BLOOD LEAD LEVELS AND SELECTED HAEMATOLOGICAL PARAMETERS AMONG AT RISK OCCUPATIONAL GROUPS AND BLOOD DONORS IN KENYASI, BRONG AHAFO REGION

BY

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DECLARATION

With the exception of duly acknowledged references, I, Veronica Agyemang, hereby declare that this research was carried out by me at the Department of Haematology, School of Biomedical and Allied Health Sciences, University of Ghana, under the supervision and direction of my supervisors and has not been presented for another degree.

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DEDICATION

I dedicate this work to the Almighty God for all that He has done for me. God, I can never thank you enough. Also to my husband, Dr. Samuel Harrison; my children, Kuukua and Kobby for your love and understanding. To my parents, Mr. and Mrs. Opuni and also to my supervisors, Professor Acquaye and Dr Olayemi for your exceptional inspiration and guidance.

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LIST OF ABBREVIATIONS

δ -ALA ……...Delta-aminolevulinic acid

δ -ALAD .....Delta-aminolevulenic acid dehydratase

δ -ALAS.....Delta-aminolevulenic acid synthase

AAS…….Atomic absorption spectrometry

ASV……..Anodic stripping voltammetry

%.....Percentage

µg/dl.....Microgram per decilitre

^{0}C.....Degree Celsius

AIDS.....Acquired Immune Deficiency Syndrome

BLLs.....Blood Lead Levels

CDC .....Centre for Disease Control and Prevention, USA

cm ……Centimetre

CNS .....Central Nervous System

CT Scan.....Computed tomography Scan

DMSA ……...2, 3-dimercaptosuccinic acid,

DNA.....Deoxyribonucleic acid

EBLL……Elevated Blood Lead Level

EDTA.....Ethylenediaminetetraacetic acid

et.al.....And others

etc .....And so on

FDA .....Food and Drugs Administration

g……Gram

H_2O_2 ……....Hydrogen peroxide

Hb ……Haemoglobin

HIV.....Human Immunodeficiency Virus

ICP-MS ……Inductively coupled plasma mass spectrometry

i.e.……That is

IQ.....Intelligence quotient

JSS……Junior Secondary School

mA……..Milliamps

mg .....Milli-gram

ml……Millilitre

mg/L …….Milligram per litre

NBTS.....National Blood Transfusion Society

nm……...Nanometre

NO_3……Nitric oxide

O^{2-} …….....Superoxide ion

OH^- ……Hydroxyl radical

Pb.....Lead

PBG…….....Porphobilinogen

PBGD……..……...Porphobilinogen deaminase

QC/QA ……..Quality control/Quality assurance

RBC .....Red blood cell

ROS …….…….....Reactive oxygen species

sec …….Seconds

-SH .....Sulphydryl group

sq km.....Square kilometre

SSS…….....Senior Secondary School

$t_{1/2}$ …….Half life

TA-GVHD.....Transfusion-Associated Graft-Versus-Host Disease

TRALI .....Transfusion-Related Acute Lung Injury

WHO.....World Health Organisation

ZPP .....Zinc protoporphyrin

ABSTRACT

Introduction: Lead poisoning has been a major public health problem for decades across the world and receiving a blood transfusion has been considered as a risk factor for lead exposure. Children and pregnant women are most vulnerable to the toxic effects of lead. However, over 40% of transfused blood in Ghana is given to children below the age of 5 years. Among adults, occupation is the leading cause of elevated blood lead levels (EBLL).

Aim: This study compared blood lead levels among selected at risk occupational groups with low risk blood donors at Kenyasi in the Brong Ahafo Region.

Methods: 200 participants made up of 40 illegal miners (galamsey), 40 painters/sprayers, 40 drivers/fuel station attendants and 40 auto-mechanics belonged to the exposed group as 40 qualified blood donors belonged to the non-exposed group. Their blood samples were taken into ethylenediamine tetraacetic acid (EDTA) tubes and profile data collected after seeking informed consent. The samples were analysed for BLL using flame atomic absorption spectrophotometry (FAAS) and full blood count (FBC) using ABX Micros 60 as well as thin blood film stained with Leishman stain for red cell morphology. Data was entered into Excel spreadsheet (2010) and transferred into STATA version 14.0 for analysis.

Results: Out of the 200 participants sampled, 186 (93%) were males and 14 (7%) were females. All females belonged to the non-exposed group. Mean age of participants was 28.58 ± 8.17 years [95% CI: 27.44 – 29.72] with a range of 18 to 57 years. The geometric mean (GM) blood lead level (BLL) of study participants was 6.34 ±1.41 µg/dl [95% CI: 6.04 – 6.65] with a minimum BLL of 1.8 µg/dl and maximum of 14.4 µg/dl. The GM BLL for the exposed group was 7.0 ± 1.8 µg/dl and the non-exposed

was 5.4 ± 1.8 µg/dl which was statistically significant (*p=0.0001*). The prevalence of EBLLs was 84.5%, 89.4% and 65% in the study population, the exposed and the non-exposed groups respectively. In the individual groups, it was 100% among painters, 97.5% among auto-mechanics, 95% in small scale miners and 65% in fuel attendants/drivers. The mean Hb of study population was 14.08 ± 1.53 g/dl with a range of 7.4 to 16.8 g/dl and that for the exposed and the non-exposed groups were 14.23 ± 1.32 g/dl and 13.68 ± 1.49 g/dl respectively. The exposed group had significantly higher Hb compared to the non-exposed group with a *p-value* of 0.0441. The prevalence of anaemia was 18.5% in the exposed group but none in the non-exposed group. Microcytic hypochromic anaemia, normocytic normochromic anaemia and macrocytic anaemia were 16.2%, 73% and 10.8% respectively. There was no significant linear correlation between haemoglobin concentration and blood lead levels measured (Correlation Coefficient=0.0492; p=0.7382). Basophilic stippling was not observed in any of the smears prepared.

Conclusion: Occupation is strongly associated with EBLL in the study area. Unusual sources of lead exposure, such as blood transfusion, deserve new attention as blood from individuals in high risk occupations could pose potential threat to the health of vulnerable populations like children and pregnant women who require frequent blood transfusion.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Lead (Pb^{2+}) poisoning is a major public health problem worldwide and work related exposure to lead is the most important source of lead poisoning among adults (Nemsadze, Sanikidze, Ratiani, Gabunia, & Sharashenidze, 2008). Lead is a non-essential element that has toxic potential for human biological systems. High levels of lead can have adverse effects on many systems in the body with the neurological, reproductive, gastrointestinal, haematopoietic and renal systems being the most affected (Nemsadze et al., 2008).

Lead has various uses and this has led to widespread environmental contamination, with humans getting exposed to significant health problems in various parts of the world (World Health Organisation, 2016). “No level of lead in human blood is considered safe” and “young children are particularly vulnerable to the toxic effects of lead” which include “profound and permanent adverse effects on the development of the brain and nervous system” (World Health Organisation, 2016).

Mining, manufacturing, smelting, and recycling activities, coupled with the sustained usage of lead-containing paint and fuel are vital sources of lead contamination in the environment in some countries (United State Centers for Disease Control and Prevention, 2009). Over three quarters of the world’s utilization of lead is for producing lead-acid batteries. Lead has also been useful in the production of paints, stained glass, pigments, ammunition, solder, jewelry, toys, ceramic glazes and in some cosmetic

products as well as traditional remedies (United State Centers for Disease Control and Prevention, 2009; Jones et al., 2009; World Health Organisation, 2016).

Individuals get exposed to lead by inhaling lead particles in the atmosphere, or by consumption of food or water contaminated with lead. Food harvested from soil contaminated with lead and some consumer products also expose people to lead. Lead was used extensively as tetraethyl and tetramethyl lead as anti-knock and lubricating agents in petrol to improve its octane rating and was the leading source of environmental exposure to lead (United State Centers for Disease Control and Prevention, 2009; Jones et al., 2009; World Health Organisation, 2016).

Efforts have been made over the past several years to reduce lead exposure from the environment and also from occupational sources among adult populations in the United States. Nevertheless, lead exposure continues to occur at unacceptable levels. Population blood lead levels (BLLs) in some countries have reduced due to a global initiative to ban leaded fuel (Cortez-Lugo, Téllez-Rojo, Gómez-Dantés, & Hernández-Avila, 2003). Leaded gasoline was in use in Ghana until the year 2004 when it was phased out (Aboh et al., 2013). Because lead is a non-degradable element, it can persist in the environment long after its discontinued use (Ab Latif Wani & Usmani, 2015).

The BLL is the best marker of recent exposure (up to 40 days) to lead (United State Centers for Disease Control and Prevention, 2015). Harmful effects are still being reported in adults as a result of low BLLs that were initially thought to be harmless. Some of these may include impaired renal function and the risk for essential tremor and hypertension at BLL less than 10 µg/dl (World Health Organisation, 2016). In acute situations, exposure to high levels of lead can result in a variety of symptoms, like

vomiting and nausea, headaches, abdominal pain, drowsiness, tremors, anaemia, irritability, convulsions, muscle weakness, ataxia, paralysis, coma and even death (World Health Organisation, 2010). Prolonged exposure to low-levels of lead is linked to reduced intelligence quotients (IQ), attention deficit disorder, aggression or hyperactivity, delinquency, subclinical hearing and balance disturbances, increased dental caries, and numerous neurobehavioral problems in addition to cognitive defects in children (Patrick, 2006; World Health Organisation, 2010). Even with EBLL in the body, some individuals may exhibit no signs and symptoms of lead poisoning (Ab Latif Wani & Usmani, 2015). Removal of exposure sources to lead is the most essential aspect of the management of lead poisoning (Kathuria, 2017).

1.2 PROBLEM STATEMENT

High lead levels can have adverse impacts on numerous systems in the body such as the neurological, reproductive, gastrointestinal, haematopoietic and renal systems (Nemsadze et al., 2008). "No level of lead in human blood is considered safe"; however, the most vulnerable group is children. Adverse effects of lead in children include permanent brain damage and other nervous disabilities (World Health Organisation, 2016).

World Health Organisation (2016) reported an estimate made by the National Institute of Occupational Safety and Health (NIOSH) that over 3 million workers in the United States are potentially exposed to lead in their workplaces. Approximately, 0.6% of the world's burden of disease is due to exposure to lead and developing countries are the most affected. It is known that nearly 600,000 newly reported cases of children suffering from intellectual disabilities annually is as a result of lead exposure (World

Health Organisation, 2016). Lead exposure also is estimated to account for "143 000 deaths per year with the highest burden in developing regions" and about "4% of the global burden of ischaemic heart disease and 5% of the global burden of stroke" (World Health Organisation, 2016).

In Ghana, a study done in the Brong Ahafo Region, recorded a 20% prevalence of lead poisoning in children less than 5 years (Report on Baseline Health Survey Report in the Newmont Mining Area, 2008). Blood transfusion is a hidden source of lead exposure (Gehrie et al., 2013). The CDC and WHO recently adopted a series of recommendations indicating that "there is no safe level of blood lead in children" (United State Centers for Disease Control and Prevention, 2012; World Health Organisation, 2016). However, these recommendations do not address strategies to avoid lead exposures occurring via blood transfusions; even though transfused lead is substantially more bio available than oral lead and a dose-response relationship between the lead concentration of transfused packed red blood cells and post-transfusion blood lead concentration is known to exist among very premature infants (Gehrie et al., 2013).

Due to these recent changes in policy by the CDC and WHO, minimization of lead exposure in children is a subject that is now being addressed with renewed urgency and therefore atypical sources of lead exposure, such as blood transfusion, deserve new attention.

Over forty percent (>40%) of blood transfused in Ghana is given to children under the age of 5years (Acquaye, 1997). It is therefore important to ensure that donated blood does not contain lead levels near the upper limit of normal. By studying the populations

that are prone to exposure to lead, one can decide whether their lead levels are high and for which reason they could be excluded from blood donation.

1.3 JUSTIFICATION

This study seeks to establish that certain occupations are more exposed to lead. Hence donor blood from such occupational groups could be a hidden source of lead poisoning if transfused. There is therefore the need to obtain data on the BLLS of some of these at risk occupational groups in Ghana. This could influence policy to exclude such at risk occupational groups from blood donation in order to reduce the potential for iatrogenic lead poisoning especially among children and pregnant women.

1.4 PRIMARY OBJECTIVE

The primary objective of this study is to compare blood lead levels among selected at risk occupational groups with low risk blood donors at Kenyasi in Brong Ahafo region, Ghana.

1.5 SECONDARY OBJECTIVES

1. To determine the average lead concentration among the occupational groups
2. To determine the prevalence of elevated blood lead levels among the occupational groups (≥5µg/dl)
3. To determine the relationship between blood lead levels of participants and their corresponding haematological parameters (Full blood count and red cell characteristics on thin blood film)
4. To determine whether there will be significant differences between lead levels among the occupational groups.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 LEAD AND LEAD POISONING

Lead has been described as being a colourless, odourless, tasteless metal that exists naturally in the environment but very toxic (Agency for Toxic Substances and Disease Registry 2007). It has been used for hundreds of years in the production of several products because it is malleable, has a lower melting point, and it is capable of forming compounds. It is however poor in electricity conduction and resistant to corrosion. For the production of batteries, alloys, exterior red lead paint, and ammunition, over 4 million tons of lead are produced each year (Kumar, Abbas, & Fausto, 2005).

Lead poisoning is described as a medical condition that arises as a result of people getting exposed to lead compounds by inhalation, swallowing, and occasionally, through the skin and it usually happens as a result of repeated exposure to small amounts of lead (Dewalere Health and Social Services, 2009). The Centres for Disease Control and Prevention (CDC) defined lead poisoning as venous blood lead level (BLL) of 10μg/dl or higher. However, current information shows that "there is no known level of lead exposure that is considered safe" (World Health Organisation, 2016), as harmful effects are still being reported in adults as a result of low BLLs that were initially thought to be harmless. Some of these may include impaired renal function and the risk for essential tremor and hypertension at BLLs below 10 μg/dl (World Health Organisation, 2016). Therefore a BLL ≥5μ/dl is now considered as an EBLL that

requires immediate public health intervention (United State Centers for Disease Control and Prevention, 2015).

2.2 SOURCES OF LEAD

Lead constitutes about 0.002% of the mineral deposits in the earth's crust (Markowitz, 2000). Most cases of lead exposure across the world come from gasoline additives, lead-based paints, food can soldering, ceramic glazes and drinking water systems. (Markowitz, 2000). A few years ago**,** gasoline that was sold in most African countries contained as much as 0.5-0.8g/L of lead (Nriagu, Blankson, & Ocran, 1996). Industries like plumbing, electrical, plastics, and nuclear found lead to be useful in their operations. As a result, products such as pipes, solder, ceramics, crystals, paints, radiation shielding, gasoline, batteries, unorthodox (e.g. Ayurvedic) medications, and cosmetic products may contain some amount of lead (Ryan et al., 2004).

Unexpectedly, household items, such as window blinds, zippers, painted furniture, and mineral supplements may contain lead. Uncontrolled emissions from metal refineries and battery recycling plants, maintenance work on bridges, and pulling down of old housing serve as significant sources of lead exposure. Target shooting in inadequately ventilated spaces using lead-containing bullets, serve as source of lead exposure to frequent visitors, probably through inhalation (Markowitz, 2000). Some alternative medications and traditional herbs used by East Indians, Indians, Hispanics and some other Asians contain lead. (Garvey, Hahn, Lee, & Harbison, 2001; Saper et al., 2004). Lead is believed to be effective in treating some conditions and can therefore be found in some traditional medicines (United State Centers for Disease Control and Prevention, 2009).

Home renovation is key to exposure to lead-based paints. This may include works carried out by the inhabitants themselves or as a result of inappropriate work practices (United State Centers for Disease Control and Prevention, 2009). Crops grown in lead contaminated soil have also been known as a source of exposure to lead (Finster, Gray, & Binns, 2004). Some imported dried fish and even spices could be contaminated with lead (Markowitz, 2000).

2.3 OCCUPATIONAL EXPOSURE TO LEAD

In 370 BC, the first workplace associated lead exposure was reported (Kazantzis, 1989). Lead poisoning became rampant among those working in the industries in the nineteenth and twentieth centuries. Thus employees who engaged in occupations like smelting, plumbing, painting, printing and many other industrial activities became exposed to lead (Kazantzis, 1989).

