic reactions such as skin rashes at the site of contact (ATSDR, 2005). Other harmful health effects from exposure to nickel include chronic bronchitis, reduced lung function, and cancer of the lung and nasal sinus. The International Agency for Research on Cancer (IARC) has found out that some nickel compounds are carcinogenic to humans and that metallic nickel is a possible carcinogen to humans (IRAC, 1986, 1990).

Manganese is known to be a health hazard to workers at high doses, but its effects at low doses is unclear (ATSDR, 2005). There are three major targets for toxicity: the brain, the

lungs, and the testes. At high doses, manganese causes a severe, degenerative neurological condition known as Manganism. Inhalation of manganese is toxic to the lungs, and produces an inflammatory reaction that increases susceptibility to pneumonia and bronchitis. Low-level air exposures have been reported to increase the prevalence of respiratory symptoms (phlegm, wheezing, sore throat) in school children (WHO, Europe, 2001). Recently, Zheng et al. (2012) conducted a study examining the relationship between three transitional metals and lung function among school children in schools close to an e-waste recycling area in China. They reported high levels of Nickel and Manganese in the blood of the school children. The concentrations of blood manganese (bMn) and serum nickel (sNi) in the exposed school children were significantly higher than the unexposed children across all the age groups. Also, the forced vital capacity value of boys aged 8-9 years was significantly lower than that of the unexposed children and so concluded that the accumulation of bMn and sNi may be risk factors for oxidative damage and decreased pulmonary function.

2.5.3 Chromium

Chromium is used to coat some electronic devices against corrosion. Chromium is a known human carcinogen (Pellerin & Booker, 2000). Increased urinary-8-hydroxyl-2-deoxyguanosine, a biomarker for oxidative DNA lesions were reported in children who were exposed to cadmium (Wong et al., 2005). E-waste recycling can result in high exposures with mean cord level of 99 μ g/L in fetuses (Li et al., 2008). Exposure causes strong allergic reaction connected to asthma, bronchitis, DNA aberrations and reduced respiratory function.

2.5.5 Cadmium

Cadmium (Cd), a by-product of zinc production is one of the most toxic elements to which man can be exposed at work or in the environment. Once absorbed, cadmium is efficiently retained in the human body, accumulating throughout life. The cadmium is primarily toxic to the kidney, especially in the proximal tubular cells, the main site of accumulation. cadmium can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. Occupational exposures to cadmium may impair lung function and increase the risk of lung cancer (Bernard, 2008). Environmental exposure to cadmium is known to cause damage to alveolar epithelial cells of the lung, impair their capacity to repair, and result in permanent structural alterations (Bernard, 2008). Cell surface heparin sulfate proteoglycans (HSPGs) modulate cell responses to injury through their interactions with soluble effector molecules (Bernard, 2008). These interactions are often sulfate specific, and the removal of sulfate groups from HS side chains could be expected to influence cellular injury, such as that caused by exposure to cadmium. Oh et al. (2014) reported that blood cadmium levels was associated with a decline in the lung function with increasing COPD trends in males when they conducted a cross-sectional study to determine the relationship between Cd levels and COPD. Chen et al (2014) also discovered that cadmium induces cytotoxicity in human bronchial epithelial cells through up-regulation of eukaryotic translation initiation factor (EIF5AI) in cell lines in the lab. Zhang et al (2012) sought to define the role 6-O-sulfate plays in cellular responses to cadmium exposure in two pulmonary epithelial cancer cell lines (H292 and A549) and in normal human primary alveolar type II (hAT2) cells. They noticed that heparin sulfate-1 sensitized these cancer cells and intensified the injury induced by cadmium suggesting that 6-O-sulfate groups on HSPGs may play important roles in protecting against certain environmental toxicants, such as

heavy metals. The finding indicated that cadmium decreased cell viability and activated apoptosis at low concentrations (Zhang et al., 2012).

2.6 Mixtures of Heavy metal exposure and respiratory function

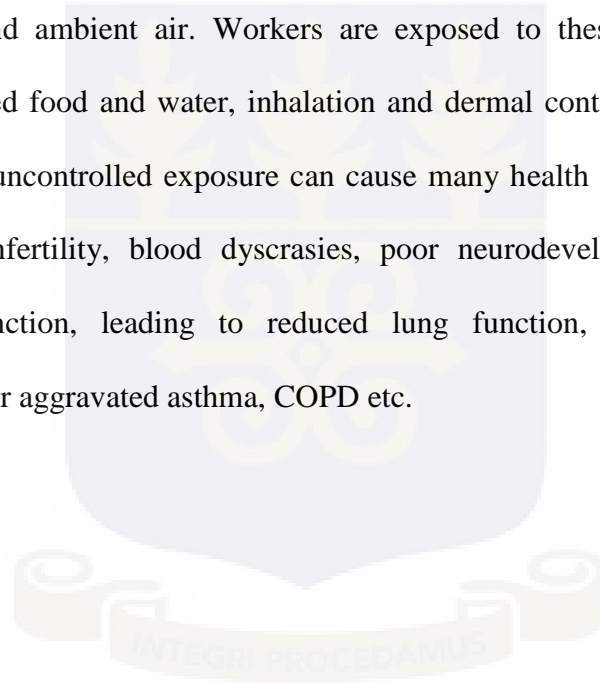
There is evidence linking occupational and environmental exposures to dusts, gases/vapours, and fumes to chronic airflow destruction, particularly exposure to mineral dust such as coal mine dust, silica, cadmium, and asbestos (Hnizdo et al., 2002; Meldrum et al., 2005). The American Thoracic Society (ATS) in 2010, issued a statement that more than 20% of both asthma and COPD is likely to be work-related whilst the National Health and Nutrition Examination Survey (NHANES III) attributed 19.2% of COPD prevalence of workplace exposures (Hnizdo et al., 2002).

Rodríguez et al. (2008) undertook a cross-sectional study of 185 male COPD patients who had baseline spirometry done and answered a questionnaire containing information on respiratory symptoms, hospitalizations for COPD, smoking habits, current employment status, and lifetime occupational history. They also assessed exposure to biological dust, mineral dust, gases and fumes using an exposure matrix and concluded that occupational exposures are independently linked with the severity of airflow limitation, respiratory symptoms, and work inactivity in patients with COPD.

Zock et al. (2005) studied the relationships between specific occupations and occupational exposure (vapors, gas, dust, or fumes); changes in lung function and symptoms of chronic bronchitis among selected young people between ages 20-45 years from the general population over a 9-year follow-up period. They noted that people exposed to dusts, gases, and fumes during

the period of follow-up did not cause a steeper decline of FEV1 than did people with consistently white-collar occupations without occupational exposures. They reasoned out that occupational exposures to dusts, gases and fumes are associated with incidence of chronic bronchitis, but did not impair lung function in this youthful population.

In summary, informal e-waste recycling release various toxic chemicals, including mixtures of heavy metals such as Pb, Ni, Cr, Cd, and Mn. These toxic chemicals discharged into the environment during the tearing down and open burning processes, contaminate the food chain, water sources and ambient air. Workers are exposed to these contaminants through ingestion of contaminated food and water, inhalation and dermal contact in the course of their work. Unprotected and uncontrolled exposure can cause many health effects including adverse pregnancy outcomes, infertility, blood dyscrasies, poor neurodevelopmental outcomes and adverse respiratory function, leading to reduced lung function, increasing the risk of occupationally induced or aggravated asthma, COPD etc.



CHAPTER THREE

METHODOLOGY

3.1 Overview

Chapter three outlines the various methods used in this study; giving descriptions of the study area, study design, sampling methods, study procedures, data collection tools and data analysis as well as ethical considerations.

3.2 Study Area

Agbogbloshie e-waste disposal site (see map in fig. 2 overleaf) is one of the biggest e-waste recycling site in Sub-Saharan Africa (Akormedi et al., 2013). The site encompasses approximately 15 acres of ground and is located west to the Odawna River and neighbouring to the busy Agbogbloshie market.

Eastward to the e- waste site at Agbogbloshie are various businesses that employ many people such as banks, pharmaceutical companies, breweries, shops, various manufacturing companies, as well as many self-employed and petty traders.

