HEALTH RISK ASSESSMENT OF LEAD EXPOSURE TO CHILDREN IN BLANTYRE, MALAWI

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Candidate's declaration

I, Wells Robert Utembe, declare that this thesis is my own work. It is being submitted for the degree of Doctor of Philosophy in the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University. I also declare that the (intellectual) content of the thesis is the product of my own work (including the literature review, proposal development and research design, sample and data collection, laboratory analysis, data analysis and manuscript preparation), except to the extent that assistance was rendered by others in the sample and data collection, laboratory analysis and presentation and linguistic expression as acknowledged in the thesis.

…15…day of August .2016

Dedication

I dedicate this to my wife Linda and my daughters Laura, Amanda and Stacy who have had to endure husband's and dad's absence for protracted periods.

Publications and presentation arising from the thesis

Conferences and seminars

1. Utembe W, Health risk assessment of lead among children in Blantyre City Malawi, National Institute for Occupational Health (NIOH) Research Forum, 16 March 2016
2. Utembe W, L Alfazema, M Kamndaya, M Gulumian, Levels of blood lead and sources of lead exposure among children in Blantyre City, Malawi, 7th Wits Cross-Faculty Symposium, 1st March, 2016
3. Utembe W, L Alfazema and M Gulumian, Health risk assessment of lead among children in Blantyre City, Malawi: Evaluation of the IEUBK model, 11th Public Health Association of South Africa (PHASA) conference in Durban, South Africa, 8th - 9th October, 2015
4. Utembe W, L Alfazema, M Kamndaya, M Gulumian, Prevalence and determinants of high blood lead levels among children in Blantyre, Malawi, poster presented at the 10th Public Health Association of South Africa (PHASA) conference in Polokwane, South Africa, 2nd - 6th September, 2014
5. Utembe W, L Alfazema, and M Gulumian, Key outstanding issues in the health risk assessment of lead: Implications for Africa, 6th Conference of the Society of Environmental Toxicology and Chemistry (SETAC) Africa, Lusaka Zambia, 2nd - 3rd September 2013
6. Utembe W, Key outstanding issues in the health risk assessment of lead and their implications for Africa, Keynote lecture, International Chemistry Conference in Africa (ICCA) 2013, University of Pretoria, 8-12 July 2013

Abstract

Although lead (Pb) is highly toxic, exposure to Pb has not been studied in Malawi. The aims of this study were therefore to:

1. Determine the levels of Pb from different sources of exposure such as food, water, and soil/house dust to predict the levels of Pb in blood (BPb) using the Integrated Exposure Uptake Biokinetic (IEUBK) model.
2. Measure the levels of BPb and compare to those predicted from the IEUBK model as an indication for its applicability in Malawi.
3. To relate the measured and predicted BPb levels as well as the prevalence of high BPb to potential health effects using the WHO and CDC guidelines.
4. Assess burden of disease using WHO spreadsheets.
5. Identify additional sources and risk factors for exposure to Pb in children in Malawi to assist the policy makers to reduce exposure to lead.

In this cross-sectional study 152 children, aged 1-6 years, were recruited. To determine sources of exposure, children's toys, domestic paints, foods, house dust, playground soil and water were collected and analyzed for Pb. A Pb exposure risk assessment questionnaire was also administered to identify potential risk factors and a 7-day food frequency questionnaire was used to collect information on food consumption. For measured BPb levels, venous blood was collected and analysed. Logistic regression was performed in STATA to evaluate the relationship between risk factors and high BPb (BPb ≥ 5 μg/dl).

The comparisons between predicted and measured blood lead showed that the IEUBK model may be used provided that the bioavailability values for lead from different sources are available as well as the food consumption rates are provided for Malawi.. There was also a high prevalence (71.7%) of high BPb that is expected to result in 8.38 cases of mild mental retardation per 1000 children aged less than five years. From the identified risk factors, only areas of residence has correlated to prevalence of high BPb in statistically significant manner ($p = 0.013$).

It can therefore be concluded that IEUBK model may be used for Malawi, that a significant proportion of children in Blantyre are exposed to levels of lead that are detrimental to their health and that exposure to lead in Blantyre require urgent intervention measures.

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List of acronyms

AALM	All-Ages Lead model
AAS	Atomic Absorption Spectroscopy
ALA	Aminolaevulinic acid
ALAD	Aminolevulinic acid dehydratase
ANOVA	Analyisis of Variance
ASV	Anodic Stripping Voltammetry
ATSDR	(US) Agency of Toxic Substances and Disease Registry
BPb	Blood lead
BMD	Benchmark Dose
BMR	Benchmark response
CARTA	Consortium for Advanced Research Training in Africa
CDC	(US) Centers for Disease Control and Prevention
CNS	Central nervous system
CSPC	(US) Consumer Product Safety Commission
CoA	Coenzyme A
DALY	Disability-adjusted life year
DTH	Delayed type hypersensitivity
DNA	Deoxyribonucleic acid
EFSA	European Union Food Authority
EP	Erythrocyte Protoporphyrin
E.U.	European Union

FAO	Food and Agriculture Organization
FEP	Free erythrocyte protoporphyrin
FFQ	Food Frequency Questionnaire
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
H_2O_2	Hydrogen peroxide
HBM	Human Biological Monitoring
HNO_3	Nitric Acid
HPLC	High-performance liquid chromatography
HQ	Hazard Quotient
HSA	Health surveillance assistants
HUD	(US) Housing and Urban Development
IARC	International Agency for Research on Cancer
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
IDPH	Illinois Department of Public Health
IEUBK	Integrated Exposure Uptake Biokinetic (model)
IgE	Immunoglobulin E
IHS	Integrated Household Survey
IQ	Intelligence Quotience
IHS	Integrated Household Survey
JECFA	Joint Food and Agriculture Organization (FAO) /World Health Organization (WHO) Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LA-ICP-MS	Laser Ablation Inductively Coupled Plasma Mass spectrometry
LACOSUS	Land Consultancy and Surveying Services

µg/dL	Microgram per deciliter
ME	Modelling Efficiency
MMR	Mild mental retardation
MOE	Margin of exposure
MOS	Margin of safety
MRC	Medical Research Council
MRLs	Minimum risk levels
NIOH	National Institute of Occupational Health
$NH_4H_2PO_4$	ammonium dihydrogen phosphate
NSE	Nash-Sutcliffe efficiency
Pb	Lead
PBTK	Physiologically based toxicokinetic
PP	Protoporphyrin
PVC	Poly Vinyl Chloride
Q-Q	Quantile-Quantile
r	Pearson correlation coefficient
RBC	Red blood cell
RFLPs	Restriction fragment length polymorphisms
ROS	Reactive oxygen species
SF	Safety factor
Th	T helper
TNF	Tumour necrosis factor
UPb	Urinary lead
USA	United States of America
USEPA	United States Environmental Protection Agency

USFDA	United States Food and Drug Authority
VDR	Vitamin D receptor
v/v	volume/volume
WHO	World Health Organization
XRF	X-ray fluorescence
YLD	Years Lived with Disability
YLL	Years of Life Lost
Zn	Zinc
ZPP	Zinc protoporphyrin

Glossary

ADI	Acceptable Daily Intake, the amount of chemical to which a person may be exposed on a daily basis for an extended period (usually for a lifetime, without suffering deleterious effects.
LD_{50}	Lethal dose 50, the amount of the substance that kills 50% of the test population of experimental animals
NOAEL	No Observed Adverse Effect Level, an experimentally determined dose at which there is no statistically or biologically significant indication of the toxic effect of concern
LOAEL	Lowest-observed-adverse-effect level, the lowest concentration or amount of a substance found by experiment or observation that causes an adverse effect
PTWI	Provisional Tolerable Weekly Intake, the weekly amount of a chemical that has been assessed to be safe for human beings on long-term basis (usually whole lifetime)

R*f*D	Reference dose, an estimate of a daily oral exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime.
TDI	Tolerable daily intake, the daily amount of a chemical that has been assessed safe for human being on long-term basis (usually whole lifetime)

1.0 Introduction

This chapter gives the context of the study, introduces lead as a toxic substance and gives the background information on lead exposure in Malawi. It also explains the focus of the paper, the main hypothesis and objectives, and provides a literature review of existing research in the area of health risk assessment of lead.

1.1 Background

Although lead poisoning has been investigated as a major public health problem, many countries, very few countries in Africa have conducted such investigations. Malawi is no exception, where there has been no exposure assessment to lead particularly with regards to children. This study takes focus on exposure of lead in children in Blantyre City, Malawi, and also explores the potential adverse health effects associated with these exposures. Blantyre City is located within the larger district of Blantyre, where in this document 'Blantyre' refers to the city and not to the district.

Exposure to lead causes many toxic effects, especially in young children, including haematological, gastrointestinal, reproductive, cardiovascular and neurological effects (Gerber et al., 1980, Lockitch, 1993). Many studies have also shown an association between blood lead (BPb) and reduction in intelligence quotient (IQ) as well as school performance, and violent behavior (Alan S, 2001a, Alan S, 2001b).

Sources of lead exposure are summarized in Figure 1 below. In the 20th century, the most important source of lead was petrol containing lead (often referred to as 'leaded petrol') petrol which by 2006 was banned in many countries including Malawi. The banning of leaded petrol was expected to reduce exposure to lead considerably. However, a large

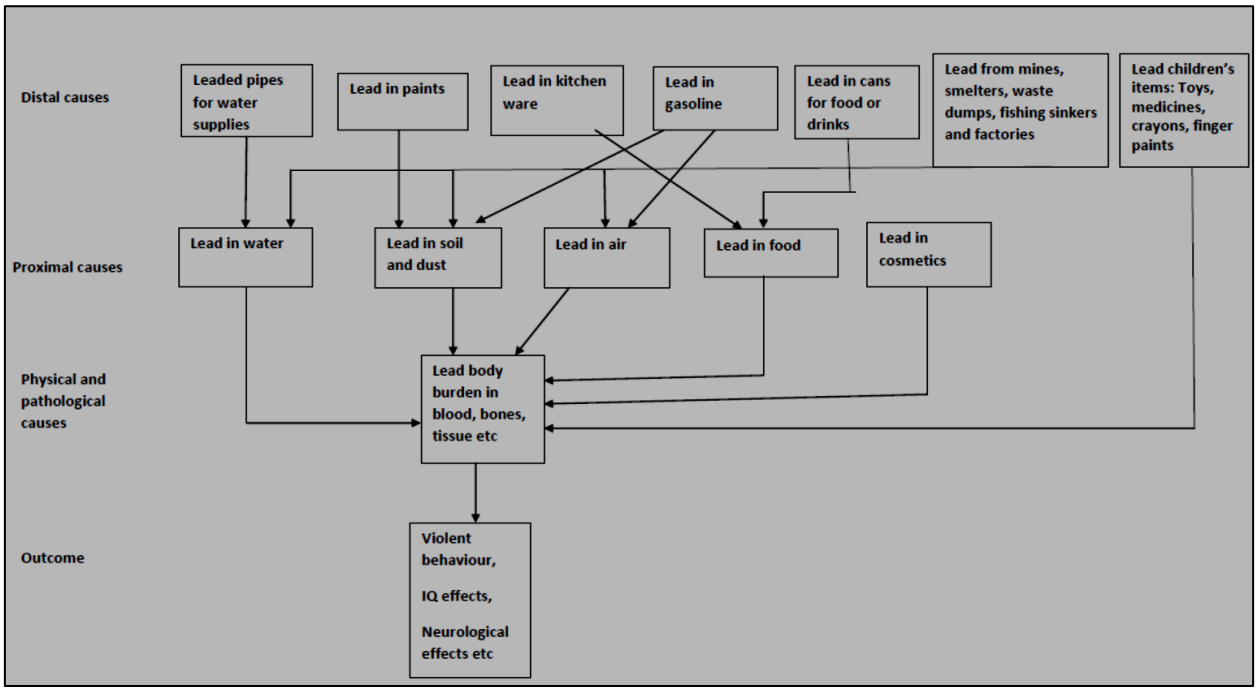

Figure 1: Sources of lead exposure in children and toxicity (WHO, 2003). Additional sources (colured red) added as per references in the text (Section 1.7)

body of evidence shows that children are exposed to lead from lead-based paint, which is distributed through contaminated dust and soil (Clark et al., 2006, Clark et al., 2009, Kumar and Pastore, 2007). Lead compounds are used as colour pigments, in addition to improve many properties of paint including, the durability, resistance to corrosion and drying. Children's toys can also contain lead from the exterior paint and poly vinyl chloride (PVC), where lead is used as a stabilizer (Kumar and Pastore, 2007). Many countries, especially developed countries, regulate the concentration of lead in domestic

paints and children's toys (Clark et al., 2006, Clark et al., 2009, Kumar and Pastore, 2007). However, Malawi does not have such regulations.

It can be seen from Figure 1 that exposure to lead may also occur from some environmental media such as air, soil, water and food (Lanphear et al., 1998, Lanphear and Roghmann, 1997). The contributions of lead from various sources and pathways differ from country to country or regions within one country.

It can also be seen in Figure 1 that exposure to lead may either be assessed through the measurement of its concentration in biological specimens, usually blood, or in various environmental media (food, water, soil, air and dust). A number of countries in Africa have therefore measured lead in blood and/or lead in various sources (Mathee et al., 2002, Mbongwe et al., 2005, Nriagu et al., 1997b). Using similar measures, the present study has assessed exposure to lead in blood and also in different media, with the aim of assessing the levels of exposure to lead in children in Blantyre and the associated risk of suffering from adverse health effects. Furthermore, the study aim is to identify external (risk) factors that may cause some children to be at a higher risk of exposure. It is hoped that by establishing the levels of exposure from different sources and the associated adverse effects, this research may influence public health policy on lead in Malawi. The study uses approaches derived from epidemiology and toxicological (health) risk assessment.

1.2 Problem statement

Although the acute and chronic toxicities of lead have already been recognized internationally, the government of Malawi only regulates the use of leaded petrol, with no other regulation. Exposure to lead, particularly in children, has also not as yet been studied in Malawi.

1.3 Overall hypothesis of the study

Children in Blantyre are exposed to lead levels that may be detrimental to their health.

1.4 Significance of study

It is hoped that this study, the first of its kind to be conducted in Malawi, will assess the possibility of exposure to lead in children from different sources. In doing so, this research will guide policy makers regarding the sources of lead and their management thereof in Malawi.

1.5 General aim

To assess the exposure to lead and potential adverse health effects among children in Blantyre.

1.5.1 Specific aims

1. Determine the levels of lead from different sources of exposure such as food, water, and soil/house dust to predict the levels of BPb using the Integrated Exposure Uptake Biokinetic (IEUBK) model.
2. Measure the levels of BPb and compare to those predicted from the IEUBK model as an indication for its applicability in Malawi.
3. To relate the measured and predicted BPb levels as well as the prevalence of high BPb to potential health effects using the World Health Organization (WHO) and United States Centers for Disease Control and Prevention (CDC) guidelines
4. Assess burden of disease using WHO spreadsheets.
5. Identify additional sources and risk factors for exposure to Pb in children in Malawi to assist the policy makers to reduce exposure to lead.

1.6 Literature review

1.6.1 Toxicokinetics of lead

Toxicokinetics of lead studies its absorption, distribution, metabolism and elimination in the body. Lead may enter the human body through ingestion, inhalation or dermal exposure to be absorbed into and transported by the bloodstream, where over 95% of lead is found in red blood cells (RBC), and about 1% in the plasma and serum (Dorman, 2012). Although only a small proportion of lead exist in the plasma, it is considered significant because it acts as a means of distribution to target organs such as kidney, lungs, brain, spleen, teeth, and bones (Papanikolaou et al., 2005). As lead can readily substitute calcium (Ca^{2+}), the skeletal system serves as a long-term storage of lead (75% in children and between 90 and 95% in adults) (Barry and Mossman, 1970). This stored lead can be

mobilized from bone to blood and other tissues, especially during periods of altered mineral metabolism such as during pregnancy and lactation (Gulson et al., 1998).

Since inorganic lead (Pb^{2+}), the most predominant form of lead in the environment, is not metabolized, the rate of excretion of lead is low, mostly through urine. Lead may also be excreted with bile through the gastrointestinal tract. An assessment of the rate of decline of BPb indicated that periods of 24.0, 20.9, 14.3, and 9.2 months are required for BPb in the ranges of 25–29, 20–24, 15–19, and 10–14 µg/dL to decline to less than 10 µg/dL (Roberts et al., 2001). On the other hand, lead in soft tissues has a mean life-time of 40 days whereas lead in bones has a lifetime of close to 30 years (Rabinowitz et al., 1976). The use of chelating agents can enhance lead excretion in urine. This is the basis of lead chelation therapy (Lowry, 2010).

The toxicokinetics of lead is affected by a number of metabolic and nutritional factors (Dorman, 2012, NRC, 1993). For example, strong negative correlations have been observed between calcium (Mahaffey et al., 1986, Blake and Mann, 1983) and iron (Wright et al., 2003, Hammad et al., 1996, Kwong et al., 2004) intake and BPb. These two elements affect absorption of lead probably because they share common transport mechanisms in the gut (Mykka and Wasserman, 1981, Abbaspour et al., 2014). Since calcium and iron affect absorption of lead, they are sometimes referred to as 'effect modifiers' of lead.

1.6.2 Mechanism of toxicity and health effects of lead

For centuries, lead was known as a neurotoxin that only affected workers in lead industries until in the 1960s when its exposure was identified in children (Marjorie, 1985). Follow-up studies on children with lead poisoning revealed neuropsychological development deficits and violent behaviour (Needleman et al., 2002, Tong et al., 2000).

Lead is a chronic poison with relatively high oral LD_{50} values, 2,000 mg/kg b.w. (EFSA, 2010) and 1200 mg/kg b.w (IPCS, 1995), reported in the literature. However, chronic exposure lead can result in many toxic effects in adults and children (Lockitch, 1993). Lead causes its toxic effects through its affinity for proteins and enzymes (Goering, 1992), particularly proteins that naturally bind Ca^{2+} and zinc (Zn^{2+}) (Godwin, 2001). This interaction is reported "to consist of reversible binding of lead to sulfhydryl groups or to other protein sites capable of binding the bivalent cations", where the binding induces conformational changes in the protein structure, leading to changes in their functioning (Landrigan et al., 2000).

Due to the physico-chemical similarities between Pb^{2+} and Ca^{2+}, lead mimics calcium in a number of processes. Ca^{2+} ions play a ubiquitous role as intracellular messengers for transducing electrical and hormonal signals. The concentration of Ca^{2+} in cell cytoplasm is normally maintained between 50 and 150 nM by the Ca^{2+} homeostasis system. Electrical signal or hormonal signals are transduced by increasing the concentration of Ca^{2+} in one or more parts of the cell (NRC, 1993). Lead is reported to interfere with this process in many cells and nerve terminals, and thereby "affecting synaptic transmission, neuronal

differentiation, permeability of brain capillaries, neuroendocrine function, protein phosphorylation, catecholamine synthesis and others" (Godwin, 2001). The impacts of Ca^{2+} on cell signals result in neurotoxicity and cardiovascular impairment (Landrigan et al., 2000).

The physico-chemical similarities between Pb^{2+} and Ca^{2+} also result in storage of lead in bones. This compromises bone cell function, which may result in a number of bone ailments, including osteoporosis (a reduction in bone mass that may lead to bone fracture) (Campbell and Auinger, 2007), delayed fracture healing and fibrous nonunions (fractures that do not heal) (Carmouche et al., 2005), and dental carries (Moss et al., 1999, Gemmel et al., 2002).

Lead has also been shown to induce apoptosis, programmed cell death, in a number of types of cells, through depolarization of rod cell mitochondria which result in cytochrome c release, caspase activation and apoptosis (He et al., 2000, Xu et al., 2006). Lead is particularly toxic to immature astrocytes and interferes with the formation of myelin, and thus disrupts the formation of the blood-brain barrier. The disruption of the formation of blood-brain barrier during foetal development and early infancy results in most of neurotoxicity of lead in children since the disruption of the central nervous system (CNS) allows molecular proteins like albumin to enter tissues of the CNS which can result in edema, increased intracranial pressure, and encephalopathy (Patrick, 2006). Lead was also shown to inhibit neurogenesis and to alter the pattern of differentiation of newly born cells in the dentate gyrus of rat hippocampus (Jaako-Movits et al., 2005) and it was shown to

cause alterations in neurotransmitter receptors, mitochondria, second messengers, cerebrovascular endothelial cells, astroglia and oligodendroglia (Sanders et al., 2009). Lead also affects the motor axons in the peripheral nervous system, which causes segmental demyelination and axonal degeneration in these fibres (Landrigan and Todd, 1994, Landrigan, 1989), In summary, effects of lead on the brain and CNS can result into a number of neurological disorders, including brain damage, nerve damage, mental retardation, behavioral problems, problems with vision and hearing, and probably neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and schizophrenia (Sanders et al., 2009, Bushnell et al., 1977, Osman et al., 1999).

As stated earlier, lead also causes its toxic effects through its affinity for proteins and enzymes, especially proteins that naturally bind Ca^{2+} and zinc (Zn^{2+}). Among the proteins that bind Zn^{2+} is the zinc enzyme δ-aminolevulinic acid dehydratase (ALAD), also known as porphobilinogen synthase. ALAD catalyzes the second reaction in the haem biosynthetic pathway (Figure 2), a process that has been shown to "begin with succinyl coenzyme A (CoA) and glycine and ends with the insertion of iron (Fe^{2+}) into a molecule of protoporphyrin (by the enzyme ferrochelatase)" (Onalaja and Claudio, 2000). In the first step, the enzyme aminolevulinic acid (ALA) synthase catalyses the formation of ALA from glycine and succinyl coenzyme A (CoA). In the second step, ALAD catalyzes the formation of porphobilinogen from two molecules of ALA. ALAD has a high affinity for lead, as lead binds to the enzyme's SH group, a group that normally binds zinc. The inhibition of ALAD activity results in the accumulation of ALA in blood and urine (Onalaja and Claudio, 2000). The disruption by lead of the biosynthesis of haem process (Figure 2) may also result in anaemia.