Exposure to lead occurs in many occupations, for instance, assembling of motor vehicles and repair, panel beating, battery recovery and manufacture, mining of lead and smelting, production of lead alloy, plastics, printing, and paint industries. Global lead consumption of more than three quarters is for the manufacture of lead-acid batteries (United State Centers for Disease Control and Prevention, 2015; World Health Organisation, 2016). Other workers potentially at risk for lead exposure include plumbers, welders, auto-mechanics and painters. Removal of paint from surfaces previously coated with lead-based paint such as bridges, residences being renovated, and structures being demolished or salvaged can also be a significant cause of lead

exposure. The possibility of being exposed to lead from paint is more common because of increased roadwork, bridge construction and repair, residential lead abatement, and residential remodeling. Lead exposure from smelting, lead mining, battery factories and other industrial activities is an important environmental problem in developing countries (CDC, 2015). Lead is mostly absorbed into the body through inhalation, ingestion and by contact. Workers may inhale lead as dust, fume, or mist while their lungs and upper respiratory tract absorb it into their bodies. Significant absorption of lead into the bloodstream also takes place through the digestive system. Occupational lead exposure in developing countries is however a common practice which is usually not regulated with a non-existent exposure monitoring (Tong, Schirnding, & Prapamontol, 2000).

Small-scale domestic secondary smelters in most low and middle-income countries are located near residential areas. Lead particles produced from such activities are significant health hazard to people in the neighbourhood. Only few middle income countries have implemented policies and guidelines to significantly fight the menace of occupational lead exposure (Tong et al., 2000).

2.4 PATHOGENESIS OF LEAD POISONING

There are several cellular targets for lead toxicity but the haematopoietic system and the central nervous system as well as the digestive and renal systems are the most affected (Carocci, Catalano, Lauria, Sinicropi, & Genchi, 2016). In the haematopoietic system, lead inhibits haem and haemoglobin synthesis and also changes the morphology and survival of red blood cells (Flora, Gupta, & Tiwari, 2012).

Haem synthesis requires sulfhydryl (-SH) dependent enzymes such as delta aminolaevulinic acid dehydratase (ALAD) and ferrochelatase, however, lead an electropositive metal has an affinity for sulfhydryl groups therefore inhibits actions of these enzymes (Gurer & Ercal, 2000). The three main enzymes involved in haem production are downregulated in a dose dependent manner. These enzymes are δ-aminolevulinic acid synthase (δ-ALAS), δ-aminolevulinic acid dehydratase (δ-ALAD) and ferrochelatase. Starting from glycine and succinyl CoA the mitochondrial enzyme, δ-aminolevulinic acid synthase (δ-ALAS) catalyzes the synthesis of δ-aminolevulinic acid (δ-ALA). From two δ-ALA molecules, in the presence of the cytosolic enzyme δ-aminolevulinic acid dehydratase (δ-ALAD) porphobilinogen is produced. Finally, the insertion of a ferrous ion (Fe^{2+}) into protoporphyrin IX to form haem is catalyzed by mitochondrial enzyme ferrochelatase (Flora et al., 2012). In lead toxicity δ-ALAD, a crucial enzyme in haem production is inhibited resulting in a decrease in haem levels with an increase in the quantity of δ-ALA. Therefore δ-ALA serves as a marker which can be found in blood and urine of subjects with lead exposure (Flora et al., 2012). A decrease in haem synthesis is observed when the activity of δ-ALAD is inhibited by 80–90 %, and this occurs at a blood lead concentration of about 55μg/dl (Ahamed, Verma, Kumar, & Siddiqui, 2006; Flora et al., 2012). It has also been shown that during lead exposure, accumulation of δ-ALA auto-oxidizes with the resulting conversion of oxyhaemoglobin to methaemoglobin (Flora et al., 2012).

Ferrochelatase inhibition by lead results in the exchange of iron by zinc producing zinc protoporphyrin (ZPP). In effect, ZPP concentration increases and its presence can be used as an indicator of lead exposure. Increased excretion of coproporphyrinogen in

urine and build-up of protoporphyrin in erythrocytes is as a result of ferrochelatase inhibition (Patrick, 2006).

Lead interferes with intracellular messenger system that is regulated by calcium and so affects endocrine and neuronal function (Needleman, 2004). Signal transduction is disrupted in the neurotransmitter and the normal organization of synaptic connections because of these disturbances (Bressler & Goldstein, 1991). There is "impaired performance on a wide variety of tests of learning and memory in a variety of animal models" and no threshold for these impairments have been recognised due to these lead-induced biochemical interferences in the brain (White et al., 2007). Immature astrocytes are more prone to the harmful effects of lead, as it interferes with "myelin formation and the integrity of the blood-brain barrier" leading to acute encephalopathy (Krigman, 1978).

Lead also affects the synthesis of collagen and therefore, vascular permeability. High doses of lead cause cerebral edema and haemorrhage (Needleman, 2004). In adult populations, the classical picture of severe lead toxicity includes bilateral wrist drop which results from segmental axonal demyelination and degeneration of the nerves leading to decreased nerve conduction velocities (Needleman, 2004).

High levels of lead generates more reactive oxygen species (ROS) that results in inactivation of endothelium-derived nitric oxide and the resultant increase in blood pressure (Dalloz, Maupoil, Lecour, Briot, & Rochette, 1997). Non-enzymatic peroxidation of arachidonic acid is caused by these ROS and results in the development of isoprostanes. This leads to vessel contraction and stimulates the production of

endothelin and the proliferation of smooth muscles of blood vessels as well as the aggregation of platelets. There is also a reaction between peroxynitrite and proteins, leading to the formation of nitrosothiols and nitrotyrosine as a consequence of inhibition of the action of prostacycline synthase. Subsequently, blood vessels contract and arterial blood pressure rises (Ahamed et al., 2006; Wójcicka, Bełtowski, & Jamroz, 2004).

High lead level causes a reduction in the volume of ejaculation, semen density, total sperm number and motility, and also increases the percentage of abnormal spermatozoa (Flora et al., 2012). Other effects of lead are reduced libido, abnormal spermatogenesis, chromosomal damage, infertility and changes in serum testosterone. Women having severe lead poisoning are more susceptible to prolonged and abnormal menstruations, infertility, miscarriage, stillbirth, premature membrane rupture, eclampsia and premature delivery (Flora et al., 2012).

Lead is capable of causing acute and chronic nephropathies (Carocci et al., 2016). Specific lead-binding proteins get bound to lead in the proximal tubular cells of the renal tubules. In acute lead nephrotoxicity, these lead-protein complexes are seen as typical intracellular inclusion bodies. These do not secrete any proteins in urine, but rather result in abnormal excretion of glucose, phosphates and amino acids causing Fanconi's syndrome (Carocci et al., 2016). Chronic lead nephropathy is much more severe and can cause irreversible morphological and functional changes, including glomerular and tubule-interstitial changes that go along with hypertension, hyperuricemia as well as renal breakdown (Rastogi, 2008). Accumulation of lead in the kidney mitochondria leads to both structural and functional changes, some of which

include swelling of the mitochondria and respiratory chain function inhibition as well as oxidative phosphorylation for ATP production. Consequently, energy-dependent processes, including tubular transport, are compromised (Carocci et al., 2016).

As Lead primarily accumulates in the bones, the metabolism of bone becomes affected (Carocci et al., 2016). Bone lead storage is in two compartments: the rapidly exchangeable lead is present at the surface of bone and the non-exchangeable lead is deeply located in the cortical bone (Carocci et al., 2016). Mobilization of lead in the bone in different physiological and pathological conditions can occur. Change in endocrine status, age, osteoporosis, and maternal age during pregnancy and lactation are examples of conditions that result in lead mobilization from the bones. (Flora et al., 2012). Low levels of lead (less than 10µg/dl) affect the immune and reproductive systems (World Health Organisation, 2010). Lead has been shown to be carcinogenic in animal models but not so in humans and so classified as a probable carcinogen (Carocci et al., 2016).

2.5 LEAD AND ANAEMIA

Anaemia is a well-known public health problem associated with an increased risk of morbidity and mortality with serious consequences for human health as well as social and economic development, especially among pregnant women and young children (McLean, Cogswell, Egli, Wojdyla, & De Benoist, 2009). Anaemia is a reduction in the total number of red blood cells, amount of haemoglobin in circulation, or circulating red blood cell mass. As a result, impaired oxygen delivery to tissues occurs, leading to

tissue hypoxia (Adamson & Longo, 2013). The accepted classification of anaemia based on haemoglobin concentration according to the WHO is displayed below.

Table 1: Classification of Anaemia by the WHO

Haemoglobin levels to diagnose anaemia at sea level (g/l)[±]

Population	Non-Anaemia*	Anaemia*		
		Mild[a]	Moderate	Severe
Children 6 - 59 months of age	110 or higher	100-109	70-99	lower than 70
Children 5 - 11 years of age	115 or higher	110-114	80-109	lower than 80
Children 12 - 14 years of age	120 or higher	110-119	80-109	lower than 80
Non-pregnant women (15 years of age and above)	120 or higher	110-119	80-109	lower than 80
Pregnant women	110 or higher	100-109	70-99	lower than 70
Men (15 years of age and above)	130 or higher	110-129	80-109	lower than 80

Source:(World Health Organisation, 2011b)

Lead inhibits haemoglobin synthesis as a result of its influence on erythroblast growth and interference with haemoglobin production. Several studies have demonstrated that lead inhibits enzymes that are involved in haemoglobin synthesis. The type of anaemia caused by lead poisoning is usually microcytic hypochromic and basophilic stippling of red cells. This occurs as a result of inhibition of pyrimidine 5'-nucleotidase, EC 3.2.2.10. This complication generally, appears only when the BLL exceeds 50µg/dl (Flora et al., 2012; Rempel, 1989). Lead poisoning also causes haemolytic anaemia and this is due to lipid peroxidation resulting from increased production of ROS (Flora et al., 2012; Rempel, 1989). However, both basophilic stippling and hypochromic anaemia are neither used as indicators of exposure to low levels of lead as haemoglobin levels do not decrease until blood lead levels are 50 µg/dl for adults and 40 µg/dl for children (Patrick, 2006).

2.6 LEAD AND PREGNANCY

Lead exposure in a sub-population of "women of child-bearing age", remains a public health problem and also for the developing foetus and infants for a number of important reasons (Bellinger, 2005). First of all, lead exposure in the pre-natal stages influences the health of the mother and infant birth and neuro-developmental outcomes (Bellinger, 2005). Additionally, lower levels of lead previously recognized as harmless are currently known to be linked with unfavourable health conditions in both children and adults (Canfield et al., 2003; Jusko et al., 2008; Lanphear et al., 2005; Navas-Acien, Guallar, Silbergeld, & Rothenberg, 2007). Another important reason is the mobilisation of bone lead stores during periods of pregnancy and lactation (Carocci et al., 2016; Flora et al., 2012). More than 90% of lead in the adult human body is stored in bone and a redistribution of cumulative lead stores from bone into blood may occur (Gulson, Mizon, Korsch, Palmer, & Donnelly, 2003). Because bone lead stores can remain for several years, women and their new-borns can be prone to the effects of lead poisoning several years after exposure to external environmental sources even when they are removed. Bone lead from the mother can also be transferred to the foetal skeleton (Patrick, 2006). Hypertension is a common complication of pregnancy and lead is a well-known risk factor for hypertension among adult populations (Kosnett et al., 2007). Lead therefore is an important risk factor in the development of hypertension and pre-eclampsia in pregnancy (Karumanchi, Maynard, Stillman, Epstein, & Sukhatme, 2005). High levels of lead can lead to spontaneous abortion (Hertz-Picciotto, 2000). During pregnancy, exposure to lead increases the danger for preterm delivery and low birth weight as studies have found an increased risk with delivering a child with neural tube defect in women exposed to high lead levels (Bound, Harvey, Francis, Awwad, & Gatrell, 1997; Irgens, Krüger, Skorve, & Irgens, 1998). Evidence also suggests that

there is an association between maternal occupational lead exposure and total anomalous pulmonary venous return (Jackson et al., 2004). The transport of lead through the placenta and also through breast milk to infants results in similarities in BLLs of the mothers and that of their infants (Ab Latif Wani & Usmani, 2015).

2.7 CHILDHOOD LEAD POISONING

Children are more prone to lead poisoning because their bodies are still undergoing development. They also absorb lead more quickly than adults and are more likely to ingest and inhale dust contaminated with lead (World Health Organisation, 2010). Lead causes asymptomatic damage to the neuro-behavioural function in the central nervous system (CNS) of children at doses that are inadequate to produce clinical encephalopathy. In younger children, whole BLL between 1–3 µg/dl is associated with sub-clinical neuro-behavioural toxicity (Canfield et al., 2003). Children with elevated body lead burden but clinically asymptomatic were found to have a four to five point deficit in average oral intelligence quotient scores (IQ) relative to children from the same locality with reduced issues of lead after a wide range of socio-economic, behavioural and biological factors were corrected for (World Health Organisation, 2010). A reduction from 10 µg/dl to 20 µg/dl in BLL may not affect IQ levels compared to an increase of <1 µg/dl to 10 µg/dl which is associated with a six Quotient score point decrease (World Health Organisation, 2010). A widespread exposure to lead in a population leads to a decrease in its mean Quotient scores. This results in significant increase in the number of children with reduced aptitude and elevated mental retardation. In addition, there is a considerable decrease in the number of children with true superior Quotient scores. These children will perform poorly in school and may

even require special teaching and other corrective measures. This becomes a limitation to their ability to make valuable contributions to humanity when they grow into adulthood and could lead to a decrease in a country's prospect leadership. A gap in socio-economic accomplishments is created between regions with elevated and low levels of lead (World Health Organisation, 2010). The detrimental effects of lead are highlighted in Figure 1.

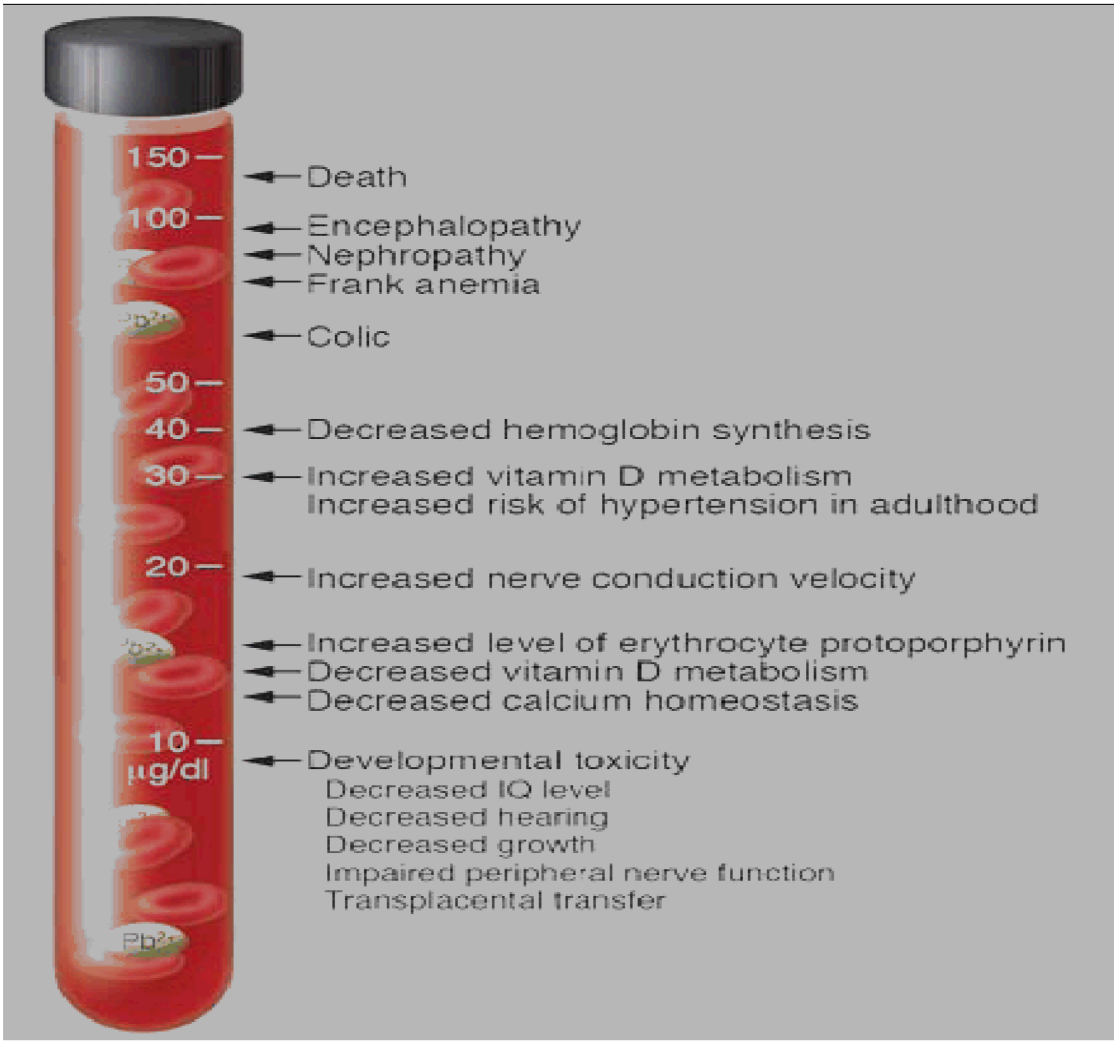


Figure 1: Effects of lead poisoning.