Located south to the e-waste site is a large residential community (about 100 meters across the Odaw River) that primarily houses Agbogbloshie workers and their families. This densely populated community consists of small, informal wooden settlements with little or no social amenities. Akormedi et al. (2013) reported that approximately 6,000 families live in this residential area. Thick bellows of irritating fumes and smoke from the burning of plastic materials to retrieve copper wires and other useful products by the e-waste workers inundate the

air continuously, contaminating the atmosphere and compromising the health of the many people who live, work or visit Agbogbloshie.



Figure 2: Map showing Agbogbloshie scrap yard, Accra. Source: Amoyaw- Osei, (2011).

3.3 Study Design

An analytical cross-sectional study was carried out from May to July 2015 to assess the effects of e-waste exposure on the respiratory function among e-waste workers engaged in burning at Agbogbloshie.

3.4 Source/ Study population

The source population included all 3000 e-waste workers at the Agbogbloshie scrap yard who were engaged in the various processes involved in e-waste recycling (personal communication, with the Chairman of e-waste association) of which 120 were burners. 89 burners available at the time of the study were all recruited to provide blood and urine samples, performed lung function tests as well as completed a respiratory questionnaire. However, at the time of writing the report,

data was available for only 22 of the burners. The analysis of the study thus would be based on the 22 burners.

3.5 Determinants and outcomes of interest

The main determinants of interest included urine Cd, Ni, Cr levels and blood Pb and Mn levels. The outcomes of interest were measures of lung function (e.g. FEV1, FVC, and FEV1/FVC) and respiratory symptoms including cough, cold, sore throat, phlegm production, breathlessness, chest pain, wheezing, itchy ear, eyes, itchy throat conditions, chest tightness and difficulty in breathing. The confounding factors were age, cigarette smoking, alcohol intake, educational level, home exposures and marital status. The selected confounding variables were based on literature (Olsson et al., 2002; Rodríguez et al., 2008).

3.6 Study procedures

All 89 e-waste burners available were actively recruited from the Agbogbloshie electronic waste site into the study. Recruitment was done randomly at various sites along the Odaw River where burning predominantly occurs. The selected participants were then sent to a mobile clinic erected on the premises of Bamson Company Limited (Sikkens) a few hundred metres away from the e-waste site for sample collection and questionnaire administration. Eligibility criteria for study participation included willingness to participate in the study, consenting and signing a consent form and employment as e-waste worker at Agbogbloshie for at least 6 months. Those who were unwilling to give consent or had any medical or surgical contraindications were excluded from the study.

After a thorough explanation of the study to the participants and receipt of informed consent, the participants completed the questionnaire administered by trained research assistants in the

language best understood by the participants with the help of interpreters were needed. The interview addressed health issues focused on respiratory health, lifestyle and habits, workplace hazards and exposures as well as health and safety at the workplace.

Following this, the participants undertook lung function measurement using spirometry performed by a qualified clinician. The forced vital capacity (FVC), Forced Expiratory Volume in one second (FEV1) and Maximum Ventilation Volume (MVV) were measured.

The participants then had their blood samples taken by qualified laboratory technicians after which they were directed to wash their hands with soap under running water before they were provided with urine containers for their urine samples.

3.7 Data Collection Tools

The data collection tools included a modified respiratory questionnaire, spirometer for lung function measurement, urine sampling kit for urine sampling, heamatology tubes for blood sampling, a weighing scale and a stadiometer for standing height measurement.

3.7.1. Questionnaire

The Interview questionnaire was in four parts. The first part ascertained information on demographic and personal data such as age, marital status (single, married, co-habiting), and highest level of formal education (No Formal Education, Primary, Junior High School, Senior High School, Part 2 asked information pertaining to work and this included the type of work (e-waste worker, non e-waste worker), job role (burning, dismantling, sorting, collecting, hawker etc.), length of service (6 month to 1 year, 2-4 years, 5-7 years, and >8 years), type of exposures at work and home (dust, smoke, liquids, and fumes) training and health and safety issues. Part 4 was about life style habits (smoking, alcohol intake, and diet) and part 5 was about respiratory

symptoms (cough, cold, sore throat, phlegm production, itching eyes, wheezing, and shortness of breath).

3.7.2 Spirometer

The highly portable Spirolab III and pulse oximeter spirometer (MIR, Italy) with a built-in printer and a coloured LED screen was used for the measurement of lung function (FVC, FEV1 and FEV1/FVC ratio). The Spirolab III spirometer is a portable, easy to read and use, rechargeable, battery powered equipment with disposable mouthpieces. Its major advantages are: the functions can be easily accessed using the high-definition color touchscreen; it offers a built-in high-resolution printer and the option to print on external printers using a USB connection and the full flow volume and volume time curves allow for inspection of maneuvers as you follow test-quality prompts on the screen. An additional advantage is that it can store up to 6,000 readings. The spirometer was used in conjunction with the Marsden HM-250P Leicester height measure (Marsden, UK) and the Tanita BWB800S weighing scale (Arlington, USA) for the measurement of weight and height required for performing accurate spirometry.

3.7.3 Urine specimen collection kit

Non-sterile, metal-free plastic urine containers (Sarstedt S-monovette, Germany) were used to collect 50 ml of urine specimen from study participants.

3.7.4 Blood specimen collection kit

Three 7.5 ml of whole blood haematology tubes two free and one containing Z-gel, an additive carrier and a clot activator (for serum separation) capable of storage at -20°C were used for the

blood sample collection. Butterfly needle (Sarstedt, S-monovette, Germany), was used for venipuncture and a tourniquet to improve vein visibility.

3.8 Sample Collection Procedure

3.8.1 Urine Sample Collection

The workers were provided with clean water and soap for hand washing before handing out to them sterile metal-free plastic urine containers for urine collection. They were instructed to void out the first portion of the urine stream before collecting 50 mls midstream urine into the plastic urine container. 10 ml of the urine were then drawn into four sterile sample tubes (Sarstedt, S-monovette, Germany) and stored in cool boxes containing ice packs (at 4-8 °C) and transported to the Noguchi Laboratory for storage at -20°C. Later, the samples were shipped to the Occupational and social medicine laboratory RWTH University Aachen, Germany for biological sample analysis for estimation of heavy metal concentration.

3.8.2 Blood Sample Collection

Following explanation of the test procedure, 7.5ml of whole blood was collected from the median cubital and cephalic veins into three separate haematology tubes (Sarstedt, S-monovette, Germany), two free and one containing Z-gel, an additive carrier and a clot activator(for serum separation) using a butterfly needle and a tourniquet. The blood samples with the additive were then centrifuged and the supernatant, the serum collected for subsequent refrigeration at 4-8 °C for onward transportation to Germany for analysis.

3.8.3 Lung function measurement (Spirometry)

Lung function measurements were performed according to the guidelines of the American Thoracic Society/ERS (1995). Standing height in centimeters and weight in kilograms were measured prior to the performance of the lung function test as measurement of stature is a prerequisite for the determination of the normal lung function and reference equations are based on stature (standing height) Renstrøm et al., 2012). The Leicester stadiometer was used to measure height of the participants in the standing position without shoes on and the shoulders in an upright position. After data entry, the participants were assisted to perform manouuvres for measuring forced vital capacity, vital capacity and maximum ventilation volume. Computation of lung function volumes were based on variations in the height, weight, sex and ethnicity of respondents. For ATS/ERS standards, the ethnic correction of 87% for African descent was applied to the predicted values of the following parameters: FVC, FEV1, FEV3, FEV6, VC (spirolab III – User Manual Code MIR 980067 REV 2.0 Page 39 / 74).

3.9 Laboratory analysis

3.9.1 General laboratory practices

Calibrated analytical balances and pipettes were used in measuring all samples as well as standard reference materials (round robin materials). All materials and instruments were washed with 1M nitric acid and demineralized water prior to analysis.

3.9.2 Urine Sample analysis for heavy metals (Cd, Ni, and Cr)

Urine samples were pretreated with nitric acid and triton. Samples were analyzed using high resolution continuum source atomic absorption spectrophotometry (HRCS-AAS ContrAA700, Analytik Jena) at the Institute of Occupational and Social Medicine Laboratory RWTH University, Aachen, Germany for nickel, chromium, and cadmium according to methods of the DFG (Angerer, 1982; Fleischer, 1988). See appendix 4 for the procedure for analysis of Cd, Ni and Cr.