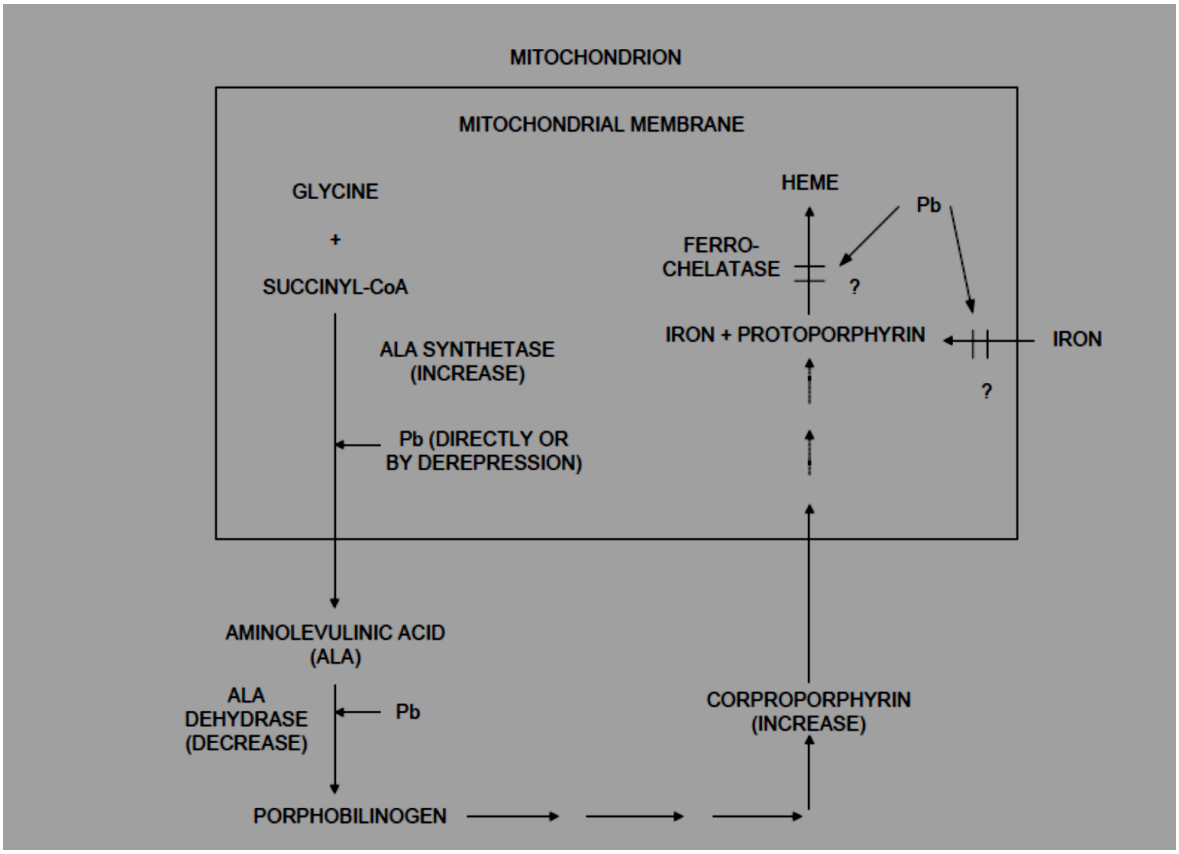

Figure 2: Schematic presentation of the enzymatic steps involved in heme synthesis pathway (USEPA, 1986)

Lead has also been shown to inhibit the activities of antioxidant enzymes, including glutathione peroxidase, catalase and superoxide dismutase, and induce oxidative stress that may lead to DNA damage, lipid peroxidation and protein oxidation (Bolin et al., 2006, Xu et al., 2008). Generation of reactive oxygen species (ROS) and depletion of antioxidant reserves have been linked with many lead induced ailments. Unlike redox-active metals that produce reactive oxidative species through Fenton–like mechanisms, redox-inactive metals such as lead generate ROS through depletion of major sulfhydryl reserves. Lead generates ROS through oxidation of ALAD, a sulfhydryl containing enzyme as shown in the following equations below, where enolization of ALA is followed by aerobic oxidation

that induces the generation of superoxide ($O_2^{\cdot -}$) and peroxide (O_2^{2-}) radicals (Ercal et al., 2001):

$$ALA\ (ketonic\ form) \leftrightarrow ALA\ (enolic\ form)$$

$$ALA\ (enolic\ form) + O_2 \rightarrow O_2^{\cdot -} + ALA^{\cdot} + H^+$$

$$ALA^{\cdot} + H_2O + H^+ \rightarrow 1{,}2 - dioxovaleric\ acid + NH_4^+$$

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$H_2O_2 + Fe^{2+} \rightarrow HO^- + HO^{\cdot} + Fe^{3+}$$

Overall, lead affect enzymes, disrupts cellular metabolism of calcium (and thus affecting conduction in nerves), and causes apoptosis and oxidative stress, processes that may result in neurotoxic, nephrotoxic and cardiovascular effects, and many other effects" (Gerber et al., 1980, Goyer, 1990, Goyer, 1993, Kaufman, 2001, Landrigan et al., 2000, Needleman, 2004, Patrick, 2006, Schwartz, 1994). Symptoms for acute lead poisoning are numerous and are not unique to lead poisoning, including headaches, vomiting, dizziness, stomach cramps, most of which appear only at high levels of exposure and long periods after exposure (Needleman, 2004). The health effects are discussed in detail in the ensuing sections.

1.6.2.1 Health effects of lead

1.6.2.1.1 Neurobehavioral effects of lead

1.6.2.1.1.1 Neurobehavioral effects of lead in children

The interruption of the processes of neural and brain development in children by lead can result in a permanently altered brain function. Consequently, lead has been linked to a number of neurobehavioral disorders in children which have often manifested in reduction in IQ, violent behavior, crime and pregnancies outside marriage in many countries including, Britain, Canada, France, Australia, Finland, Italy, West Germany, and New Zealand (Needleman et al., 2002, Needleman et al., 1996, Nevin, 2000, Nevin, 2007). However, it is important to note that since neurobehavioural effects of lead in children do not have easily detectable symptoms, and since they have prolonged latency periods, they have offered special challenges to analysts (Weiss, 1988). Furthermore, these effects are affected by many other factors including but not limited to, parenting skills, parenting styles of child rearing, parental time spent with the child, the skills and styles of key caretakers other than the parents, genetic factors, levels of education of parents and socio-economic status. These factors are often not controlled for in many studies (Kaufman, 2001).

Nevertheless, despite of the challenges and short comings in many studies, there is enough evidence to show a link between lead exposure and IQ reduction. For example, a correlation between lead exposure and IQ has been shown in a meta-analysis in which an increase in BPb from 10 µg/dL to 20 µg/dL is associated with a mean reduction of 2.6 IQ (Schwartz, 1994).

There is also enough evidence in both animal and human studies to establish a link between lead exposure and attention-deficit/hyperactivity disorder (ADHD) in children. ADHD is a neurobehavioral disorder that manifests through inattentiveness, hyperactivity, and impulsiveness (Sagvolden et al., 2005). ADHD is subdivided into three main diagnostic subtypes: predominantly inattentive, predominantly hyperactive/impulsive subtype, and the combined subtype. Predominantly inattentive children appear dreamy and inert, with poor focus to attention and less accuracy in information processing. Predominantly hyperactive/impulsive children have inattention specifically related to distractibility and reduced persistence (Taylor, 1998, Sagvolden et al., 2005). They are shown to "have memory retrieval problems, exhibit aggressive, oppositional behavior leading to adolescent delinquency and substance abuse, and suffer peer rejection" (Sagvolden et al., 2005).

Associations between levels of BPb with ADHD have been shown in many countries. In the United States of America (USA), children with BPb ≤ 5 μg/dL were about 3 times more likely to have ADHD than children with BPb below 5 μg/dL, with 290,000 of the 1.8 million cases of ADHD among U.S. children of attributed to lead exposure (Braun et al., 2006). A dose-dependent association between BPb and ADHD was also established in Korea, where ADHD could be attributed to lead even at very low BPb (1.5 μg/dL) (Ha et al., 2009). Associations between BPb and BPb have also been established in China, where children with BPb ≥ 10 μg/dL were 6 times more likely to develop ADHD compared to children with BPb ≤ 5 μg/dL, and children with BPb in the range 5–10 μg/dL were 5 times more likely to develop ADHD compared to children with BPb ≤ 5 μg/dL (Wang et al., 2008). Lead induced ADHD (and IQ reduction) manifest as a reduction in school

performance especially at BPb levels above 10 µg/dL as reported in many studies (Chandramouli et al., 2009, Miranda et al., 2009, Miranda et al., 2007).

1.6.2.1.1.2 Neurobehavioural effects in adults

Similar to children, neurotoxic effects have also been observed in adults, although in adults they appear to be initiated at BPb levels of about 18 µg/dL, whereas in children they are observed at BPb levels below 10 µg/dL (Murata et al., 2009). The main neurotoxic effect in adults is the decline in cognitive functions, which has been reported following both environmental and occupational exposures (Schwartz et al., 2005, Shih et al., 2006, Dorsey et al., 2006). Neurological effects can also be detected in adults 20 years after childhood environmental exposure (Stokes et al., 1998).

Effects of lead on the CNS also result on adverse effects on sensory faculties such as eye sight and hearing. In this regard, lead has been shown to cause impairment of scotopic vision (night blindness) in monkeys (Bushnell et al., 1977, Lilienthal et al., 1994). Similarly in humans, an association was established between chronic lead exposure and the age-related risk of cataract in men (Schaumberg et al., 2004). In addition, occupational and environmental lead exposure also causes impairment in hearing (Forst et al., 1997, Choi et al., 2012).

Since it is now recognized that parameters of fetal, infant, and childhood growth may be predictors of disease in later life, there have been speculations on the possible role of lead

in neurodegenerative disease such as Alzheimer's disease and Parkinson disease (Prince, 1998, Landrigan et al., 2005). Indeed, it has been reported that exposure to Pb early life can reprogram gene expressions that can result in both upregulation and down-regulation of genes that may contribute to neurodegeneration in old age, leading to Alzheimer's disease (Bihaqi et al., 2011, Wu et al., 2008). However, a meta-analysis on retrospective occupational exposures to lead found not association between lead and the disease (Graves et al., 1991). These conflicting results are expected as studies on early exposures to lead and the onset of Alzheimer's disease are beset by long latency periods and lack of validated circulating epigenetics biomarkers and retrospective biomarkers of Pb exposure (Bakulski et al., 2012).

Associations have also been found exposure to lead and the development of Parkinson's disease, where more than 20 years of co-exposures to lead and copper and lead and iron were found to be risk factors for Parkinson's disease, with odd ratios 5.24 and 2.83 respectively (Gorell et al., 1997). Divalent metals such as Pb^{2+} accelerates the rate of formationof α-synuclein fibril, which is involved in Parkinson's disease (Uversky et al., 2001).

1.6.2.1.2 Reproductive and developmental effects of lead

1.6.2.1.2.1 Reproductive and developmental effects of lead in children

Reproductive and developmental effects of lead administered *ad libitum* to rats in *utero,* pre-pubertally, or post-pubertally included a decrease in weights of sex organs and suppression of serum testosterone levels in males, and delayed vaginal opening and

disrupted estrus cycling in females. The effects on reproductive physiology and growth appeared to involve actions at multiple sites on the hypothalamic–pituitary–gonadal axis, probably resulting from disruption of calcium-dependent secondary messenger systems (Ronis et al., 1996). Indeed, epidemiological studies have shown environmental exposure to lead may delay growth and pubertal development in girls (Selevan et al., 2003, Naicker et al., 2010a).

1.6.2.1.2.2 Reproductive and developmental effects of lead in adults

In male mice lead was shown to inhibit spermatogenesis and sperm development (Wang et al., 2013). Similarly in men, lead can impair the production of sperms and reduce concentrations of androgens, and consequently "decreased sperm count, volume, and density, impaired sperm motility and morphology in male workers" have been reported among workers exposed to lead at BPb levels higher than 40 µg/dl (Landrigan et al., 2000). Reproductive effects of lead have also been observed among the general population at average BPb concentrations of about 5 µg/dl (Telišman et al., 2007).

Lead also significantly suppressed circulating levels of luteinizing hormone (LH), follicle stimulating hormone, estradiol in monkeys, without inducing overt signs of menstrual irregularity (Foster, 1992). Similarly in women, lead can cause spontaneous abortions and low birth weight in infants. For example, odds ratios of spontaneous abortions of 2.3, 5.4, and 12.2, for BPb levels of 5-9, 10-14 and ≥ 15 ug/dL, respectively, in comparison to < 5 ug/dL, have been reported in Mexico (Borja-Aburto et al., 1999). Increased frequency of

spontaneous abortions and decreased birth weights in children have also been reported in Sweden (Nordstrom, 1979).

1.6.2.1.3 Effects of lead on the immune system

Lead affects the immune system, where it suppresses the T helper (Th1)-dependent delayed type hypersensitivity (DTH) response, increases production of immunoglobulin E (IgE), and increases production of the proinflammatory cytokines tumor necrosis factor (TNF) α and IL-6 (Dietert and Piepenbrink, 2006, Dietert et al., 2004).

1.6.2.1.4 Lead and anemia

Lead interferes with heme synthesis and production of RBC, where reduction of RBC production has been observed at concentrations above 7.0 μg/dl (Iavicoli et al., 2003). In addition, lead shortens the life spans of RBC (Hernberg et al., 1967). For these reasons anaemia is often observed with Pb poisoning. A strong non-linear dose-response relationship between BPb level and hematocrit was observed in a cross-sectional study involving children (Schwartz et al., 1990). In India, children with BPb levels above 10 μg/dl were 1.3 times as likely to have moderate anemia as children with BPb levels below 10 μg/dl. Furthermore, children with BPb levels above 10 μg/dl were at least 1.7 times more likely to develop severe anaemia than children with BPb below 10 μg/dl (Jain et al., 2005).

1.6.2.1.5 Lead and renal effects

1.6.2.1.5.1 Lead and renal effects in children

Nephrotoxic effects can result from both acute and chronic exposure to lead. Acute lead nephrotoxic effects include a deficit of tubular transport mechanisms and degenerative changes in the tubular epithelium, manifested in children by glycosuria and aminoaciduria and changes in specific ion transport. These changes appear to result from effect of lead on mitochondrial respiration and phosphorylation (Goyer, 1989). On the other hand, chronic lead nephrotoxic effects include renal dysfunction, characterized by glomerular and tubule-interstitial changes, and culminating in chronic renal failure, hypertension and hyperuricemia (Rastogi, 2008). While acute nephrotoxic effects are reversible upon reduction of lead exposure or treatment with chelating agent, chronic nephrotoxic effects are irreversible (Goyer, 1989).

Nephrotoxic effects have been associated with lead exposure in children in some epidemiological studies. For example, environmental exposure to lead in the vicinity of a lead smelter caused slight effects on the proximal tubule function in children in Prague (Bernard et al., 1995). The pattern of nephrotoxic effects in children appear to be similar to that observed in adults, although they occur at lower BPb levels than in adults (Fels et al., 1998).

1.6.2.1.5.2 Lead and renal effects in adults

Lead-induced nephrotoxic effects have also been detected among workers in many countries. In Singapore, decreased glomerular function was observed among lead-exposed

workers (Chia et al., 1995). In the USA, a significant decrement in kidney function was detected in lead smelter workers, and in Taiwan BPb and tibia lead were significantly associated with changes in renal function among lead workers (Weaver et al., 2009).

Studies have also indicated nephrotoxic effects from environmental exposure. For example, in Taiwan, longitudinal studies established a correlation between BPb and progressive renal insufficiency (Lin et al., 2001, Yu et al., 2004). Similarly, in the USA, an association was obtained between BPb and chronic kidney disease (Muntner et al., 2003), while in Scotland, a highly significant correlation was established between lead BPb and renal insufficiency (Campbell et al., 1977).

1.6.2.1.6 Carcinogenic and genotoxic effects of lead

1.6.2.1.6.1 Carcinogenic and genotoxic effects of lead in children

Substitution of Zn^{2+} and Ca^{2+} by lead in enzymes that are involved in deoxyribonucleic acid (DNA) processing and repair can inhibit DNA repair. Lead may also produce reactive oxygen species which can cause oxidative damage to DNA (Silbergeld et al., 2000). The inhibition of DNA repair or oxidative damage to DNA may result in genotoxic effects. Therefore, lead is likely to be mutagenic or it is a mutagen (Ariza and Williams, 1996, Zelikoff et al., 1988).

The direct damage of DNA or inhibition of DNA synthesis or repair may also cause lead to be carcinogenic. Indeed, lead has been reported to cause kidney and brain tumors in rats

and mice (Mulware, 2013). However, "because of the *limited and inadequate evidence* in humans and *sufficient evidence* in experimental animals", the International Agency for Research on Cancer (IARC) classifies inorganic lead compounds as *probably carcinogenic to humans (Group 2A)* (IARC, 2004). Indeed, DNA damage has been detected in children exposed to lead around a mining site (Yáñez et al., 2003), and lead exposure was found to cause an increase in micronuclei in children (Kapka et al., 2007), which is an indication of DNA damage. This notwithstanding, there appears to be no literature on the prevalence or incidence of cancer resulting from lead exposure in children.

1.6.2.1.6.2 Carcinogenic and genotoxic effects of lead in adults

The direct damage of DNA or inhibition of DNA synthesis or repair by lead may also result in cancer in adults. Indeed, lead has been linked to stomach and lung cancers in workers (Fu and Boffetta, 1995), brain cancer in workers (Cocco et al., 1998) and breast cancer among the general population (Alatise and Schrauzer, 2010).

1.6.2.1.7 Cardiovascular effects of lead

1.6.2.1.7.1 Cardiovascular effects of lead in children

Exposure to lead has been shown to be "associated with blood pressure, hypertension and increased incidence of cardiovascular complications such as coronary heart disease, stroke and peripheral arterial disease, left ventricular hypertrophy and alterations in cardiac rhythm" (Pirkle et al., 1985, Navas-Acien et al., 2007, Park et al., 2006). The mechanism for cardiovascular dysfunctions is reported to occur through "impairing nitric oxide signaling, augmentation of adrenergic activity, increasing endothelin production, alteration

of the renin-angiotensin system, raising of vasoconstrictor prostaglandins, lowering of vasodilator prostaglandins, disturbance in vascular smooth muscle Ca^{2+} signaling, reduction in endothelium-dependent vasorelaxation, and modification of the vascular response to vasoactive agonists" (Vaziri, 2008). An association between prenatal lead exposure and blood pressure has been established in children (Zhang et al., 2012).

1.6.2.1.7.2 Cardiovascular effects of lead in adults

In contrast to children where there are only very few studies linking lead exposure and cardiovascular effects, there are a number of studies that indicate the link between lead exposure and cardiovascular effects in adults both in the general population (Hu et al., 1996) and among workers (De Kort et al., 1987). These effects have also been observed in animal studies (Nowack et al., 1993, Staessen et al., 1994). On the other hand, there are studies also which could not establish an association between lead exposure and cardiovascular effects in the general population (Staessen et al., 1996) and among workers (Parkinson et al., 1987, Kirkby and Gyntelberg, 1985, Maheswaran et al., 1993). Because of these contradictions, a systematic review on lead and cardiovascular effects concluded that the evidence for a causal relationship of lead exposure with clinical cardiovascular outcomes was only suggestive but not sufficient (Navas-Acien et al., 2007).

1.7 Sources of lead

Children are exposed to lead from a number of sources, including food (47%), dust and soil (45%), water (6%) and air (1%) (Prüss-Üstün et al., 2006). Concentrations of lead in tap water have been shown to be associated and BPb in many countries (Fertmann et al.,

2004, Edwards et al., 2009). As most sources of water have naturally very low lead levels, drinking water from the source is usually free of lead. Use of lead for soldering pipes and plumbing has in the past been a major source of high lead levels in water. Therefore, water may be contaminated as it is distributed from the water treatment plant through lead water pipes and across lead-soldered joints into homes (Renner, 2009). As soft, acidic water has the greatest tendency to dissolve lead (plumbosolvency), plumbosolvency is decreased when the pH and water hardness are increased through the addition of lime (Fergusson, 1986). Indeed, when lead pipes were still in use it was shown in England that towns that had soft water supplies had the highest water lead concentrations and consequently the highest mean BPb concentrations (Pocock et al., 1983). Lead pipes are no longer used for water distribution throughout the world.

Food can be a significant contributor to BPb, contributing as much as 72% of the total daily lead intake (Wilheim et al., 2003). Lead in food can come from environmental contamination of crops through atmospheric deposition, soil or water. Since there are spatial variations in environmental contamination of lead, there can be variations in lead concentrations in foods from different regions of the same country (Cuadrado et al., 2000). The variations can also result from the methodological challenges in sampling and dietary exposure assessment (Elwood, 1986).

Lead contamination of food can also result from use of contaminated utensils/apparatus for food preparation or storage. As an example, lead-glazed ceramics are an important source of lead contamination in some countries (De Mejía and Craigmill, 1996), where a

correlation could be found between use of lead-glazed ceramics and BPb among women in Mexico (Avila et al., 1991). In addition, food and drink cans soldered with lead-based solder can also be an important source of lead (Sherlock, 1987).

Lead exposure can also result from paint since lead pigments are often used in domestic and industrial paints. Paint is essentially composed of a binder, pigment and solvent. Binders are usually polymeric substances that are added to the paint to hold the pigment to the surface of the wall. Pigments are used primarily to give the paint its colour and finish, as well as protecting the surface underneath from corrosion and weathering (Clark, 2000). Common lead-based pigments include white lead ($2PbCO_3.Pb(OH)_2$)), vivid yellow lead chromate ($PbCrO_4$), basic lead chromate ($PbCrO_4.Pb(OH)_2$), lead molybdate ($PbMoO_4$), lead carbonate ($PbCO_3$), red lead (Pb_3O_4), leaded zinc oxide (white lead + ZnO), basic lead sulphate ($2PbSO_4.PbO$), litharge (PbO) and basic lead silicate ($PbO.SiO_2$) (Sturges and Harrison, 1985). Lead pigments are often preferred to other paint pigments because of their durability and bright colours (Filippelli and Laidlaw, 2010, RSC, 2007).

Lead compounds may also be added to enamel (oil-based) paints to act as driers (sometimes called drying agents or catalysts). The driers serve as catalysts that speed up the polymerization, and thus make paints dry faster and more evenly (UNEP, 2013). Lead compounds that are commonly used as driers include lead octoate, lead acetate and lead naphthenate. Lead compounds are also sometimes added to paints used on metal surfaces to inhibit rust or corrosion, where the most common of these is lead tetroxide (UNEP, 2013). Children can be exposed to lead when lead-based paints are applied in their

environments, where they can be exposed when they play with walls, through dust when they play on the floor and through direct eating of paint chips in a habit known as pica. In this regard, paint flakes make significant contributions to lead in house dust and street dust (Gulson et al., 1995, Gulson et al., 1997, Sturges and Harrison, 1985). Consequently, house dust has been found to be a significant source of BPb (de Freitas et al., 2007, Dixon et al., 2008, Kumar and Scott Clark, 2009, Lanphear et al., 1998, Lanphear and Roghmann, 1997).

Lead-based paint is also often applied to children's toys through which children can be exposed to lead via mouthing behavior. Children's toys made of PVC can also contain lead through the use of lead as a stabilizer in PVC. For these reasons, studies on lead in toys in many countries have shown lead levels above permissible limits (Greenway and Gerstenberger, 2010, Kumar and Pastore, 2007, Omolaoye et al., 2010), which is currently 100 mg/kg in the USA (CPSC, 2008). Heavy metals, including lead, are found to be bioaccessible from PVC toys (Guney and Zagury, 2014).

Lead can also be found in air. Sources of lead emissions include traffic (Monna et al., 2006), industries and waste combustion (Sun et al., 2006), thermal power stations (Liang et al., 2010), lead smelters (Roels et al., 1980, Kalač and Stašková, 1991), lead-zinc smelters (Gulson et al., 2004), copper smelters (Carrizales et al., 2006) and mining (Davies and White, 1981, Zheng et al., 2013). Atmospheric concentrations of lead were high in areas where there was use of leaded petrol or industries that dealt with lead, especially mining. However, the most important source of lead in air appears to have been leaded petrol,

which is now banned in almost all countries of the world, leading to a significant reduction in atmospheric lead. Internationally, after the introduction of regulations on lead in gasoline, concentrations of lead air have been reported to be reduced to about 0.2 $\mu g/m^3$ (Thomas et al., 1999). In the USA BPb levels were observed to decline as atmospheric lead declined (Hayes, 1994).

There are also many numerous sources of lead that can be termed as 'miscellaneous sources' because they are not well documented or characterized. These include crayons (CUS, 2004), finger paints (Rastogi, 1992), jewels (CDC, 2015), cosmetics (Bocca et al., 2014) and fishing sinkers (Mathee et al., 2013). Occupational sources of lead are not within the scope of the present study except in the cases where parents may bring lead from work through clothes and equipment. The most important sources of lead exposure to children at home are summarized in Figure 3 below.