(Source: Adapted from Belinger and Belinger, 2006).

2.8 METABOLISM OF LEAD

Four main isotopes of lead namely, ^{204}Pb (1.4%), ^{206}Pb (24.1%), ^{207}Pb (22.1%) and ^{208}Pb (52.4%) exist as a mixture in nature. These isotopes act identically with regards to their metabolism (Markowitz, 2000). Children absorb more lead than adults and therefore become more susceptibility to its toxic effects. Among adults, lead absorption is mainly through the respiratory tract where about 30 to 70% of inhaled lead enters circulation (Markowitz, 2000). Lead is deposited into three major pools in the body. The first is the blood pool that is rapidly exchangeable but constitutes only 2% of the body's total lead burden. Lead levels in the blood is the most important component biochemically, as it reflects recent exposure (up to 40 days) (Ab Latif Wani & Usmani, 2015). The remaining lead is distributed between an intermediate pool consisting of the skin and muscle, and a stable pool in dentine and the skeleton. Over 95% of the total body load of lead is found in the skeletal pool and has a biological half-life of 20–30 years. The main means of lead removal from the body is through the urine (Gordon, Taylor, & Bennett, 2002).

2.9 EPIDEMIOLOGY OF LEAD POISONING

An estimation by the National Institute of Occupational Safety and Health (NIOSH), revealed that over 3 million people within the working class in the United States are prone to contact with lead in their workplaces (World Health Organisation, 2016). Lead exposure is also estimated to account for about 0.6% of the world's burden of disease, with developing countries having the highest burden. Lead exposure among children is also estimated to contribute to about 600 000 new cases of children that develop intellectual disabilities and 143, 000 deaths per year with the highest burden in

developing countries (World Health Organisation, 2016). In Ghana, a study reported a 20% prevalence of lead poisoning in children less than 5 years in the Brong Ahafo Region (Report on Baseline Health Survey Report in the Newmont Mining Area, 2008).

2.10 BLOOD TRANSFUSION ASSOCIATED LEAD POISONING

Hazards of blood transfusion include any adverse event that may occur in a patient during or after the transfusion of blood or blood products (Negi, Gaur, & Kaur, 2015). "Blood transfusion is like marriage: it should not be entered upon lightly, unadvisedly or want only or more often than is absolutely necessary" (Alter & Klein, 2008). "The safest blood transfusion is the one not administered" (Galel & Fontaine, 2006). These statements are underlined by the numerous hazards of transfusion. These hazards are categorised based on different criteria. The ones of most interest are those caused by antigen-antibody reactions (immune reactions) due to genetic differences, and contamination by and transmission of various microbes (Negi et al., 2015). A study done in Ghana found hepatitis C virus antibodies in 5.2% of blood donors (Acquaye & Tettey-Donkor, 2000). Another study in Ghana has also demonstrated the possible risk of transfusion-transmitted human T-cell lymphotrophic virus type 1, hepatitis C virus and *Treponema pallidum* (Ampofo et al., 2002).

Infectious disease transmission through blood transfusion has been minimised but not eliminated using specific donor screening assays and other interventions. Other transfusion hazards that may persist are human error leading to the unintended transfusion of incompatible blood, acute and delayed transfusion reactions, transfusion-

related acute lung injury (TRALI), transfusion-associated graft versus-host disease (TA-GVHD) and transfusion-induced immunomodulation (Alter & Klein, 2008).

Blood transfusion has been considered as "a hidden source of lead exposure" and studies have shown the possible exposure to lead from blood transfusion to premature infants (Bearer, O'riordan, & Powers, 2000). Kameaka in 2010 stated that, in a study of lead exposure in premature infants, packed red cells, a product of blood "could actually double the lead concentration of the unit of blood". The removal of plasma (which contains just about 1% of the total blood lead volume) during packed cell preparation results in doubling of lead concentration as lead is bound to red cell. This therefore suggests that blood recipients can be at risk of lead poisoning if their corresponding donors have high lead concentrations especially in children as no ''safe'' threshold for BLL in young children is known (Duncan, Reid, & Voutchkov, 2010; World Health Organisation, 2016).

2.11 CLINICAL MANIFESTATIONS OF LEAD POISONING

Excess lead causes neurologic effects in adults and children with peripheral neuropathies predominating in adults (such as bilateral wrist or foot drop). Among children, CNS effects are more common. Severe neurological damage from lead poisoning could lead to cerebral oedema and encephalopathy. The neurological effect of lead in children is usually severe encephalopathy (Gordon et al., 2002). Long term exposure to lead in children include a lesser intellectual capability exhibited by low IQs, in addition to behavioural issues such as hyperactivity and poor organizational skills (Jin et al., 2006; Jusko et al., 2008). Excess lead also interferes with the normal

remodelling of calcified cartilage and primary bone trabeculae in the epiphyses in children, leading to increased bone density, which is detected as radio dense "lead lines" on radiographs. Lead again inhibits the healing of fractures by increasing chondrogenesis and delaying mineralization of cartilage (Kumar et al., 2005).

Lead inhibits normal haem biosynthesis resulting in the formation of zinc-protoporphyrin instead of haem. Elevated levels of zinc-protoporphyrin or its products in the blood, as well as free erythrocyte protoporphyrin, are vital indicators of lead poisoning. Characteristically, a microcytic, hypochromic, haemolytic anaemia results (Rempel, 1989).

Lead nephropathy was comparatively common among industrial workers and the distillers of illegal ‘moonshine’ whisky in America (Gordon et al., 2002). Long term exposure to lead causes interstitial nephritis and chronic renal failure. Acute and severe lead exposure could lead to proximal tubular dysfunction accompanied by glycosuria, hyper-phosphaturia, and aminoaciduria (Gordon et al., 2002). Lead colic, which includes sporadic vomiting, intermittent abdominal pain, and constipation, may occur with blood lead level of 60µg/dl (Agency for Toxic Substances and Disease Registry 2007). Other health effects as a result of chronic low-level exposure to lead among adults include hypertension, cardiac failure, renal failure and adverse reproductive outcomes such as impotence (Agency for Toxic Substances and Disease Registry 2007).

Lead could negatively influence the sexual maturation in a growing female causing a decrease in fertility. A study done in 2003 however, reported that BLLs as low as 3µg/dl

were related to 2-6 months delay in Tanner stage measurements (breast and pubic-hair development) and menarche in both African-American and Mexican-American girls (Selevan et al., 2003). Again, elevated blood lead levels were associated significantly with delayed menarche and pubic hair development, but not breast development. Even though scientific study is limited, it is speculated that relatively low BLLs may lead to changes in inception of sexual maturation and decreased fertility (Wu, Buck, & Mendola, 2003).

2.12 DIAGNOSIS OF LEAD POISONING

The BLL is the readily available biomarker for the determination of recent exposure (up to 40 days) to lead (Kumar et al., 2005). The BLL can be determined by a number of laboratory methods. These may include atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS) and anodic stripping voltammetry (ASV). Atomic absorption spectrometry uses either a flame (flame atomic absorption spectrometry, FAAS) or electro-thermal source, usually a graphite furnace (graphite furnace atomic absorption spectrometry, GFAAS) (World Health Organization, 2011a). Free red cell protoporphyrin IX levels or determination of zinc-protoporphyrin (ZPP) levels in addition to EBLLs are required for definitive diagnosis (Gordon et al., 2002). Anaemia may be the most apparent anomaly detected in some milder cases of lead poisoning (Kumar et al., 2005). Umbilical cord whole blood lead may be determined at delivery for neonatal lead exposure (Harville et al., 2005). Abdominal radiography may show the presence of radiopaque flakes. Head CT scanning could be needed in patients who present with impaired mental function to eliminate the possibility of cerebral oedema and structural lesions (Kumar et al., 2005).

2.13 LABORATORY DERTERMINATION OF LEAD IN WHOLE BLOOD.

Several laboratory methods can be employed to determine the amount of lead in whole blood. These methods may include the following:

2.13.1 Atomic absorption spectrometry (AAS)

The principle of the AAS is that free atoms absorb light at specific wavelengths. The amount of absorbed light can be correlated in a linear fashion to the concentration of the analyte present in the sample. In order to perform an AAS measurement for lead, the sample must first be processed to generate ground-state atoms as vapour within the light path of the instrument. This process is known as atomization and can be done with a flame, (flame atomic absorption spectrometry, FAAS) or electro-thermal source, most often a graphite furnace (graphite furnace atomic absorption spectrometry, GFAAS) (World Health Organization, 2011a). The flame atomic absorption spectrometry makes use of an acetylene–air or a nitrous oxide–acetylene–air laminar flame to atomize lead at temperatures ranging between 2000–3000 °C (World Health Organization, 2011a). This method is relatively cheaper, simple to use and free from interferences. The GFAAS uses an electrically heated graphite tube to vaporize and atomize the analyte at temperatures up to 3000 °C prior to its detection. It is a very sensitive method but subject to interferences. These interferences have been reduced by enhanced instrument design in addition to using matrix modifiers. Improved instrument design also makes it possible to detect BLLs as low as 1µg/dl with the AAS. Smaller sample volumes between 10–50 µl can also be measured by this method (World Health Organization, 2011a).

2.13.2 Anodic stripping voltammetry (ASV)

With the ASV method of estimating blood lead, a standard electrode and a thin-film mercury graphite electrode are placed in the blood sample, followed by a negative potential application to the mercury electrode for few seconds causing the lead in the blood sample and other cations present to accumulate on the surface of the mercury electrode which is negatively charged (World Health Organization, 2011a). The potential's direction is then reversed to yield a progressively higher potential over several minutes. All other ions are released from the electrode when a specific characteristic lead voltage is reached. A measureable current which is directly proportional to the number of lead ions released is produced. This is then compared with calibration solutions to evaluate the lead concentration in the blood sample (World Health Organization, 2011a). This method requires sample preparation because the lead has to be available as free Pb^{2+} aqueous cation. These ASV devices have a detection limit between 1–100µg/dl. Portable ASV devices (example, leadcare device) are available for use on the field and also as a point of care device. It is recommended that BLLs greater than 10µg/dl are confirmed with either the AAS or ICP-MS. The presence of co-reducible metals and chelating agents or elevated copper concentrations can create interferences. Small sample volumes of 50µl are used (World Health Organization, 2011a).

2.13.3 Inductively coupled plasma mass spectrometry (ICP-MS)

This method involves the use of inductively coupled plasma to atomize and subsequently ionize the atoms of interest in the sample. Extraction of the ions from the plasma is done and they are passed through a mass spectrometer. In the mass

spectrometer, the ions are separated and measured based on their mass-to-charge ratio. The ICP-MS has a high selectivity of filters of ions in addition to highly amplified ionic signals that strike the detector. It also has low background interferences with noise and offers very minute detection limits (part per trillion to part per billion) for majority of elements (World Health Organization, 2011a). The ICP-MS method has a detection limit of nearly 0.2 µg/dl for direct analysis of lead in blood. Blood sample dilution before aspiration into the plasma is very vital because ICP-MS is less tolerable relative to AAS of heavy matrices. Based on this, highest standard of operation by skilled personnel is required for the operation of ICP-MS. The ICP-MS can evaluate several elements from a lone sample volume as low as 50–100 µl and also permits the measurement of the lead isotope ratio present in the blood sample. This makes it feasible in making out whether the lead was from a specific location. The ICP-MS is expensive though highly productive and therefore, becomes relatively economical in analysing many samples or elements. (World Health Organization, 2011a).

The AAS method for the detection of blood lead was adopted for this study. The reason being that it is relatively cheaper and available and also has a high detection limit as well as a low detection limit of above 100µg/dl and 1µg/dl respectively. It can also analyse smaller sample volumes of 10 to 50µl. Improvement in instrument designs has also reduced the problem with interferences.

2.14 MANAGEMENT OF LEAD POISONING

The most vital aspect concerning the treatment of lead poisoning involves the identification and elimination of source of exposure (Gordon et al., 2002). In situations

where the blood lead level is greater than 25µg/dl, chelation therapy using any one of the following compounds, succimer, D-pennicillamine, edetate calcium disodium, dimercaprol can be done. (Kathuria, 2017). These chelating agents form complexes with lead, prevent its binding to cell constituents and because they are hydrophilic, are eliminated in the urine (Gordon et al., 2002). Exchange transfusion together with chelation therapy may be used in neonates (Gordon et al., 2002). In acute exposure, gastric lavage, cathartics or whole bowel irrigation may be used to reduce lead absorption (Kathuria, 2017). Dietary measures which includes improved mineral (Like Calcium, Iron, Zinc) intake to prevent lead absorption also helps (Kathuria, 2017).

CHAPTER THREE

3.0 METHODS

3.1 STUDY DESIGN

The study is a cross sectional comparative study of blood lead levels among four distinct at risk occupational groups and apparently healthy blood donors at Kenyasi in the Brong Ahafo Region of Ghana.

3.2 STUDY SITE DESCRIPTION

The study was carried out at Kenyasi, a mining town located in Asutifi North district of Brong Ahafo region of Ghana. Kenyasi (appendix 7 – map of Ghana showing Kenyasi) is the capital town of the district with a population of 52,259 which represents 2.7% of the region's total population (Ghana Statistical Service, 2014). Kenyasi is known for the mining of gold with the presence of Newmont Ghana (A mining company), operating in the area. However, illegal mining activities have been rampant in the area for several years now. A news report on GhanaWeb, accredited to the Ghana News agency, way back in 2009, even puts the population of illegal miners (popularly known as galamsey operators in Ghana) at 10,000 and interestingly, it is the most booming economic activity in the area. The town has diverse occupations including office workers and at risk occupations such as small scale miners, auto-mechanics, fuel station attendants/automobile drivers, painters and sprayers thus making it an ideal locality for this study. Control participants were recruited from Hwidiem St. Elizabeth Hospital which is the nearest hospital with a blood bank serving the people of Kenyasi and its environs.

The hospital on average gets about 102 donations per month (Records from Hwidiem St. Elizabeth Hospital blood bank, 2016). Laboratory analyses were carried out at the Kintampo Health Research Centre (KHRC) laboratory (full blood count and blood smears) and the Chemistry laboratory of the Ghana Atomic Energy (Blood lead estimation) because these facilities have the equipment and resources needed for the various analyses.

3.3 STUDY POPULATION

The study population was apparently healthy individuals from among four at risk occupations namely auto-mechanics, fuel station attendants/automobile drivers, painters/Sprayers and small scale miners who met the eligibility criteria at Kenyasi in the Brong Ahafo Region of Ghana. Blood donors from Hwidiem St. Elizabeth Hospital, who had been medically screened and selected to donate blood, but fell outside these selected at risk occupations served as control group.

3.4 SAMPLE SIZE

The sample size for this study was determined by assuming a mean blood lead concentration (µg/dl) of 8.5, 8.5, 6.5, 6.5 and 5.0 for Small scale miners, Auto-mechanics, Painters/Sprayers, Gasoline attendance/Commercial drivers and blood donors respectively. [This is based on the recommendation by the CDC that BLL ≥5µg/dl is elevated and therefore requires a public health intervention (United State Centers for Disease Control and Prevention, 2015)]. With a common standard deviation of 4.5, a 5% type I error rate and a power of 90%, an estimated sample size of 36

participants within the ages of 18-60 years will be required for each distinct occupational group using a power formulation in SAS. Adjusting for a 10% non-response rate in each group, a total sample size of 200 thus, 40 in each group was required to compare the differences in their blood lead concentration as well as some pre-disposing factors. Below is the formula for the sample size calculation.

$$n = 2\frac{\left(\sigma Z_{1-(\frac{\alpha}{2\tau})} + Z_{1-\beta}\right)2}{\mu_A - \mu_B}$$

Where;

n=Sample Size

Ϭ is the standard deviation

α is the type I error rate

τ *is the number of comparisms*

β *is the type II error, meaning* $1-\beta$ *is Power*

μ *is the estimated mean lead concentration for a particular occupational group*

Z is standard normal distribution

A and B - any of the occupational group

3.5 INCLUSION CRITERIA

i. All apparently healthy individuals, not suffering from any underlying acute or chronic disease within each of the selected at risk occupational groups – auto-mechanics, fuel station attendants, small scale miners, painters/sprayers.

ii. Apparently healthy blood donors who do not belong to these selected occupations and have passed the blood donation screening examination.

iii. Must be within the age range of 18-60 years.

iv. Those who consented to participate in the study.