3.9.3 Blood Sample Analysis for heavy metals (Pb and Mn)

The HRCS-Atomic Absorption spectrophotometer (HRCS-AAS ContrAA700, Analytik Jena) was initially, thoroughly cleaned with methyl alcohol (meOH) followed by washing of the beakers and pipettes with 1M HNO₃ and demineralized water. The blood samples were thoroughly mixed using a vortex for about 10-20 seconds. 100 μL of triton (C₈H₁₇C₆H₄(OCH₂CH₂)_nOH) was pipetted into a sample holder followed by 100 μL of the blood sample. The Triton reduces the surface pressure of the red blood cells to aid pyrolysis and atomization. The mixture was mixed thoroughly and loaded into the atomic absorption spectrometer together with a standardized sample (round robin standardized material for occupational and environmental ranges) for quality assurance. The desktop based programme was then activated for the analysis of the sample. See appendix 4 for complete procedure for analysis of individual metals in blood.

3.10 External quality control

The following measures were undertaken to ensure that the data collected were of superior quality so as to assure its validity: Research assistants received training in questionnaire

administration, urine and blood sampling and spirometry performance. Participants were asked to wash their hands with soap under running water before urine sample collection. Urine samples were packed in cool boxes containing ice packs to maintain the integrity of samples sent to laboratory for storage. The spirometer was disinfected with mild soapy solution before and after daily use and kept dry and clean.

The laboratory participated regularly and successfully at the German external quality assessment scheme (EQUAS) for analyses in biological materials of the Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg, Germany and at the EQUAS of INSTAND e.V., WHO Collaborating Centre for Quality Assurance and Standardization in Laboratory Medicine, Düsseldorf, Germany. (<http://www.eu-hbm.info>, Angerer et al., 2007, Angerer et al., 1997, Angerer and Schaller, 2008)

3.11 Data Processing and Analysis

The Data were crossed checked to identify missing values and to correct inconsistencies in the data. Data entry was done using Epi- Info 7 (Centre for Disease Control, CDC, USA).

For continuous variables, means and standard deviations were computed and for categorical variable proportions were computed also. Correlation analysis was used to compare the relationship between heavy metal levels and measures of lung function. Multiple regression analysis models were carried out, controlling for potential confounders. All data analyses were undertaken using SPSS® v.16 (IBM, Armonk, USA and Excel v14 (Microsoft, Redmond, USA) spreadsheet.

4.11 Ethical Considerations

Ethical clearance was sought from the Ethical Review Board of the Ghana Health Service. Permission was also sought from the leaders of the community and the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana. Oral or written consent was obtained from every participant. Before the individual respondent gave consent, the participant information leaflet and the consent form, which contained the benefits, risks and the procedures for the research was read out and explained to each participant before they appended their signatures or thumbprints. They had the liberty to ask questions, and to seek clarifications or withdraw unconditionally.



CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of participants

The demographic characteristic of the participants is shown in **Table 4.1** below.

Table 4.1: Demographic Characteristics of E-waste Burners (n=22)

Characteristics of Study Participants	Mean	Standard deviation
Age (years)	22.40	7.02
Height (cm)	167.50	6.02
Weight (kg)	65	10.01

	Frequency (N= 22)	Percentage (%)
Gender		
Male	22	100
Marital Status		
Single	16	72.7
Married	6	27.3
Highest level of Education		
No formal education	14	63.6
Primary	7	31.8
Secondary	1	4.5
Region		
Ashanti	2	9
Northern	19	86.4
Upper East	1	4.5
Ethnic Group		
Asante	2	9.1
Dagomba	17	77.3
Frafra	1	4.5
Gonja	2	9.1
Alcohol use		
User	3	13.6
Non-user	19	86.4
Alcohol use		
User	3	13.6
Non-user	19	86.4
Cigarette smoking		
Non-smoker	4	18.1
smoker	18	81.8

The age of the e-waste burners ranged from 16 to 45 years with a mean (standard deviation) of 22.4 (7.02) years. The height also ranged from 158 to 183cm with a mean (standard deviation) of 167.5 (6.02) cm; and the weight also ranged from 48 to 88 kg with a mean (standard deviation) of 65 (10.01) kg. All the participants were males, mostly singles (72.7%) with no formal education (63.6%) and mostly from the northern region (86.4%) and of Dagomba tribe (77.4%). 59.1% (n=13) smoked cigarette while 22.7% were previous smokers. 86.4% were non-users of alcohol whilst 18.1% were users. Most of the participants (59.10%) had engaged in e-waste burning for more than 5 years and 4% had worked for 6 months, about 28 % had worked for 4 to 5 years, 9% had worked for 2 to 3 years and approximately 4% had worked for 6months to 1 year (**Fig. 4.1**).

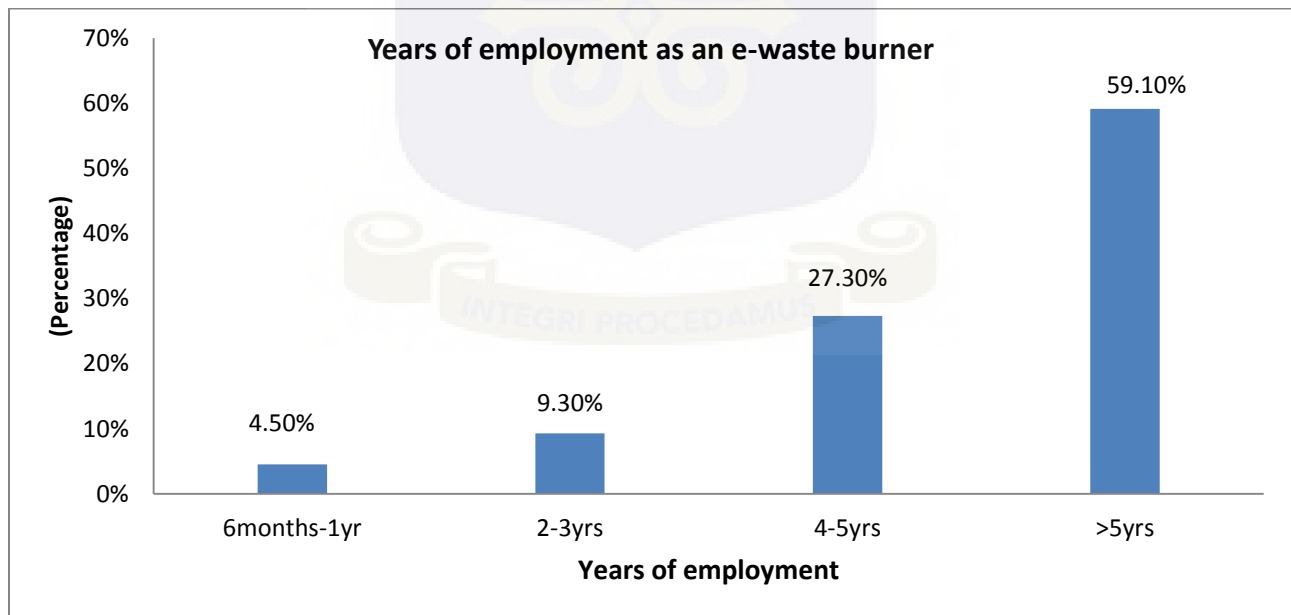


Figure 4.1: Number of years of Employment as an e-waste burner.

4.2 Awareness of exposure to respiratory hazards

The participants were asked whether they were fully aware that the work they do can harm their health. The majority, representing 90.9% answered in the affirmative whilst the remaining 9.1% said they were not aware that it could have any health consequences. Subsequently, when they were asked whether they used any type of personal protective equipment (PPE) at work, majority, and representing 72.7% did not use any type of PPE during the execution of their work. The remaining 27.3%, however, used safety gloves, safety shoes and nose mask. The views of the participants were sought, whether they go for any safety training; only 9.1% of them go for training of which they reported as informal training whilst the remaining majority (90.9%) does not go for any safety training.

4.3 Prevalence of Respiratory Symptoms

When the respondents were asked to report the respiratory health symptoms they experienced, most of them cited easy tiredness (63.6%), and sore throat (50%). The remaining cited cold (36.4%), chest pains (45.5%), excessive phlegm (45.5%), and chronic cough (37%). There were few cases of wheezing, prolonged sneezing, shortness of breath and respiratory infections (Fig.4.2).