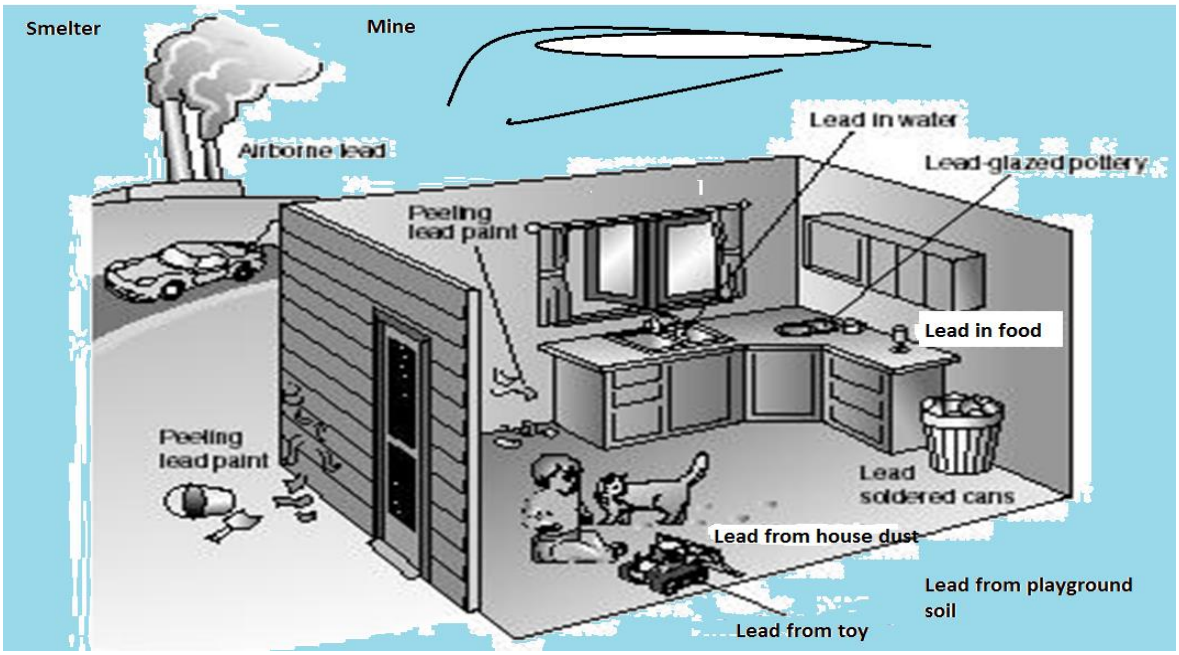

Figure 3: Sources of lead at home (adopted from http://www.faqs.org/health/Sick-V3/Lead-Poisoning-Prevention.html)

1.8 Factors that affect levels of exposure to lead among children

In addition to the previously described sources of lead, there are factors that have been found to affect the levels of exposures among children from these identified sources. These factors are often referred to as 'risk factors', defined as factors that increase the likelihood of developing a disease, a condition or injury (WHO, 2016). They are also sometimes termed as 'determinants' (Menezes-Filho et al., 2011) or 'predictors' (Friedman et al., 2005) of lead exposure. They are usually demographic, socio-economic, nutritional and genetic factors that are often different among countries and even within regions of the same country. These factors are the reasons why within the same environment some children may have higher BPb than others. An understanding of these factors is important for rational and effective design of control measures (Burstyn and Teschke, 1999).

Some risk factors may easily be linked to the various sources of lead, including living near an industrial site (Menezes-Filho et al., 2011), use of glazed kitchenware (Isidra et al., 2003), distance of house from a smelter, proximity of a house to busy streets, applying of surma to eyes, having a father who works with lead (Rahbar et al., 2002), mouthing behaviours, or residing in mining towns (Malcoe et al., 2002). On the other hand risk factors such as age, levels of income and education (socio-economic status), and gender (CDC, 2001, Olaiz and Fortoul, 1996) and having a teenage mother at birth (Naicker et al., 2010b) may be linked to some habits that directly or indirectly increase the risk of exposure. Genetic and nutritional factors affect the toxicokinetics of lead in the body, and therefore can be viewed as effect modifiers, factors that can affect the magnitude of the effect of exposure. In this regard, zinc deficiency increases lead absorption, while calcium and iron deficiency increase the retention of lead and thus increases the severity of effects (Mason et al., 2014). The nutritional factors partly accounts for relationship between socio-economic status and lead exposure. The role of nutritional factors on lead toxicity has been discussed in *Section 1.6.1,* while the role of genetic factors is discussed in more detail in *Sections 1.10.3.1.3.*

Risk factors that are associated with high exposure to lead are often utilized in *lead exposure risk assessment questionnaires* in many developed countries, particularly by the CDC. Lead exposure risk assessment questionnaires serve as initial screening tools for identifying subjects, especially children, who may be at a high risk to exposure to lead. In this type of assessment children that are found to be at risk are subjected to blood test for lead. In this regard, an evaluation of one such questionnaire in Illinois indicated that the questionnaire would identify most children with high BPb (Binns et al., 1999).

1.9 Studies on exposure to lead in Sub-Saharan Africa

In Sub-Saharan Africa there have been relatively few studies on exposure to lead in blood and/or sources of lead including food, air, water, soil, toys, paint, crayons, medicines (herbal and western) and cosmetics. Most studies have been conducted in South Africa and Nigeria, where most of these studies were on the identification of the sources of lead and levels of exposure, often with no accompanying risk assessment resulting from the exposures.

In South Africa, prior to regulations on lead in petrol in 1996, a 13% of urban children of mixed race had BPb levels above 25 μg/dL (von Schirnding et al., 1991). These levels of BPb in urban areas ware also confirmed to be greater than 25 μg/dL, compared to children in rural areas, where only 2% of the children had BPb levels greater than 10 μg/dl (Nriagu et al., 1996, Nriagu et al., 1997a). Subsequent to the introduction of regulations on lead in petrol in 1996 BPb, BPb levels were reported to be within the range of 1.0 and 24.5 μg/dL, with 10% of children having BPb levels ≥ 10 μg/dL (Mathee et al., 2006). The impact of the introduction of regulations on lead in petrol was confirmed in another study where the average BPb was 4.9 μg/dL, with 49% of the population having BPb ≥ 5.0 μg/dL and only 1% > 10.0 μg/dL (Naicker et al., 2010a).

In Nigeria, prior to the banning of leaded petrol the mean BPb in children aged 1-6 years was found to be 10.6 μg/dL (Nriagu et al., 1997b). There appear to be no other studies on BPb in Nigeria that may indicate the impact of introduction of regulations on lead. However, as a confirmation of the importance of other sources of lead, investigations into

the fatal lead poisoning outbreaks in Nigeria showed that 97% of children had BPb ≥ 45 μg/dL, resulting from gold ore processing (Dooyema et al., 2012, Lo et al., 2012).

Studies on lead in blood were also conducted in other countries, such as Botswana, where at the time regulations on leaded petrol were introduced, 31% of children were shown to have BPb ≥ 10 μg/dL (Mbongwe et al., 2005), and in Uganda, where the mean BPb after the introduction of regulations on leaded petrol was 7.15 μg/dL, with 20.5% of the children having BPb above10 μg/dL (Graber et al., 2010). In addition to studying lead in blood, there are many studies in sub-Saharan Africa on sources of lead. For example, there were a number of studies on levels of lead in paint. In South Africa, 48% of paint in public playgrounds in the municipalities of Johannesburg exceeded the reference level of 1 mg/cm^2 (Mathee et al., 2009). Hazardous levels of lead were also found in domestic paint in Nigeria (Adebamowo et al., 2007, Clark et al., 2006, Clark et al., 2009), and Cameroon (Gottesfeld et al., 2013).

The presence of lead was also found in medicines in Nigeria, where the use of herbal medicines is reported to result in "lead intakes ranging from 250 μg/day to 27,000 μg/day, which were higher than the Provisional Tolerable Weekly Intake (PTWI) of 25 μg/week of lead that was being used at the time" (Obi et al., 2006). In Nigeria lead was not only found in herbal medicines but also in imported western medicinal syrups (Orisakwe and Nduka, 2009), indicating that both herbal and 'western' medicines may be a potential source of lead.

Studies have also been conducted on lead in air where, lead concentrations of 1 μg/m^3 in air and 3620 mg/kg in dust were found in air in industrial, commercial, park/beach and residential areas of South Africa (Nriagu et al., 1996, Nriagu et al., 1997a) and in Ghana, where the air in areas surrounding a battery, an electronic repair, a welding and e-waste recycling workshops, and a waste disposal site exceeded the contemporary WHO standard of 50 μg/m^3 (Dartey et al., 2010, Caravanos et al., 2011).

Studies on lead in food in Uganda indicated that vegetables grown along heavily trafficked streets were heavily contaminated with lead (Nabulo et al., 2006), while in Kenya, fish and vegetables were also found to be contaminated with high levels of lead (Makokha et al., 2008). In Zimbabwe, high levels of lead were found in vegetables that were irrigated using mixtures of wastewater and sewage sludge containing high levels of lead (Muchuweti et al., 2006). Lead was also found in processed foods. For example, in Nigeria, lead was found in canned and non-canned beverages (Maduabuchi et al., 2006).

There were also studies on lead in other commodities such as toys, crayons and cosmetics. For example, in Nigeria, lead was shown to be present in toys (Sindiku and Osibanjo, 2011, Omolaoye et al., 2010), and in South Africa, lead was present in crayons (Okonkwo and Maribe, 2004). In South Africa, levels of lead in lipsticks, lip gloss, and foundation ranged from below detection limit to 73.1 ± 5.2 mg/g, 4.7 to 11.7 ± 2.8 mg/g, and 7.8 to 32.9 ± 1.4 mg/g, respectively, with the majority exceeding the United States Food and Drug Administration (USFDA) maximum permissible concentration of 0.10 mg/g for lead

in cosmetics (Brandao et al., 2012). High levels of lead were also present in cosmetics in Nigeria (Orisakwe and Otaraku, 2013).

These and other studies in Africa confirm the possibility of exposure to lead from these sources among children in Africa. They also confirm that although banning the use of leaded petrol had a significant impact on BPb in children, other sources of lead may still remain, and therefore they may warrant further investigation.

1.10 Risk assessment of chemicals

Risk assessment has been defined as 'the systematic scientific characterization of potential adverse health effects resulting from (human) exposures to hazardous agents or situations' (NRC, 1983). In reference to chemicals the process is specifically referred to as health risk assessment (of chemicals) (WHO, 2010b, Filipson et al., 2003), toxicological risk assessment (Nielsen et al., 2008), and risk assessment of chemicals or chemical risk assessment (Fryer et al., 2006, Nielsen et al., 2008). Risk assessment of chemicals is conducted to establish permissible exposure levels for human beings and other species, and to assess the health risks resulting from a particular exposure (Filipson et al., 2003). Risk assessment is important for risk management, which is defined as the process by which policy or other management actions are implemented in order to control hazards identified in the risk assessment process.

In risk assessment it is important to distinguish between the terms risk and hazard, where risk is defined as the probability of the occurrence of an adverse outcome, and the term hazard is defined as the intrinsic toxic properties of a chemical. There is a risk when there is exposure to a hazardous substance. The risk assessment process is said to comprise of "four components namely, *hazard identification, dose response assessment, exposure assessment* and *risk characterization"* (Nielsen et al., 2008).

1.10.1 Hazard identification

Hazard identification aims at "determining if a chemical has an inherent potential to cause harm in an experimental animal or in the human body" (Rudén 2006). A chemical is declared hazardous only if it produces adverse effects in humans or in experimental animals (Abernathy and Roberts, 1994). For human health risk assessment in many countries there are lists of tests intended for assessment of toxic effects. Lead has been shown to be a hazardous chemical in *in vivo* and *in vitro* toxicological studies and also in epidemiological studies discussed in *Section 1.6*.

1.10.2 Dose-response assessment and safe levels for lead

The *Dose-response assessment* process evaluates responses at particular exposure levels (USEPA, 2005). A response can be any "detectable adverse alteration of morphology, functional capacity, growth, development or life span of the target organism" (Nielsen et al., 2008). The increase in incidence or severity of adverse effect depends on the dose, which can be defined as the amount of the test substance that comes in contact with the organism or part of the organism. However, it is important to note that a chemical enters

the body in two steps, the first of which involves contact with the boundary or barrier (gastrointestinal tract, skin or lungs) followed by actual crossing of the barrier (absorption). The amount of chemical available at the absorption barrier is referred to as the *applied dose,* administered *dose*, potential *dose* or *intake*. Absorption results in the availability of the chemical to physiologically significant sites, and the amount of chemical that has been absorbed is referred to as the *internal dose* (Paustenbach, 2010). The internal dose is calculated from the applied dose using *bioavailabilities* (discussed later in *Section 1.10*.3.2).

For most chemicals, especially for non-cancer effects, there exists a threshold below which exposure to the chemical produces no adverse effect (response) and above which adverse effects are experienced. Therefore, the dose-response assessment aims to identify the No-Observed-Adverse-Effect Level (NOAEL), the Lowest Observed-Adverse-Effect Level (LOAEL), or the benchmark dose (BMD). The NOAEL is defined as the greatest concentration or amount of a substance, found by experiment or observation, which causes no response in the target organism under defined conditions of exposure (WHO/IPCS, 1994, Nielsen et al., 2008). The LOAEL is defined as the lowest concentration or amount of a substance, found by experiment or observation, which causes a response under defined conditions of exposure. A typical dose-response curve demonstrating a hypothetical NOAEL and LOAEL is shown in Figure 4 below. This type of dose-response curve, where there is no response at lower dose levels, is often referred to as an S-shaped curve. However, it is important to note that there are other shapes of dose-response curves, including J-shaped, U-shaped and inverted U shaped dose-response curves depending on the manner of response (Nielsen et al., 2008).

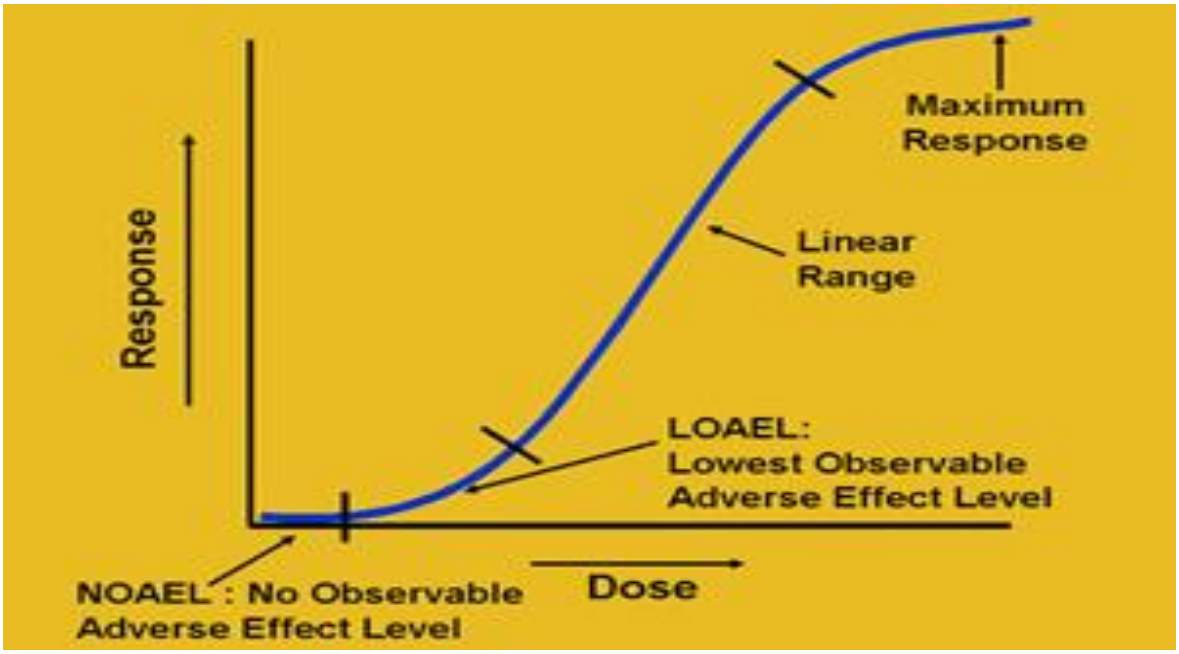

Figure 4: A typical dose-response curve

The BMD is not a ‘no response level but the “dose that produces a predetermined change in response (referred to as benchmark response [BMR])” (Gephart et al., 2001). It is calculated by fitting experimental data to a dose–response curve, as shown in Figure 5, often through the use of a number of dose–response models that are incorporated in some BMD software.

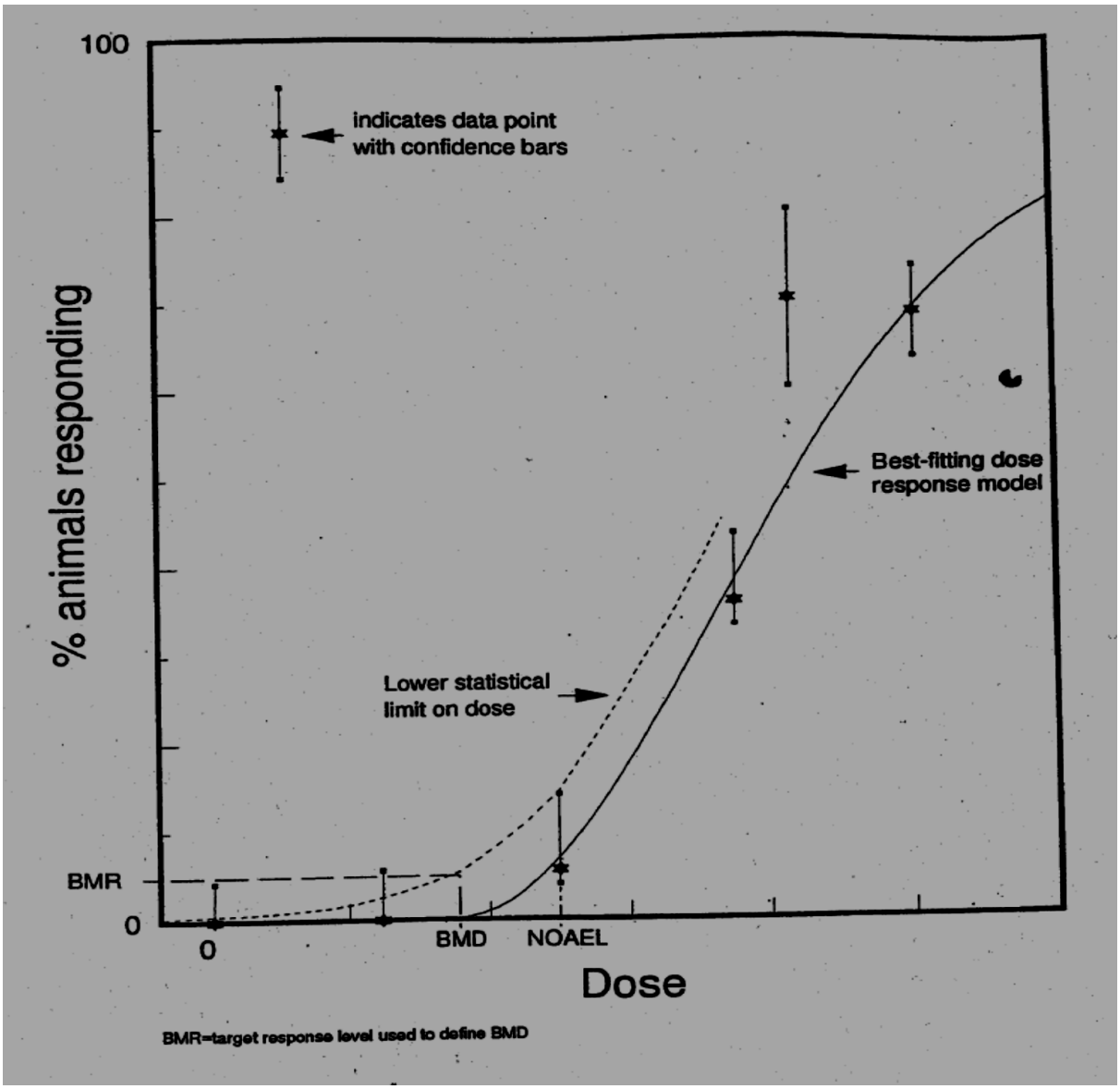

Figure 5: Calculation of a BMD (USEPA, 1995b)

Using the NOAEL, LOAEL or BMD and with the use of uncertainty or safety factors, the international scientific committees such as the Joint Food and Agriculture Organization (FAO)/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), regional scientific committees such as the European Food Safety Authority (EFSA), and national regulatory agencies such as the United States Environmental Protection Agency (USEPA) could establish acceptable or

tolerable intakes of substances that exhibit thresholds of toxicity. For example, for non-cancer effects, the WHO derives the Acceptable Daily Intake (ADI), the Tolerable Daily Intake (TDI) or the PTWI, whereas the USEPA derives the reference dose (R*f*D) and the Agency of Toxic Substances and Disease Registry (ATSDR) derives minimum risk levels (MRLs). The ADI, TDI and R*f*D stand for ' a daily intake of a chemical which, during an entire lifetime, appears to be without appreciable risk'(Lu and Sielken Jr, 1991, Herrman and Younes, 1999). TDI is often used for chemicals that are intentionally added or 'in cases where exposure can be controlled, such as for food additives and residues of pesticides' (WHO/FAO, 2009). The PTWI, which represents the weekly intake of a chemical to which a person can be exposed for their entire lifetime without appreciable risk, is used for contaminants that may accumulate in the body such as lead or cadmium (WHO/FAO, 2009). An MRL is defined as 'an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure' (ATSDR, 2015).

As an example, the R*f*D is calculated in the following manner:

$$RfD = \frac{NOAEL}{UF_1 x UF_2 x \ldots} \qquad \textit{Equation 1}$$

where UF is the uncertainty factor. The number of the uncertainty factors depends on the number of uncertainties involved, where a "factor of 10 is used to account for intra-species variation, a factor of 10 to account for interspecies variation, a factor of 10 to account for uncertainty resulting from the use of a LOAEL, if a NOAEL cannot be determined, a factor of 10 for uncertainty resulting from use of sub chronic toxicity instead of chronic

toxicity, and extra uncertainty factors for other sources of variation" (Gaylor, 1992, Vermeire et al., 1993, Pohl and Abadin, 1995).

ADIs and TDIs are calculated in a similar manner using uncertainty factors. The use of R*f*D, ADI, TDI and PTWI has a number limitations which include, 'dependence on the background incidence of the health outcome on unexposed animals, dependence on the spacing of the doses, and inability to use all available data' (Barnes et al., 1995). In contrast, the BMD does not have these limitations as the BMD results from statistical calculations that utilize all the data, and not just a single point such as NOAEL or a LOAEL, makes use of the sample size in its calculation and takes consideration of the shape of the dose-response curve (Crump et al., 1995).

1.10.2.1 Safe levels for lead

The derivation of acceptable or tolerable intakes is applicable to chemicals that have a threshold of response such as a NOAEL or LOAEL. Since lead appears to have no NOAEL, acceptable or tolerable intakes could not be derived for lead. However, as early as the 1970s it was recognized that dose-response relationships could be established in terms of BPb (Zielhuis, 1975, IPCS, 1977). Consequently, in 1972 the WHO established a PTWI of 50 µg/kg body-weight (b.w.) (JECFTA, 1972), which was meant to keep the level of BPb below 10 µg/dL. However, since the 1980s there has been a great number of literature that showed that adverse effects of lead may occur at well below BPb levels of 10 µg/dL, especially in children (Herbert, 2009, Lockitch, 1993, Marjorie, 1985, Nation and

Gleaves, 2001). In order to address this, in 1986 the JECFA set the PTWI for children at 25 µg/kg b.w. (EFSA, 2010).

In the USA, reference values issued by the CDC as guidance on levels of BPb in young children have also been changing from 40 µg/dL in 1970, to 30 µg/dL in 1975, to 25 µg/dL in 1985, and to 10 µg/dL in 1991 (CDC, 1991). However, since lead appears to have no threshold, the USEPA could not derive an oral R*f*D for lead (USEPA, 2004). The ATSDR also published the Toxicological profile for lead in 2007 without MRLs (ATSDR, 2007).