3.6 EXCLUSION CRITERIA

i. Those suffering from acute (example cold, fever, headaches) or chronic (e.g. diabetes, hypertension) disease within each of the selected at risk occupations.

ii. Those outside the age range 18-60 years.

iii. Those who did not give consent to participate in the study.

3.7 SAMPLE AND DATA COLLECTION

Convenience sampling procedure was used to select study participants. Interaction with the various groups and the blood donors was done to explain the study to them and their consent sought. Those who agreed to be part of the study were given consent forms (appendix 3) to sign in the presence of a witness. The witness also signed the consent form after which the investigator also signed. Participants who could not provide signatures were given a stamp pad for thumb printing. They were then converged at a suitable location within their various premises for the sample collection. Prior to the sample collection, a profile form was issued to each consented participant for the collection of background information such as age, sex, occupation, source of drinking water etc. (appendix 4). The investigator filled the form as the participant provided the answers.

3.7.1 Blood sample collection

Materials for blood sample collection

- Two (2) 500µl EDTA tubes
- 5ml syringes and needles
- 70% isopropyl alcohol
- Tourniquet
- Cotton wool
- Adhesive tapes
- Gloves
- Sharps container
- Biohazard waste bin

3.7.2 Procedure for sample collection

- Each participant was made to sit in a comfortable chair and well positioned in the chair.
- The arm of the participant was hyper extended and a suitable vein located.
- This was done by palpating and tracing with the index finger.
- The tourniquet was then applied above the venepuncture site (about 3inches). To avoid venostasis, the tourniquet was not left on the arm for more than 2 minutes.
- The participant was then asked to make a fist and cotton wool soaked in 70% alcohol was used to clean the puncture site in a circular manner, beginning from the puncture site and moving outwards.
- The alcohol was allowed to dry to disinfect the site of puncture.

- The sterile needle and syringe was then taken and opened at the full glare of the participant to acknowledge that it is sterile.
- The needle was then positioned on the syringe and the bevel was aligned with the graduations on the syringe.
- The arm of the patient was then held and the needle gently inserted in the vein at an angle of about 20 degrees while soothing their pain with kind words.
- One millilitre (1ml) of blood was drawn into the syringe.
- The tourniquet was released and the needle, gently removed by a backward motion.
- Clean cotton was then applied to the site of the puncture, and the participant made to apply adequate pressure to avoid excessive bleeding as well as formation of a haematoma.
- The needle cap was placed on a table (to avoid needle pricks) and the needle carefully inserted into the needle cap and pressed to close. It was twisted and removed from the syringe and placed in the sharps container.
- The blood was then dispensed into the EDTA tubes from the syringe and not through the needle (to avoid haemolysis).
- 500µl of the blood was dispensed into each of two EDTA tubes after uncapping and recapping.
- The samples were then mixed immediately to avoid clotting.
- They were then placed in a rack and put in an ice chest containing ice packs at temperature between 2 to 8 degrees Celsius to preserve sample integrity.
- The above procedure was repeated for each participant until all 200 participants were sampled.

- The samples collected were preserved at 2 to 8 degrees Celsius on site in the laboratory refrigerator at St. Elizabeth Hospital until transported to the Kintampo Health Research Centre (KHRC) laboratory and the Ghana Atomic Energy commission's Chemistry laboratory for analysis. All samples were transported to their various destinations within three days of collection.
- During transportation, the samples were kept in an ice chest containing ice packs and temperature maintained between 2 to 8 degrees Celsius.
- One tube was sent to Kintampo Health Research Centre (for full blood count, and blood smear preparation) and the other to the Ghana Atomic Energy Commission (for blood lead analysis).

3.8. LABORATORY ANALYSIS

3.8.1 Full blood count measurement using the ABX Micros 60 Haematology analyser.

Materials and equipment:

- Sample mixer
- ABX Haematology analyser
- QC samples – low, normal and high.
- Reagents – Miniclean, Minidil, Minilyse
- Printer
- A4 sheets
- Gloves and protective clothing

The ABX Micros 60 Haematology analyser (Horiba-ABX, Montpellier, France), is a three-part differential analyser. It provides count for lymphocytes, monocytes and puts all the granulocytes together (neutrophils, eosinophils and basophils).

3.8.1.1 Start-up analysis

Upon switching on the analyser, it takes 3 minutes to do a self-check and then automatically runs a start-up. This process checks the background to ensure no particles interfere with real sample measurements. Upon completion, the results for the main blood cell measurements (haemoglobin, red blood cells, haematocrit, white blood cells and platelets) are displayed. This usually shows 0.00 for all parameters and the start-up will be deemed as passed. The results are printed automatically, signed by the operator and double-checked by a supervisor. If the start-up fails, the machine automatically repeats until it passes. If the failure persists after a couple of repeats, a signal will be given that the start-up has failed. Samples are not analysed in this mode because every result will have the inscription **"**start-up failed''. Troubleshooting is done in such instances and this may include, concentrated cleaning using 75% percent bleach, back flushing, needle rinsing, auto-cleaning etc. The assistance of a servicing technician may be sought in instances where the problem is above the operator.

3.8.1.2 Quality control:

After start-up has passed, the next stage of quality check is the running of the quality control (QC) material. The manufacturer, ABX, commercially prepares the QC material. Three levels of controls (low, normal, high) are run on a daily basis. The following steps illustrate how the QC sample is analysed.

The three levels of controls are brought out of the fridge and allowed to attain room temperature.

- On the screen of the analyser display window, the main menu is selected, then the ‘up’ and ‘down’ arrows are used to select the ‘QC’ and then ‘Enter’ is pressed to access the ‘QC’ mode.
- Upon entering the ‘QC’ mode, ‘Automatic’ is selected, followed by the level of ‘QC’ to be run.
- The QC card, which contains information about the various levels of controls and their reference ranges, is inserted in the designated space in front of the analyser. Each lot of controls come with its own QC card.
- Series of steps to enable confirmation of the lot number, date of expiry are followed and then the ‘Enter’ key is pressed. This automatically gets the machine ready for sampling as the probe comes out.
- The level of control to be run is mixed by inverting the tube 10 times, following standard operating procedures in the KHRC laboratory.
- The sample is then uncapped and the tube is placed beneath the sampling needle.
- The tube is moved upwards to allow the needle to be submerged in the sample.
- The sampling bar is then pressed and about 10µl of the sample is aspirated and automatically analysed.
- After about I minute of analysis, the display window shows the results of the control sample analysed.

- The letters H or L may appear beside any values that fall outside the upper or lower limit respectively.
- The results are automatically printed and scrutinized for accuracy.
- The same procedure is repeated for the other two levels.
- If any parameter falls outside the reference ranges, a repeat is done. If this persists, trouble shooting is done to identify the cause of the problem and the problem is rectified.
- The printouts are signed and then crosschecked by a supervisor.
- They are then filed in their appropriate files.

Calibration of the analysers are done along with routine servicing, every three months by service engineers or after a corrective maintenance and also whenever the need arises by the operator. Calibration materials are provided by the manufacturer.

3.8.1.3 Sample analysis for FBC

- The sampling mode was selected from the main menu of the analyser display window.
- The 'Enter' key was pressed to enable capturing of participant identification number.
- The tube was uncapped after flicking the bottom and inverting the tube 10 times.
- It was placed beneath the probe and moved upwards to allow the needle to be submerged in the sample.
- The sampling bar is then pressed and about 10µl of the blood is aspirated and analysed.
- The analyser display screen displays the results after about a minute and it is further printed automatically. Results are printed in duplicates. One to be given to the participant.
- The results are validated and if there is a need for a repeat, it is done.
- It is then checked and counter checked by a colleague in the laboratory.

3.8.2 Preparation of smears for blood film morphology

Materials and equipment

- Frosted end microscope glass slides – clean, dry and grease-free
- Pencil for labelling
- Pasteur pipette
- Spreader

Procedure:

- The frosted end of a clean dry grease-free microscope slide was labelled with the identification number of the sample using a pencil.
- The EDTA anticoagulated whole blood was well mixed by inverting the tube 10 times.
- A drop of the blood was placed at about 1cm from the frosted end of the microscope glass slide.
- A clean spreader (a glass slide with chipped ends) was used to spread the blood to the other end of the slide by raising the spreader at an angle of about 45 degrees and applying swift forward movement making sure a smooth feathered end is produced.
- The slide was waved back and forth and then allowed to air-dry.

3.8.3 Leishman staining procedure

Materials and equipment needed:

- Leishman stain
- Staining rack
- Pasteur pipette
- Phosphate buffer – pH 6.8
- Draining rack
- Tissue paper or Cotton
- Timer
- Gloves

Procedure

- Batches of 10 slides were stained at a time.
- The slides were arranged on a staining rack making sure they were not tilted to one side.
- Counted drops (10) of the Leishman stain was used to cover the entire smear and allowed about 3 minutes to fix and stain the smear.
- The stain was then diluted with a double volume (20 drops) of phosphate buffer (pH 6.8), and well mixed by sucking the stain at one end and dispensing at the other end while ensuring there is no spill over.
- Slides were left to stain for about 10 minutes after which the stain was washed off with the buffer.
- The buffer was left on the slide for about 2 minutes to allow for differentiation.
- The buffer was drained off, back of the slides wiped with tissue paper and arranged in a draining rack to air dry.

3.8.4 Microscopic examination of slides for Basophilic stippling and microcytosis

Materials and equipment needed:

- Microscope
- Immersion oil
- Lens cleaning tissue
- Pen and data sheet for recording of results

Procedure:

- The air dried smear was mounted onto the microscope stage. The 40x objective lens was used to scan through the smear with the condenser iris sufficiently closed to provide good contrast. A good area is selected at the tail end of the smear where red cells are just touching.
- The microscope stage was then moved downwards and the objective lens changed to 100x oil immersion.
- A drop of immersion oil was then placed on the smear and the stage moved upwards.
- The coarse and fine adjustments were then used to obtain a sharp focus.
- Basophilic stippling was identified as red cells with dark-purple-staining granules in their cytoplasm.
- Microcytes were identified as red cells smaller than the nucleus of a small lymphocyte, in addition to MCV less than 80fl from the FBC results.

3.8.5 Measurement of blood lead levels

Blood lead measurements were done at the Ghana Atomic Energy Commission's Chemistry laboratory using AAS (VARIAN AA 240FS- Atomic Absorption Spectrometer in an acetylene- air flame). The principle of this technique is that, by exposing a sample to a strong acid and moderate temperature which leads to thermal decomposition of the sample and the solubility of heavy metals in solution, it becomes possible to quantify the sample through elemental techniques.

Materials and equipment needed:

- 50ml measuring cylinder

- 100ml class A beaker
- Test tube
- Fume chamber
- Cling film
- Hot plate
- A dropper
- Wash bottle
- Weighing balance and standard weight
- Forceps
- Protective gloves

Procedure:

3.8.5.1 Weighing of sample

- Upon receipt in the laboratory, the samples were removed from the ice chest and placed on the bench to attain room temperature.
- The balancing knobs on the sides of the weighing balance were adjusted to ensure that the spirit or the liquid level is centred to ensure accuracy of measurement.
- A standard weight of 2g was then picked with the forceps and placed on the balance to calibrate it.
- This measured 2g and implied that the balance was ready for measuring the weight of the blood samples.
- The beaker to be used for each sample was labelled with the sample identification number and then placed on the balance and its weight zeroed.

The dropper was then used to transfer drops of well mixed EDTA anticoagulated whole blood into the beaker until it measured 2g.

- The measured samples were then transferred into a fume chamber for the addition of the nitric acid and hydrogen peroxide for the digestion process.

3.8.5.2 Digestion:

- Inside the fume chamber, 10mls of 67% nitric acid was added to the sample followed by 2mls of 30% hydrogen peroxide.
- The mixture was gently swirled and covered with cling film
- It was then placed on a hot plate for 3 hours at a temperature of 45^{o}C.
- After the digestion, the sides of the beaker were washed with double distilled water using a wash bottle to make a final volume of 20mls.
- This was then transferred into a test tube for AAS measurement.

Note: Volume adjustments were made depending on the weight of blood measured (example, using 5ml of nitric acid and 1ml of hydrogen peroxide and making up to 10mls of final volume for 1g of whole blood)

3.8.5.3 Atomic absorption spectrophotometric analysis

Blanking and Calibration:

- A series of steps were followed to select the type of element to be measured (Pb), the lamp current (10.0mA), and wavelength (217.0nm). Other settings include the time for measurement (2sec), minimum reading (0.00mg/L), lower valid limit for standard concentration (0.00mg/L) and upper valid limit of

(11.0mg/L) – (i.e. +10% of the higher standard which is 10.0mg/L), and number of decimal places of measurement (3).

- After inputting the required information into the analyser, the OK button was pressed and the start button is clicked.
- Double distilled water which serves as the blank was aspirated and its concentration determined (0.000mg/L). The first standard concentration with a known value of 2.0mg/L was also aspirated and the concentration measured was 2.000mg/L. The second standard concentration with a known value of 5.0mg/L was also aspirated and the concentration measured was 5.000mg/L. The third standard concentration with a known value of 10.0mg/L was also aspirated and the concentration measured was 10.000mg/L. The calibration curve was automatically drawn by the analyser and it was acceptable. The rate of absorbance is directly proportional to the concentration of the element. The analyser was then ready for the samples to be analysed.
- Prior to sample analysis, a worksheet was created on the computer screen that is attached to the analyser and labelled with the laboratory identification codes for the samples to be analysed.
- Lead concentrations in samples were measured one after the other by clicking the 'Read' button after aspiration. Concentrations of the samples were measured seven times within two seconds and the average given. The level of precision for acceptable results is between 1-25%. Precision above 25% requires a repeat.
- The final concentration of the sample is calculated as: the concentration measured, multiplied by the nominal volume (final volume after digestion, that is, 20ml) all divided by weight of sample in grams (2g).

- If any dilution was made, the dilution factor is multiplied by the concentration before calculating the final concentration in milligrams per litre (mg/L). The results in mg/L were converted to micrograms per decilitre (µg/dl) by multiplying by 1000. Samples were read in duplicates and their averages were taken.
- For every ten samples that are run, two standards are read, one QC standard is read and a blank is also read.
- A QC standard is a sample with a known concentration that is treated the same way as the sample and its percentage recovery is calculated as: (measured value/expected value) x100%. The percentage recovery should be between 77% and 120%.
- The blank checks for contamination during the sample preparation, the standard checks for the efficiency of the equipment and the repeats check the reproducibility of the method used.
- These are all QC/QA measures to check work quality.
- Reference standards used are from FLUKA ANALYTICAL, Sigma-Aldrich Chemie GmbH, a product of Switzerland.

Table 2: Working Conditions For AAS For The Determination Of Blood Lead.

Element	Wavelength (nm)	Lamp Current (mA)	Fuel	Support
Pb	217.0	5	Acetylene	Air

(Procedure adopted from VARIAN. Publication No 85- 100009-00 Revised March 1989).

3.9 DATA MANAGEMENT

Confidentiality of data obtained for all study participants was ensured. Hard copies have been stored in locked cabinets with access limited to the investigators. Unique identification numbers were assigned to each person instead of names. All data were coded and double entered into Microsoft Excel to reduce the occurrence of missing values. Data verified for accuracy was transferred into STATA version 14.0 for analysis.

3.10 DATA ANALYSIS

Stata version 14.0 was used in the data analysis. Participants' lead levels were categorized as either elevated or not elevated. As blood lead levels (BLLs) were not normally distributed, data of BLLs were expressed as geometric mean and geometric standard deviation (GSD). Statistical analysis was done after logarithmic transformation. Elevated lead levels were blood lead concentration ≥5µg/dl. This binary indicator was used as the response variables. Occupation was used as the main predictor. Participant's age, sex, marital status, number of years of house painting, type of cooking material, etc. were used as confounders. Descriptive measures were provided for all study variables. For continuous variables such as age, haemoglobin concentration, etc. means and standard deviations were provided. Categorical data such as occupation, educational level, etc. were presented using frequency tables. For studying the association between each of the predictors and the binary response (elevated or non-elevated lead levels) a univariate logistic regression model was used. A multiple logistic regression model was used to study the effect of occupation on lead levels, while accounting for the effect of the possible confounders. ANOVA was used

to test for differences in BLLs among the different occupational groups. The Tukey's procedure was applied for the set of all pairwise comparisons. All statistical tests were two sided and the level of significance was 0.05.

3.11 ETHICAL APPROVAL

Ethical approval was sought from the Ethical and Protocol Review Committee of the, College of Health Sciences, University of Ghana (appendix 2) as well as from the Institutional Ethics Committee of the Kintampo Health Research Centre (appendix 1). Permission was also sought from the leaders of occupational groups involved in the study and from the Head of blood bank in the area.