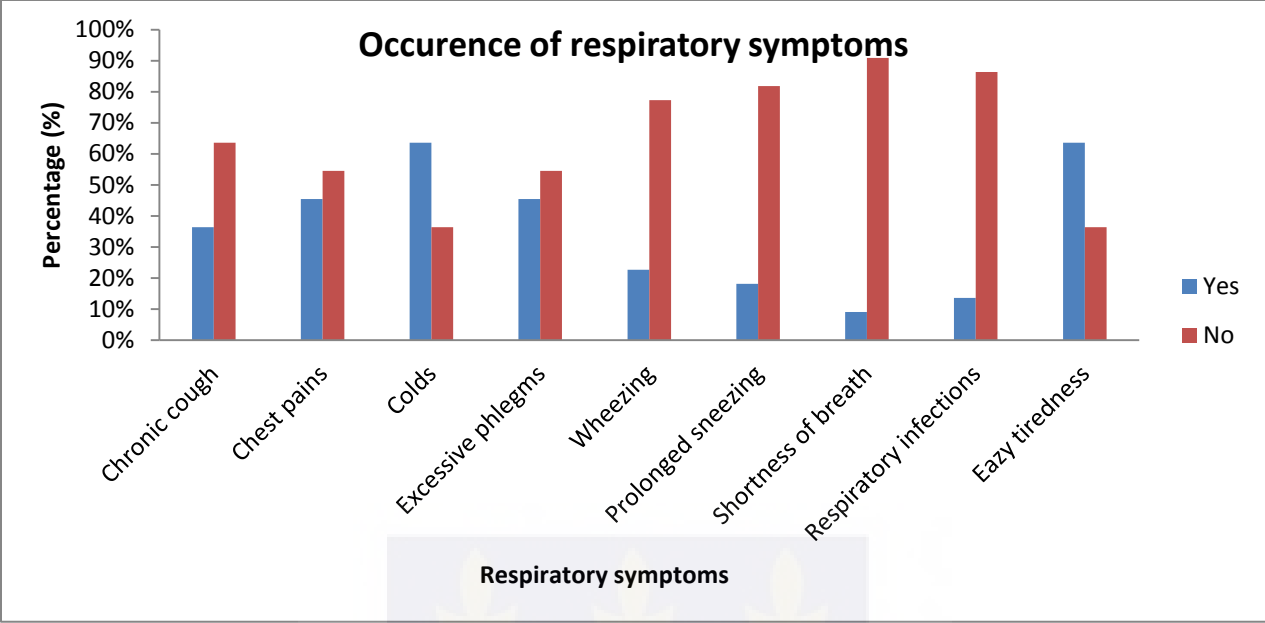


Figure 4.2: Respiratory symptoms of E-waste burners in the study area.

4.3 Mean values of Heavy Metals in Blood and Urine and Pulmonary Function of Participants

The mean (standard deviation) concentrations and the ranges of heavy metals are presented in Table 4.2. The mean concentration of the metals varies from 119.1577µg/L (47.10µg/L) for Pb in blood, 9.6095 µg/L (3.23µg/L) for Mn in blood, to 8.7745 µg/L (1.26 µg/L) for Ni in urine.

The mean values of the pulmonary function test ranged from 3.34L (0.599L), 2.78L/s (0.620L/s) and 83.5s⁻¹ (15.01 s⁻¹) for FVC, FEVI and FEV1/FVC% respectively (**Table 4.3**).

Table 4.2: Summary of mean heavy metal concentrations in urine and blood samples

Heavy metal	Concentration ($\mu\text{g/L}$)		
	Minimum	Maximum	Mean (SD)
Pb Blood	43.76	192.88	119.16 (47.10)
Mn Blood	6.16	20.29	9.61 (3.23)
Cd Urine	0.10	0.94	0.39 (0.02)
Ni Urine	0.10	49.98	8.77 (1.26)
Cr Urine	0.10	1.35	0.20 (0.03)

Table 4.3: Summary of mean values of pulmonary function measures

Pulmonary function parameters	Mean (SD)
FVC (L)	3.3432 (0.60)
FEV1 (L/s)	2.7800 (0.62)
FEVI/FVC (%)	83.5500 (15.02)

4.4 Associations between heavy metals and respiratory function (FVC, FEVI and FEVI/FVC)

A correlation analysis was conducted to exploit the relationship among the study variables. Except for Ni in urine and Cr in urine (-0.808, $P < 0.01$), there was no correlation between the study variables (**Table 4.4**). Furthermore, multivariate regression analysis revealed that Pb in blood reduced FVC by 0.20L, Cd in urine reduced FVC by 0.143L, Ni in urine reduced FVC by 0.294L. However, none of the associations between the heavy metals and FVC was significant (**Table 4.5**).

Table 4.4: Pearson’s product moment correlation between heavy metals and pulmonary function

Variable	Pb Blood	Cd Urine	Ni Urine	Mn Blood	Cr Urine	FVC	FEV1	FEV1/FVC
Pb Blood	1	-0.095	-0.179	0.170	-0.279	-0.048	-0.107	-0.035
Cd Urine		1	-0.034	-0.136	0.082	0.048	-0.082	-0.103
Ni Urine			1	-0.164	-0.808*	-0.216	-0.411	-0.399
Mn Blood				1	-0.239	0.307	0.353	0.127
Cr Urine					1	-0.088	-0.192	-0.181
FVC						1	0.549**	-0.040
FEV1							1	0.751**
FEV1/FVC								1

** Significant at 0.01 level

Table 4.5: Association between heavy metal concentration and FVC (L) among e-waste burners at Agbogloshie (n=22)

Heavy Metal parameters	Crude β (95% CI)			Adjusted β (95% CI)		
	β	95% CI	P-value	β	95% CI	P-value
Pb Blood	-0.009	-0.008, 0.005	0.707	-0.203	-0.009, 0.004	0.410
Cd Urine	0.046	-1.239, 1.490	0.847	-0.143	-1.928, 1.140	0.583
Ni Urine	-0.420	-0.067, 0.022	0.300	-0.294	-0.060, 0.029	0.458
Mn Blood	0.332	-0.032, 0.155	0.181	0.003	-0.095, 0.096	0.989
Cr Urine	0.301	-1.180, 2.451	0.469	0.203	-1.380, 2.237	0.613

*Adjusted for age, smoking, educational level and alcohol use

There was also a reduction in FEV1 for Pb in blood ($\beta = 0.339L$), Cd in urine ($\beta = 0.338L$), and Ni in urine ($\beta = 0.512L$) (see **Table 4.6**). None of the results was significant.

Table 4.6: Association between heavy metal concentration and FEV1 (Ls^{-1}) among e-waste burners at Agbogloshie (n=22)

Heavy Metal parameters	Crude β (95% CI)			Adjusted β (95% CI)		
	β	95% CI	P-value	β	95% CI	P-value
Pb Blood	-0.184	-0.008, 0.003	0.386	-0.339	-0.012, 0.003	0.225
Cd Urine	-0.116	-1.556, 0.890	0.572	-0.338	-2.738, 0.802	0.254
Ni Urine	-0.776	-0.082, -0.003	0.036*	-0.512	-0.080, 0.023	0.255
Mn Blood	0.356	-0.015, 0.152	0.102	0.346	-0.044, 0.177	0.212
Cr Urine	0.478	-0.583, 2.672	0.193	0.238	-1.565, 2.606	0.594

*Adjusted for age, smoking, educational level and alcohol use

Again, adjusted levels of Pb in blood, Cd in urine and Ni in urine resulted in a reduction of FEV1/FVC but the association was not significant (**Table 4.7**).