Internationally, there have also been calls to reduce the levels that are deemed safe for lead. At the Brescia Workshop in 2006 an action level of 5 µg/dL was recommended the worldwide reduction of lead (Landrigan et al., 2007). Within the same period, some researchers also made the recommendation that threshold for BPb should be reduced from 10 µg/dL to 2 µg/dL (Gilbert and Weiss, 2006). For this reason in 2010, the German Commission on Human Biological Monitoring (HBM) suspended the HBM values which it had set in 1996 (10 µg/dL for children of equal to or less than 12 years and females of a reproductive age, and 15 µg/dL for adults) (Wilhelm et al., 2010). In the same year, the EFSA concluded that the PTWI of 25 µg/kg b.w. that was being used then was no longer appropriate (EFSA, 2010) and the WHO also decided to withdraw the PTWI of 25 µg/kg b.w. for lead after realizing that the PTWI could no longer be considered protective for health (WHO, 2011, Zheng et al., 2013). In 2012 the CDC adopted a new guideline of 5 µg/dL (Betts, 2012). Although many countries have adopted the CDC reference value of

of 5 µg/dL, an international pooled BMD analysis calculated BMDs ranging from 0.1 µg/dL to 1.0 µg/dL, indicating that adverse effects could still occur below the CDC reference value of 5 µg/dL (Budtz et al., 2013).

The proposed intake limit values such as the PTWI and RfD that are established by different international agencies are aimed to achieve acceptable levels of lead in blood. These agencies have also proposed safe values of lead in different commodities and environmental samples with the assumption that exposure to lead through these sources may not produce high levels of BPb in exposed individuals. These values are presented in Table 1 below.

Table 1: Safe levels for lead in various countries

Medium/product	Safe level	Organization/country
Paint	90 mg/kg	CSPC*, USA
Paint	1 mg/cm^2	USEPA
Paint	600 mg/kg	South Africa
Toys (total lead)	100 mg/kg	CSPC*, USA
Toys (paint)	90 mg/kg	CSPC*, USA
Soil	400 mg/kg	USEPA
Dust	400 mg/kg	USEPA
Dust	40 µg/ft^2	USEPA
Water	10 µg/dL	EU[#]
Air	0.5 µg/m^3	EU
Air	1.5 µg/m^3	USEPA
Canned fruits and vegetables	0.1 mg/kg	Codex Alimentarius
Fruit juices	0.03 mg/kg	Codex Alimentarius
Infant formula	0.01 mg/kg	Codex Alimentarius

*CSPC Consumer Product Safety Commission [#] EU European Union

1.10.3 Exposure assessment

The *Exposure assessment* process determines the magnitude, frequency and duration of exposure (USEPA, 2005). There are two main approaches in exposure assessment: In the bottom-up approach chemicals are measured in environmental media such as air, water and food. This approach gives information on external exposures and their sources, but gives no information on internal doses. In the top-down approach chemicals are measured in bodily fluids or other specimens. This approach, often referred to as biomonitoring, gives information on the actual concentrations of a chemical in specified fluids, tissues or specimens from an organism. Although this approach gives some information about the internal dose, it, however, does not provide information about sources of exposure (Rappaport, 2011). Both approaches (i.e. measurement of lead in environmental media as well as biomonitoring) are used in the exposure and risk assessment of lead. The former approach is used to give an indication on the level of contamination of particular media (food, water, air, soil etc), which can in turn be converted to potential doses of lead. The latter is used in occupational settings or epidemiological studies to give an indication of the level of exposure to lead among the participants involved. In order to obtain a complete indication of the levels of exposure and risks, measurement of lead in food, air, water, and soil should be coupled with measurements of lead in the body.

1.10.3.1 Assessment of exposure to lead by assessing internal doses through top-down approaches

Most studies on exposure to lead appear to be based on biomonitoring. Biomonitoring is based on the use of biological markers or biomarkers which are defined as ‘systems that specifically measure interactions between biological systems and chemical, physical, or

biological agents' (Sanders et al., 2009). There are three types of biomarkers in toxicology termed as biomarkers of exposure, biomarkers of (toxic) effect and biomarkers of susceptibility (Timbrell, 1998). Biomarkers of exposure, biomarkers of effect and biomarkers of susceptibility are all used in the biomonitoring of lead, to different extents.

1.10.3.1.1 Biomarkers of exposure

A biomarker of exposure is defined as 'an exogenous substance or its metabolite or the product of an interaction between an exogenous agent and some target molecule or cell that is measured in a compartment within an organism to confirm and assess exposure' (Mross et al., 2007). An exogenous substance is a 'substance that enters the body from exogenous sources such as air, water, diet, drugs, and radiation, as compared to an endogenous substance that is produced from processes in the human body such as inflammation and lipid peroxidation' (Rappaport, 2011).

Biomarkers of exposure are further subdivided into biomarkers of internal dose and biomarkers of effective dose. Biomarkers of internal dose give an indication that exposure to a particular substance has taken place by measuring the concentration of the substance or its metabolite(s) in a body fluid or specimen. Biomarkers of effective dose give an indication that exposure to a substance has resulted in the substance reaching the target organ or cell (Timbrell, 1998). Biomarkers of exposure to Pb give information on current lead body burden in an individual. Since the body burden of lead is a function of recent and/or past exposure, there is need for selection of the most appropriate biomarkers that

suit the intended objective (Barbosa et al., 2005). Biological samples that are used to assess biomarkers of exposure to lead include blood, urine, hair, saliva, bone and teeth.

1.10.3.1.1.1 Blood

BPb reflects past exposure from the past few last months. It is an indication of relatively recent exposure in young children that were not excessively or chronically exposed in the earlier part of their lives. The amount of BPb in heavily exposed children and adults is an integration of BPb of concentration recent and older exposures (NRC, 1993). Therefore, BPb measurement is also used as a measure of chronic lead exposure. Lead can be measured in whole blood, serum or plasma, although lead in whole blood is the most widely used biomarker of exposure to lead (Bergdahl et al., 1997c).

Blood for lead measurements can be drawn from the vein or capillary. However, most capillary blood specimens have been shown to have falsely elevated lead levels that can be traced to contamination (Delves, 1996). For this reason, the CDC recommends that 'capillary BPb measurements may be used for initial screening purposes, whereas venous BPb is appropriate for diagnostic evaluation and for initiating an environmental investigation or chelation therapy' (Parsons and Chisolm, 1997).

Analysis of BPb concentrations involves chemical modification (by ammonium phosphate, or dibasic form of ammonium phosphate), electrothermal excitation (in a graphite furnace),

and measurement using atomic absorption spectroscopy (GFAAS) (Shuttler and Delves, 1986, Parsons and Chisolm, 1997).

1.10.3.1.1.2 Urine

Since some of the absorbed lead is predominantly excreted in urine, urinary lead (UPb) may be used to indicate exposure. The relationship between UPb and exposure is curvilinear upward at high doses (NRC, 1993), making it difficult to directly relate UPb and the internal dose of lead. Furthermore, UPb does not depend only on the body burden but also the kidney function. For this reason the amounts of lead in urine are very variable, requiring creatinine correction (Barbosa et al., 2005). Despite these shortcomings, measurement of UPb is a favoured non-invasive approach in long-term biomonitoring occupational studies. UPb is usually determined by GFAAS or anodic stripping voltammetry (ASV) after modification with nitric acid (2% volume/volume (v/v)) and ammonium dihydrogen phosphate ($NH_4H_2PO_4$) (Hodges and Skelding, 1981, Parsons and Chisolm, 1997).

1.10.3.1.1.3 Skeletal system (bones and teeth)

Since lead accumulates in bones, bones and teeth can be used for biomonitoring of lead. Bone lead gives information on lead accumulation and retrospective exposure over a fairly long period (NRC, 1993). Non-invasive *in vivo* bone-Pb measurements using the X-ray fluorescence (XRF) methods are becoming increasingly common (Barbosa et al., 2005).

Teeth also accumulate lead over the long term. Therefore, shed teeth can be used to measure chronic exposure to lead. Biomonitoring for lead using teeth has many advantages including easy collection (after exfoliation) and stability for preservation (Barbosa et al., 2005). However, the disadvantage is that interpretation of the data depends on the type and part of tooth (IPCS, 1995). Lead in teeth can be analyzed using atomic absorption spectrometer (AAS), XRF and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) (USEPA, 2006)

1.10.3.1.1.4 Hair

Hair is an attractive biomonitoring specimen because it can be easily and noninvasively collected at a minimal cost. Furthermore, it can be easily stored and transported to the laboratory for analysis (Barbosa et al., 2005). However, hair lead measurement is less sensitive and less accurate than BPb measurement (Esteban and Rubin, 1999). In addition, the lead that is incorporated into the hair matrix cannot be distinguished from the lead that is originating from external sources. The dose response for hair lead is also not well characterized (Barbosa et al., 2005). Lead in hair can be measured using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) after digestion in nitric acid (HNO_3) –hydrogen peroxide (H_2O_2) (Rao et al., 2002) or Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) (Rodushkin and Axelsson, 2000).

1.10.3.1.1.5 Finger nails

Similar to hair, finger nails can be easily and non-invasively collected and can be easily stored and transported to the laboratory for analysis. Concentration of nail lead reflects

long-term exposure since nails are isolated from metabolic activities in the body. Toe nails are preferred to finger nails because they are said to be "less affected by environmental contamination than fingernails" (Barbosa et al., 2005).

Use of fingers or toe nails lacks reproducibility as there is high variability in lead levels measured in the same fingernails or toenails of various subjects (Gulson, 1996). This lack of reproducibility is a serious limitation for using nail lead as a biomarker for lead. Lead in nails can be measured using ICP-MS (Rodushkin and Axelsson, 2000).

1.10.3.1.1.6 Saliva

Saliva has also been proposed as a biological specimen that can be used to assess levels of exposure to lead. However, its utility is limited by uncontrolled variation in salivary flow rates, lack of standard or certified reference materials, and absence of reliable reference values for human populations, and the very low levels of Pb present in saliva (Barbosa et al., 2005). Whereas a clear relationship could be shown between saliva lead and environmental contamination (de Almeida et al., 2009), only a weak correlation could be shown between lead in saliva and lead in blood (Barbosa Jr et al., 2006, Costa de Almeida et al., 2010). Lead has been successfully determined in human saliva using combined cloud point extraction–capillary zone electrophoresis with indirect ultra violet (UV) detection (Luconi et al., 2006) and ICP-MS (Costa de Almeida et al., 2010).

1.10.3.1.2 Biomarkers of effect

A biomarker of effect is defined as 'a measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease' (Mross et al., 2007). Biomarkers of effect reflect actual biologic responses of the body. The critical effects first appear when the concentration reaches a critical level in critical organs or tissues (Sakai, 2000). In lead exposure, critical organs include the central and peripheral nervous system, bone marrow, kidney and the digestive system. Critical concentrations of lead in the bone marrow can be observed by assessing perturbations in the heme biosynthetic pathway and nucleotide metabolisms as per the discussion below.

1.10.3.1.2.1 ALAD

As it was presented in Figure 2, lead directly inhibits the activity of the cytoplasmic enzyme ALAD. There is a negative exponential relationship between ALAD and BPb over the range of 3–34 μg/dL (ATSDR, 2007). Therefore, the ALAD concentration (as a biomarker of effect) in blood can be used to indicate toxic effects of lead and consequently indicate exposure (Sakai, 2000).

1.10.3.1.2.2 Extractable protoporphyrin (EP) and Zinc protoporphyrin (ZPP)

In the final step of the hematopoietic cycle, as shown in Figure 2, the enzyme ferrochelatase introduces iron into the protoporphyrin (PP) molecule to form heam. Lead inhibits the activity of ferrochelatase and therefore prevents incorporation of iron into haemoglobin (NRC, 1993, Barbosa et al., 2005). This results in the increase of the

concentration of erythrocyte protoporphyrin (EP) in blood. This reaction also leads to the binding of zinc by protoporphyrin, producing zinc protoporphyrin (ZPP) (Onalaja and Claudio, 2000). Therefore, the accumulation of PP or ZPP is a measure of the disturbance of the hematopoietic cycle and an indication of the effect of lead in the bone marrow (Barbosa et al., 2005). EP can be measured as free EP (FEP) or ZPP using absorption spectrophotometry or fluorometry (Lamola and Yamane, 1975, Sakai, 2000).

1.10.3.1.2.3 ALA

As lead inhibits ALAD activity (Figure 2), ALA accumulates in blood and urine. Therefore, ALA in blood, plasma and urine has been used as biomarkers of effects of lead. Methods used in the determination of ALA include ion exchange column chromatography, high-performance liquid chromatography (HPLC) with fluorescence detection and other solvent extraction procedures (Sakai, 2000).

1.10.3.1.3 Biomarkers of susceptibility

A biomarker of susceptibility is defined as 'an indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance' (Mross et al., 2007). These are often genetic factors that modify effects in exposed individuals.

1.10.3.1.3.1 ALAD gene

Over 99% of BPb accumulates in erythrocytes and more than 80% of this is bound to ALAD. The gene that encodes ALAD exists in two polymorphic forms (alleles), ALAD-1 and ALAD-2 (Bergdahl et al., 1997b). This polymorphism may have an effect on lead toxicokinetics, and therefore can affect the susceptibility of an individual to lead poisoning (Barlow et al., 2001). Individuals that have the ALAD-2 allele have been shown to have higher BPb levels than those that have the ALAD-1 allele (Wetmur et al., 1991, Schwartz et al., 2000). The reason for this is that the presence of ALAD-2 appears to increase the retention of lead in blood (Wetmur et al., 1991, Bergdahl et al., 1997b). In addition, ALAD-2 appears to reduce kidney function, which in turn reduces excretion of lead (Bergdahl et al., 1997a). The impact of ALAD genotyope on BPb was also shown in two strains of mice that differ in their expression of the ALAD gene. DBA/2 mice, which have a duplication of the ALAD-2 gene, were shown to accumulate twice the amounts of lead in blood than C57BL/6 mice (Onalaja and Claudio, 2000). Consequently, the determination of the ALAD genotype is sometimes used to identify individuals that are genetically susceptible to higher BPb levels.

1.10.3.1.3.2 Vitamin D receptor

The vitamin D receptor (VDR), which is responsible for many of the biological actions of vitamin D, exists in many genetic variations which are referred to as Taq I, Fok I, and BsmI. These genes are said to be ‘defined by the restriction fragment length polymorphisms (RFLPs) that result from cutting the DNA with three different restriction enzymes’(Onalaja and Claudio, 2000). For example, the polymorphism defined by the restriction enzyme BsmI results in three genotypes denoted as bb, BB, and Bb, where the

capital letter signifies the absence of the restriction site. Since these genotypes were shown to affect calcium and bone density, they were also suspected to play a role in lead bioaccumulation. Indeed, humans with the BB genotype for the BsmI polymorphism or with the V genotype for the FokI polymorphism have been shown to have lower BPb than those in the other genotype groups (Schwartz et al., 2000, Rezende et al., 2008). Therefore, VDR polymorphism can be used as a biomarker of susceptibility.

1.10.3.2 Exposure assessment using bottom-up approach by assessing lead in food, water, toys, house dust and soil

Exposure assessment can also be conducted through measurement of the concentration of a chemical in the relevant media followed by the estimation of intake. The intake, which is related to the concept of dose, which has been defined in section *1.10.2*, is also of much importance in exposure assessment studies. The daily intake is calculated from ingestion or consumption data and the concentration of chemicals in the media (food, soil, water, air, etc) as follows:

$$D = C \; x \; IR \qquad \textit{Equation 2}$$

where *D* is the intake (dose) from ingestion of food (mg/kg-day), *C* is the chemical concentration in food (mg/kg), *IR* is the food ingestion rate (mg/day) (Liu, 1994). Therefore, assessment of exposure to a chemical through dust and soil involves sampling of dust or the soil, laboratory analysis of the dust and estimation of intake. Calculations of intake from soil and house dust make use of soil ingestion rates from international studies conducted on the amounts of dust or soil that children ingest in a day. Children are

reported to ingest 50–200 mg soil/day (Calabrese et al., 1989, Calabrese et al., 1997), and the USEPA recommends 100 mg/day as the average soil ingestion rate and 400 mg/day as the upper 95^{th} percentile (USEPA, 2002).

Since toys are not ingested, exposure assessment of chemicals from toys would ideally be conducted by sampling the toys, analysis of the concentration of the chemical in the toy and an estimation of the rate at which the chemical would be released from the toy when a child puts the toy in the mouth. This is a challenging exercise that involves studies of speciation of the chemical in the toy matrix, leaching rates of the chemical from the matrix, use patterns of the toys and mouthing behavior ((Bosgra et al., 2005). When information on leaching rates, use patterns of the toys and mouthing behavior is available, the computer Consumer Exposure (ConsExpo) model can be used to estimate the exposure of a chemical from a toy (Bremmer and Veen, 2002). However, since these are usually not available, most studies are only limited to the analysis of the concentration of lead in the toy and assessing their safety by ascertaining if the concentration of the lead exceed permissible levels indicated in standards (Greenway and Gerstenberger, 2010, Kumar and Pastore, 2007, Omolaoye et al., 2010).

Dietary exposure assessment involves determination of the concentration of the chemical in individual foods and the determination of intake using food consumption data (Kroes et al., 2002). In order to determine the concentration of the chemical in the food, food samples are usually collected from local markets (Rubio et al., 2005, Schuhmacher et al., 1991) or from portions of food taken from foods eaten by participants (Muñoz et al., 2005,

Schrey et al., 2000). The food samples from the market can be analyzed as collected or they can be used to prepare foods using the most common recipes (Lee et al., 2006).

Determination of intake or dose requires food consumption data (or food ingestion rates) presented as food consumed per capita per day. Food consumption data (for exposure assessments) are usually obtained through retrospective methods (such as twenty-four-hour recall surveys, food frequency questionnaires (FFQs), and diet history surveys) and prospective methods such as duplicate diet (portion) studies and food record surveys (FAO/WHO, 2005). Among the retrospective methods, the most widely used are the 24-hour recall and FFQ.

In the 24- or 48-hour recall method participants are asked to describe the types and amounts of all foods and beverages that were consumed in the past 24 or 48 hours, using household measures, food models, or photographs (Kroes et al., 2002). However, one disadvantage of using this method is that it does not deal very well with the considerable variations in individual's food intake between days (Thompson and Byers, 1994). Despite of these challenges, the 24- (48-) hour recall is often used for dietary exposure assessment of many chemicals.

The FFQ consists of a structured list of foods and a frequency of its consumption by respondents (Żukowska and Biziuk, 2008). This method assesses food intake over more extended periods of time such as 5 days or 7 days. Portion size aids that are in the form of

common household measures such as cups and teaspoons are commonly used to help respondents estimate portion size (Thompson and Byers, 1994). However, food intake assessments using FFQs are prone to errors arising from the reliance on memory, or on estimation of frequency and portion size (Beerman and Dittus, 1993). Nevertheless, the FFQ has been used for dietary exposure assessment of lead and other chemicals (Ihedioha and Okoye, 2013, Kwon et al., 2012).

Whereas the 24 hour recall and FFQ involve collection of information retrospectively, prospective methods such as food record surveys and duplicate portion studies involve provision of dietary information prospectively. For example, duplicate portion studies require the preparation of an exact sample of food consumed by an individual for a period of 3 to 7 days. This approach provides not only information on the exact types and amounts of food consumed by an individual, but also an exact sample for laboratory analysis (WHO, 1985). Although prospective dietary assessment methods are not prone to errors that arise from the reliance on memory, or on estimation of frequency and estimation of amounts, they are expensive and challenging to conduct. In addition, they suffer from bias as participants change dietary habits when they recognize that they are under observation. Despite these challenges, duplicate portion studies are often used for dietary exposure assessment of lead (Lacey et al., 1985, Stanek et al., 1998, Wilheim et al., 2003).

1.10.3.3 Conversion of measurements from the bottom-up approach to BPb

Health risk assessment of lead requires conversion of the information on lead intake (from food, water, soil etc) into BPb (for the reasons that are explained later in *Section 1.10.3.4***)**.

This conversion is achieved through the use of biokinetic models, an example of which is the IEUBK model. The IEUBK model attempts to predict BPb concentrations for children between 1 and 6 years of age exposed to lead in their environment. The model allows the user to input relevant absorption parameters (e.g., the fraction of lead absorbed from food) as well as intake and exposure rates. A schematic representation of the model is shown in Figure 6 below.

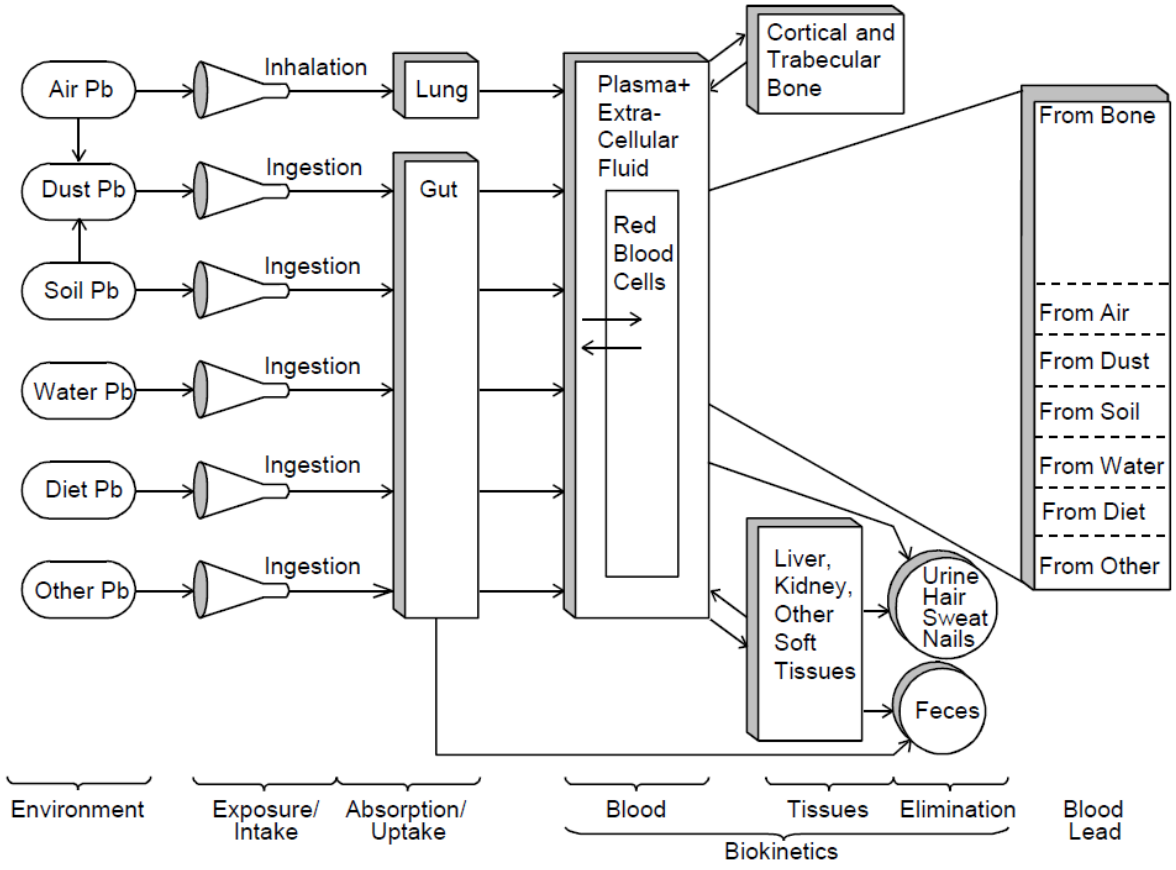

Figure 6: Schematic representation of the IEUBK model (USEPA, 1994a)

Conversion of administered dose (or intake) to BPb also requires use of bioavailability. This is important because not all of the lead that is ingested or inhaled is absorbed. Bioavailability is defined as the 'proportion of lead considered to be extracted in the gastrointestinal tract or lungs compared with the total lead that has been ingested or

inhaled' (Gulson and Davis, 1994). Lead can exist in various forms including lead from cars (PbClBr and $PbSO_4$), lead from minerals(PbS and $PbCO_3$), and lead from paint ($PbCO_3 \cdot Pb(OH)_2$ and $PbCrO_4$) (Chaney et al., 1989). Different forms of lead appear to have different bioavailabilities (Freeman et al., 1992). Different bioavailabilities are reported in the literature for dietary lead: a 10% or lower (Rabinowitz et al., 1980, Heard et al., 1983), around 20% (Kostial and Kello, 1979), around 31% (Hallén and Oskarsson, 1995), around 40% (Ziegler et al., 1978) and about 70% (Kostial et al., 1971). The IEUBK model uses a very conservative default figure of 50% for lead bioavailability in food (USEPA, 1994a). Ideally, the bioavailabilities of lead from soil or food should be determined empirically for each site.