3.12 CONSENTING PROCESS

Written informed consent (appendix 3) was sought from all participants in this study, after the background, goal and objectives of the study were explained to them. Prospective participants were given copies of the written informed consent forms bearing their signature/thumbprint (for illiterate respondents) and the signature of the researcher or a designated person. Only participants who agreed to participate and provided a written consent were enrolled to take part in the study.

3.13 PRIVACY AND ANONYMITY

Study identification numbers were assigned to all respondents and data analysis was done based on these numbers without referring to any particular participant's name.

3.14 RISK

Venous blood sampling involves the risk of minimal pain at the site of venepuncture, discomfort, infection. Qualified and well-trained laboratory personnel as well as sterile syringes and needles were used to ensure these risks were avoided. Alcohol swab (antiseptic) was used to adequately clean the area of puncture to avoid introducing external microorganisms into the blood stream.

3.15 BENEFIT

Volunteers benefited by getting to know their blood lead levels (BLLs) and haemoglobin (Hb) values so that those with high levels and/or anaemia were prompted to seek medical attention to avoid complications. This was communicated to only the study participants and nobody else within a week of sample collection.

3.1 6 COMPENSATION

Participants did not receive any monetary or material compensation for taking part in this study.

3.17 VOLUNTARINESS AND THE RIGHT TO WITHDRAW.

Participation in this study was absolutely voluntary. Participants had the right to decline participation, or withdraw their consent at any time. Refusing or declining participation did not affect participants in any way.

3.18 CONFLICT OF INTEREST

The Principal Investigator and all other investigators declare no conflict of interest in the conduct of this research.

CHAPTER FOUR

4.0 RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS

A total of 200 participants made up of 186 (93%) males and 14 (7%) females were enrolled in the study. Forty (40) participants belonged to each of the selected at risk occupational groups which included auto-mechanics, fuel attendants/drivers, small scale miners and painters/sprayers (the lead exposed group) while forty (40) apparently healthy blood donors made up of office workers, teachers, bankers, traders, health workers served as the control (non-exposed) group.

The mean age of participants was 28.58 ± 8.17 years [95% CI: 27.44 – 29.72] with a range of 18 to 57 years. The average age of participants in the occupationally exposed groups was 28.07 ± 8.02 years and 31.35 ± 8.53 years for the non-exposed apparently healthy blood donors (Tables 3 and 4). The major sources of drinking water included pure water (sachet water) (97%, n=194), pipe borne water (74.5%, n=149) and water from well/boreholes (32.5%, n=65). The relationship between the source of drinking water and belonging to a particular group as well as among the exposed and non-exposed groups are demonstrated in Tables 7 through to 16.

The level of education of respondents were such that 56.5% of participants had attained only middle school or Junior secondary education with 21.5% and 2.5% of participants having attained Senior Secondary and University level education respectively. Those with University education were in the non-exposed group.

Table 3: Mean age of participants in the various study groups with minimum and maximum limits.

Study Group	Mean age	Min - max
Blood Donors	31.6	18 - 57
Small scale miners	29.4	19 - 42
Fuel attendants/Drivers	29.3	20 - 47
Auto-mechanics	25.4	18 - 40
Painters/Sprayers	27.2	18 - 55

Table 4: A comparison of mean age differences between the various study groups.

Comparison	Difference in mean ages	*p-value*	[95% Confidence	Interval]
Small scale miners Vs Blood donors	-2.175	0.223	-5.686786	1.336786
Fuel attendants/Drivers Vs Blood donors	-2.275	0.203	-5.786786	1.236786
Auto-mechanics Vs Blood donors	-6.25	***0.001***	-9.761786	-2.738214
Painters/Sprayers Vs Blood donors	-4.4	***0.014***	-7.911786	-0.88821
Fuel attendants/Drivers Vs Small scale miners	-0.1	0.955	-3.611786	3.411786
Auto-mechanics vs Small scale miners	-4.075	***0.023***	-7.586786	-0.56321
Painters/Sprayers vs Small scale miners	-2.225	0.213	-5.736786	1.286786
Auto-mechanics vs Fuel attendants/Drivers	-3.975	***0.027***	-7.486786	-0.46321
Painter/Sprayer Vs Fuel attendant/Driver	-2.125	0.234	-5.636786	1.386786
Painters/Sprayers vs Auto-mechanics	1.85	0.3	-1.661786	5.361786

Comparing the differences in mean ages among the study groups, there were significant differences between blood donors with auto-mechanics and with painters/ sprayers. There was also significant difference between auto-mechanics and small scale miners, and also with fuel attendants/drivers.

Table 5: Educational level of study Participants.

Study group	No formal education	Primary school	Middle/ continuation school/JSS	Technical/ commercial/ SSS	Post SSS	Total
Blood donors	0 0%	0 0%	9 22.5%	12 30%	19 47.5%	**40** **100%**
Small scale miners	1 2.5%	4 10%	28 70%	7 17.5%	0 0%	**40** **100%**
Fuel attendants/Drivers	0 0%	3 7.5%	22 55%	15 37.5%	0 0%	**40** **100%**
Auto-mechanics	1 2.5%	9 22.5%	30 75%	0 0%	0 0%	**40** **100%**
Painters/Sprayers	0 0%	7 17.5%	24 60%	9 22.5%	0 0%	**40** **100%**
Total	**2** **1%**	**23** **11.5%**	**113** **56.5%**	**43** **21.5%**	**19** **9.5%**	**200** **100%**

JSS=junior secondary school, SSS=senior secondary school

Table 6: Educational level of exposed and non-exposed groups.

Occupation status	No formal education	Primary school	Middle/ continuation school/JSS	Technical/ commercial/ SSS	Post SSS	Total
Unexposed	0 0%	0 0%	9 22.5%	12 30%	19 47.5%	**40** **100%**
Exposed	2 1.25	23 14.37%	104 65.00%	31 19.38%	0 0%	**160** **100%**
Total	**2** **1%**	**23** **11.50%**	**113** **56.50%**	**43** **21.50**	**19** **9.50%**	**200** **100%**

JSS=junior secondary school, SSS=senior secondary school

Table 7: Pure (sachet) water as source of drinking water among study groups.

	Pure Water (Sachet water)		
Study Group	Those who do not drink	Those who drink	**Total**
Blood donors	0	40	**40**
Small scale miners	2	38	**40**
Fuel attendants/Drivers	0	40	**40**
Auto-mechanics	3	37	**40**
Painters/Sprayers	1	39	**40**
Total	**6**	**194**	**200**

There was no significant relationship between taking sachet water and belonging to a particular group *(p = 0.211).*

Table 8: Sachet/pure water intake as source of drinking water among exposed and non-exposed groups.

	Pure Water (Sachet water)		
Status	Those who do not drink	Those who drink	**Total**
Non-exposed	0	40	**40**
Exposed	6	154	**160**
Total	**6**	**194**	**200**

No difference between the exposed and non-exposed as far as drinking pure water is concerned (*p-value=0.214*).

Table 9: Pipe borne water as source of drinking water and belonging to a particular group.

	Pipe borne water		
Study Group	Those who do not drink	Those who drink	**Total**
Blood donor	21	19	**40**
Small scale miner	3	37	**40**
Fuel attendant/Driver	15	25	**40**
Automechanic	6	34	**40**
Painter/Sprayer	6	34	**40**
Total	**51**	**149**	**200**

Table 10: Logistic regression for drinking pipe borne water and belonging to a particular group.

Study Group	Odds Ratio	*p-value*	[95% Confidence	Interval]
Control	ref			
Small scale miner	13.63158	***<0.0001***	3.60454	51.55162
Fuel attendant/Driver	1.842105	0.179	0.7552983	4.492731
Auto-mechanic	6.263158	***0.001***	2.154926	18.20348
Painter/Sprayer	6.263158	***0.001***	2.154926	18.20348

The logistic regression analysis for drinking pipe borne water and belonging to a particular group shows that Small scale miners, Auto-mechanics and Painter/sprayers have higher significant odds of drinking pipe borne water compared to the blood donors.

Table 11: Pipe borne water as source of drinking water among exposed and non-exposed groups.

	Pipe borne water		
Status	Those who do not drink	Those who drink	**Total**
Non-exposed	21	19	**40**
Exposed	30	130	**160**
Total	**51**	**149**	**200**

Table 12: Logistic regression for pipe borne water as source of drinking water and status (exposed versus non-exposed).

Status	Odds Ratio	*p-value*	[95% Confidence	Interval]
Non-exposed	ref			
Exposed	4.789	*<0.0001*	2.292709	10.00522

The exposed group has almost 5 times (4.789) the odds of drinking pipe borne water compared to the non-exposed group.

Table 13: Well/borehole as source of drinking water and belonging to a particular group.

	water from well/borehole		
Study Group	Those who do not drink	Those who drink	Total
Blood donor	39	1	40
Small scale miner	15	25	40
Fuel attendant/Driver	26	14	40
Auto-mechanic	32	8	40
Painter/Sprayer	23	17	40
Total	**135**	**65**	**200**

Table 14: Logistic regression for drinking from well/borehole and belonging to a group.

Study Group	Odds Ratio	*p-value*	[95% Confidence	Interval]
Control	ref			
Small scale miner	64.99999	***<0.0001***	8.075143	523.2104
Fuel attendant/Driver	21	***0.004***	2.601165	169.5394
Auto-mechanic	9.749999	***0.036***	1.15777	82.10822
Painter/Sprayer	28.82608	***0.002***	3.595589	231.1007

All the other groups have higher odds of drinking from well/borehole compared to the control group.

Table 15: Well/borehole water as source of drinking water among exposed and non-exposed groups.

	Water from well/borehole		
Status	Those who do not drink	Those who drink	**Total**
Non-exposed	39	1	**40**
Exposed	96	64	**160**
Total	**135**	**65**	**200**

Table 16: Logistic regression for well/borehole water for exposed and non-exposed groups.

Status	Odds Ratio	*p-value*	[95% Confidence	Interval]
Non-exposed	ref			
Exposed	26	***0.001***	3.483796	194.041

The exposed groups are 26 times more likely to drink water from well/borehole compared to the non-exposed group.

4.2 BLOOD LEAD LEVEL

Forty participants each from the five selected groups were screened for blood lead levels (BLLs). The geometric mean (GM) blood lead level (BLL) of study participants was 6.3 (GSD- geometric standard deviation 1.4) µg/dl [95% CI: 6.0 – 6.7] with a minimum BLL of 1.8 µg/dl and maximum of 14.4 µg/dl. The GM BLL for the exposed group was 7.0 ± 1.8 µg/dl and the non-exposed was 5.4 ± 1.8 µg/dl which was statistically significant (*p=0.0001*). Auto-mechanics recorded the highest geometric mean BLL of 8.1 ± 1.8 µg/dl while Blood donors recorded the lowest geometric mean BLL of 5.4 ± 1.8 µg/dl. Fuel Attendants/Drivers also recorded marginal increase in the geometric mean BLL of 5.5 ± 1.6 µg/dl. The distribution of GM blood lead levels of the various groups is demonstrated in Figure 2. The *p-value* of the F-test from the test of variance (ANOVA), was 0.000 (Table 18), and this shows that there is significant difference between the GM of BLLs of the exposed group and the non-exposed group. The Tukey's procedure was applied for the set of all pairwise comparisons (Table 19).

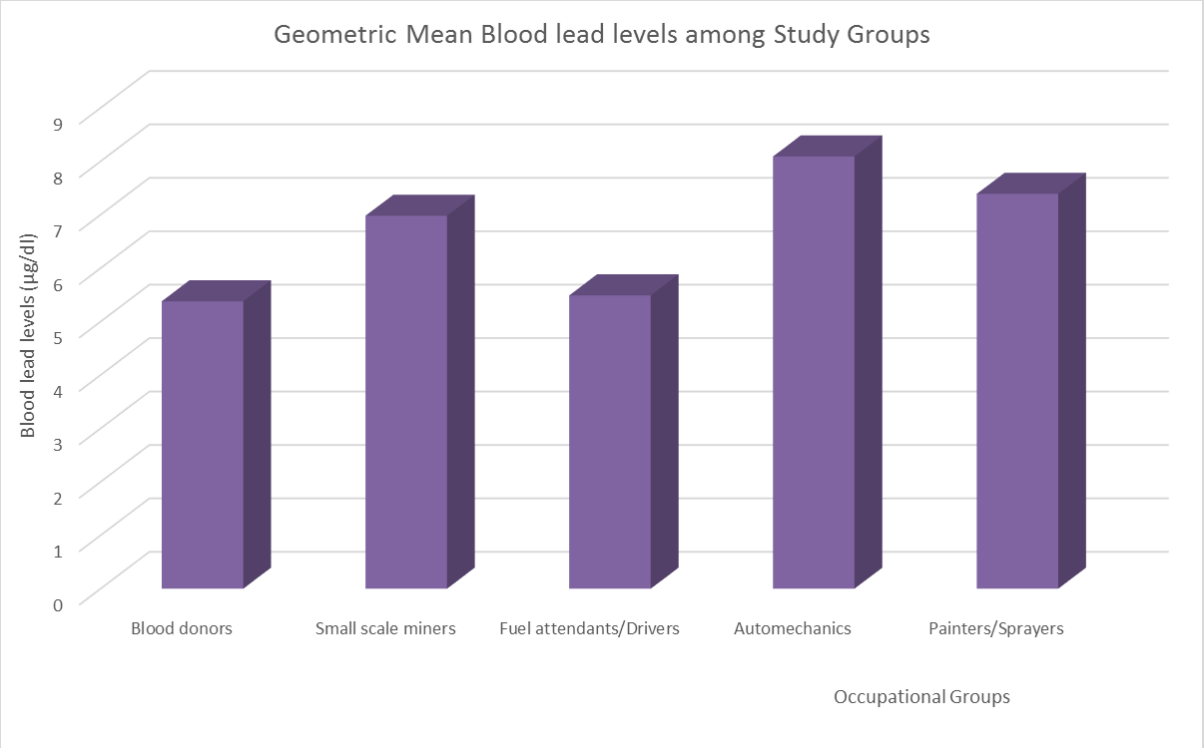


Figure 2: Geometric mean Blood lead levels (BLLs) among study groups.

Auto-mechanics registered the highest measured BLL of 14.4 µg/dl followed by Painters and Sprayers with 10.2 µg/dl. Fuel attendants recorded a maximum BLL of 7.7 µg/dl compared to Blood donors and Small Scale miners who had maximum values of 9.2 µg/dl. Blood donors also recorded the minimum BLL of 1.8 µg/dl. In general, the measured BLLs ranged from 1.8 to 14.4 µg/dl. These are highlighted in Table 17.

Based on the revised CDC/NIOSH reference value for elevated BLL being ≥ 5 µg/dl, the prevalence of elevated blood lead level (EBLL) among the study participants was 84.5%. All the painters/sprayers screened had EBLL followed by 97.5% for auto-mechanics, 95% for small-scale miners and 65% for apparently healthy blood donors and fuel attendants/Drivers. Table 20 shows the significant differences between the

distribution of EBLLs across the various groups. The prevalence of elevated BLL based on various study groups is shown in figure 3.

Table 17: Geometric Mean blood lead levels for the different study groups with ranges.

Study Group	Geometric Mean	Standard Deviation	Min-max
Blood Donors	5.4	1.81	1.8 - 9.2
Small scale miner	7.0	1.51	4.5 - 9.2
Fuel attendant/Driver	5.5	1.58	2.1 - 7.7
Auto-mechanic	8.1	1.75	2.0 - 14.4
Painter/Sprayer	7.4	1.32	5.5 - 10.4

Table 18: Test of variance (ANOVA) for the GM of BLLs of the exposed group and the non-exposed group.

Source	**ss**	**df**	**ms**	**F-test**	**p-value**
Between groups	227.910498	4	56.9776244	22.17	0.0000
Within groups	501.244493	195	2.57048458		
Total	**729.15499**	**199**	**3.66409543**		

***ss =sum of squares, **df=degree of freedom, ***ms=mean square**

Table 19: Tukey's procedure for the set of all pairwise comparisons for significant differences in GM of BLLs.