Table 4.7: Association between heavy metal concentration and %FEV1/FVC among e-waste burners at Agbogloshie (n=22)

Heavy Metal parameters	Crude β (95% CI)			Adjusted β (95% CI)		
	β	95% CI	P-value	β	95% CI	P-value
Pb Blood	-0.078	-0.178, -0.128	0.004	-0.300	-0.276, 0.084	0.267
Cd Urine	-0.160	-43.542, 21.346	0.479	-0.430	-71.509, 11.913	0.144
Ni Urine	-0.772	-2.081, -0.023	0.055	-0.417	-1.774, 0.661	0.336
Mn Blood	0.102	-1.739, 2.690	0.655	0.207	-1.642, 3.564	0.434
Cr Urine	0.459	-18.905, 67.441	0.251	0.097	-44.021, 54.289	0.822

*Adjusted for age, smoking, educational level and alcohol use

CHAPTER FIVE

DISCUSSION

5.1 Summary of Main Findings

Most of the e-waste burners were aware of the respiratory hazards associated with their work, sadly though, over 72% of the workers do not use any personal protective equipment (PPE). The most commonly self-reported respiratory symptoms included easy tiredness and colds; and few cases of chronic cough and chest pains. Heavy metal concentration in urine and blood samples did not significantly influence the respiratory function parameters (FVC, FEV1 and FEV1/FVC). This was because the study was under-powered to detect any significant effect.

5.2 Methodological validity

This is the first study to have looked at the associations between occupational exposure to heavy metals and lung function measures among e-waste workers. Data on exposure to heavy metals and the measures of pulmonary function were collected objectively thus, minimizing the influence of information bias. The study also controlled for important confounders such as age, cigarette smoking, alcohol use and educational level. The main challenge was language barrier. Most of the workers spoke and understood mainly Dagbani, a local Ghanaian dialect not spoken nor understood by the researcher. To overcome this hurdle, a native Dagomba was employed to assist with interpretation of questionnaire and general communication. The major limitation of the study however, was the small sample size thereby, reducing the power of the study to detect any effect. The study was also a cross-sectional design thus, precluding any causal association.

5.3 Comparing study results with previous findings

The workers were mainly young Ghanaian men of Dagomba ethnicity, single, had no formal education and worked on average for 10 hours per day. Akormedi et al., (2013) also reported that electronic waste recycling work in Ghana was a male dominant activity due to the physical strength required. Most of the burners were young men in their late teens and early twenties, youthful with no or low formal education and thus have reduced employability and vulnerable, with very low and erratic income levels, a fact supported by (Oteng-Ababio, 2012b). They were also aware of respiratory hazards associated with their work, however, only a small number of them used any respiratory protective equipment. Caravanos et al. (2011) also reported a high perception of hazards associated with e- waste recycling. The workers recognize the usefulness of PPEs, however only a few used any form of PPE which were mainly safety boots, noise mask and trousers. This finding brings to the fore the need for education of the workers on respiratory health to prevent respiratory ill-health among the burners. Chest pains, excessive phlegm production, sore throat, chest tightness and easy fatigability were common among the workers. About 45% of workers reiterated that symptoms become better when they are away from their work an assertion consistent with report by Caravanos et al. (2013). Phlegm production has been observed in men exposed to mineral dust, gases and fumes (Zock et al., 2012).

The mean concentration of lead was the highest of all the heavy metals analyzed, about 2 times that of the German background (MAK) level of 70 $\mu\text{g/L}$, and similar to the findings of Feldt et al. (2014) who reported mean blood lead (107.10 v 44.24, $p < 0.001$), but lower than mean Pb levels in the serum reported by Caravanos et al. (2013) reported among e- waste workers at

Agboglobshie. The dichotomy between the present study and that of Caravanos et al. (2012) could be attributed to analysis of Pb in serum samples instead of whole blood. On the contrary, the mean concentration of Mn reported in the present study was within the accepted workplace biological exposure limit of 15 $\mu\text{g/L}$ (MAK, Germany), but lower than that reported by Caravanos et al (2012). The mean concentration/ $\mu\text{g/L}$ of nickel in urine observed in the present study was higher than the German background (MAK) value of 3.0 $\mu\text{g/L}$. Feldt et al., (2014) also measured levels of trace metals in the urine of e-waste workers at Agboglobshie and observed significantly higher mean concentrations of urine nickel (5.62 $\mu\text{g/L}$) though lower than the mean concentration observed in this study, pointing to a possible heightened environmental contamination of nickel at the scrap yard. The low concentration levels of Cd (0.39 $\mu\text{g/L}$) in urine and Cr (0.20 $\mu\text{g/L}$) in urine observed in this study was consistent with the MAK biological exposure limit value of 0.8 $\mu\text{g/L}$ for Cd and 0.6 $\mu\text{g/L}$ for Cr 0.20 $\mu\text{g/L}$ (0.03); MAK value, 0.6 $\mu\text{g/L}$; and also consistent with the observations made by Caravanos et al. (2012) and Feldt et al. (2014).

Caravanos et al. (2011) also reported high levels of Pb in ambient air (0.72 BDL 0.15, 4x above the permissible USEPA ambient air quality standard) and the breathing zones of the e-waste workers at Agboglobshie above the ACGIH threshold Limit Values (TLVs). Whilst no air sampling testing was undertaken in this study, their findings are a good proxy for gaining an understanding of the possible air contaminants exposure among the e-waste workers at Agboglobshie.

The regression results revealed that Pb in blood and Cd and Ni in urine may influence respiratory function by increasing airway resistance. Due to inadequate sample size, none of these

associations was significant. This finding is consistent with the finding observed by (Leung et al., 2013). The authors studied the relationship between Secondhand Smoking (SHS) and urinary metals and lung function among preschool children (n=2763) in China. They measured urinary Pb, Cd and cotinine using immunoassay and indirect coupled plasma mass spectrometry. They reported that second hand smoke (SHS) was not associated with any spirometric indices such as reduced FEV1, FVC or FEV1/FVC ratio, whilst cotinine-to-creatinine ratio was inversely associated with forced expiratory volume in 0.5-sec ($\beta = -0.093$, $P = 0.003$), forced expiratory flow between 25% and 75% of expiration ($\beta = -0.138$, $P = 0.002$) and peak expiratory flow ($\beta = -0.106$, $P = 0.002$). Cd exposure was not associated with reported respiratory symptom or spirometric indices. Their study showed, however, that SHS exposure defined by urinary cotinine is a strong risk factor for lung function impairment measured by spirometry in Chinese preschool children. Although high urine Cd was common in the preschoolers, it was not associated with any clinical or spirometric outcomes. Zock J et al. (2005) also reported that individuals exposed to dusts, gases, and fumes over a period of follow up did not have a steeper decline of FEV1 than did individuals with unexposed consistently white-collar occupations without occupational exposures, or an increase of prevalence or incidence of airway obstruction defined as an FEV1/FVC ratio of less than 0.7.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, this study showed elevated levels of lead in the blood and nickel in the urine of the e-waste burners. However, there was no significant association between heavy metals (Pb, Cd, Mn, Cr, and Ni) and lung function parameters among the study participants.

6.2 Recommendation

The projections made by StEP Initiative indicate that about 65.7 million tons of e-waste would be produced worldwide by 2017 (StEP Initiative –Solving the e-waste problem, 2014). Since this environmental and health risk is likely to remain, there is the urgent need for regulation of e-waste recycling activities and training of the workers on standard operating procedures so as to prevent environmental contamination and to reduce health effects on the workers and the residents at Agbogbloshie. Workers involved in e-waste burning should be educated on the harmful effects of indiscriminate burning of e-waste to their own health and those around them. Finally, the study must be conducted on a population with larger sample size to improve the study outcomes.

Finally, there is the need for further studies to examine the effect of organic contaminants from e-waste exposure, such as PCBs, POPs and PAHs on the respiratory function.