The applications of the IEUBK model can be summarized as follows:

- Determination of predicted values of BPb resulting from exposures to different sources (Wang et al., 1997, Khoury and Diamond, 2003, Lynch et al., 2000);
- Estimation of the contribution of a single source of lead (e.g. tap water) to BPb (Li et al., 2016, Sathyanarayana et al., 2006, Wang et al., 2011) and subsequent determination of exposure routes (Cornelis et al., 2006); this is often achieved by entering information on a single source only into the model;
- Estimation of the concentration of lead in soil that would result in a predetermined percentage of children (such as 10%) not having BPb 5 µg/dL(Cornelis et al., 2006, von Lindern et al., 2003, Rasmuson et al., 2012); this estimation is often performed for remediation of contaminated sites.

In addition to the IEUBK model, there are also other models that are used for simulating BPb that can be used in the exposure assessment to lead from different sources. For example, the Carlisle and Wade model is used by the California EPA to predict BPb from food, drinking water, soil and dust. The model is more suitable for adults than for children (LaKind, 1998). The California Department of Toxic Substances Control LeadSpread model is used to estimate BPb concentrations that may result from exposure to lead via inhalation, ingestion or dermal contact with contaminated media (CDTSC, 2007). The All-Ages Lead model (AALM) simulates lead concentration in body tissues and organs from a lifetime of exposure to lead (USEPA, 2012). The O'Flaherty physiologically-based toxicokinetic (PBTK) model can be used to estimate the distribution of lead in the body especially in blood and bones (Oflaherty, 1993).

1.10.4 Risk characterization of lead

In the traditional risk assessment paradigm, assessment of intake or dose is followed by risk characterization, a step that integrates information from the hazard identification (*Section 1.10.1*), dose-response assessment (*Section 1.10.2*) and exposure assessment (USEPA, 1995a). For most chemicals, risk characterization for non-cancer effects is achieved by calculating the Hazard Quotient (HQ) as follows:

$HQ = DI/RfD$ *Equation 3*

where: $HQ = Hazard\ Quotient$

DI = Daily Intake (mg/kg-day)

RfD = Reference Dose (mg/kg-day)

There is no risk if the HQ for a chemical is equal to or less than one. If the HQ exceeds 1, there is some possibility that some effects may occur (EPA, 2011, Williams and Paustenbach, 2002). Risk can also be characterized using the margin of exposure (MOE) or margin of safety (MOS) where

$$MOE = NOAEL/dose \qquad \textit{Equation 4}$$

MOEs in the range of 100–1000 are considered safe (USEPA, 2000).

These approaches may not be applicable to lead for the fact that lead has no R*f*D, ADI or PTWI. Many dose-response relationships have however been identified for many adverse effects of lead that could be correlated to the levels of BPb as indicated in Table 2. Using these thresholds or cut-off points, the predicted (or measured) BPb are correlated with potential health effects. In other words, 'BPb concentrations are the metric used to integrate exposure estimates and predict the likelihood of health hazards associated with lead exposure' (Mahaffey, 1998).

Table 2: BPb thresholds for health effects of lead (WHO, 2003a)

Outcome	BPb threshold
Reduction in IQ	5 µg/dL
Increased systolic blood pressure	ND
Gastro-intestinal effects	60
Anaemia	70

ND No documented effects or insufficient evidence

1.10.4.1 Estimation of the burden of disease of mild mental retardation (MMR) attributable to lead exposure to children

An estimation of the burden of disease is a quantification of the burden of premature mortality and disability for major diseases or disease groups (Mathers and Woodward, 2003). The burden of disease is an 'indicator that helps in identifying disadvantaged groups and targeting of health interventions, and setting of priorities in health service and research' (Murray, 1994). The burden of disease can be assessed at a global level, national level or sub- national level such as a city or district (Prüss-Üstün et al., 2003a). In the case of lead, the burden of disease from Pb exposure converts BPb distribution to actual impacts of the lead exposure in figures with which policy makers are conversant. It highlights the magnitude of disease burden that could be avoided so that information on the disease burden can enable policy makers to correctly direct interventions on the specific risk factors.

Burden of disease estimates are usually reported as measures of population health, such as the disability-adjusted life year (DALY) (Murray and Lopez, 1996). The DALY is the sum of years of life lost due to death and years of life with disability, where each disease condition is attributed a defined severity weight. The DALY therefore measures the health of a population by combining data on mortality and morbidity outcomes into a single number (Prüss-Üstün et al., 2003b). It attempts to combine disease occurrence and severity and it attempts to quantify the impacts of a disease on the health, psycho-social and economic well being of individuals and populations in one measurement unit. According to the WHO 'one DALY can be thought of as one year of "healthy" life lost whereas the sum of DALYs across the population can be thought of as a measurement of

the gap between current health status and an ideal health situation' (WHO, 2015). Calculation of DALYs for various diseases can enable comparison of the impacts of various diseases, which is important for prioritization of resources.

The WHO has produced general guidelines on methods for estimating burden of disease (Mathers and Woodward, 2003) and also specifically a guideline for estimating the burden of disease attributable to exposure to lead (WHO, 2003a). The guideline has been used to estimate the burden of disease in various contexts. A global estimate of the burden of disease from exposure to lead was made in 2004 (Fewtrell et al., 2004) and a number of estimates for burden of disease attributable to lead have also been performed for various countries and regions (Jarosińska et al., 2006, Landrigan et al., 2002, Norman et al., 2007). The guideline is under revision by the WHO in order to incorporate recent findings on the effects of lead (WHO, 2003b). This guideline is however been used in the present study to obtain conservative estimates only.

The main toxicity end points for lead in children include MMR (at BPb $\geq$ 5 µg/dL), gastro-intestinal problems (60 µg/dL) and anaemia (at BPb $\geq$ 70 µg/dL) (WHO, 2003a). Most studies on Pb focus on IQ losses resulting in MMR. In this regard, IQ loss is not considered to be a disease by itself. Instead IQ losses are converted into cases of MMR where MMR is defined as having an IQ score of 50–69 (Fewtrell et al., 2004). IQ in human populations has a normal distribution with a mean of 100 and a standard deviation of 15 IQ points (Lezak, 2004). Children with an IQ scores just above 69 are at the greatest risk of lead-induced MMR. Therefore, as portrayed in Figure 7 below, it is important to calculate

the number of children with IQ just above the threshold of IQ score 70, whose IQ would shift to the MMR range through IQ reduction due to lead exposure.

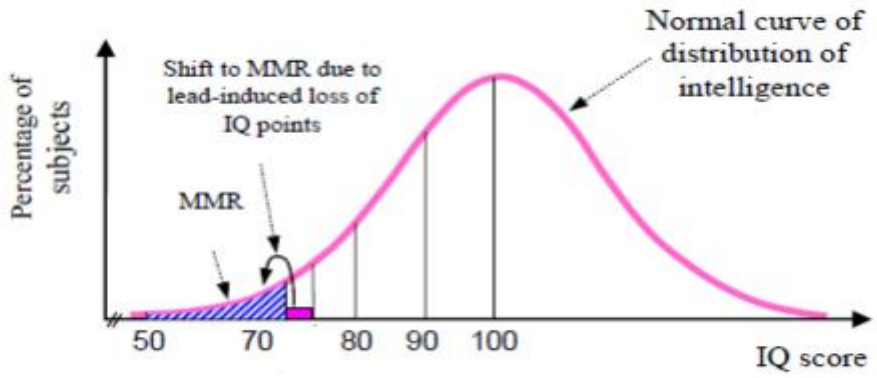

Figure 7: Shift to MMR as a result of lead-induced IQ loss (WHO, 2003a)

In the dose-response relationship for lead, loss in IQ points vary according to BPb levels: The average loss of IQ points for the 5–10 µg/dl interval is taken as 0.65, that for <10 to 15 as 1.95 IQ points, that for the < 15 to 20 as 3.25 IQ points and that for blood lead < 20 is taken as 3.5 IQ points. This information is incorporated into the *WHO lead burden of disease spreadsheets* that are used to calculate the burden of disease. The prevalence of MMR is subsequently estimated by multiplying the number of children within the IQ points loss category with the respective percentage of the population within that concentration range.

1.10.5 Summary of approaches in the exposure assessment of lead

The advantages and disadvantages of the approaches used in the exposure assessment of lead have been summarised in Table 3 below. The table indicates that utilization of top-down approaches (biomonitoring) alone does not provide much information on the sources of exposure. Utilization of bottom-up approaches (measurement in environmental media) on the other hand provides information of the sources without indicating the internal dose among participants. Therefore, in the present study both top-down approaches and bottom-up approaches were utilised to obtain a complete understanding of internal exposure levels and the sources of exposure to lead. Furthermore, biomonitoring using blood has been utilised in this study because it is the most widely used specimen in the risk assessment of lead, and also because it reflects past exposure from the past few months. As a widely used specimen in the risk assessment of lead, BPb has well-characterized dose-response relationships and is more easily comparable to other studies. Also, since BPb reflects past exposure from the past few months, BPb offers a wider picture of the levels of exposure to lead among children in a cross-sectional study that can only give a snap-shot of the exposure levels.

Table 3 also shows the need for conversion of levels of lead in different environmental media to BPb. This is necessary for risk characterization lead as lead has no RfD. In the present study the conversion of environmental lead to BPb was achieved using the IEUBK model because it is the most widely used in literature, it has been validated and has been shown to give accurate results, and because it is freely available on the internet.

Table 3: Summary of approaches in the exposure assessment of lead

Method	Advantages	Disadvantages
Biomonitoring using blood	Reflects past exposure from the past few months. Easy to collect and determine	Does not indicate the sources
Biomonitoring using urine	Non-invasive and painless collection	Does not indicate the sources UPb also depends on kidney function
Biomonitoring using the skeletal system	Indicates accumulated lead. Non-invasive collection and measurements	Does not indicate the sources
Biomonitoring using hair	Easily and noninvasively collected	Does not indicate the sources Not very sensitive and accurate
Biomonitoring using finger nails	Easily and noninvasively collected	Does not indicate the sources lacks reproducibility
Biomonitoring using saliva	Easily and noninvasively collected	Does not indicate the sources Uncontrolled variation in salivary flow rates, lack of standard or certified reference materials and reference values
Biomonitoring using ALAD	Biomarker of effect indicates level of damage	Does not indicate the sources Lack of reference values
Biomonitoring using EP and ZPP	Biomarker of effect indicating level of disturbance of the hematopoietic cycle	Does not indicate the sources Lack of reference values
Biomonitoring using ALA	Biomarker of effect indicating level inhibition of ALAD activity	Does not indicate the sources Lack of reference values
Biomonitoring using ALAD gene	May be used to identify genetically susceptible individuals	Does not indicate the sources
Biomonitoring using VDR	May be used to identify genetically susceptible individuals	Does not indicate the sources
Environmental measurement of lead in media (food, air, water, soil)	Indicate level of contamination in the sources	Does not indicate internal exposure
24 hour recall	Easy for participants to remember food consumed in the past 24 hours Cost-effective	Has been shown to have large intra-individual and inr-individual variability May not show foods consumed on weekends or during the week Depends on recall Susceptible to bias
FFQ	Captures foods	Very difficult for participants to

Method	Advantages	Disadvantages
	consumed over many days Cost-effective	remember foods consumed in the past few days Depends on recall Susceptible to bias
Food diary	Captures foods consumed prospectively Does not depend on recall	Susceptible to bias Time consuming Requires more resources
Conversion of environmental measurements in media to BPb	Indicates internal exposure that can permit risk characterization	It is data intensive
IEUBK model	Validated Specifically for children Widely used Freely available Available in software format	Data intensive
Carlisle and Wade model		Not available in software format Validation status not clear Primarily for adults Not freely available on the internet Not widely used Data intensive
The All-Ages Lead model (AALM)	For all ages Freely available Available in software format	Not widely used Validation status not clear Data intensive
The O'Flaherty PBTK	For all ages	Not available in software format Validation status not clear Not widely used Data intensive
WHO burden of disease spreadsheet	Estimates burden of disease of mild mental retardation (MMR) attributable to lead	It is still under revision

2.0 Methodology

This chapter aims to describe the methods and approaches used to recruit participants, collect samples and specimens, collect food consumption data, collect information on potential risk factors, analyze for lead in the samples and specimens, statistically analyze the data, predict blood lead from food, water, house dust and soil, assess the applicability of the model to Malawi, correlate predicted and measured blood lead levels to potential health effects, assess the burden of disease and identify risk factors.

2.1 Study design

This study is a descriptive cross-sectional epidemiological study conducted within the paradigm of toxicological (health and environmental) risk assessment of chemicals. However, it should be noted that the risk assessment for lead follows a slightly different approach from the traditional risk assessment approach because of the lack of R*f*D, ADI, TDI or PTWI for lead. The risk assessment of lead that has been followed in the present study involved the following steps:

1. Measurement of the levels of BPb of the participants.
2. Measurement of the levels of lead present in different sources.
3. Calculation of the predicted BPb values resulting from exposure to lead from the identified sources.
4. Evaluation of the applicability of the IEUBK model by assessing the agreement between the measured to predicted BPb values.
5. The identification of the sources of exposure to lead, once the model has been evaluated.
6. The identification of potential risk factors using the measured BPb levels.

2.2 Study setting and study population

Blantyre City is the commercial capital city of Malawi, which is situated in Southern Africa (Figure 8).

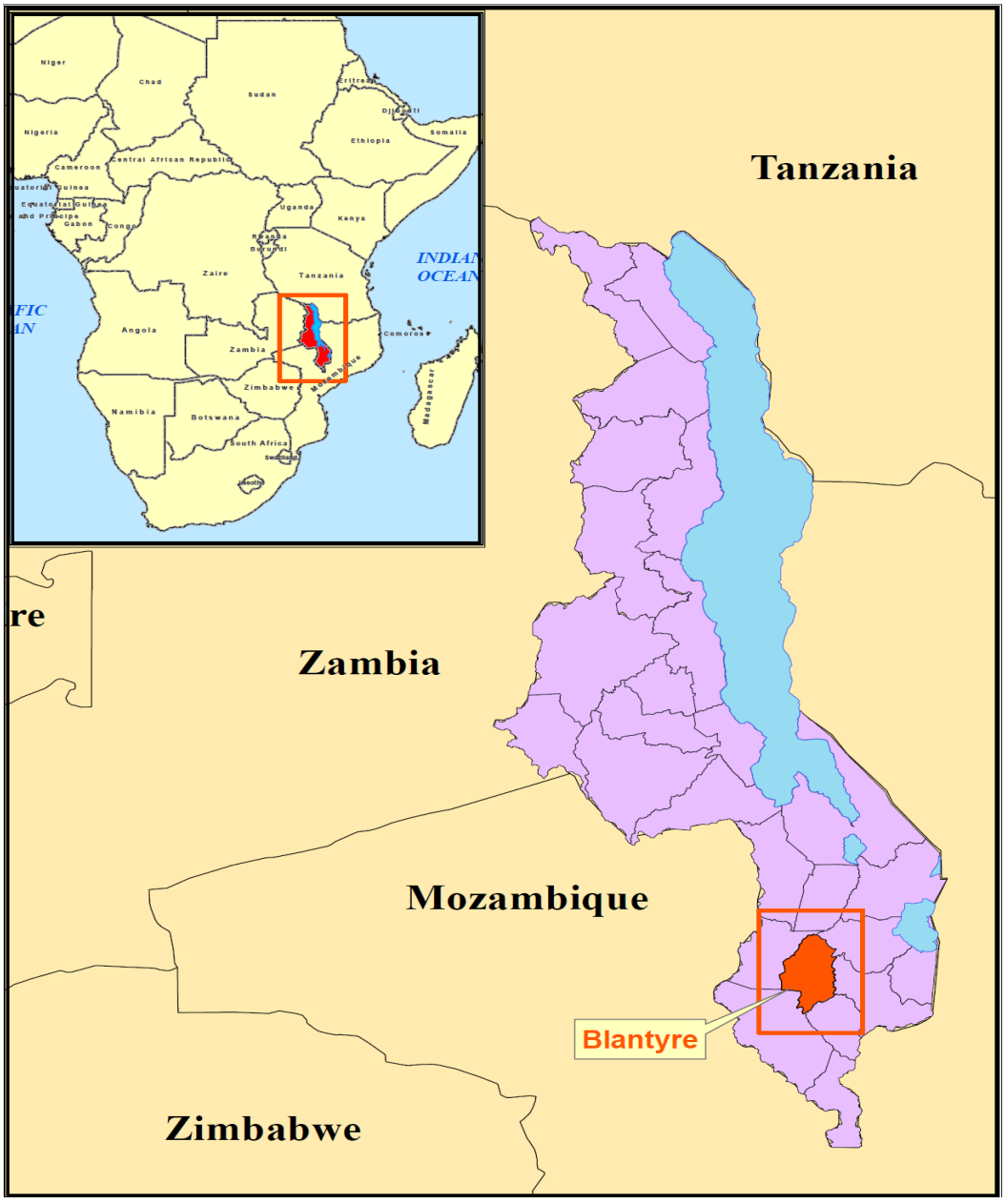

Figure 8: Map showing the location of Malawi in Africa and the location of Blantyre in Malawi (Courtesy of LACOSUS)

The study population included children between 1-6 years of age, who had lived at the residence for at least six months prior to enrollment. Children that were still under breast feeding were excluded. According to the 2008 census Blantyre has around 81,717 children

in the 1-4 year bracket and 83,747 children in the 5-9 year bracket (NSO, 2008). The population of the age group 1-6 years can therefore be estimated to be over 100,000.

Blantyre is divided into 6 health catchment areas which are served by six main health centres. These areas are Machinjiri, Chilomoni, Ndirande, Limbe, Zingwangwa and Bangwe (Figure 9). Blantyre has many residential, commercial and industrial areas, with some of the residential areas in close proximity to industrial areas. However, there are no known industrial or mining activities (such as lead mining and smelting, thermal power stations, incinerators or waste recycling) in Blantyre that can result in occupational and environmental exposure to lead. Furthermore, in spite of other possible industrial sources of lead, the introduction of regulations on lead in gasoline in many countries has been shown to reduce the concentrations of lead in air (Thomas et al., 1999, Li et al., 2016). For this reason air lead concentrations in the present study were not determined and the IEUBK default value of 0.1 $\mu g/m^3$ was utilized.

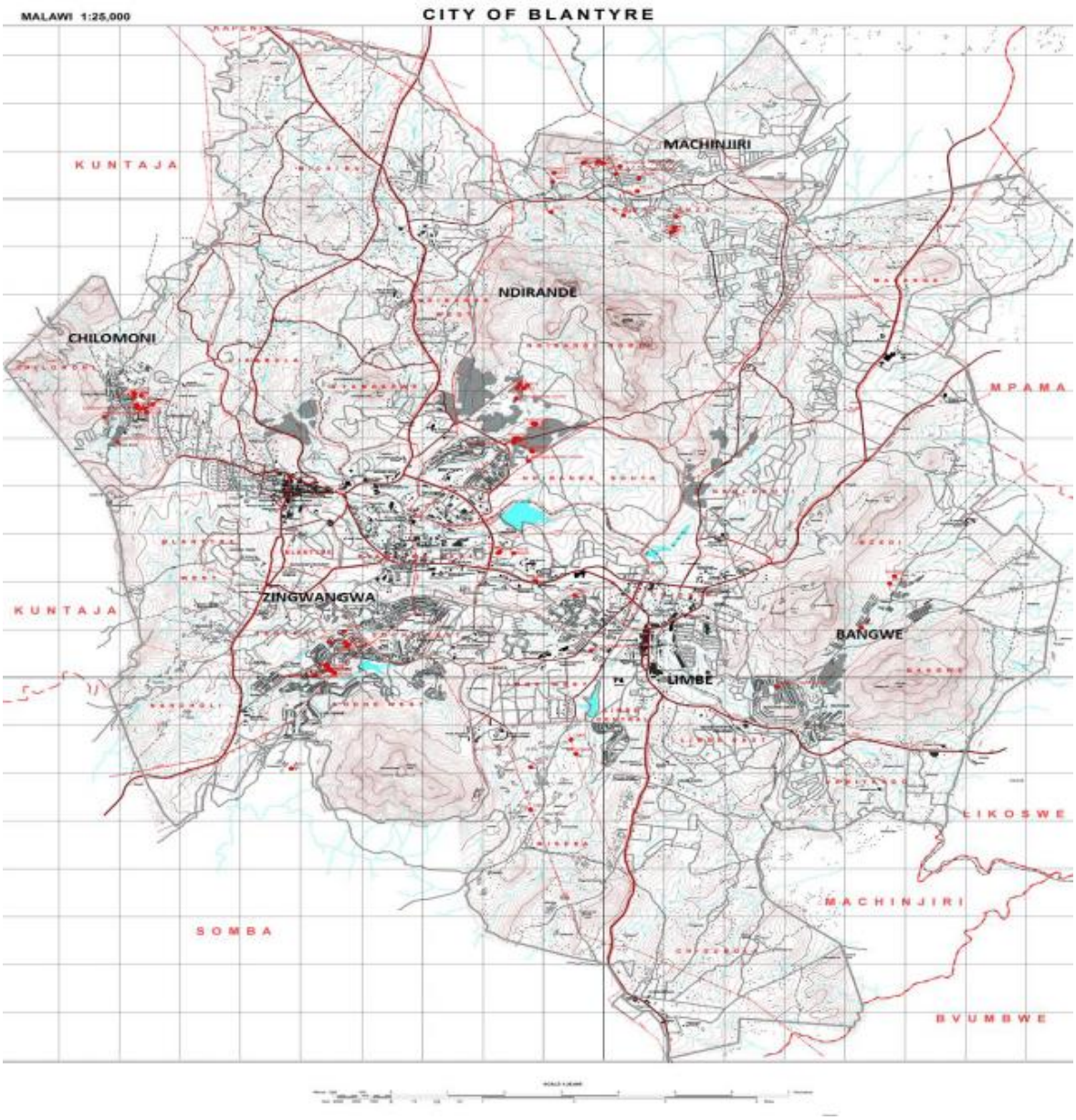

Figure 9: Map of Blantyre showing the 6 health catchment areas (Courtesy of Land Consultancy and Surveying Services (LACOSUS)). The red dots are the locations where the participants resided.

2.3 Recruitment of children

Ethical approval was sought from the University of the Witwatersrand Committee for Research on Human Subjects (*No M120662*) (Appendix 3) as well as from Malawi College of Medicine Research Ethics Committee (COMREC) (*No. P.09/12/1282*) (Appendix 3).