Comparison	mean difference	p-value
Small scale miners Vs Blood donors	1.5975	0.0000
Fuel attendants/Drivers Vs Blood donors	0.1125	0.9980
Auto-mechanics Vs Blood donors	2.7150	0.0000
Painters/Sprayers Vs Blood donors	2.0125	0.0000
Fuel attendants/Drivers Vs Small scale miners	-1.4850	0.0000
Auto-mechanics vs Small scale miners	1.1175	0.0180
Painter/Sprayers vs Small scale miners	0.4150	0.7750
Auto-mechanics vs Fuel attendants/Drivers	2.6025	0.0000
Painters/Sprayers Vs Fuel attendants/Drivers	1.9000	0.0000
Painters/Sprayers vs Auto-mechanics	-0.7025	0.2900

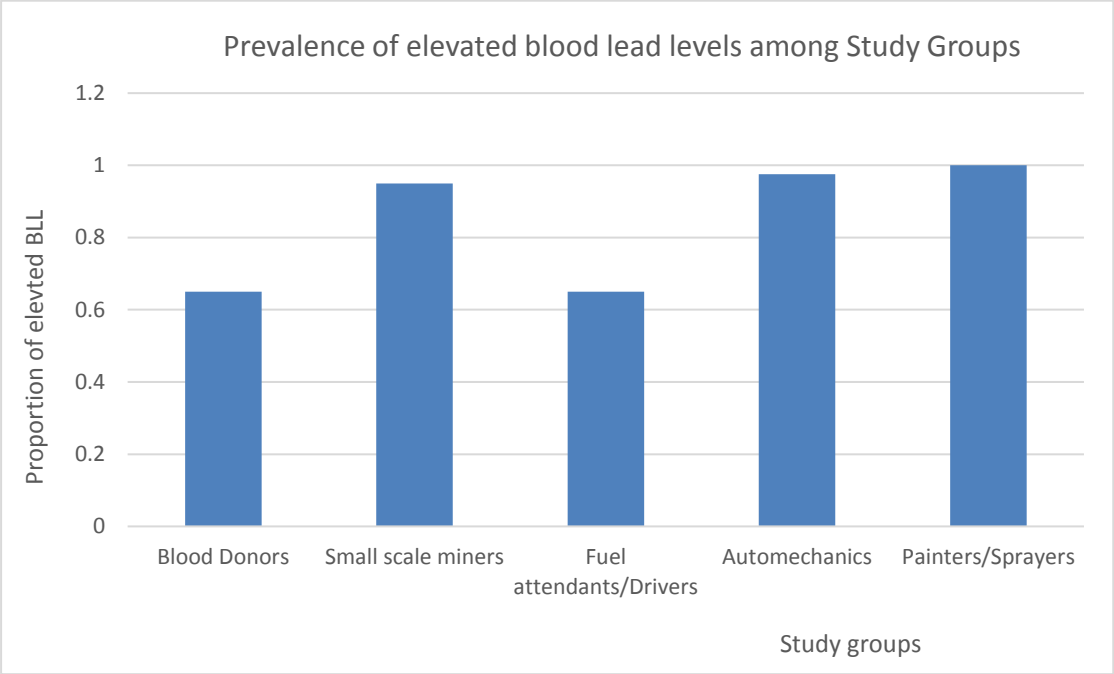


Figure 3: Prevalence of elevated Blood lead levels by study groups.

With the exception of fuel attendants/drivers, the odds of any of the occupational groups having an EBLL was higher compared to the control group (blood donors). This is highlighted in Table 20.

Table 20: Testing for significant differences in EBLLs across the various study groups.

Occupation	Odds ratio	95% Conf. Int.	p-value
Blood donor	ref	-	-
Small scale miner	10.2308	2.1427 - 48.8481	***0.004***
Fuel attendant/Driver	1.0000	0.3980 - 2.5064	1.000
Auto-mechanic	21	2.6012 - 169.5394	***0.004***
Painter/sprayer	Predicts EBLL perfectly		

In terms of BLLs and gender, males generally recorded higher BLLs than females. The GM BLL for males and females were 6.8 ± 1.9 µg/dl [95% CI: 6.5- 7.1] and 5.1 ± 1.8 µg/dl [95% CI: 4.0 – 6.1] respectively. The difference between the GM BLL for males and females was statistically significant (*p-value* = 0.0006).

Factors that could possibly predict elevated blood lead levels such as age, sex, marital status, educational status, source of drinking water and occupation were analysed. Of these individual variables, age, Junior secondary school (JSS) education and occupation showed statistical significance. These are illustrated in Table 21.

After adjusting for all possible confounders (age, sex, educational level, marital status, source of drinking water), the significant predictor for EBLL in the study area was occupation. Specifically, auto-mechanics, painters/sprayers and small scale miners had significantly EBLLs. This is demonstrated in Table 22.

Given all other predictors of EBLL, small scale miners had 10 times the odds of elevated blood lead level compared to apparently healthy controls. Again, holding all other predictors constant, the odds for an auto-mechanic to have EBLL was 22 times higher than the odds of apparently healthy controls.

Table 21: Results of univariate analysis for each of the proposed predictors of elevated BLLs.

Variable	Odds Ratio	95% CI	p-value
Sex			
Female	Reference	-	-
Male	2.3556	0.6891 - 8.0525	0.172
Age	0.9565	0.9162 - 0.9986	0.043
Marital status			
Unmarried	reference	-	-
Married	0.5107	0.2350 - 1.1098	0.090
Educational level			
Post SSS/post technical	reference	-	-
Primary education	3.8889	0.8419 - 17.9628	0.082
JSS/Middle school/ etc.	6.7407	2.1250 - 21.3827	0.001
SSS/Technical/commercial/ etc.	1.5069	0.4791 - 4.7394	0.483
Source of drinking water			
Pipe borne water	2.1053	0.9399 - 4.7156	0.070
Water from well/borehole	1.4632	0.6158 - 3.4764	0.389
Occupation			
Blood donors	reference	-	-
Small scale miners (galamsey)	10.2308	2.1427 - 48.8481	0.004
Fuel attendants/Drivers	1.0000	0.3980 - 2.5064	1.000
Auto-mechanics	21	2.6012 - 169.5394	0.004
Painters/sprayers	Predicts elevated BLLs perfectly		

Table 22: Results of multiple logistic regression controlling for all possible confounders of EBLLs.

Variable	Odds Ratio	95% Conf. Interval	p-value
Sex			
Female	Reference	-	-
Male	0.8523	0.1993 - 3.6455	0.829
Age	0.8279	0.5977 - 1.1467	0.256
Marital status			
Unmarried	reference	-	-
Married	0.3937	0.1429 1.0842	0.071
Educational level			
Post SHS/post technical	reference	-	-
Primary education	0.2937	0.0284 - 3.0326	0.304
Middle/continuation school/JSS	2.3311	0.4560 - 11.9161	0.309
Technical/commercial/SSS etc.	0.7251	0.1676 - 3.1369	0.667
Occupation			
Blood donors reference	- -		
Small scale miners	10.7348	1.5878 - 72.5747	0.015
Fuel attendants/Drivers	1.0536	0.2759 - 4.0243	0.939
Auto-mechanics	22.4049	1.6170 - 310.4479	0.020
Painter/sprayer	Predicts elevated BLL perfectly		

4.3 HAEMATOLOGICAL PARAMETERS

The mean haemoglobin (Hb) concentration for the entire study participants was 14.1 ± 1.5 g/dl with a range of 7.4 to 16.8 g/dl and that for the occupationally exposed group and the non-exposed groups were 14.2 ± 1.3 g/dl and 13.7 ± 1.5 g/dl respectively. The exposed group had significantly higher Hb compared to the non-exposed group with a *p-value* of 0.0441. However, between the different study groups, there was no significant difference in their mean haemoglobins as the *p-value* was 0.1076. The distribution of haemoglobin concentration in terms of individual study groups is shown in Table 23 below. Table 24 shows the test of variance for the mean Hb across the various study groups.

Table 23: Mean haemoglobin concentrations (Hb) for individual study groups.

Occupation	Mean Hb (SD)	Min-max
Blood donors	13.7 (1.49)	12 .1 - 16.1
Small scale miners	14.6 (1.37)	10.9 - 16.8
Fuel attendants/Drivers	14.3 (1.36)	11.5 - 16.8
Auto-mechanics	14.1 (1.74)	10.0 – 15.7
Painters/Sprayers	14.0 (1.84)	7.4 - 16.1

Table 24: Test of variance (ANOVA) for the means of Hbs across the various study groups.

Source	Partial ss	df	ms	F-test	p-value
Model	19.064803	4	4.7662007	1.93	0.1076
Occupation	19.064803	4	4.7662007	1.93	0.1076
Residual	482.567	195	2.4747026		
Total	501.6318	199	2.5207628		

***ss =sum of squares, **df=degree of freedom, ***ms=mean square**

Based on the WHO classification of haemoglobin levels, the prevalence of anaemia among study participants was 14.5% (29/200). The proportions of mild, moderate and severe anaemia were 11% (22/200), 3% (6/200), and 0.5% (1/200) respectively. Table 25 shows the severity of anaemia among the various study groups. There was no significant association between study groups and anaemia, *p-value* = 0.060.

Table 25: Presence or absence of anaemia and severity among the study groups.

Study group	No anaemia	mild	moderate	severe	Total
Blood donor	40	0	0	0	**40**
Small scale miner	36	3	1	0	**40**
Fuel attendant/Driver	31	9	0	0	**40**
Auto-mechanic	30	9	1	0	**40**
Painter/Sprayer	34	1	4	1	**40**
Total	**171**	**22**	**6**	**1**	**200**

Severe anaemia characterized by haemoglobin concentration < 8g/dl was found only among Painters/sprayers. Among participants who were anaemic, the red cell parameters were such that 65.5% (19/29) had normocytic normochromic anaemia, 20.7% (6/29) had microcytic hypochromic anaemia and 13.8% (4/29) had macrocytic anaemia. There was no significant linear correlation between haemoglobin concentration and blood lead levels measured (Correlation Coefficient=0.0492; *p=0.7382*). Blood smears prepared did not show any basophilic stippling.

CHAPTER FIVE

5.0 DISCUSSION

Lead poisoning is an environmental disease that is the result of human activities (Garza, Vega, & Soto, 2006). Occupational exposure to lead is one of the main sources of EBLLs which could cause severe health effects that might be irreversible. This study sought to compare BLLs among selected at risk occupational groups with low risk blood donors at Kenyasi in the Brong Ahafo region of Ghana.

The geometric mean (GM) blood lead level (BLL) obtained for participants in the study was 6.3 (GSD 1.41) µg/dl with a range of 1.8 to 14.4. That for the exposed group was 7.0 µg/dl as against 5.4 µg/dl for the non-exposed group *(p-value = 0.0001*). The prevalence of EBLL among the study participants was 84.5% based on CDC's cut off BLL of 5 µg/dl. It is important to state that the significant predictor for EBLL in the study area was occupation *(p=0.004).* This lends credence to studies that have associated significant EBLLs and lead poisoning with occupational exposure to lead especially among lead acid battery recycling workers and smelters (Needleman, 2004; Nemsadze et al., 2008).

Blood transfusion has been considered as a hidden source of lead exposure (Bearer et al., 2000; Gehrie et al., 2013). It is worrying however that in this study, the geometric mean BLL for apparently healthy blood donors was 5.4 µg/dl (GSD=1.81) which is higher than CDC's accepted level of less than 5 µg/dl. They also had a 65% prevalence in EBLLs. The impact of such transfused blood on infants and pregnant women cannot be underestimated. A mother's exposure to lead in pregnancy influences her health and

that of the infant with neurodevelopmental outcomes. Spontaneous abortion, prematurity, miscarriages and low birth weight may also occur (Bellinger, 2005). Lead stores in bones are released into circulation during periods of increased bone turnover such as pregnancy and breastfeeding and this may impact on the health of the foetus and the infant (Carocci et al., 2016; Flora et al., 2012). The transfer of lead through the placenta and the mother's milk results in similarities between blood lead levels of the mothers and infants (Ab Latif Wani & Usmani, 2015). Sub-clinical neuro-behavioural toxicity are associated with BLLs that may be as low as 1–3 µg/dl in younger children. (Canfield et al., 2003).

Prolonged, exposure to low-levels of lead as recorded in this study is associated with lowered intelligence quotients (IQ), attention deficit disorder, aggression or hyperactivity, delinquency, subclinical hearing and balance disturbances, increased dental caries, and several neurobehavioral problems as well as cognitive defects in children (Patrick, 2006; World Health Organisation, 2010).

The danger with giving blood that has substantial amount of lead could result in children who have diminished intelligence and mental retardation with substantial decrease in the number of intelligent children. Consequently, the number of children who do poorly in school and may require special education and other remedial programmes may rise. This becomes a limitation to their ability to contribute fully to society when they become adults and thus a reduction in a country's future leadership. This creates a widening gap in socioeconomic attainment between countries with high and low levels of population exposed to lead (World Health Organisation, 2010).

Indeed the BLL of Fuel Attendants/Drivers were comparable to healthy blood donors (*p-value = 0.9980)*. The increase could be attributed to background soil contamination from the longstanding activities of illegal mining in the area. The phasing out of leaded gasoline since 2004 in Ghana (Aboh et al., 2013), could have contributed to the relatively low BLL of Fuel attendants and Drivers compared to Auto-mechanics and Painters/Sprayers. The use of leaded acid batteries by auto-mechanics and the use of lead containing chemicals by small scale miners could explain the relatively high BLLs recorded in these occupational groups. It could also be possible that during the gold mining process lead is encountered as it also forms part of the earth's crust.

The fact that this study recorded significant increases in BLL of Painters and Sprayers with geometric mean of 7.4 ± 1.32 µg/dl raises concerns over possible use of leaded paints in the study area and Ghana as a whole and as to whether these paints/sprays are locally made or imported. It is important that attention is given to such neglected sources of lead exposure as lower BLLs such as recorded in this study (<10µg/dl) are associated with decreased renal function and increased risk for hypertension and essential tremor (Ab Latif Wani & Usmani, 2015) among adult populations**.** In acute situations, high-dose exposure to lead can cause a variety of symptoms, including nausea, abdominal pain, drowsiness, anaemia, headaches, convulsions, muscle weakness, ataxia, tremors, paralysis, coma and even death (World Health Organisation, 2010).

In terms of lead levels and gender, females rarely engaged in auto-mechanics, painting, spraying and small scale mining commonly referred to as 'galamsey' due mainly to socio-cultural limitations. Additionally, blood donation is even male dominated. This

resulted in relatively few females participating in this study. Therefore, the difference between the GM BLL for males and females which was statistically significant *(p-value = 0.0006)* could be due to chance.

Studies have demonstrated that anaemia is one of the earliest manifestations of elevated blood lead levels due to its effect on haemoglobin synthesis (Flora et al., 2012; Rempel, 1989). The prevalence of anaemia among study participants was 14.5%. It is important to state that all the participants who were anaemic were males possibly due to the relatively small numbers of female study participants. In addition, the significant difference between the means of the Hb of exposed as against non-exposed (14.2g/dl, 13.7g/dl: *p=0.0441*) could be attributed to the number of females in the non-exposed group as women have relatively lower Hb levels. It is also important to note that regular blood donors are at risk of iron deficiency (Shuchman, 2014), and currently in Ghana, it is not a policy to give iron supplements to blood donors. This could also explain the lower mean Hb of the non-exposed group as compared to the exposed group. Nevertheless, the study did not find significant linear correlation between haemoglobin concentration and blood lead levels (correlation coefficient=0.0492; *p=0.7382*). This could be explained by the relatively low BLLs recorded among participants. Studies have shown that haem synthesis does not decrease until the activity of δ-ALAD is inhibited by 80–90 %, and this occurs at a blood lead level ≥ 50µg/dl (Ahamed et al., 2006; Flora et al., 2012).

The fact that haemoglobin levels were not significantly reduced in this study, is in agreement with Cremer (1989), who did not observe significant haemoglobin reduction in industrial workers whose BLLs were below 50 µg/dl. However, Ribarov, Benov, and

Benchev (1981), demonstrated that lead significantly increases the rate of haemoglobin-catalyzed lipid peroxidation similar to Hu, Watanabe, Payton, Korrick, and Rotnitzky (1994), who also studied the relationship between bone lead and haemoglobin and concluded that patella bone lead levels were associated with decreased haematocrit and haemoglobin levels despite the presence of low BLLs. This finding may reflect the subclinical effect of bone lead stores on haematopoiesis and is the first epidemiological evidence that bone lead may be an important biological marker of ongoing chronic lead toxicity.