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APPENDICES

Appendix 1: Components of a Personal Computer (PC)

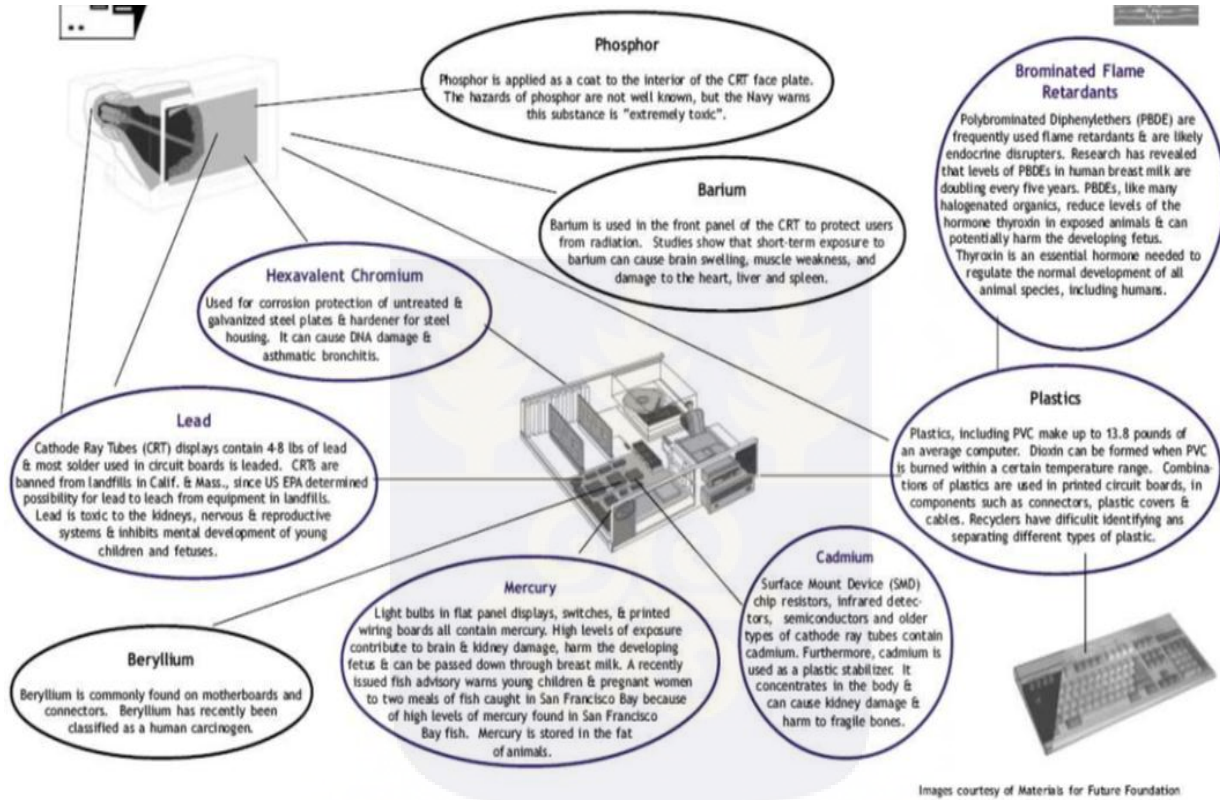


Image courtesy of materials for the future foundation. Adapted from Sussane Dittke of EnviroSense

Appendix 2: Questionnaire

Firstly, could you please tell us about yourself?

General Information

- 1. In which year were you born? Year
- 2. What is your gender? Male Female
- 3. What is your current marital status? Single Married Divorced Co-habiting
- 4. What is your highest level of education? No formal education Primary secondary

The next set of questions is about your work

- 5a. Are you an e-waste worker? Yes No
- 5b. if yes, do you work at the Burning site Dismantling site collector
- 6. How long have you worked in this job? 6 mths- 1 year 2-3 years 4-5years > 5years.
- 7. Does your work expose you to any of the following hazards? Tick (√) as appropriate

Dust	
Smoke	
Irritating gases and liquids	
Heat	
Fumes	
Aerosols	

8. Are you exposed to any of the following hazards at home? Please kindly tick (✓) as appropriate.

Dust	
Smoke	
Irritating gases and liquids	
Heat	
Fumes	
Aerosols	
Molds/ dampiness	

9a. Do you think the work you do can harm your health in any way? Yes No Don't know

9b) If yes please specify how

9c. Do you wear any personal protective equipment when you are working?

Never Sometimes Don't know what it is

9d. Have you received any training on how to do your work in a healthy and safe way?

Yes No

This set of questions is about your health in relation to your work?

8a. Do you have any illness you know about? Yes No

8b. Have you had any respiratory illnesses? Never During childhood All the time

8c. was it diagnosed by a doctor? Yes No

8d. is the illness brought on or made worse by the work you do?

Yes No

9. Do you suffer from any of the following in the course of your work? Please tick (✓)

Symptoms	Always	Sometimes	Never
Cough			
Colds			
Prolonged or repeated sneezing			
Easy tiredness			
Chest pains			
Sore throat			
Bringing out excessive phlegm			
Itchy ears and throat			
Itchy and watery eyes			
Wheezing			
Shortness of breath			
Difficulty in breathing			
Chest tightness			
Skin irritation or skin disease			
If you answered yes to any question please give details:			

10. Do these symptoms stop when you are away from your work place? Yes No

The next set of questions is about your habits/ lifestyle

11a. Do you smoke cigarettes? Yes No In the past

11b. If yes how many sticks do you smoke per day? < 5 sticks 5-10 sticks > 10sticks.

11c.How long have you being smoking? 1-5 years 6-10 years >10 years

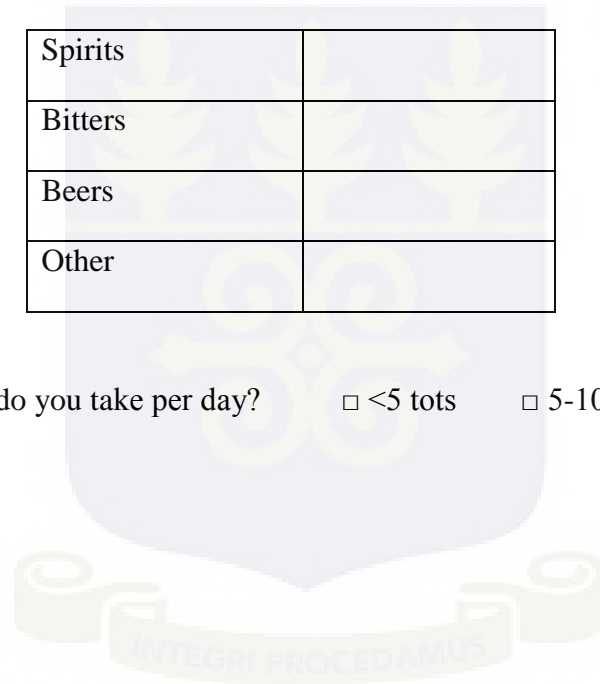
12a. Do you take alcohol? Yes No

12b. how long have you being taking alcohol? 1-5 years 6-10 years >10 years

12c.Which type of alcohol do you usually take? Please tick (√) as appropriate.

Spirits	
Bitters	
Beers	
Other	

12d. How much alcohol do you take per day? <5 tots 5-10 tots > 10 tots.



Appendix 3: Consent Form

Title: Effects of e-waste on respiratory function among e-waste workers engaged in burning at Agbogloshie, Accra.

Principal investigator: Afua Asabea Amoabeng Nti

Qualification: Bsc. Medicine and Surgery.

Address: School of Public Health, University of Ghana, Legon.

General information about the research

This research is being conducted to collect data on the effects of e-waste recycling activities on the respiratory function of e-waste workers at Agbogloshie, Accra.

The activities involved in the recycling processes of e- waste, especially the dismantling and open air burning releases dust, noxious gases and fumes which contain various toxic materials such as heavy metals polyvinyl chloride etc into the atmosphere and these contaminants are deleterious to human health.

The study is purely an academic research which forms part of the researcher's work towards the award of a Master Degree in Occupational Medicine.

Possible risk of discomfort

There are no major risks associated with participating in this study. The procedures involved in this study are non-invasive and will not cause any discomfort to the participants. However, a few participants may experience dizziness during the lung function measurement.

Description of level of research burden

Study participants would be asked to answer a questionnaire, participant in lung function test and provide urine for laboratory analysis for heavy metals.

Possible benefits

There will be no direct benefit to the participants. However, the information given will guide government and other relevant agencies for any future interventions on e-waste management in Ghana.

Confidentiality

Data security

All study recordings and field notes will be kept in locked files by the principal investigator. The field notes will be expanded and typed into computer files with secured pass codes.

Plans for record keeping

The study materials (laboratory data and results, questionnaires, informed consents) will not be labeled with participant's names but rather a unique identification number for each study participant.

Person responsible and phone number

The person responsible for the data storage will be

Afua Asabea Amoabeng Nti (Student)

School of Public Health, University of Ghana, Legon.

Mobile number: 0208521653.

Voluntary participation and the right to leave the research

Potential study participants will be told that participating in the study is entirely voluntary and that declining to enter the study, answer a question or terminating the interview will have no negative consequences.