The sample size was calculated based on lead exposure from Botswana (Mbongwe et al, 2005) because it is a country in the region with similar socio-economic characteristics. Consequently, with an expected average BPb of 8.8 µg/dl, the sample size was calculated as follows (Daniel, 1995, Naing et al., 2006):

$$n = \frac{Z_\alpha \sigma^2}{d^2} \qquad \textit{Equation 5}$$

where

n = number of children

Z_α = standard normal deviate corresponding to a 2 sided level of significance of 5% = 1.96

σ = standard deviation of lead level from previous study = 5.6 µg/dl

d = level of precision = 1 µg/dl

n = 120

Therefore, the minimum sample size was 120. After adjusting for non-response rate of 50%, the targeted minimum number of children in the study was 240, which comprised of 40 children from each of the 6 health catchment areas of Blantyre. Therefore, after introducing the project to community leaders, 40 names of eligible children were randomly chosen from the community nurses' and health surveillance assistants' (HSAs) register in each health catchment area. Study information sheets written in both English and the vernacular Chichewa were given to the children's parents or guardians. The study information sheets were read by the researchers to parents or guardians that could not read. Only individuals that consented to take part in the study were enrolled. In addition,

children between 4 and 6 years were asked to assent to take part in the study. Randomly allocated numbers were used to maintain confidentiality.

The refusal rate was very high with the result that extra names were drawn from the registers. There were large differences in the willingness to participate in the study according to health catchment area. In total, a152 children were recruited from 310 participants that were contacted in all the catchment areas. Therefore, the refusal rate was about 51%, with more children participating in some catchment areas than others.

2.4 Sampling and sample collection of blood, paint, food, water, house dust and soil

2.4.1 Blood

One millimetre venous samples of whole blood were drawn into Vacutainer tubes with techniques designed to ensure minimal extraneous lead contamination, as is recommended in the literature (Parsons and Chisolm, 1997, WHO, 2010a). These samples were stored at 4-6 ^{o}C at the College of Medicine laboratory awaiting transportation to Lancet laboratories in South Africa for analysis.

2.4.2 Paint (fresh paint and paint chips)

One sample each of the common colours of paint (red, orange, yellow, green, blue, black, and white) available for each common brand (both imported and locally made) were

purchased from paint hardware and building supply stores that are used by the general public. This approach has also been followed in similar studies (Clark et al., 2006).

The sampling approach for paint chips was similar in many aspects to the approaches recommended by the United Stated Housing and Urban Development (USHUD, 1997, MDCH, 2004). Wherever owners of the house would allow, paint chips were scraped from a small area using a builder's knife, which was then washed and wiped dry after each use to avoid cross contamination. The paint chips were collected into 50 ml sample bottles. Lime (CaO) or white wash was not considered as paint.

2.4.3 Toys

Children's toys were acquired from main markets in Blantyre, i.e. Blantyre market and Limbe, using similar approaches as in the literature (Greenway and Gerstenberger, 2010).

2.4.4 Food and water

Samples of the most commonly consumed foods were acquired from the market in the health catchment area concerned, as recommended in the WHO guidelines (WHO, 1997). Wherever necessary, the food was prepared by a few women from each catchment area using the most common methods of preparation. The foods were then stored at -20 ^{o}C at the College of Medicine cold room.

Water (250 ml) sample was drawn from the homes of the participants at any random time of the day, as is commonly practiced in literature (Haider et al., 2002). The samples of water were then stored at -20 ^{o}C at the College of Medicine cold room.

2.4.5 House dust and soil

Floor dust samples were collected from the children's bedroom wherever possible or in the lounge, using a broom or brush from each particular home. Use of broom or brush for sampling dust is a method that is also recommended in the literature (Lewis et al., 1994, Li et al., 2016). Other methods for sampling house dust that are also found in the literature include wipe sampling methods and use of vacuum cleaners (Farfel et al., 1994, Sterling et al., 1999). In some cases, samples of dust were obtained from the school that the children were attending. The dust samples were not touched with bare hands to avoid contamination, as is recommended by the US Department of Housing and Urban Development (HUD) (USHUD, 1997).

Two samples of surface soil from the children playground at home were collected into a sample container (60 ml bottles) by a scoop. In some cases, samples of soil were also collected from the playground at the school that the children were attending. Once again, the soil samples were not touched with bare hands to avoid contamination,

2.5 Laboratory analysis of lead in different samples

Blood samples were analyzed at Lancet Laboratory in Johannesburg South Africa whereas dust, soil, food, water, paint and toy samples were analysed at Protechnik laboratory (a Division of Armscor SOC Ltd) in Pretoria South Africa, both of which being accredited commercial laboratories that participate in national and international quality control programmes.

2.5.1 Lead in blood

Whole blood samples were diluted ten times by adding 100 µl of each blood sample to 900 µl of diluent (10% Triton X-100). Analysis was performed on a Varian SpectrAA 220Z GFAAS. The instrument was calibrated with calibration standards prepared in sheep blood for matrix matching. Aliquots of each sample were analyzed in duplicate at 283.3 nm. Two certified reference controls, UTAK Metals Control in whole blood (UTAK Laboratories Inc., Valencia, CA, USA) were analyzed with every analytical run in intervals of 10 samples for quality assurance of the measurement. The detection limit (three times standard deviation of all blank samples) for lead in whole blood was 1 µg/dL and the uncertainty of reading was 14.5%.

2.5.2 Lead in paint

Analysis of lead in paint was conducted in line with the American Consumer Product Safety Commission (CSPC) guidelines (CPSC, 2011). Fresh paint was stirred and applied by brush to pre-cleaned petri dishes and left to dry for a minimum of 72 hours and further

dried in the oven at 105 ^{o}C. The paint was then carefully removed from the petri dishes using a clean unused knife.

Fresh paint samples and paint chips from home were analyzed using In-house ICP/MS method based on NIOSH 7300, EPA 2007.7 and 8 and ISO 15202-3.

2.5.3 Lead in toys

Analysis of lead in toys was conducted in line with the CSPC guidelines for analysis of lead in toys (CPSC, 2008). Samples were ashed to break down the plastic material and then digesting it in accordance with EPA SW-846 3050 (digestion with HNO_3 and H_2O_2. The final processed samples were quantitatively analyzed using ICP-MS.

2.5.4 Lead in food and water

A known weight (approximately 2g) of sample was dry-ashed at 420 ^{o}C. Ashed samples were dissolved in 10 ml 0.25% HNO_3. The lead concentrations were then determined by ICP-MS. Water samples were acidified before determination of lead using an ICP-MS method based on NIOSH 7300, EPA 2007.7 & 8 and ISO 15202-3.

2.5.5 Lead in house dust and soil

House dust and soil lead were digested in nitric and perchloric acids. The filtered solutions were analyzed for lead by graphite furnace AAS at a wavelength of 217 nm using an ICP-MS method based on NIOSH 7300, EPA 2007.7 & 8 and ISO 15202-3.

2.6 Food consumption data

Food consumption data was collected using a 7-day FFQ (Appendix I). The questionnaire was adopted from the Birth-to-Twenty cohort study with the types of food consumed taken from the Malawi Second Integrated Household Survey (IHS) household characteristics, income and expenditure questionnaire (NSO, 2004). Food conversion factors were adopted from the South African Medical Research Council (MRC) Food Photo manual (Senekal and Steyn, 2004) and the Malawi Third IHS 2010/11 Data (NSO, 2013). In the cases where the conversion factor for some food standard portions were not available from these sources, the most commonly used unit portions (such as the flat wooden serving spoons locally known as chipande) were measured at least 5 times and used in the conversion factor.

2.7 Collection of data on risk factors

Information on potential risk factors was collected using a lead exposure risk assessment questionnaire attached in Appendix 1, which was adopted from Illinois Department of Public Health (IDPH) Public Health Home Visit Form for Environmental Health and Lead Assessment (IDPH, 2011)

2.8 Data Processing and analysis

Data were entered into Microsoft Excel 2007 spreadsheets, cleaned and then transferred to STATA version 12 statistical package spreadsheets for analyses.

2.9 Predicting blood lead from food, water, house dust and soil

The USEPA recommends that when calculating exposures from ingestion, the unit of weight used to measure intake should be consistent with the unit used in measuring the contaminant concentration in the produce (USEPA, 1997). In the present study, the analysis of Pb in food was based on dry weight. However, the food consumption rates as given by parents were based on wet foods. For this reason it was important to analyze the moisture content of the foods. Wherever the Pb content of a food item was expressed per mass of the food item, moisture content was corrected using the following equation (USEPA, 1997, Jang et al., 2014):

$$IR_{Dry} = IR\ \frac{(100-M)}{100} \qquad \textit{Equation 6}$$

Where IR_{Dry} is the average consumption rate of the food item on a dry basis, IR is the average consumption of the food item as given in the FFQ and M is the moisture content of the food.

The calculated IR_{Dry} could then be used in equation 2 in section 1.10.3.2 to calculate the dietary intake (dose). These dietary intake values were then used in the IEUBK, in the dialogue data window, an example of which is shown in Figure 10 below. The IEUBK

model default bioavailability of 50% and a 31% value from the literature were used in the present study to assess which bioavailability would result in a better agreement between predicted and measured BPb. The default value is the value recommended for the model.

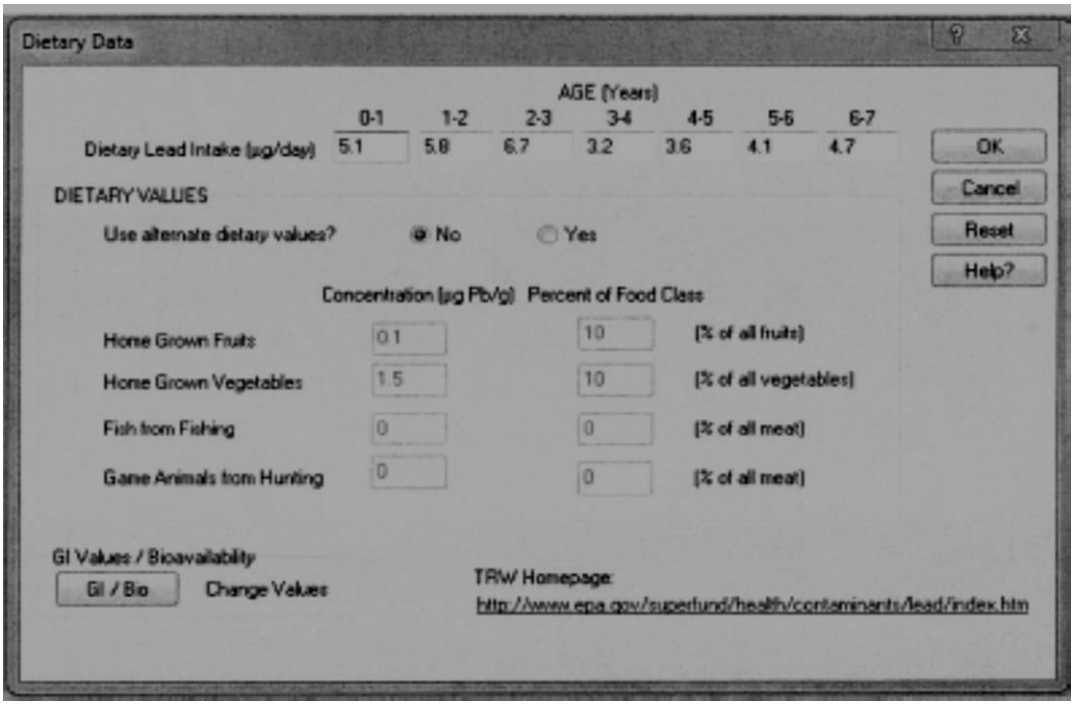

Figure 10: IEUBK window for dietary data

The soil concentrations, the IEUBK model default bioavailability of 30% and the soil ingestion data (Table 3) were utilized in the IEUBK model soil data dialogue window, an example of which is shown in Figure 11 below.

Table 4: IEUBK Soil/Dust Ingestion Defaults by Age (USEPA, 1999)

Age Group (years)	**IEUBK Model Defaults (g/day)**
0-1	0.085
1-2	0.135
2-3	0.135
3-4	0.135
4-5	0.100
5-6	0.090
6-7	0.085

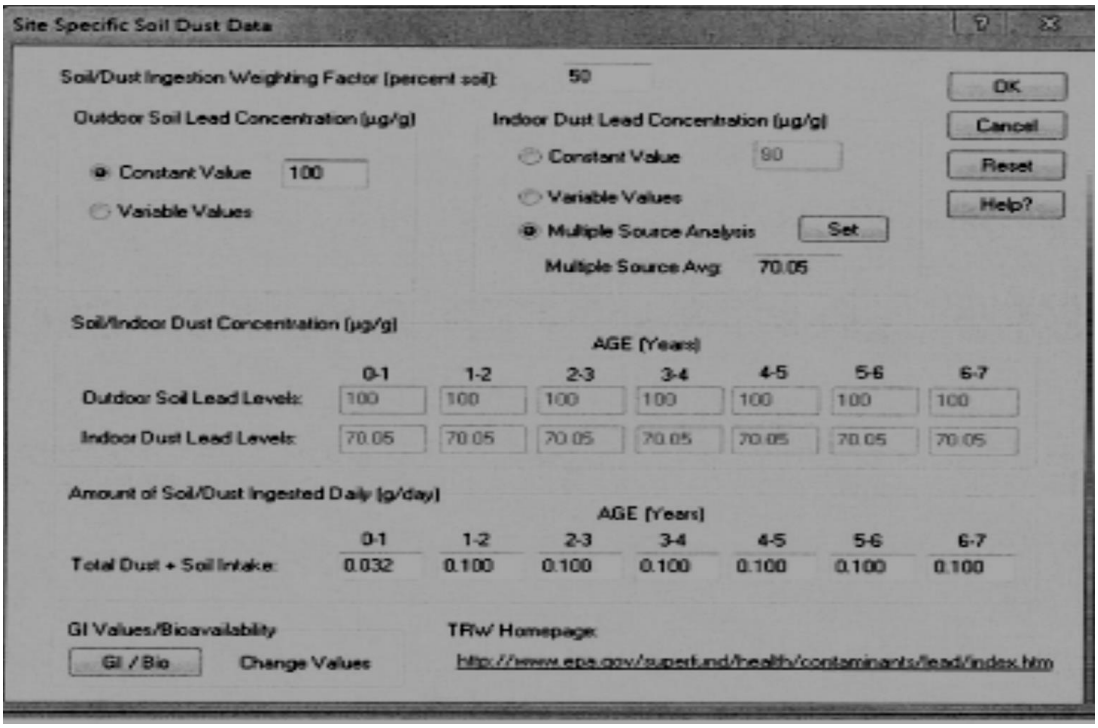

Figure 11: IEUBK window for soil and house dust data

2.9.1 Assessment of model performance

Model performance was assessed by calculating the Pearson (product-moment) correlation coefficient, modelling efficiency (ME), the Nash-Sutcliffe efficiency (NSE), the Root Mean Deviation (RMD), the '95% limits of agreement method', and by using the paired student's *t* test. Since each one of model performance criteria emphasizes on different aspects of model performance, it is important to use a combination and not just one criterion.

The Pearson correlation coefficient (r) is used to determine if there is a relationship between two sets of paired numbers, in this case the measured and the predicted BPb values. It measures the degree of collinearity between predicted and measured data, with values ranging from +1 to -1, where a value of 0 indicates lack of correlation between two variables, + 1 indicates a positive correlation and -1 indicates a negative correlation with the implication that the latter two values are positioned on the same line (Bruning and Kintz, 1987). The Pearson Correlation coefficient used in the present study was calculated using STATA Version 12. It has often been argued, with good reason, that the Pearson Correlation coefficient is not a good measure of agreement. For this reason, it was used in this study as a starting point, and was supplemented with other approaches.

ME compares the efficiency of the model to the efficiency of describing the data using the mean of the observations. Since the normal distribution curve has an optimum at the mean, the mean value has the highest probability of occurrence. Consequently, the mean is often called the "expected value" since it is a value most expected in random observations.

In this regard, the mean BPb for a population describes the expected or most likely value from a child selected randomly. The modelling efficiency is a criterion that measures whether the model provides a better prediction of BPb than merely using the sample or population average. The ME is calculated as follows:

$$ME = \frac{\sum_{i=1}^{n}(O_i - O_{Av})^2 - \sum_{i=1}^{n}(P_i - O_i)^2}{\sum_{i=1}^{n}(O_i - O_{Av})^2} \qquad \textit{Equation 7}$$

Where P_i is the predicted BPb value, O_i is the observed or measured BPb value, O_{Av} is the average of the observed values and n is the number of values (Scorza Júnior and Boesten, 2005). The Values of ME range from -1 to +. A positive value of ME indicates that the predicted values provide a better trend than the mean of observed values, whereas a negative value of ME indicate that the predicted values do not describe the trend better than the mean of observed values (Smith et al., 1997, Li et al., 2016).

It is important to note that the ME, is based on the arithmetic mean, which does not perfectly describe the central tendency for data that are not normally distributed. On the other hand, the ME analyses the effect of outliers and variability (spread) by considering the difference between observed BPb and the average BPb (O_i - O_{av}) and/or the difference between predicted BPb and average BPb (P_i - O_{av}). Nevertheless, the ME may result in overestimation of large values and under-estimation of lower values since the differences between the observed and predicted values are calculated as squared values

NSE is a "statistic that determines the relative magnitude of the residual variance compared to the measured data variance" (Moriasi et al., 2007). It can be computed using the following equation:

$$NSE = 1 - \frac{\sum_{i=1}^{n}(O_i - P_i)^2}{\sum_{i=1}^{n}(O_i - O_{av})^2} \qquad \textit{Equation 8}$$

Where O_i is the observed or measured BPb value at a particular place or time i, P_i is the predicted BPb value at a particular place or time i and O_{Av} is the average of the observed values. NSE indicates how well the plot of measured versus predicted data fits the '1:1 line'. NSE values range between $-\infty$ and 1.0, with an optimal value of 1, where "values between 0 and 1 are generally viewed as acceptable levels of performance, whereas values <0.0 indicates that the mean observed value is a better predictor than the simulated value, which indicates unacceptable performance" (Moriasi et al., 2007). Since the closer the NSE is to 1, the more accurate the model is, NSE therefore provides a measure of accuracy for a model. The NSE shares the shortcomings of the ME due to the use of an arithmetic mean and squared values of the differences between the observed and predicted values (Krause et al., 2005). On the other hand, the NSE also analyses the effect of outliers and variability (spread) by considering the difference between observed BPb and the average BPb (O_i - O_{av}) and/or the difference between predicted BPb and average BPb (P_i - O_{av})

The RMD is a parameter that evaluates systematic bias in the model, with values close to 0 indicating absence of bias (Li et al., 2016). It can be calculated using the following equation:

$$RMD = \frac{100}{O_i}\sum_{i=1}^{n}\frac{P_i - O_i}{n}$$ *Equation 9*

Similar to the ME and the NSE, the RMD is based on the arithmetic mean which does not perfectly describe the central tendency for data that are not normally distributed. On the other hand, the RMD analyses the effect of outliers and variability (spread) by incorporating the difference between predicted BPb and observed BPb ($P_i - O_i$) for all the ranges of measurements including outliers.

The 95% agreement method is a simple statistical approach in which the difference between measurements on the same individual from two methodologies implemented is plotted against the mean of the two measurements. In this approach, the 95% of differences between measurements are expected to be within the mean difference of ± 2 standard deviations (Bland and Altman, 2010). Furthermore, the paired student's t test determines if there is a significant difference in the means of independent paired data.

In addition to the assessment of accuracy, bias, level of agreement between predicted and measured BPb values, a sensitivity analysis was conducted to assess the sensitivity and specificity of the model, where sensitivity is defined as "the proportion of true positives that are correctly identified by the test", whereas specificity is defined as "the proportion of true negatives that are correctly identified by the test"(Altman and Bland, 1994).

2.9.2 Estimating the contribution of soil and food to measured blood lead

The IEUBK model was used to assess the contribution of soil and food to BPb by entering the required data for each source (i.e. food and soil) separately into model and taking the percentage of the resulting BPb against measured BPb.

2.10 Relating the resulting BPb levels to potential health effects

Potential health effects were assessed in terms of BPb concentrations using cut-off points or thresholds for BPb concentrations for various health outcomes, as shown in Table 2, presented in *Section 1.10.3.4.* The table shows that reduction of IQ starts at BPb levels of 5 µg/dl, gastro-intestinal effects at 60 µg/dL and anaemia at70 µg/dL. The prevalence data from the measured and predicted BPb was used to estimate the percentage of children that would be at a risk to produce these health effects.

2.11 Assessment of the burden of disease using the WHO spreadsheets

The expected IQ reduction and cases of MMR that may result from BPb distribution were calculated using WHO spreadsheets and guidelines for burden of disease from lead exposure (WHO, 2003a, Prüss-Üstün et al., 2003a). The spreadsheets are based on the (linear) relationship between IQ points lost and BPb from the meta-analysis by Schwartz (1994) (WHO, 2003a). For this purpose the WHO has given estimates of proportions or expected incidence rates of children who are at risk for various BPb lead intervals. The spreadsheet calculates the number of children just above the MMR threshold of 70 IQ points who would drop into the MMR range due to lead-induced loss of IQ points. The spreadsheet requires use of a regional adjustment ratio for MMR to account for variations

in the incidence of MMR from such causes as anaemia and meningitis. For this study, a regional adjustment ratio of 2.0 was used (WHO, 2003a).

The incidence of MMR from the first WHO spreadsheet was entered into the second WHO spreadsheet together with population data in order to estimate the DALYs attributed to childhood exposure to lead. DALYs comprise of the sum of Years Lived with Disability (YLD) and Years of Life Lost (YLL) from a disease or health state. YLD, which represents the morbidity from a disease, are calculated by multiplying the duration of the disease in years with a disability weight. The disability weight is a value from zero to one that estimates the severity of the disease, where zero indicates perfect health and one would indicate a full year lost to death (Caravanos et al., 2014). MMR due to lead has a disability weight of 0.361 (WHO, 2003b).

2.12 Identification of risk factors for high blood lead

Proportions and means were generated and compared using Mann-Whitney test and Kruskal Wallis tests at 5% level of significance. Quantile- quantile (Q-Q) plots of log transformed data were prepared to assess differences in distribution of BPb for some of the potential risk factors. Multivariate logistic regression analysis was conducted to evaluate the relationship between the explanatory variables and high blood lead (BPb $\geq$ 5 µg/dl). Adjusted odds ratios (ORs) and their corresponding 95% confidence intervals were then calculated.

3.0 Results

This chapter summarizes the major findings of the study, which include laboratory results on blood lead and concentrations of lead in various samples, statistical analyses of the laboratory results, and results on predicted BPb values, assessment of the applicability of the IEUBK model, correlation of measured and predicted BPb values to health effects, assessment of the burden of disease, and assessment of the risk factors

3.1 Socio-demographics

There were 152 subjects between 1 to 6 years of age, with 82 male and 70 female.

3.2 Lead in blood

The raw data for BPb is presented in Table 10 in Appendix II. The average BPb was 6.9 ± 5.3 µg/dl, where 71.7% of the children had high BPb i.e. BPb ≥ 5 µg/dL and 22.8% had BPb ≥ 10 µg/dl. However, it is important to note that because of the uncertainty in BPb measurements of 14.5% BPb values of 5 µg/dl may actually range from 4.3 to 5.7 µg/dl. Therefore, the uncertainty in BPb measurement may slightly affect the prevalence of high BPb. The descriptive statistics for the BPb, without including the uncertainty, are presented in Table 5 below.

Table 5: Descriptive statistics of blood lead

Parameter	**Value (µg/dL)**
Range	2.0 – 50.4
Arithmetic mean	6.9 ± 5.3
Geometric mean	6.5 ± 10*
25^{th} percentile	4
75^{th} percentile	8
95^{th} percentile	13
Median	6
BPb ≥ 5 µg/dl.	71.7%
BPb ≥ 10 µg/dl.	22.8%

* Geometric mean ± geometric standard deviation

3.3 Lead from other sources

3.3.1 Lead in paint

The USEPA defines lead-based paint as hazardous if contains lead above a concentration of 90 mg/kg (CPSC, 2008), whereas the limit for lead in paint in most countries is 600 mg/kg (Clark et al., 2009). The concentrations of lead in fresh paint are presented in Table 12 in the Appendix II. It can be seen that the values have ranged from 1.1 mg/kg to 7139 mg/kg, where 56 % of the samples were above 90 mg/kg while 37.5% were above 600 mg/kg.