Again, basophilic stippling is another important haematological indicator of lead poisoning (Patrick, 2006). However, blood smears prepared for participants in this study did not show any basophilic stippling possibly due to the relatively low levels of blood lead recorded in this study. Basophilic stippling do not appear in red cells until BLL is ≥50 µg/dl for adults and ≥40 µg/dl for children (Patrick, 2006)

The BLLs recorded in this study are relatively lower compared to other studies among similar populations. The mean BLLs in blood donors in Egypt, Morocco, and Italy were 17.0 µg/dl, 8.7 µg/dl and 14.8 µg/dl respectively but that in the blood bank in the USA was 1.0 µg/dl (Ahmed et al., 2015; Bulleova, Rothenberg, & Manalo, 2001) compared to 5.4 µg/dl for blood donors in this study. In a study by Gharaibeh, Hasan Alzoubi, Falah Khabour, Saleh Khader, and Khalid Matarneh (2014), the blood lead levels of hospital health workers, shop workers, taxi drivers, mechanics and wood workers in Jordan were 9.5 µg/dl, 13.9 µg/dl, 28.8 µg/dl, 24.6 µg/dl, and 22.3 µg/dl respectively as compared to 5.5 µg/dl for drivers and 8.1 µg/dl for auto-mechanics in this study. Another study by Al-Rudainy (2010) Basrah City, Iraq, recorded a median lead level

of 14.1 µg/dl among fuel station workers as against 6.5 µg/dl in the control group of farmers. A mean BLL of 33.6 µg/dl and 8.1 µg/dl were also recorded in another study by Eltayeb, Nageeb, and Ali (2014) in fuel station workers and non-exposed workers respectively in Khartoum City, Sudan. The mean lead concentration in auto-mechanics in Nigeria was 36.1µg/dl (Ibeh, Aneke, Okocha, Okeke, & Nwachukwuma, 2016) which is also far higher than the 8.1 µg/dl recorded in this study among auto-mechanics.

The expectation was to find higher BLLs among the study participants because of the illegal mining activities and the indiscriminate use of lead and other harmful substances like mercury in their activities. However, a mean BLL of 6.3µg/dl was measured in the study population. This could be attributed to the recent ban on illegal mining activities in Ghana by the Ghana government. Most of the illegal miners were idle at the time of sample collection. As BLL is indicative of recent exposure; up to 40 days (Ab Latif Wani & Usmani, 2015), the possibility that most of the lead in the blood has moved into the storage pool in bone and soft tissue could be a factor. The same could be said for the auto-mechanics and the painters/sprayers because low economic activity in the area as a result of the ban has affected their businesses. The exclusion criteria for this study could have also excluded people with higher BLLs as only apparently healthy individuals who did not have any health complaints qualified to participate in the study.

5.1 LIMITATIONS OF THE STUDY

Bone lead levels which represent a lifetime retained cumulative dose of lead levels were not measured in this study.

5.2 CONCLUSION

Occupational exposure to lead is strongly associated with elevated blood lead levels. Unusual sources of lead exposure, such as blood transfusion, deserve new attention since blood transfusion could be a major hidden source of lead exposure especially among vulnerable populations such as children and pregnant women. In our society today, replacement blood donation by family and friends is common and the people we fall on could belong to these high-risk occupations like auto-mechanics, painters/sprayers and small scale miners whose occupations put them at increased risk of lead overload.

5.3 RECOMMENDATIONS

In the light of the findings of this study, the following are recommended:

a) There should be concerted effort to screen blood for lead where possible or exclude auto-mechanics, painters/sprayers and small scale miners from donating blood.

b) Larger studies on occupational exposure to lead and long term impact on haematological parameters should be carried out. Also a prospective study comparing the incidence of intellectual disability among a cohort of infants receiving blood transfusions in the study area is needed to guide policy on blood transfusion.

c) Protective measures should be enforced to reduce occupational exposure to lead especially among auto-mechanics, painters, sprayers and small scale miners.

d) There is the need to look at the lead levels in imported as well as locally manufactured paints/sprays.

REFERENCES

Ab Latif Wani, A. A., & Usmani, J. A. (2015). Lead toxicity: a review. *Interdisciplinary toxicology, 8*(2), 55.

Aboh, I. J. K., Sampson, M. A., Nyaab, L. A.-K., Caravanos, J., Ofosu, F. G., & Kuranchie-Mensah, H. (2013). Assessing levels of lead contamination in soil and predicting pediatric blood lead levels in Tema, Ghana. *Journal of Health and Pollution, 3*(5), 7-12.

Acquaye, J. (1997). Blood transfusion practice in district hospitals. *Ghana Medical Journal, 31*, 859-863.

Acquaye, J., & Tettey-Donkor, D. (2000). Frequency of hepatitis C virus antibodies and elevated serum alanine transaminase levels in Ghanaian blood donors. *West African journal of medicine, 19*(4), 239-241.

Adamson, J. W., & Longo, D. L. (2013). *Anaemia and Polycythaemia. In: Harrison's Haematology and Oncology* (2nd edition ed.). New York: McGraw-Hill Inc.

Agency for Toxic Substances and Disease Registry , U. D. o. H. a. H. S. (2007). Case Studies in Environmental Medicine (CSEM): Lead Toxicity Cover Page. . Retrieved March 11, 2010., from http://www.atsdr.cdc.gov/csem/lead/pbcover_page2.html

Ahamed, M., Verma, S., Kumar, A., & Siddiqui, M. K. (2006). Delta-aminolevulinic acid dehydratase inhibition and oxidative stress in relation to blood lead among urban adolescents. *Human & experimental toxicology, 25*(9), 547-553.

Ahmed, M., Amr, A. S., Ibrahim, O. A., Tarek, M. S., Ahmed, B., & Mostafa, F. B. (2015). Estimation of lead in blood donors: Removal of lead associated with blood transfusion using carboxymethyl cellulose

Scholars Academic Journal of Biosciences (SAJB), 3(6), 558-562.

Al-Rudainy, L. A. (2010). Blood lead level among fuel station workers. *Oman medical journal, 25*(3), 208.

Alter, H. J., & Klein, H. G. (2008). The hazards of blood transfusion in historical perspective. *blood, 112*(7), 2617-2626.

Ampofo, W., Nii-Trebi, N., Ansah, J., Abe, K., Naito, H., Aidoo, S., . . . Ofori-Adjei, D. (2002). Prevalence of blood-borne infectious diseases in blood donors in Ghana. *Journal of clinical microbiology, 40*(9), 3523-3525.

Bearer, C. F., O'riordan, M. A., & Powers, R. (2000). Lead exposure from blood transfusion to premature infants. *The Journal of pediatrics, 137*(4), 549-554.

Bellinger, D. C. (2005). Teratogen update: lead and pregnancy. *Birth Defects Research Part A: Clinical and Molecular Teratology, 73*(6), 409-420.

Bound, J. P., Harvey, P. W., Francis, B. J., Awwad, F., & Gatrell, A. C. (1997). Involvement of deprivation and environmental lead in neural tube defects: a matched case-control study. *Archives of disease in childhood, 76*(2), 107-112.

Bressler, J. P., & Goldstein, G. W. (1991). Mechanisms of lead neurotoxicity. *Biochemical pharmacology, 41*(4), 479-484.

Bulleova, S., Rothenberg, S. J., & Manalo, M. A. (2001). Lead levels in blood bank blood. *Archives of Environmental Health: An International Journal, 56*(4), 312-313.

Canfield, R. L., Henderson Jr, C. R., Cory-Slechta, D. A., Cox, C., Jusko, T. A., & Lanphear, B. P. (2003). Intellectual impairment in children with blood lead

concentrations below 10 μg per deciliter. *New England journal of medicine, 348*(16), 1517-1526.
Carocci, A., Catalano, A., Lauria, G., Sinicropi, M. S., & Genchi, G. (2016). Lead toxicity, antioxidant defense and environment *Reviews of Environmental Contamination and Toxicology Volume 238* (pp. 45-67): Springer.
Centers for Disease Control and Prevention, U. S. (2009). Children with elevated blood lead levels related to home renovation, repair, and painting activities--New York State, 2006-2007. *MMWR. Morbidity and mortality weekly report, 58*(3), 55.
Centers for Disease Control and Prevention, U. S. (2012). Response to Advisory Committee on Childhood Lead Poisoning Prevention recommendations in "Low level lead exposure harms children: a renewed call of primary prevention". from http://www.cdc.gov/nceh/lead/ACCLPP/CDC_Response_Lead_Exposure_Recs.pdf
Centers for Disease Control and Prevention, U. S. (2015). Adult Blood Lead Epidemiology & Surveillance (ABLES). Retrieved 17/01/2017, from https://www.cdc.gov/niosh/topics/ables/description.html
Cortez-Lugo, M., Téllez-Rojo, M. M., Gómez-Dantés, H., & Hernández-Avila, M. (2003). Trends in atmospheric concentrations of lead in the Metropolitan Area of Mexico City: 1988-1998. *Salud Pública de México, 45*, 196-202.
Cremer, J. (1989). A study of action lead–alkyls in the solvents. *Industrial Med, 81*(6), 325-330.
Dalloz, F., Maupoil, V., Lecour, S., Briot, F., & Rochette, L. (1997). In vitro studies of interactions of NO· donor drugs with superoxide and hydroxyl radicals. *Molecular and cellular biochemistry, 177*(1-2), 193-200.
Dewalere Health and Social Services, D. o. P. H. (2009). Lead Poisoning; Frequently Asked Questions.
Duncan, K. J., Reid, H., & Voutchkov, M. (2010). Lead concentrations in Jamaican blood donors in relation to occupation. *Continuing Education, 117*.
Eltayeb, T., Nageeb, A., & Ali, K. T. (2014). Blood lead level among fuel station workers at Khartoum city. *American Journal of Research Communication, 2*(6), 74-82.
Finster, M. E., Gray, K. A., & Binns, H. J. (2004). Lead levels of edibles grown in contaminated residential soils: a field survey. *Science of the Total Environment, 320*(2), 245-257.
Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: a review with recent updates. *Interdisciplinary toxicology, 5*(2), 47-58.
Galel, S. A., & Fontaine, M. J. (2006). Hazards of neonatal blood transfusion. *NeoReviews, 7*(2), e69-e75.
Garvey, G. J., Hahn, G., Lee, R. V., & Harbison, R. D. (2001). Heavy metal hazards of Asian traditional remedies. *International Journal of Environmental Health Research, 11*(1), 63-71.
Garza, A., Vega, R., & Soto, E. (2006). Cellular mechanisms of lead neurotoxicity. *Medical science monitor, 12*(3), RA57-RA65.
Gehrie, E., Keiser, A., Dawling, S., Travis, J., Strathmann, F. G., & Booth, G. S. (2013). Primary prevention of pediatric lead exposure requires new approaches to transfusion screening. *The Journal of pediatrics, 163*(3), 855-859.

Gharaibeh, M. Y., Hasan Alzoubi, K., Falah Khabour, O., Saleh Khader, Y., & Khalid Matarneh, S. (2014). Lead exposure among five distinct occupational groups: a comparative study. *Pakistan journal of pharmaceutical sciences, 27*(1).

Gordon, J., Taylor, A., & Bennett, P. (2002). Lead poisoning: case studies. *British journal of clinical pharmacology, 53*(5), 451-458.

Gulson, B. L., Mizon, K. J., Korsch, M. J., Palmer, J. M., & Donnelly, J. B. (2003). Mobilization of lead from human bone tissue during pregnancy and lactation—a summary of long-term research. *Science of the Total Environment, 303*(1), 79-104.

Gurer, H., & Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biology and Medicine, 29*(10), 927-945.

Harville, E., Hertz-Picciotto, I., Schramm, M., Watt-Morse, M., Chantala, K., Osterloh, J., . . . Rogan, W. (2005). Factors influencing the difference between maternal and cord blood lead. *Occupational and environmental medicine, 62*(4), 263-269.

Hertz- Picciotto, I. (2000). The evidence that lead increases the risk for spontaneous abortion. *American journal of industrial medicine, 38*(3), 300-309.

Hu, H., Watanabe, H., Payton, M., Korrick, S., & Rotnitzky, A. (1994). The relationship between bone lead and hemoglobin. *JAMA, 272*(19), 1512-1517.

Ibeh, N., Aneke, J., Okocha, C., Okeke, C., & Nwachukwuma, J. (2016). The influence of occupational lead exposure on haematological indices among petrol station Attendants and automobile mechanics in Nnewi, South-East Nigeria. *J Environ Occup Sci, 5*(1), 1.

Irgens, Å., Krüger, K., Skorve, A. H., & Irgens, L. M. (1998). Reproductive outcome in offspring of parents occupationally exposed to lead in Norway. *American journal of industrial medicine, 34*(5), 431-437.

Jackson, L. W., Correa- Villaseñor, A., Lees, P. S., Dominici, F., Stewart, P. A., Breysse, P. N., & Matanoski, G. (2004). Parental lead exposure and total anomalous pulmonary venous return. *Birth Defects Research Part A: Clinical and Molecular Teratology, 70*(4), 185-193.

Jin, Y., Liao, Y., Lu, C., Li, G., Yu, F., Zhi, X., . . . Yang, J. (2006). Health effects in children aged 3–6 years induced by environmental lead exposure. *Ecotoxicology and environmental safety, 63*(2), 313-317.

Jones, R. L., Homa, D. M., Meyer, P. A., Brody, D. J., Caldwell, K. L., Pirkle, J. L., & Brown, M. J. (2009). Trends in blood lead levels and blood lead testing among US children aged 1 to 5 years, 1988–2004. *Pediatrics, 123*(3), e376-e385.

Jusko, T. A., Henderson Jr, C. R., Lanphear, B. P., Cory-Slechta, D. A., Parsons, P. J., & Canfield, R. L. (2008). Blood lead concentrations< 10 μg/dL and child intelligence at 6 years of age. *Environmental health perspectives, 116*(2), 243.

Karumanchi, S. A., Maynard, S. E., Stillman, I. E., Epstein, F. H., & Sukhatme, V. P. (2005). Preeclampsia: a renal perspective. *Kidney international, 67*(6), 2101-2113.

Kathuria, P. (2017). Lead Toxicity Treatment & Management. Retrieved 24 April 2017, from http://emedicine.medscape.com/article/1174752-treatment#d8

Kazantzis, G. (1989). Lead: Ancient Metal—Modern Menace? *Lead Exposure and Child Development* (pp. 119-128): Springer.

Kosnett, M. J., Wedeen, R. P., Rothenberg, S. J., Hipkins, K. L., Materna, B. L., Schwartz, B. S., . . . Woolf, A. (2007). Recommendations for medical

management of adult lead exposure. *Environmental health perspectives, 115*(3), 463.
Krigman, M. R. (1978). Neuropathology of heavy metal intoxication. *Environmental health perspectives, 26*, 117.
Kumar, V., Abbas, K. A., & Fausto, N. (2005). *Robbins and Cotran: Pathologic Basis of Disease* (7th ed.): Elsevier.
Lanphear, B. P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., . . . Greene, T. (2005). Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. *Environmental health perspectives, 113*(7), 894.
Markowitz, M. (2000). Lead Poisoning. *Paediatrics in Review, 21*, 327-335.
McLean, E., Cogswell, M., Egli, I., Wojdyla, D., & De Benoist, B. (2009). Worldwide prevalence of anaemia, WHO vitamin and mineral nutrition information system, 1993–2005. *Public health nutrition, 12*(4), 444-454.
Navas-Acien, A., Guallar, E., Silbergeld, E. K., & Rothenberg, S. J. (2007). Lead exposure and cardiovascular disease—a systematic review. *Environmental health perspectives, 115*(3), 472.
Needleman, H. (2004). Lead poisoning. *Annu. Rev. Med., 55*, 209-222.
Negi, G., Gaur, D. S., & Kaur, R. (2015). Blood transfusion safety: A study of adverse reactions at the blood bank of a tertiary care center. *Advanced biomedical research, 4*.
Nemsadze, K., Sanikidze, T., Ratiani, L., Gabunia, L., & Sharashenidze, T. (2008). Mechanisms of lead-induced poisoning. *Georgian medical news*(172-173), 92-96.
Nriagu, J. O., Blankson, M. L., & Ocran, K. (1996). Childhood lead poisoning in Africa: a growing public health problem. *Science of the Total Environment, 181*(2), 93-100.
Patrick, L. (2006). Lead toxicity, a review of the literature. Part I: exposure, evaluation, and treatment. *Alternative medicine review, 11*(1), 2-23.
Rastogi, S. (2008). Renal effects of environmental and occupational lead exposure. *Indian journal of occupational and environmental medicine, 12*(3), 103.
Rempel, D. (1989). The lead-exposed worker. *JAMA, 262*(4), 532-534.
Ribarov, S. R., Benov, L. C., & Benchev, I. C. (1981). The effect of lead on hemoglobin-catalyzed lipid peroxidation. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 664*(3), 453-459.
Ryan, J. A., Scheckel, K. G., Berti, W. R., Brown, S. L., Casteel, S. W., Chaney, R. L., . . . Maddaloni, M. (2004). Peer reviewed: reducing children's risk from lead in soil: ACS Publications.
Saper, R. B., Kales, S. N., Paquin, J., Burns, M. J., Eisenberg, D. M., Davis, R. B., & Phillips, R. S. (2004). Heavy metal content of ayurvedic herbal medicine products. *JAMA, 292*(23), 2868-2873.
Selevan, S. G., Rice, D. C., Hogan, K. A., Euling, S. Y., Pfahles-Hutchens, A., & Bethel, J. (2003). Blood lead concentration and delayed puberty in girls. *New England journal of medicine, 348*(16), 1527-1536.
Shuchman, M. (2014). Frequent blood donors risk iron deficiency: Can Med Assoc.
Tong, S., Schirnding, Y. E. v., & Prapamontol, T. (2000). Environmental lead exposure: a public health problem of global dimensions. *Bulletin of the World Health Organization, 78*(9), 1068-1077.