Contacts for additional information

Please call the person responsible for this study Afua Asabea Amoabeng Nti, on 0208521653 if you have questions about the study. If you have any questions about your rights as a research participant or feel you have not been treated fairly, **call the Institutional Review Board (IRB) of the Ghana Health Service administrator, Madam Hannah Frimpong on the telephone numbers 0243235225 or 0507041223 to seek further clarification or redress.**

Your rights as a participant

This research has been reviewed and approved by the Ghana Health Service Ethical

Review Board. If you have any further questions about your rights as a research participant, you may contact the chairman of the Board.

Volunteer agreement

The above document describing the benefits, risks and the procedures for the research title (“Effects of e-waste on the respiratory function among e-waste workers at Agbogloshie”) has been read and explained to me. I have been given the opportunity to ask questions and all the questions that I have asked about the research have been answered to my satisfaction. I agree to participate as a volunteer.

.....

.....

Date

Signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

.....

.....

Date

Signature of witness

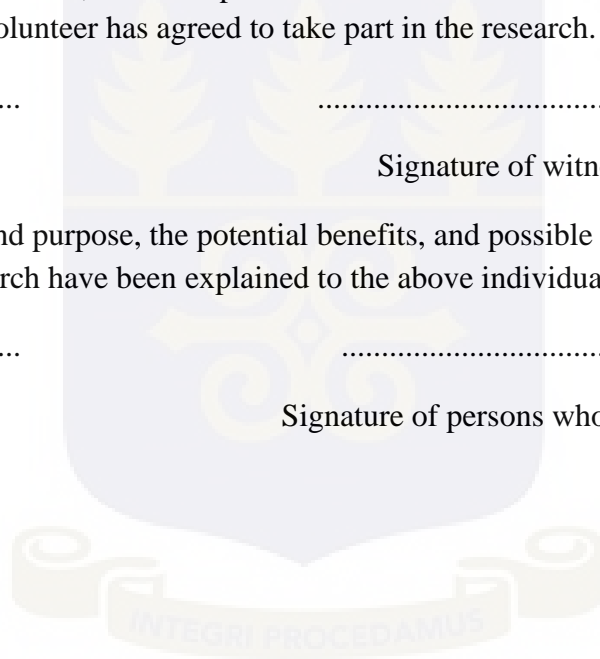
I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

.....

.....

Date

Signature of persons who obtained consent



Appendix 4: Ethical Clearance Letter



Appendix 5: Analysis of heavy metals

Parallel determination of cadmium and nickel in urine for Ghana 09.06.2015

Cadmium (228, 8018nm), nickel (232, 003nm)

Method: Parallel CdNi

CD: BLW: 7µg/L urinary

NI: (1.1 2.7) 3µg/L urine (not strictly representative collective)

Sinks: All glassware with 1 * 1 M HNO₃ and 3 * with Millipore. Also dishes and measuring cylinders! Use white vials;

Storage urine :-20 ° C

Urine with 96% acetic acid to pH 3-4 place: adding 100 µL acetic acid on 5mL urine

Glutathionloesung:

134mg glutathione 200 ml Millipore (1 week shelf life)

1% acetic acid:

2.5 ml 96% acetic acid at 250mL Millipore

Wässr. extraction solution WE:

0.275 g HMA HMDC in 100 mL of Millipore. Best release in the US bad (stability approx. 2-3Tage)

Organic extraction solution:

Release 1, 36 g HMA HMDC in 30 mL of xylene using warm running tap water; Let it cool and fill up to 100 ml with Diisopropylketon. (Stability approx. 1 week)

CD:

SL1: 1mL CD-standard (1 g / L) to 10 mL of Millipore c(CD)= 0, 1 g / L

SL2: 100 µL SL1 10 ml Millipore c(CD)=1 mg/L

SL3: 1mL 50 mL Glutathionlösung SL2 c(CD)=20µg/L

NI:

SL1: 50 µL (1 g / L) NI standard to 10 ml 1% acetic acid c(NI)=5mg/L

SL2: 200 µL SL1 on 20 ml of 1% acetic acid c(NI)=50µg/L

Common standards:

In a 20 ml volumetric flask: per 2 ml 1% CH₃COOH

	Vol 50µg/L NI	c(NI) in µg/L	Vol 20µg/L CD	c(CD) in µg/L
Std1	400	1	1000	1
Std2	600	1.5	2000	2
Std3	800	2	4000	4
Std4	1000	2.5	5000	5
Std5	1200	3	6000	6
Std6	1400	3.5	8000	8

And fill it with **Glutathionlösung** . Standard 2-3 days shelf life

Sample preparation:

Standards

- Blank value: 4 ml Glutathion
- CD/NI QC 5: 2ml QK + 2 ml 1% CH₃COOH VF2
- CD/NI QK 20: 0, 4ml QK + 3.6 ml 1% CH₃COOH VF10
- **4 ml** Standard solution

Sample dilution

- 4 ml urine VF1
- 2ml urine + 2 ml 1% CH₃COOH VF2

To all standards and Urines **1 ml** 1% CH₃COOH +**1 ml** GR → **60s** Vortex

Then Vortex **1,5 ml** ö → **60 s**. Then **1 h** in Shaker, then centrifuge min **10** at **4500 rpm**.

Phases must be separated otherwise errors.

Method rule lead in blood: (283, 3060nm) Status: 18.06.15

Method: "BleiBlutenvironmentAJau10" = 253 s 4min13s

$\Lambda = 283, 3060\text{nm}$ NWG: 0.1-1 $\mu\text{g/L}$

Method: "BleiBlut testwork" for work (**SL: 50 $\mu\text{g/l}$**) if $c(\text{Pb}) \geq 300 \rightarrow \text{VF40}$

Sample vessel cleaning

10mL glass flask overnight soak in 1 M HNO₃. Vessels 1xl with 1M HNO₃ and 3 x with Millipore. fill-> air dry

All before washing with 1 M HNO₃ and 3 x Millipore.

AAS

Caution: do not damage PIN scratch out,

0.1% Triton

100 μl Triton in 100 ml Millipore / 1 ml Triton in 1000 ml of Millipore

1M HNO₃

650 μl 69% HNO₃ Fluka trace select in 10 ml of Millipore

0,1M HNO₃

1 ml of 1m HNO₃ in 10 ml of Millipore

Modifier 0.25 g / l PD-mg-modifier

250 μl PD modifier + populate 250 μl mg modifier in **10 ml** PFA vessels with Millipore.

625 μl PD modifier + populate 625 μl mg modifier in **25 ml** PFA vessels with Millipore.

1ml / 4ml depending on the number of samples

Stock solution environment

SL1: 100 μl PB standard [1 g / L PB] + 1ml 1M HNO₃ in 10 ml Millipore-> $c(\text{Pb}) = 10 \text{ mg / l}$

SL2: 100 μl SL1 + 1ml 1M HNO₃ in 10ml Millipore. -> $c(\text{Pb}) = 100 \mu\text{g/l}$

SL3: 1ml SL2 + 1ml of 1M HNO₃ in **10 ml** Millipore-> $c(\text{Pb}) = 10 \mu\text{g/l}$

2ml SL2 + 2ml of 1M HNO₃ in **20 ml of** Millipore-> $c(\text{Pb}) = 10 \mu\text{g/l}$

Blank value

900 μl 0, 1% Triton + 100 μl 0,1M HNO₃ 1 ml

3600 μl 0, 1% Triton + 400 μl 0,1M HNO₃ 4 ml

Samples

800 μl 0.1% Triton + 100 μl 0,1M HNO₃ + 100 μl blood VF 10

850 μl 0.1% Triton + 100 μl 0,1M HNO₃ + 50 μl blood VF 20

875 μl 0.1% Triton + 100 μl 0,1M HNO₃ + 25 μL blood VF 40

Added vol/Konz stock solution environment: 10, 15, 20 μl / 5; 7.5; 10 $\mu\text{g/l}$

Added vol/Konz stock solution work: / 25; 37.5; 50 $\mu\text{g/}$

Method rule chromium in urine ($\lambda = 357,8687$ nm) 18.06.15 (for environmental or OK5)**Sample vessel cleaning**

10ml glass flask overnight soak in 1 M HNO₃. Vessels 1 x mit 1M HNO₃ and 3 x with Millipore rinse, air dry