From Table 13 in Appendix II it can also be seen that the concentrations of lead in paint chips from houses ranged from 0.62 mg/kg to 6458 mg/kg. Only 2 of the 23 samples had hazardous amounts of lead.

3.3.2 Lead in toys

It can be seen in Table 14 in Appendix II that lead content in toys ranged from 0.091 mg/kg to 9.00 mg/kg, indicating that all the toys contained lead in amounts that were much lower than the CPSC limit value of 100 mg/kg (CPSC, 2008).

3.3.3 Lead in food and water

Concentrations of lead in food are presented in Table 15 in Appendix II. In summary, the values ranged from 0.01 mg/kg in chicken to 3.3 mg/kg in chips. These concentrations are much higher than those values published for Spain (10 µg/kg to 350 µg/kg) (Heard et al., 1983, Cuadrado et al., 2000), Germany (18.7 µg/kg to 52 µg/kg) (Wilheim et al., 2003), Chile (below detection limit to 251 µg/kg) (Muñoz et al., 2005) and Nigeria 102.25 µg/kg to 125 µg/kg (Akinola and Ekiyoyo, 2006). On the other hand, the concentrations of lead in food from Blantyre are of a similar order of magnitude to those published in Korea (below detection to 0.54 mg/kg) (Lee et al., 2006) and Mexico (<0.003–66.32 mg/kg) (García-Rico et al., 2007).

All of the water samples contained lead in amounts that were below the detection limit of 0.000018 mg/L (results not provided).

3.3.4 Lead in house dust and soil

Results of lead in soil and dust are presented in Table 16 in Appendix II. The concentrations of lead in house dust ranged from 2.3 mg/kg to 265 mg/kg, with an outlying

figure of 17179 mg/kg. Apart from the latter outlying figure, the concentrations of lead in house dust in most homes are much lower than the US limit of 400 mg/kg for lead in soil in playgrounds. From the same table, it can also be seen that the concentration of lead in soil ranged from 1.5 mg/kg to 482 mg/kg, with only one sample of soil containing lead above the limit of 400 mg/kg. Therefore, it can be concluded that in general the concentrations of lead in house dust and playground soil in many homes in Blantyre were very low.

In addition, a paired student's t test indicated that there is no significant difference between corresponding concentrations of lead in house dust and lead in the soil samples taken from yard on which the children often play. Similarly, one factor Analysis of variance (ANOVA) indicated that there were no significant differences in lead concentration in samples of house dust and the corresponding soil samples taken from various health catchment areas.

3.4 Food consumption rates

Food consumption rates are provided in Table 17 in the Appendix II. Results indicated within the table indicate that foods that are consumed by children in significant rates include: include the following: Mealie meal or Msima (520.3 g/day), bread (31.35g/day), mangoes (86.78 g/day), green maize (34.82 g/day), rice (105.6 g/day), maize flour porridge (109.4 g/day), soya porridge (121.6 g/day), chicken (66.65g/day), chips (38.5 g/day), beans (30.88 g/day), and drinks such as Sobo (96.25g day) and freezes (68.83 L/day) . Then

food items were also included within the table which were not consumed in significant rates

3.5 Predictions by the IEUBK model and comparison with measured blood lead

The IEUBK model is designed to predict BPb levels from exposure to lead from a limited number of external sources including food, water, air, soil and house dust due to the fact that the exposure rates from these external sources are possible to assess. For the same token, it is therefore not possible to use this model to predict BPb from external sources such as toys and paint as exposure rates to lead from these sources are not practically possible to assess.

In the present study, as lead in air was not measured and also the concentrations for lead in water were below detection limit, it was only possible to use the lead levels assessed in food, soil and house dust. Using these latter values, in addition to the default and published values for availability of lead from these sources, it was possible for us to predict the BPb levels resulting from exposure to lead from the aforementioned three sources. For example, using the default bioavailability value for lead in food (50%) and a default bioavailability value for lead in soil (30%), the obtained predicted values of BPb ranged from 10.5 µg/dL to 39.2 µg/dL, with a geometric mean of 12.5 µg/dL, compared to the measured BPb values which ranged from 2.0 µg/dL to 50.4 µg/dL, with a geometric mean of 6.5 µg/dL (Table 18, Appendix II). The comparison of the geometric means of predicted BPb with the geometric means of observed BPb segregated by age of children is provided in Figure 12 below.

It is important to note that the IEUBK model is designed to predict BPb values below 30 µg/dL and is set calculate geometric means for populations. Therefore, all BPb values above 30 µg/dL have been removed in the calculation of the averages for comparisons. Consequently, the skewness of the data brought by values above 30 µg/dL has been reduced. Furthermore, as the BPb values were still not normally distributed even after dropping BPb values above 30 µg/dL, geometric means were calculated to reduce effects of outliers. The geometric mean, which is equivalent to log-normally transformed data, is less subject to distortion caused by positively skewed data, and is thus a better measure of central tendency of skewed data than the arithmetic mean (Olivier et al., 2008, Bland and Altman, 1996).

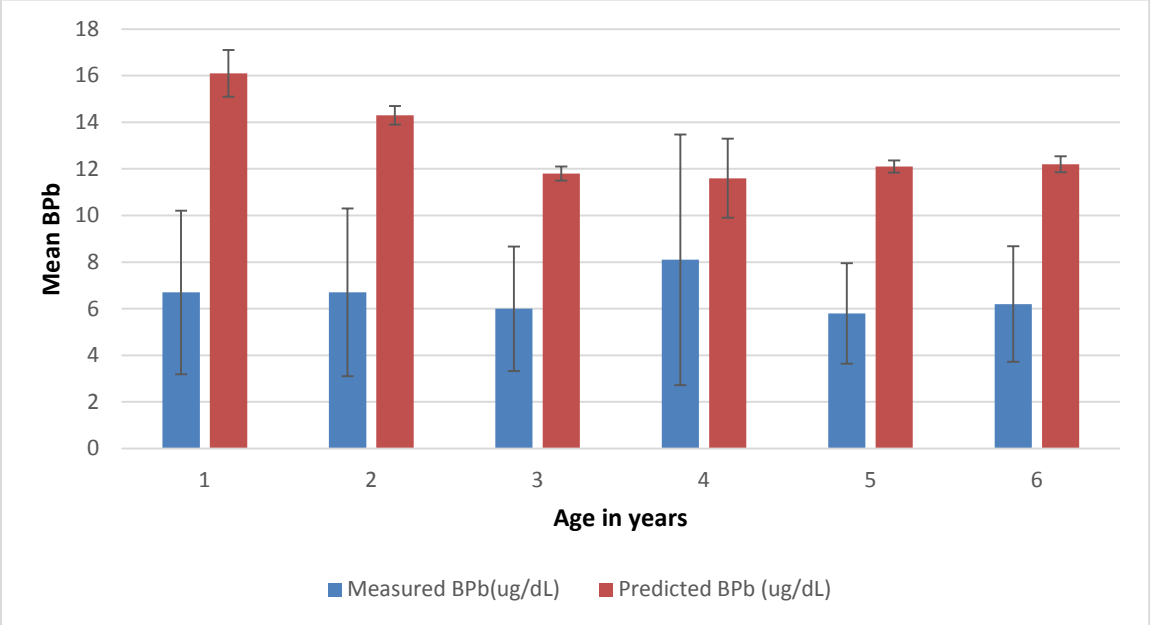

Figure 12: Comparison of the geometric means predicted and mean observed BPb against age

The Pearson correlation coefficient of -0.07 between the predicted and measured BPb values, NSE of -3.30, ME of -2.24, RMD of 88 and the students' t-test have indicated poor agreement between these two values, bias in the model and that the measured mean was a better description than the values predicted by the model. Subsequently, it could be said that there was significant difference between measured and predicted BPb values when a default bioavailability of 50% for lead in food and 30% for lead in soil were used, indicating that the predicted values were on average two-fold higher than the measured values.

Furthermore, using the 95% agreement method, the averages of the measured and predicted BPb values were plotted against the differences between predicted and measured values for each individual is given in Figure 13 below. The differences between predicted and measured values have a mean of 5.96 μg/dL and a standard deviation of 3.65. Therefore, as the 95% limits are 5.96 ± 1.96 x 3.65 (i.e. 13.1 and -1.11) it can be concluded that for 95% of children, prediction by the IEUBK model would be between about 1 μg/dL less and about 13 μg/dL higher than measured values (Bland and Altman, 2003). Since the critical value for BPb in children is only 5 μg/dL, the model would tend to over-predict most BPb values when 50% dietary lead bioavailability is used. This over-prediction is consistent with the results displayed in Figure 12 above.

The over-prediction is also shown by the sensitivity analysis of the IEUBK model as a diagnostic tool, where it could predict all children to have BPb above 5 μg/dL (100%), and

as yet the measured values could show that only 71.7% of children had BPb above 5 µg/dL (false positives 28.3%).

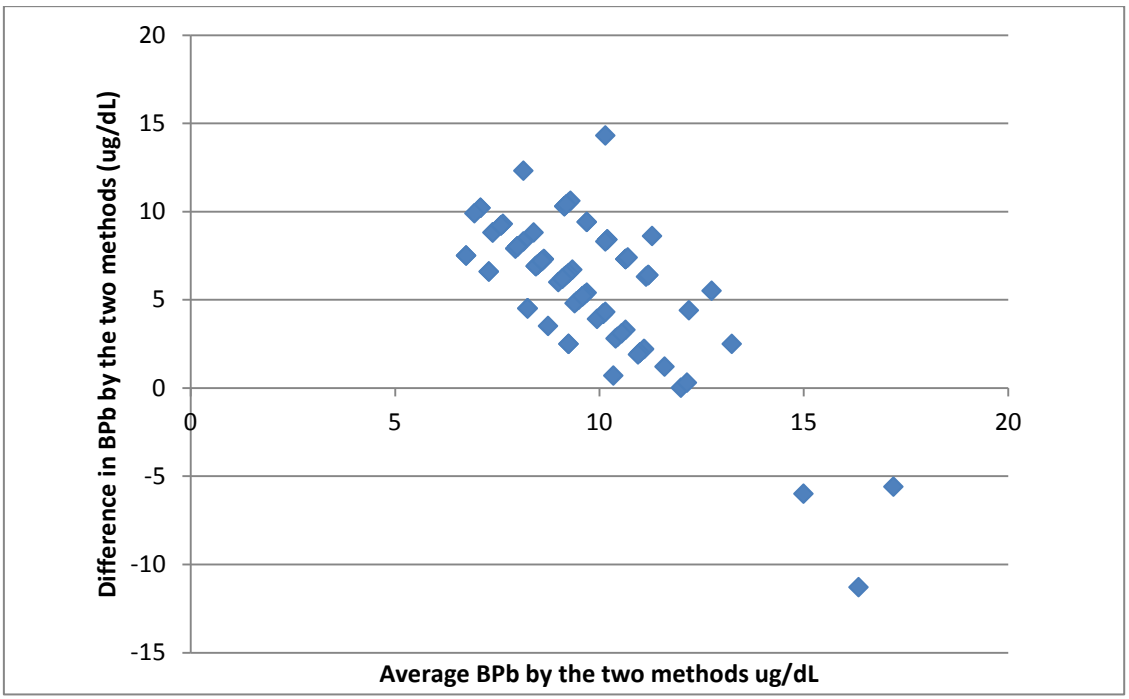

Figure 13: A plot of the average of the measured and predicted BPb against the differences between predicted and measured BPb values (for 50% food bioavailability)

On the other hand, using a bioavailability of 31% for lead in food obtained from the literature and a default bioavailability of 30% for lead in soil, the predicted BPb values obtained have ranged from 6.8 to 33.9 µg/dl, with geometric mean of 8.30 µg/dl, once again compared to measured BPb values which ranged from 2.0 µg/dL to 50.4 µg/dL, with a geometric mean of 6.5 µg/dL. These predicted and measured values are presented in Table 17 in Appendix II. The comparison of the geometric means of predicted BPb with the geometric means of observed BPb segregated by age of children is provided in Figure 14 below.

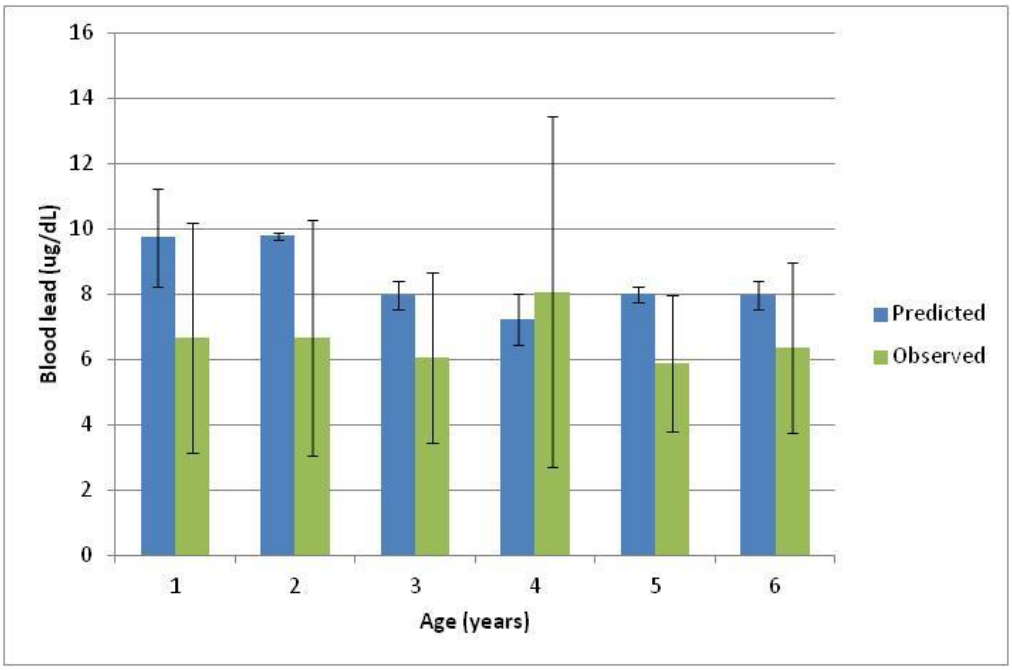

Figure 14: Comparison of the geometric means predicted and mean observed blood lead against age

In this instance, the Pearson correlation of 0.079 between the predicted and measured BPb values, an NSE of -0.3, and a paired t-test values have indicated poor agreement between the predicted and measured BPb values. On the other hand, an RMD value of 25.7 indicated slight bias in the model and an ME value of +0.49 has shown that the predicted values are better indicators of BPb levels than the mean of measured BPb. As such, the ME and RMD values have provided an acceptable agreement between the two sets of values, where the predicted values were on average only 1.3-fold higher than the measured BPb values.

For the 95% agreement method, a plot of the average of the measured and predicted BPb against the differences between predicted and measured values for each individual (for

31% dietary lead bioavailability) is provided in Figure 15 below. The differences between predicted and measured values have a mean of 1.74 µg/dL and a standard deviation of 3.44. Therefore, as the 95% limits are 1.74 ± 1.96 x 3.44 (i.e. -5.0 and 8.48) it can be concluded that for 95% of children, prediction by the IEUBK model would be between about -5 µg/dL less and about 8 µg/dL higher than measured values. Predictions of 5 µg/dL less or 8 µg/dL higher than measured values may result in misallocation of a child's BPb, especially since the critical value for BPb in children is only 5 µg/dL. Nevertheless, there is much better agreement between predicted and measured BPb than in the case when 50% dietary lead bioavailability was used, which indicates that the value of bioavailability used in the model can have a significant impact on the level of agreement between predicted and measured BPb values.

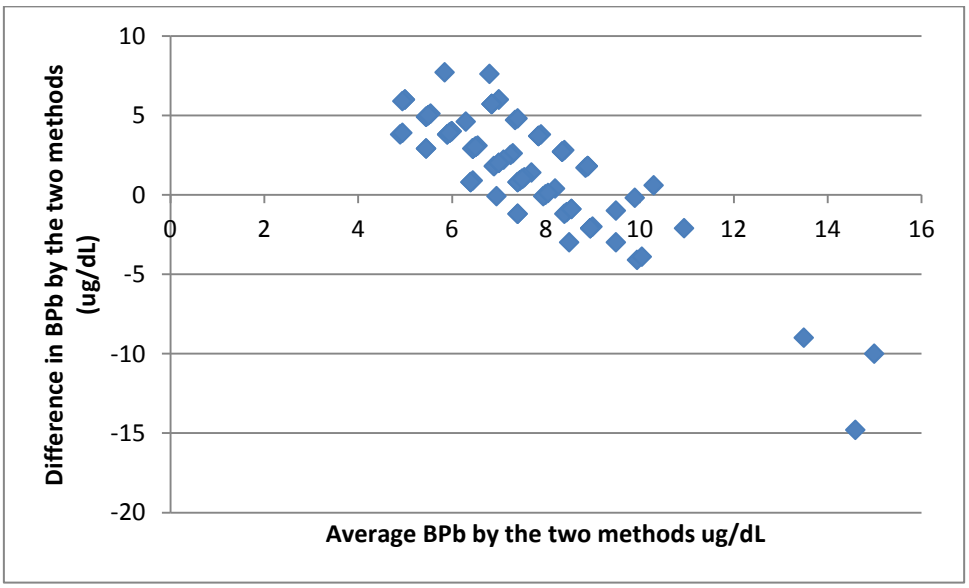

Figure 15: A plot of the average of the measured and predicted BPb against the differences between predicted and measured BPb values (for 31% dietary lead bioavailability)

Furthermore, as was the case when 50% dietary lead bioavailability was used, the IEUBK model could correctly predict the BPb of all children that had BPb above 5 μg/dL, with false positive results for 28.3% of the children that had BPb below 5 μg/dL.

3.6 Assessment of the contribution of soil and food to measured blood lead

The IEUBK was also utilised to assess separately the contribution of each food and soil as external sources of exposure to the measured BPb values. For the assessment of the former, the dietary intakes and the bioavailability values of 50% or 31% of lead in food were utilised to obtain the predicted BPb values as well as the percentage contribution of the dietary lead intake to the measured BPb values (Table 19, Appendix II). Although the concentration of lead in food was constant, food consumption was different depending on the amount of food consumed per child depending on age. When either 50% or 31% bioavailability of lead from total food consumed per child was calculated, different % contribution was estimated for each % lead bioavailability. However, although a quotient of 1.6 (i.e. 50%/30%) (Table 19) would have been expected, different values of this quotient could be observed per child. A quotient of 1.6 would ideally be expected if uptake was linear function of concentration or bioavailability of lead. Contrary to this expectation, lead absorption has been observed to occur through saturable and non-saturable components that resulted in a non-linear function of total uptake. Consequently, BPb concentrations did not increase proportionately with dose (Aungst et al., 1981), and according to the USEPA, "there are significant non-linearities in the empirical relationship between lead intake and observed BPb that can be attributed to saturation of lead uptake from the gut as well as the nonlinear binding in red cells" (USEPA, 1994b). Furthermore, renal lead clearance and BPb clearance resulting from distribution of lead to other

compartments (tissues, bone, teeth, hair, and finger and toe nails are not linearly related to the magnitude of the dose (Aungst et al., 1981, Rabinowitz et al., 1976). It is not surprising, therefore, that the quotient of % contribution for 50% vs 31% in Table 19 is not a single value of 1.6 but ranges from 1.4 to 1.7, with an average of 1.48.

It can also be seen in Table 19 that the dietary contribution to total BPb ranged from 58% to 610% (for 50% bioavailability) and from 31% to 410% (for 31 % bioavailability). Therefore, there were many instances of overestimation for both bioavailabilities, arising from the inaccuracies in the food consumption rates as reported by parents. Nevertheless, despite of the inaccuracies in the food consumption rates it can be concluded that food is a significant contributor of BPb.

For the assessment of contribution of soil as an external source, a similar exercise was followed and the results are presented in Table 20, Appendix II. In this case, a default bioavailability value of 30% was applied and the contribution of lead intake from this source was calculated to be ranging from 1% to 23% with an outlier of 582%. From the corresponding predicted BPb values presented in the same table, it can be seen that the mean predicted BPb values were much lower than the measured values which ranged from 0.1 µg/dL to 3.0 µg/dL with a mean of 0.5 µg/dL, and subsequently were much lower than the measured BPb values, and thus translating to an average of 1.4-fold lower than the measured BPb values.

3.7 Relating of BPb to adverse health effects

Adverse health effects that may result from exposure to lead were determined by relating the relevant health effects to the percentage of children above the relevant threshold values presented earlier in Table 2. The results are presented in Table 5 where it can be seen that 71.7% of children had BPb above 5 μg/dL, indicating the possibility that they may be at risk of suffering from IQ reduction. On the other hand, none of the children had BPb levels above 60 μg/dL, and hence the risk of suffering from lead-induced anaemia and gastro-intestinal effects amounted to zero.

Table 6: Adverse health effects that may arise from exposure to lead among children in Blantyre

Adverse effect	**BPb threshold (μg/dL)**	**% at risk**
IQ reduction	≤ 5	71.7
Anaemia	≤ 60	0
Gastro intestinal effects	≤ 70	0

3.8 Estimation of burden of disease

As discussed earlier in *Section 1.10.3.2.1*, children with pre-existing conditions of low IQ are at a risk of suffering from MMR. Consequently, through the use of WHO burden of disease spreadsheets and guidelines for burden of disease from lead exposure (WHO, 2003a, Prüss-Üstün et al., 2003a), it could be determined that the distribution of BPb among children in Blantyre would result in 8.38 cases of MMR per 1000 children between 1 and 4 years old. A similar estimate could not be performed for children aged between 5 and 6 years due to lack of population data.

Using the prevalence rates of MMR, the DALYs for children aged 0-4 years attributable to lead-induced MMR were calculated to be 7314 DALYs. Because MMR is not inherently fatal, the analysis does not consider YLL but only YLD Therefore, the DALY calculations represent only the morbidity and does not incorporate any premature mortality associated with lead exposure. As a way of interpretation, 'a child who develops MMR from lead exposure at birth and has a life expectancy of 80 years is said to have lived 28.9 YLDs (80 years x 0.361 (the disability weight for MMR)') (Caravanos et al., 2014). As expected, the burden of MMR of 7,314 estimated in the present study for a small city such as Blantyre is much lower than the national burden of MMR of about 44,000 attributed to lead in South Africa (Norman et al., 2007).

3.9 Risk factors for high blood lead

The descriptions of the participants in relation to the (potential) risk factors are presented in Table 7 below. The risk factors that have been considered in the present study included age, sex, living in a painted house, residential/catchment area, mouthing behavior, use of pottery, use of the toys. As indicted in this table, there were more male than female children. In addition, the number of subjects was not equally distributed within the same catchment area and that most of the parents/guardians did not know the use of toys, pottery and mouthing behaviour of their children

Table 7: Description of participants with respect to the risk factors

Characteristics	Number of children (%) (N=152)
Age (mean ± SD)[a]	4.07 ± 1.59
Sex	
Male	82 (53.9%)
Female	70 (46.1%)
Living in painted house	
No (Ref)	55 (36.2%)
Yes	37 (24.3%)
Not reported	60 (39.5%)
Area of residence	
Chilomoni	29 (19.1%)
Ndirande	28 (18.4%)
Bangwe	18 (11.8%)
Limbe	7 (4.61%)
Zingwangwa	31 (20.4%)
Machinjiri	39 (25.7%)
Guardian observing mouthing behaviour of child	
No (Ref)	21 (31.8%)
Yes	64 (41.3%)
Don't know	67 (44.1%)
Guardian observing use of pottery by child	
No (Ref)	66 (43.4%)
Yes	19 (12.5%)
Don't know	67 (44.1%)
Guardian observing use of toys by child	
No (Ref)	44 (28.9%)
Yes	41 (27.0%
Was not sure	67 (44.1%)

[a] The children were aged 1 to 6 years. Ref = Reference category

The prevalence of high BPb was calculated in relation to different risk factors (Table 8), where it can be seen that there were differences in prevalence values of high BPb in relation to residence or catchment areas, especially for the area of Chilomoni which was substantially difference from the other areas. However, it is important to note that although

Machinjiri in Table 8 has the least prevalence of high BPb, it had a number of children with BPb values above 20 µg/dL.