White, L., Cory-Slechta, D., Gilbert, M., Tiffany-Castiglioni, E., Zawia, N., Virgolini, M., . . . Basha, M. R. (2007). New and evolving concepts in the neurotoxicology of lead. *Toxicology and applied pharmacology, 225*(1), 1-27.

Wójcicka, G., Bełtowski, J., & Jamroz, A. (2004). Stres oksydacyjny w nadciśnieniu tętniczym Oxidative stress in hypertension. *Postepy Hig Med Dosw (online), 58*, 183-193.

World Health Organisation, W. (2010). Childhood lead poisoning. Retrieved 09/07/2017, 2017, from http://www.who.int/ceh/publications/leadguidance.pdf

World Health Organisation, W. (2011b). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity Retrieved 12 July, 2017, from http://www.who.int/vmnis/indicators/haemoglobin.pdf

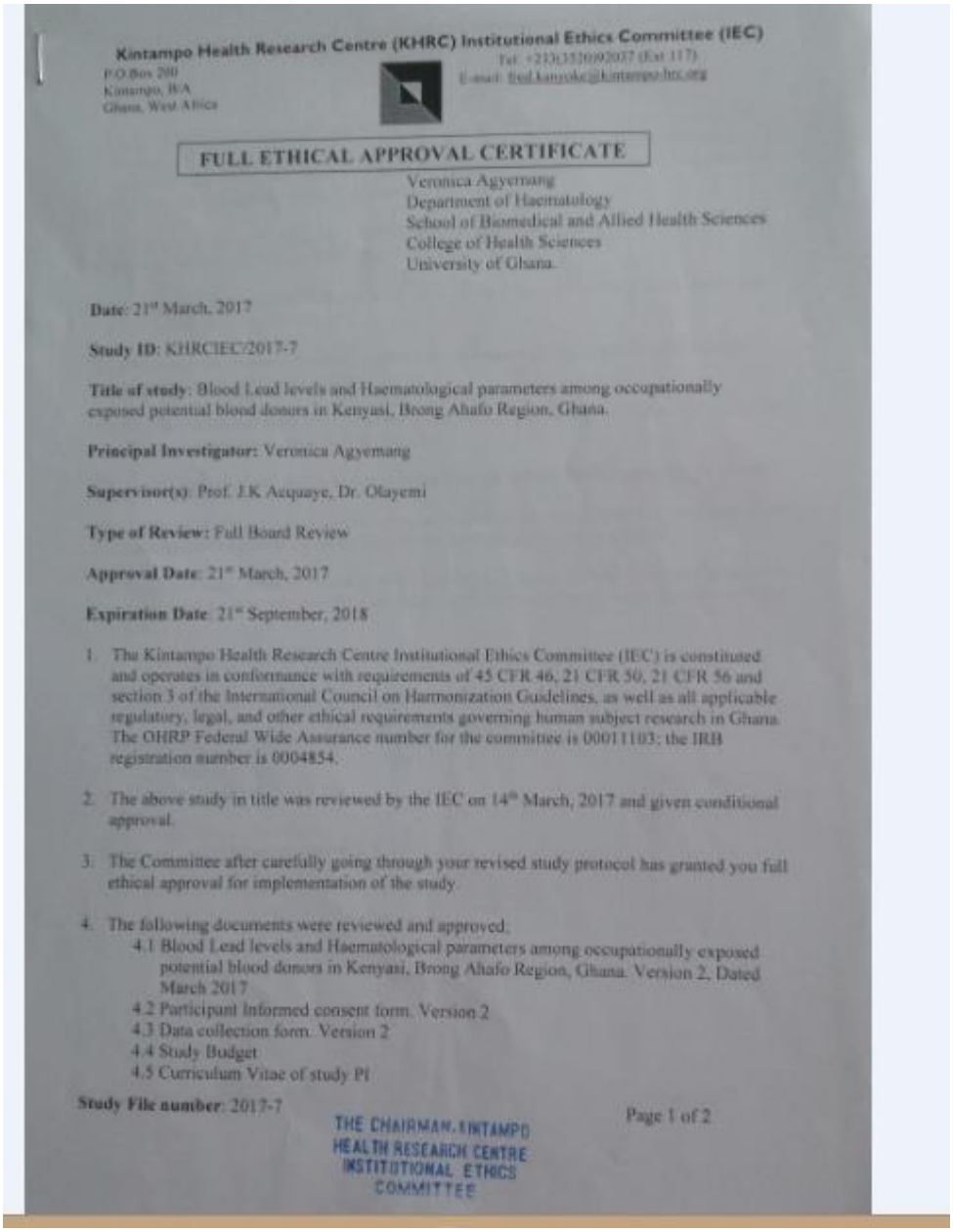
World Health Organisation, W. (2016). Lead poisoning and health. Fact sheet on Lead Retrieved July 15, 2016., from http://www.who.int/mediacentre/factsheets/fs379/en/

World Health Organization, W. (2011a). Brief guide to analytical methods for measuring lead in blood.

Wu, T., Buck, G. M., & Mendola, P. (2003). Blood lead levels and sexual maturation in US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. *Environmental health perspectives, 111*(5), 737.

APPENDICES

APPENDIX 1: Ethical approval from Kintampo Health Research Centre



Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)

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FULL ETHICAL APPROVAL CERTIFICATE

Veronica Agyemang
Department of Haematology
School of Biomedical and Allied Health Sciences
College of Health Sciences
University of Ghana.

Date: 21st March, 2017

Study ID: KHRCIEC/2017-7

Title of study: Blood Lead levels and Haematological parameters among occupationally exposed potential blood donors in Kenyasi, Brong Ahafo Region, Ghana.

Principal Investigator: Veronica Agyemang

Supervisor(s): Prof. J.K Acquaye, Dr. Olayemi

Type of Review: Full Board Review

Approval Date: 21st March, 2017

Expiration Date: 21st September, 2018

1. The Kintampo Health Research Centre Institutional Ethics Committee (IEC) is constituted and operates in conformance with requirements of 45 CFR 46, 21 CFR 50, 21 CFR 56 and section 3 of the International Council on Harmonization Guidelines, as well as all applicable regulatory, legal, and other ethical requirements governing human subject research in Ghana. The OHRP Federal Wide Assurance number for the committee is 00011103; the IRB registration number is 0004854.

2. The above study in title was reviewed by the IEC on 14th March, 2017 and given conditional approval.

3. The Committee after carefully going through your revised study protocol has granted you full ethical approval for implementation of the study.

4. The following documents were reviewed and approved.
   - 4.1 Blood Lead levels and Haematological parameters among occupationally exposed potential blood donors in Kenyasi, Brong Ahafo Region, Ghana. Version 2, Dated March 2017
   - 4.2 Participant Informed consent form. Version 2
   - 4.3 Data collection form. Version 2
   - 4.4 Study Budget
   - 4.5 Curriculum Vitae of study PI

Study File number: 2017-7

Page 1 of 2

THE CHAIRMAN, KINTAMPO
HEALTH RESEARCH CENTRE
INSTITUTIONAL ETHICS
COMMITTEE

Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
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5. During study implementation, the IEC must be informed within 72 hours by the principal investigator (PI) of learning of any (a) unexpected, serious, study related adverse events; (b) disclosed adverse events, or (c) unanticipated problems with the study which may pose risk to study participants or others, if applicable.

6. All safety monitory reports, including DSMB summaries and reports, must be submitted to the IEC as soon as they become available to PI(s).

7. Changes or modifications to this research activity must be submitted and approved by the IEC before they are implemented.

8. PI(s) would be required to submit application for renewal of this approval certificate (if necessary) plus a progress report.

9. PI(s) is required to notify the IEC of study completion (end of data collection/last follow-up) or early termination of the research project.

10. Submit final report of the study one month after approval certificate expires (study closure)

11. Before conduct of the study, submit original/final copy of your informed consent form for an **authentication stamp** before making photocopies for your consent process.

12. Regulated study records, including IEC approvals and signed consent form must be securely maintained by PI(s) and available for audits for three years after the study is closed with the IEC.

Sincerely,

THE CHAIRMAN, KINTAMPO
HEALTH RESEARCH CENTRE
INSTITUTIONAL ETHICS
COMMITTEE

Dr. Damien Punguyire
Chair
Institutional Ethics Committee
Kintampo Health Research Centre

Study File number: 2017-7

APPENDIX 2: Ethical approval from the College of Health Sciences, University of Ghana

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.:.....

18th May, 2017.

Veronica Agyeman
Dept. of Haematology
SBAHS
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.8 – P 4.1/2016-2017**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 30th of March, 2017 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Lead Levels and Haematological Parameters among occupationally exposed Potential Blood Donors in Kenyasi, Brong-Ahafo Region, Ghana".**

PRINCIPAL INVESTIGATOR: **Ms. Veronica Agyeman**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 22nd May, 2018.

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

Signed: .....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS
Head of Department

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• Email: administration@chs.edu.gh / provost@chs.edu.gh • Website: www.chs.ug.edu.gh

APPENDIX 3: Informed consent form

Kintampo Health Research Centre/ College of Health Sciences University of Ghana

Blood Lead Levels And Haematological Parameters Among Occupationally Exposed Potential Blood Donors In Kenyasi, Brong Ahafo Region, Ghana	Form Number:
Information and Informed Consent Form	

PURPOSE OF STUDY/BACKGROUND

Lead poisoning is acute or chronic intoxication of lead and its compounds and receiving a blood transfusion has been considered as a risk factor for lead exposure. This study seeks to know the concentration of lead (Pb) in the blood of some at risk occupational groups in this area so that appropriate measures can be taken to prevent lead poisoning from receiving blood transfusion.

PROCEDURES

If you agree to participate in this study, you will be invited to a central location where a profile form will be used to collect basic demographic information like age, sex, occupation etc. A few drops of blood (1ml), which is less than a teaspoon will be collected from you. This will be sent to the laboratory and analyzed for lead concentration, full blood blood count (FBC) and blood smear for morphology.

RISKS/DISCOMFORTS

Venous blood sampling involves the risk of minimal pain at the site of puncture, discomfort, infection. However, qualified and well trained laboratory personnel as well as sterile syringes and needles will be used to ensure these risks are avoided

BENEFITS By

Participating in this study, you will benefit by getting to know your blood lead level (BLL) and haemoglobin (Hb) values so that if your BLL is high with or without anaemia, you will be prompted to seek medical attention to avoid complications. This will be communicated to only the consented individuals and nobody else within a week of sample collection.

CONFIDENTIALITY Study

identification numbers will be assigned to all respondents, data analysis will be done based on these numbers without referring to any particular participant's name. This will be password protected and used by only named investigators. A summative report of the results of this study is what will be given out.

VOLUNTARINESS

Participating in this study is completely voluntary. Participants have the right to decline participation, or withdraw their consent at any time. Refusing or declining participation will not affect participants in any way

Contact persons
You can contact me, Principal investigator, Veronica Agyemang, for any clarification on the study on 0243388503. You can also contact my supervisors: Prof. J.K. Aquaye on 0244374025 and Dr. Olayemi on 0246968211.

If you have any ethical concerns during or after your participation in this study, please contact the Chairman of the Kintampo Health Research Centre Institutional Ethics Committee, Dr. Damien on 0244525538.

Statement of consent:
" I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and questions I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study and understand that I have the right to withdraw from the study at any time or decline to answer any questions without it affecting my further medical care. I will be given a copy of this consent form''

……………………..	…………….......	……………
Name of Participant	Signature	Date
Contact		

Witness statement:
''I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely''.

....	....	……………… ……..
Name of Witness	Signature	Date
Contact		

……………………………….....	……………………..	………………
Name of investigating team member	Signature	Date

APPENDIX 4: Data collection form

DATA COLLECTION FORM

KINTAMPO HEALTH RESEARCH CENTRE/COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA **OCCUPATIONAL LEAD PROJECT** PROFILE AND SAMPLE COLLECTION FORM (20/03/17)	**PROFILE Form No.**

FORM NO

1.0 BACKGROUND:

1.1 Participant's number | | | | | | PATNMB

1.2 Participant's residence | | PATRSD

1.3Participant's age: …… | | | PATAGE

1.4 Participant's Sex

1. Male	2. Female	SEX

1.5 Date of visit: …… | | | | | | | DATE VISIT

1.6 Staff code: …… …… …… ……			SC

1.7 Do you feel sick today?	1. Yes	2. No	SICK

1.8 Do you have any chronic disease?	1. Yes	2. No	CHRONIC

1.9 Are you pregnant or nursing a child?	1. Yes	2. No	PRGNRS
	3. N/A		

1.10 Status	1. Consented	2. Refused	3. Withdrawn	STATUS

2.0 SOCIO-DEMOGRAPHIC CHARACTERISTICS:

2.1 Highest educational level reached

1. None	2. Primary school	3. Middle/continuation school/JSS	MEDLEV
4.Technical/commercial/SSS Secondary school	5. Post-middle college – teacher training, secretarial	6. Post secondary – nursing, teacher, polytechnic, etc.	
7. University	8. Not known		

2.2 Marital Status

Married	2. Living together	3. Widowed	MARRIED
4. Divorced	5. Separated	6. Single, unmarried	

2.3 What is your source of drinking water?

1. Bottled water	2. Pure water (sachet)	3. Pipe borne water	SDWTER
4. Water from well/borehole	5. Water from stream/river	6. Pot water	
7. Not known	8. Other, specify		

3.0 SOCIO-ECONOMIC

3.1 Your occupation

1. Blood Donor (office worker, trader, Banker, teacher, health worker etc)	2. Small scale miner (galamsey worker)	3. Fuel attendant/Driver	OCCPTN
4. Automechanic	5. Painter/Sprayer		

3.2 Number of years in current occupation | | | OCPTNYRS

3.3 Do you do any part time job?

1. No	2. Yes	3. If yes, Specify	OTHJOB

3.4 Do you rent or live in your own house?

1. Rent	2. Own house	3. Other, Specify	HOUSE

3.5 Type of building material used

1. Cement with roofing sheets	2. Mud with roofing sheet	3. Mud with thatch roof	MBDN
4. Corrugated sheets	5. Burnt bricks	6. Other, specify	

3.6 Number of years of house painting

1. Built and painted more than 35 years ago	2. Built and painted less than 35 years ago	3. Built but not painted more than 35 years ago	PNTAGE
4. Built but not painted less than 35 years ago	5. Built and painted but number of years unknown	6. Not applicable	

3.7 Type of material used for cooking

1. Gas	2. Charcoal	3. Electric cooker	MCKN
4. Saw dust	5. Fire wood	6. Other, specify	

4.0 SAMPLE COLLECTION

4.1 Was sample collected?

1. Yes	2. No	SMPLCLN

4.2 If No, reason

1. Refusal	2. NA	3. Other, specify	SMPNCD

5.0 LAB RESULTS

5.1 Blood Lead Results

		.		(µg/dl)	BLRSLT

5.2 Haemoglobin Results

		.		(g/dl)	HBRSLT

5.3 Blood film Results

	BFRSLT

APPENDIX 5: Preparation of Leishman Stain

Leishman powder, 0.3g was weighed and transferred into a 500ml capacity conical flask. 200ml of absolute methanol was added and placed in a water bath at 37 degrees celcius with intermittent mixing for about 30 minutes. The mixture was allowed to cool and filtered into a container with a tight fitting lid and stored away from sunlight after labeling with name of reagent, date of preparation and expiry and the initials of the person who prepared it.

APPENDIX 6: Preparation of Phosphate Buffer (pH 6.8)

Di-Sodium hydrogen phosphate (Na_2HPO_4)), 0.47g was weighed in addition to 0.46g Potassium di-hydrogen phosphate (KH_2PO_4) and transferred into a beaker containing about 500ml of distilled water, the mixture was stirred with a clean stirring rod and made up to the 1 litre mark with distilled water. The pH meter was used to check the pH to ensure it was 6.8. It was then transferred into a leak proof container and labelled with name of reagent, date of preparation and expiry and initial of the person who prepared it.

Appendix 7: Map of Ghana Showing Kenyasi