0.1% Triton

100 μ l Triton in 100 ml of Millipore

1M HNO₃

720 μ l 65% HNO₃ (Merck) in 10 ml of Millipore

0, 05 M HNO₃

500 μ l 1M HNO₃ in 10 ml of Millipore

Blank value

100 μ l 0.1% Triton + 900 μ L of 0.05 M HNO₃ 1 ml

400 μ l 0.1% Triton + 3600 μ L of 0.05 M HNO₃ 4 ml

*** Stock solution 8 μ g/l CR**

SL1: 100 μ l CR standard [(1g/l) in 10 ml of Millipore]

SL2: 100 μ l SL1 + 500 μ l 1M HNO₃ in 10ml of Millipore

SL3: 800 μ L SL2 + 500 μ l 1M HNO₃ in 10ml of Millipore \rightarrow 8 μ g/L

Stock solution 2 μ g/l CR

SL1: 100 μ l CR standard [(1g/l) in 10 ml of Millipore]

SL2: 100 μ l SL1 + 500 μ l 1M HNO₃ in 10ml of Millipore

SL3: 200 μ l SL2 + 500 μ l 1M HNO₃ in 10ml of Millipore \rightarrow 2 μ g/L

Sample preparation

Samples with 69% to pH 2-3: HNO₃-trace select

0, 35ml conc. \rightarrow HNO₃ / 100 ml urine 17, 5 μ l HNO₃/5 ml urine

Samples

100 μ l 0.1% Triton + 100 μ l of 0.05 M HNO₃ + 800 μ l urine VF1, 25

100 μ l 0.1% Triton + 500 μ l of 0.05 M HNO₃ + 400 μ l urine VF2, 5

100 μ l 0.1% Triton + 700 μ l of 0.05 M HNO₃ + 200 μ l of urine VF5

100 μ l 0.1% Triton + 850 μ l of 0.05 M HNO₃ + 50 μ l urine VF20

100 μ l 0.1% Triton + 875 μ l of 0.05 M HNO₃ + 25 μ l urine VF40

Adding stock solution: Work area 2, 4, 6 μ g/l; Environmental 0.5, 1, 1.5 μ g/l

Method rule manganese in blood: Status: 18.06.2015 ($\lambda = 279,5$ nm NWG: 0.6-1 $\mu\text{g/L}$)

Sample vessel cleaning

10ml glass flask overnight soak in 1 M HNO₃. Filling vessels with 1M HNO₃ and 3 x with Millipore 1xl-> air dry
Rinse the pipette tips with 1 M HNO₃

Modifier 2 g / l Pd(NO₃)₂- modifier

fill the 2ml PD-modifier [10 g / l] in 10ml PFA tube with Millipore.
1 ml / 4 ml depending on the number of samples

0, 1% Triton:

Stock solution

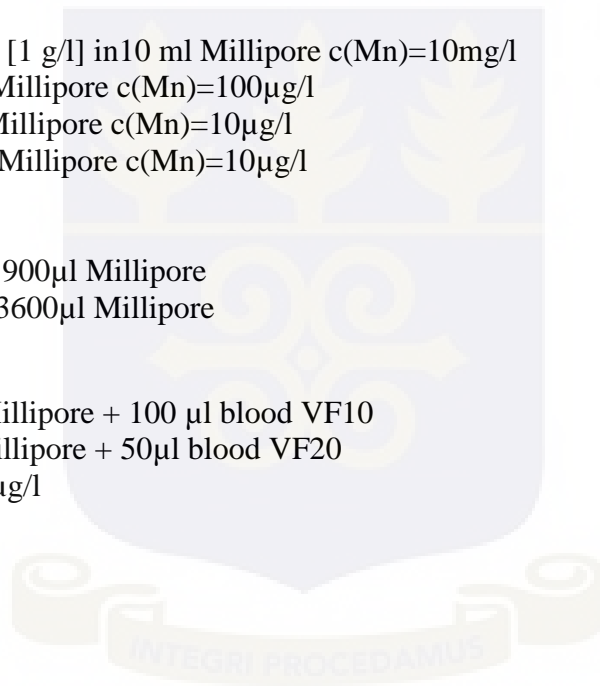
SL1: 100 μl MN standard [1 g/l] in 10 ml Millipore c(Mn)=10mg/l
SL2: of 100 μl to 10 ml Millipore c(Mn)=100 $\mu\text{g/l}$
SL3: from 1ml to 10ml Millipore c(Mn)=10 $\mu\text{g/l}$
of which 2ml in 20ml of Millipore c(Mn)=10 $\mu\text{g/l}$

Blank value

100 μl 0.1% Tritonlsg. + 900 μl Millipore
400 μl 0.1% Tritonlsg. + 3600 μl Millipore

Samples

100 μl Triton + 800 μl Millipore + 100 μl blood VF10
100 μl Triton + 850 μl Millipore + 50 μl blood VF20
Be added + 3 / + 6 / + 9 $\mu\text{g/l}$



Method rule nickel in urinary Status: 18.06.15 ($\lambda = 232,003$ nm NWG: 0,2 $\mu\text{g/L}$)

Sample vessel cleaning

10mL glass flask overnight soak in 1 M HNO₃. Vessels with 1M HNO₃ 1 x and 3 x with Millipore. fill-> air dry also rinse the scales, graduated cylinder, etc.!

Use white vials!

1% CH₃COOH

1 ml of 96% owned to 100ml Millipore CH₃COOH

Wässr. Extraction solution WE

0,1375 g HMA HMDC 50 ml Millipore; In the US bad (stability approx. 2-3Tage)

Organic extraction solution ö

Remove 0,68 g HMA HMDC in 15 ml of xylene under warm, running water; Let cool and fill it with Diisopropylketon on 50 ml. (Stability about 2-3 days)

Stock solution

SL1: 100 μl NI standards (1 g / l) to 20 ml 1% populate CH₃COOH, $c(\text{Ni})=5\text{mg/l}$.

SL2: of 200 μl 20 ml 1% CH₃COOH populate $c(\text{Ni})=50\mu\text{g/}$

STD. 1: $c(\text{Ni})=1,0\mu\text{g/L}$: 200 μl STD. 1: $c(\text{Ni})=1,0\mu\text{g/L}$: 400 μl

STD. 2:; $c(\text{Ni})=1,5\mu\text{g/L}$: 300 μl STD. 2:; $c(\text{Ni})=1,5\mu\text{g/L}$: 600 μl

STD. 3:; $c(\text{Ni})=2,0\mu\text{g/L}$: 400 μl STD. 3:; $c(\text{Ni})=2,0\mu\text{g/L}$: 800 μl

STD. 4:; $c(\text{Ni})=2,5\mu\text{g/L}$: 500 μl STD. 4:; $c(\text{Ni})=2,5\mu\text{g/L}$: 1000 μl

STD. 5:; $c(\text{Ni})=3,0\mu\text{g/L}$: 600 μl STD. 5:; $c(\text{Ni})=3,0\mu\text{g/L}$: 1200 μl

STD. 6: $c(\text{Ni})=3,5\mu\text{g/L}$: 700 μl STD. 6: $c(\text{Ni})=3,5\mu\text{g/L}$: 1400 μl

A populate uf **10 ml** with 1% CH₃COOH. Auf **20 ml** with 1% CH₃COOH populate.

Blank:

4 ml 1% acetic acid

Sample preparation

Samples with 1ml of CH₃COOH 96%, Suprapur / 100 mL urine put \rightarrow pH 3-4

\rightarrow Add 100 μl of adding acetic acid on 5mL urine

Qk2 $\mu\text{g/l}$: 4 ml urine

Qk5 $\mu\text{g/l}$: 2 ml urine + 2 ml 1% CH₃COOH VF2

Qk20 $\mu\text{g/l}$: 1 ml urine + 3 ml 1% CH₃COOH VF 0.5 ml urine + 3,5 ml 1% CH₃COOH VF8

4 ml standard solution/BW/urine + **1 ml** 1% CH₃COOH + **1 ml** GR \rightarrow **60s** Vortex

Then **1, 5 ml** ö to Vortex \rightarrow **60 s**

Then **1 h** in Shaker .Then min **4500** rpm centrifuge at **10** (9 hoch_4 down).