Table 8: Prevalence of high BPb based on different risk factors

Category	Prevalence (%)	95% CI
Age category (years)		
1-3	72.6	62.9 – 82.3
4-6	70.6	59.6 – 81.6
Sex		
Male	69.5	59.4 - 79.6
Female	74.2	63.8 – 84.6
Living in painted house[a]		
No	69.1	56.6 – 81.6
Yes	78.4	64.7 – 92.0
Area of residence		
Machinjiri	64.1	48.7– 79.4
Zingwangwa	77.4	62.3– 92.5
Ndirande	67.9	50.1 – 85.6
Limbe	71.4	35.0 – 108
Bangwe	66.7	44.1 – 89.3
Chilomoni	82.8	68.7 – 96.9
Guardian observing mouthing behaviour of child[a]		
No	66.7	45.7 – 87.6
Yes	73.4	62.4 – 84.5
Guardian observing use of pottery by child[a]		
No	72.7	61.7 – 83.7
Yes	68.4	46.6 – 90.2
Guardian observing use of toys by child		
No	77.3	64.5 – 90.0
Yes	65.9	50.9 – 80.8

[a] the proportions excludes the missing values.

In addition, differences could also be seen when the total number of BPb levels were plotted against the area of residential/catchment area using Q-Q plots (Figure 16). Since the plots were not identical, it can be said that each residential/catchment area had its unique distribution of BPb levels.

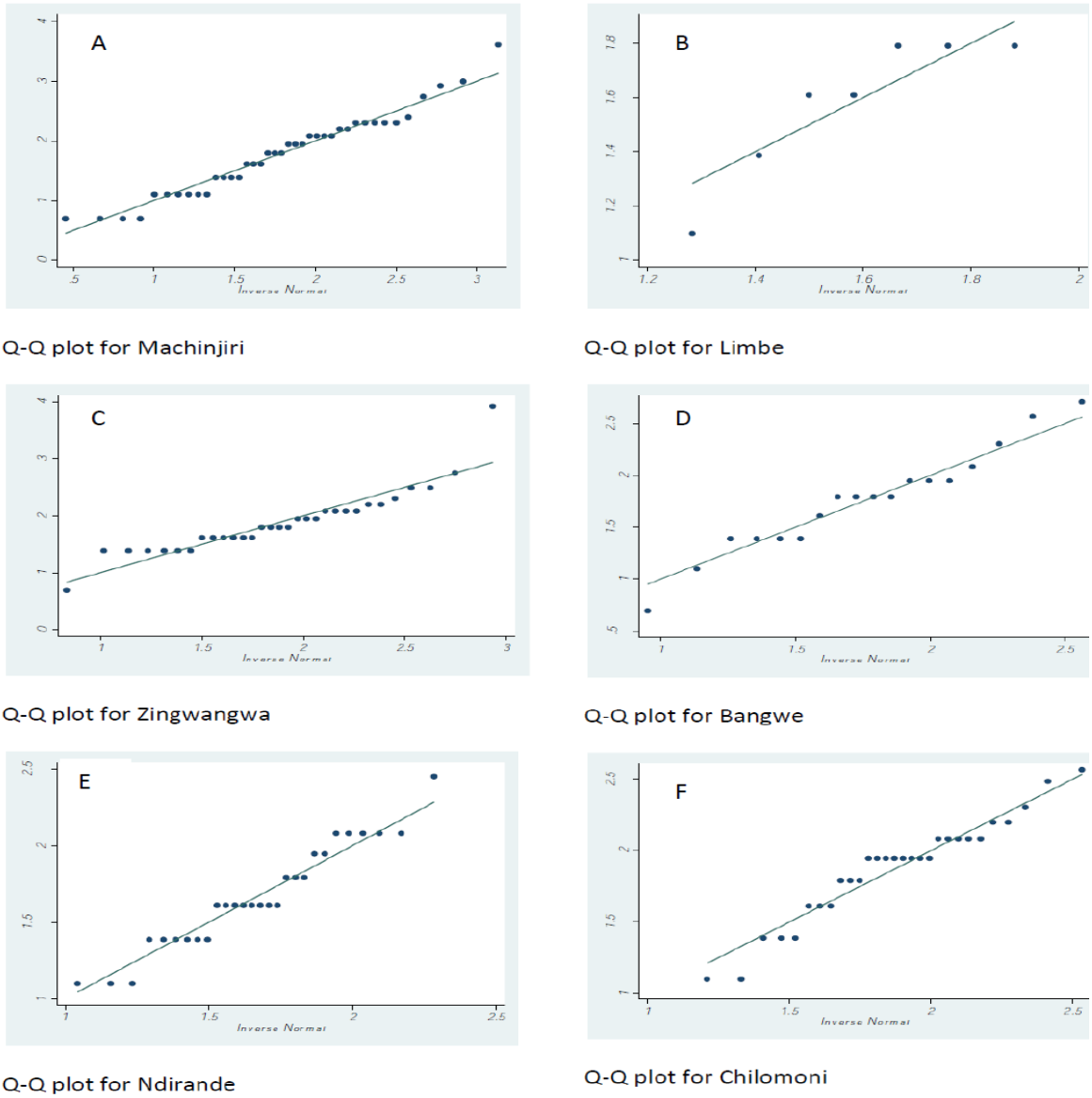

Figure 16: Inverse normal distribution Q-Q plot of BPb for children in the various residential/catchment (A Machinjiri, B Limbe, C Zingwangwa, D Bangwe, E Ndirande and F Chilomoni)

3.9.1 Bivariate and multivariate logistic regression

The differences in mean BPb levels in the previous section within different residential/catchment areas were further analysed, in addition to other risk factors, using bivariate and multivariate logistic regression to establish associations between these variables (Tables 9 and 10).

Table 9: Mean values for high BPb for each characteristic and results of Wilcoxon rank-sum/Kruskal-Wallis test for significant differences within each characteristic

Characteristic	Mean high BPb (µg/dL)	p-value
Age category (years)		0.978
1-3	8.54 (±6.25)	
4-6	8.13 (±4.97)	
Sex		0.559
Male	7.87 (± 2.98)	
Female	8.88 (± 7.65)	
Living in painted house		0.797
No	7.90 (±3.60)	
Yes	7.41 (± 2.01)	
Area of residence		0.013
Machinjiri	10.2 (±6.77)	
Zingwangwa	9.38 (±9.14)	
Ndirande	6.51 (±1.77)	
Limbe	5.60 (±0.547)	
Bangwe	8.00 (±3.10)	
Chilomoni	7.58 (±1.98)	
Guardian observing mouthing behaviour of child		0.733
No	8.26 (±4.81)	
Yes	7.50 (±2.43)	
Guardian observing use of pottery by child		0.893
No	7.65 (±3.20)	
Yes	7.77 (± 2.83)	
Guardian observing use of toys by child		0.318
	No	7.80 (±3.85)
	Yes	7.52 (±1.85)

Results presented in Table 8 indicate that indeed there were statistically significant differences in relation to residence/catchment areas, with a p value of 0.013, while no such significant differences could be obtained between mean BPb and the other risk factors considered. On the other hand, because of the very wide confidence intervals, it could not be determined from Table 10 whether or not, sex, age, living in a painted house and mouthing behaviour as well as residential/catchment areas are risk factors for high BPb. Nevertheless, as the p-value for area of Chilomoni was the only one having p-value of

0.049 (< 0.05), it should then be considered that some areas but not all residential/ catchment areas may be risk factors for high BPb.

Table 10: Results for multivariate analysis

	Odds ratio	95% CI	p-value
Age (mean years)	1.10	0.815 – 1.50	0.52
Sex			
Male	1.00	-	
Female	1.17	0.9 -1.61	0.9
Living in painted house			
No	1.00	-	-
Yes	2.06	0.66 – 6.52	0.22
Area of residence			
Machinjiri	1.00	-	-
Zingwangwa	2.34	0.52 - 10.5	0.26
Ndirande	2.76	0.571 - 13.3	1.26
Limbe	3.83	0.284 - 51.5	0.312
Bangwe	0.304	0.0200 - 4.63	0.392
Chilomoni	6.94	1.01 - 47.6	0.049
Mouthing behaviour			
No	1.00	-	-
Yes	2.04	0.506 - 8.24	0.316
Use of pottery			
No	1.00	-	-
Yes	0.725	0.1979 - 2.65	0.627
Use of toys			
No	1.00	-	-
yes	0.346	0.104 - 1.14	0.083

4.0 Discussion

This chapter discusses the results on levels and sources of exposure to lead, the risk factors for high blood lead, the applicability of the IEUBK model in Blantyre, potential health effects and potential burden of diseases that are associated with the levels of exposure to lead among children in Blantyre, study limitations as well as recommendations that can be drawn from the study.

This present study aimed at assessing levels of exposure to lead, sources of lead and the risk factors for high BPb, the associated risks of suffering from adverse health effects, and the applicability of the IEUBK model for predicting BPb among children in Blantyre.

The assessment of the levels of BPb in this cross sectional study has indicated that indeed there were high levels of exposure to lead among children in Blantyre, where 71.7% of children had BPb ≥ 5 µg/dL, and 23 % had BPb ≥ 10 µg/dL. Similar investigations that were conducted in other countries in the region have also shown high levels of exposure to lead among children. These have included Botswana, where 31% of the children had BPb levels ≥ 10 µg/dL (Mbongwe et al., 2005) and South Africa, where 78% of children having BPb ≥ 10 µg/dL BPb (Mathee et al., 2002). However, it needs to be pointed out that these two studies were conducted soon after the introduction of regulations on leaded petrol in these countries, whereas the present study has been conducted about ten years after the introduction of these regulations. Other investigations conducted in countries outside the region have also indicated that the levels of BPb found in the present study for children in Blantyre were much higher than those reported for example for China, where only 1.32% of children had BPb above 5 µg/dL (Li et al., 2014a). The results obtained in the present study had therefore warranted us to investigate further on the sources of lead that may have contributed to such high levels of BPb in Blantyre. Subsequently, a number of possible sources of lead were investigated including food, soil and house dust, paint, toys and water.

Food was the first source to be investigated, where the present study could ascertain that food is the major contributor to the high BPb in children. Investigations in the literature

have also shown that food has been the major contributor to BPb in China (83.4%) (Li et al., 2016) and Belgium (over 75%) (Cornelis et al., 2006). Present results and those presented in the literature thus reiterate the importance of food as a major source of lead of BPb and hence it is recommended further investigations to elucidate the origins of high lead contained in food. Dietary lead originates from environmental sources such as soil, water and air, as well as food processing, food handling, and food packaging. According to the CODEX Alimentarius, "sources of lead from food processing include lead paint and lead-containing equipment, such as piping and lead-soldered machinery, whereas sources from packaging include lead-soldered cans coloured plastic bags and wrapping papers, cardboard containers lead foil capsules on wine bottles, and lead-glazed ceramic, lead crystal and lead-containing metal vessels used for packaging or storing food" (JECFA, 2004). There is need for a systematic investigation of the contribution of these sources to dietary lead.

In the present study soil and house dust were shown to be minor contributors (11.2%). These results were in agreement with those reported for China (15%) (Li et al., 2016) but in disagreement with those reported for Australia (54%) (Zheng et al., 2013). In contrast, water samples in the present study have been shown to contain undetectable amounts of lead, and hence it could be said that contribution of water to BPb was minimal. Our results were, however, not in agreement with those reported for the USA (Edwards et al., 2009) and Germany (Fertmann et al., 2004).

The contributions of the sources of lead (food, soil and house dust) to BPb reported in the preceding paragraphs were estimated through the use of the IEUBK model. However, prior to the use of this model for these determinations, it had to be evaluated, by comparing predicted BPb with measured BPb. The comparisons showed that this model may be used provided that the bioavailability values for lead from different sources are available as well as the food consumption rates are provided for Malawi. Similar comparisons in other countries such as China (Li et al., 2016), Kazakhstan (Rasmuson et al., 2012), Belgium (Cornelis et al., 2006) and Mexico (Gersberg et al., 1997) have also shown that the model may be applied for prediction of BPb in children in the respective countries.

Other sources of lead were also investigated including toys and paint chips from the residences of participants, where both of these samples were found to contain very low levels of lead. These results are contrary to those reported in the literature for toys for India (Kumar and Pastore, 2007), Nigeria (Omolaoye et al., 2010), USA (Greenway and Gerstenberger, 2010) and Turkey (Aliyev et al., 2011) and for paint chips for Nigeria (Nduka et al., 2008). In contrast to toys and paint chips, our investigation on lead in fresh paint has shown that 56% of the samples of fresh paint contained high levels of lead, which is in agreement with studies conducted in other countries including China, India, Malaysia, Brazil, Singapore and Nigeria (Clark et al., 2006, Clark et al., 2009).

The high levels of exposure to lead among children in Blantyre from numerous sources has warranted further investigation on the risk factors for high BPb, which are thought to affect exposure to lead from the previously identified sources of lead. The risk factors which

were investigated in the present study have included age, sex, living in a painted house, residential/catchment area, mouthing behavior, use of pottery and use of the toys. Among these potential risk factors, the residential/catchment area of Chilomoni was found to be a significant risk factor for high BPb. Similar investigations have shown that residential area is a risk factor for high BPb, often as a result of differences in (industrial and traffic) sources (Chen et al., 2012). However, as Chilomoni is not a heavy industry area (as per discussion of page 82), industrial sources are not likely to be the source of lead in Chilomoni. Usually contribution of lead from these industrial sources will manifest itself with a high content of lead in soil. However, one factor ANOVA in *Section 3.3.4* indicated that there were no significant differences in lead concentration in samples of house dust and the corresponding soil samples taken from various health catchment areas. It can be concluded that as yet, sources could not be identified that may explain why Chilomoni residential/catchment area is to be a significant risk factor for high BPb.

On the other hand, due to very wide confidence intervals it could not be determined whether sex, age, living in a painted house and mouthing behavior were significant risk factors for high BPb in Blantyre. Nevertheless, in the literature age (Menezes-Filho et al., 2011), sex (Li et al., 2014b), living in a painted house (Schwartz and Levin, 1991), and mouthing behavior (Kranz et al., 2004) have been reported to be significant risk factors for high BPb. Therefore, there is need for further investigations on the roles of these potential risk factors to lead exposure in Blantyre using studies with greater samples (statistical power).

The establishment of high BPb as well as the main sources of exposure and risk factors has enabled us to assess the potential health effects that are likely to result from these exposures. In this regard, the present study has ascertained that 71.7% of children were at risk of suffering from IQ reduction, which was already proven in the literature by many investigations (Nevin, 2000, Schwartz, 1994). Furthermore, the present study has shown that the children who are at risk to develop IQ reduction, an incident rate of 8.38 children out of 1000 between the ages of 1 and 4, will suffer from MMR. This estimated incidence rate of MMR for Blantyre is about 40-fold higher than those reported for Poland (Jarosińska et al., 2006), and about 9-fold higher than those reported for South Africa in 2007 (Norman et al., 2007) and about 1.4-fold higher than those reported for those who live near toxic waste sites in low and middle income countries (Chatham-Stephens et al., 2014). Lead-induced IQ reduction and MMR in Blantyre are additional impediments to early stimulation and learning in a population where 27% of under-five children are underweight and about 50% are stunted (Madise and Mpoma, 1997). Therefore, based on our results and those reported in the literature it is imperative that measures are taken to reduce the levels of exposure to lead in children in Blantyre.

4.1 Limitations of the study

The present cross-sectional study was subject to number of limitations, including use of a cross-sectional study design, use of a modest sample size, lack of (national) food consumption data, lack of air lead measurements and use of default input data in the IEUBK model for some of the parameters.

In its conceptual design, a cross-sectional study only captures a snapshot of exposure levels, on the assumption that levels of exposure to each individual remain constant with time. Contrary to this assumption, however, levels of lead in dust, soil and food, may vary with time, and thereby affecting the cumulative lead in blood. These variations may have significantly contributed to the poor agreement between predicted and measured BPb values. In contrast to cross-sectional studies, a longitudinal study design captures the variations in levels exposure. Therefore, the IEUBK model could be more effectively evaluated using longitudinal studies that measure lead in the same participants and their environments periodically over time. However, longitudinal studies are much more costly than cross-sectional studies.

The use of a modest sample size in the study also placed a limitation on the study. This limitation was imposed by the huge costs of laboratory analyses of a large number of samples. In this study, a sample size of 152 participants is only a very small proportion of the entire population of children in Blantyre, and therefore limits the generalizability of the results to the whole population. In addition, the sample size used in the study was not very adequate for the multivariate logistic modelling for the identification of the risk factors. In this regard, use of a modest sample size may have resulted in the lack of firm associations between BPb and some risk factors as well as the wide confidence intervals for the odds ratios. Use of a small sample size was exacerbated by missing data for some of the risk factors.

The study was also impacted by a high refusal or non-participation rate that may have resulted in aselection bias. Refusal to participate in a study can be influenced by age, levels of education, level of income, marital status (Vrijheid et al., 2009, DeMaio, 1980, Keeter et al., 2000) and other factors. Non-participation will especially introduce a selection bias in cases where refusal to participate is determined by a certain socio-economic factor that is correlated with the attribute under consideration. This study had a high refusal rate probably because most respondents were not aware of the issue of lead poisoning. Therefore, future risk assessments of lead in Malawi require more awareness of the issue of lead poisoning in order to reduce refusal rates.

Lack of (national) food consumption data was also a great limitation in the study. Assessment of food intake would require a study on its own, covering a long period of time and involving thousands of participants. Consequently, lack of food consumption data resulted in the poor agreement between predicted and measured BPb values.

The study was conducted without measuring concentrations of lead in air, on the assumption that banning of leaded petrol has reduced its concentrations in air to negligible levels (Thomas et al., 1999). This may not exclude the possibility that there may still be some industries that may use lead and therefore such investigations may be warranted in the future.

Finally, the use of default input data in the IEUBK model may have resulted in the poor agreement between predicted and measured BPb values. The default parameters that were used in the study for bioavailabilities were obtained from studies in America and Europe, and therefore may not be appropriate for all children and for every site-specific application. Bioavailability is known to be affected by nutritional status of the subjects, and has also been shown to depend on the food matrix (Peijnenburg and Jager, 2003). Therefore, prediction of BPb using biokinetic models such as the IEUBK model should ideally be conducted using site-specific bioavailabilities.

5.0 Conclusion

This chapter presents the main theoretical and policy implications, conclusions and recommendations that can be drawn from the study.

Exposure to lead and related health effects

The present study sought to assess levels of exposure to lead among children in Blantyre city, the sources and the risk factors for exposure. The study has established that a substantial proportion of children in Blantyre are exposed to levels of lead that are detrimental to their health. The study could also establish that food is a significant contributor of lead and that the residential/catchment area of Chilomoni is a risk factor for lead. Consequently, from the finding of the present study it can recommended further studies should be undertaken to determine the origin of lead in food and methods for reducing it. Further studies are also recommended to establish the sources for high exposure to lead in Chilomoni.

Applicability of the IEUBK model to children in Blantyre

The present study has also established that the IEUBK model may be applied in Malawi. However, better results could be obtained by use of national food consumption data and site-specific bioavailability values. Therefore, there is need to acquire accurate (national) food consumption data and other site-specific data for future risk assessments

6.0 References

ABBASPOUR, N., HURRELL, R. & KELISHADI, R. 2014. Review on iron and its importance for human health. *Journal of Research in Medical Sciences,* 19.

ABERNATHY, C. O. & ROBERTS, W. C. 1994. Risk assessment in the Environmental Protection Agency. *Journal of Hazardous Materials,* 39**,** 135-142.

ADEBAMOWO, E. O., SCOTT CLARK, C., RODA, S., AGBEDE, O. A., SRIDHAR, M. K. C. & ADEBAMOWO, C. A. 2007. Lead content of dried films of domestic paints currently sold in Nigeria. *Science of The Total Environment,* 388**,** 116-120.

AKINOLA, M. & EKIYOYO, T. 2006. Accumulation of lead, cadmium and chromium in some plants cultivated along the bank of river Ribila at Odo-nla area of Ikorodu, Lagos state, Nigeria. *Journal of Environmental Biology,* 27**,** 597-599.

ALAN S, K. 2001a. Do low levels of lead produce IQ loss in children? A careful examination of the literature. *Archives of Clinical Neuropsychology,* 16**,** 303-341.

ALAN S, K. 2001b. How dangerous are low (not moderate or high) doses of lead for children's intellectual development? *Archives of Clinical Neuropsychology,* 16**,** 403-431.

ALATISE, O. I. & SCHRAUZER, G. N. 2010. Lead exposure: a contributing cause of the current breast cancer epidemic in Nigerian women. *Biological Trace Element Research,* 136**,** 127-139.

ALIYEV, V., BOZALAN, M., GÜVENDIK, G. & SÖYLEMEZOĞLU, T. 2011. The potential health risk assessment of lead levels in children's toys. *Toxicology Letters,* 205**,** S255.

ALTMAN, D. G. & BLAND, J. M. 1994. Diagnostic tests. 1: Sensitivity and specificity. *BMJ: British Medical Journal,* 308**,** 1552.

ARIZA, M. E. & WILLIAMS, M. V. 1996. Mutagenesis of AS52 cells by low concentrations of lead (II) and mercury (II). *Environmental and molecular mutagenesis,* 27**,** 30-33.

ATSDR 2007. Toxicological Profile for Lead, retrieved 04/07/2013, from http://www.atsdr.cdc.gov/toxprofiles/tp13.pdf.

ATSDR. 2015. *Minimal Risk Levels (MRLs)* [Online]. Available: http://www.atsdr.cdc.gov/mrls/index.asp [Accessed 2/2/2016].

AUNGST, B. J., DOLCE, J. A. & FUNG, H.-L. 1981. The effect of dose on the disposition of lead in rats after intravenous and oral administration. *Toxicology and Applied Pharmacology,* 61**,** 48-57.

AVILA, M. H., ROMIEU, I., RIOS, C., RIVERO, A. & PALAZUELOS, E. 1991. Lead-glazed ceramics as major determinants of blood lead levels in Mexican women. *Environmental Health Perspectives,* 94**,** 117.

BAKULSKI, K. M., ROZEK, L. S., DOLINOY, D. C., PAULSON, H. L. & HU, H. 2012. Alzheimer's disease and environmental exposure to lead: the epidemiologic evidence and potential role of epigenetics. *Current Alzheimer Research,* 9**,** 563.

BARBOSA, F., TANUS-SANTOS, J. E., GERLACH, R. F. & PARSONS, P. 2005. A Critical Review of Biomarkers Used for Monitoring Human Exposure to Lead: Advantages, Limitations, and Future Needs. *Environmental Health Perspectives* 113**,** 1669-1674.

BARBOSA JR, F., RODRIGUES, M. H. C., BUZALAF, M. R., KRUG, F. J., GERLACH, R. F. & TANUS-SANTOS, J. E. 2006. Evaluation of the use of salivary lead levels as a surrogate of blood lead or plasma lead levels in lead exposed subjects. *Archives of toxicology,* 80**,** 633-637.

BARLOW, S. M., KOZIANOWSKI, G., WÜRTZEN, G. & SCHLATTER, J. 2001. Threshold of toxicological concern for chemical substances present in the diet. *Food and Chemical Toxicology,* 39**,** 893-905.

BARNES, D. G., DASTON, G. P., EVANS, J. S., JARABEK, A. M., KAVLOCK, R. J., KIMMEL, C. A., PARK, C. & SPITZER, H. L. 1995. Benchmark Dose Workshop: Criteria for Use of a Benchmark Dose to Estimate a Reference Dose. *Regulatory Toxicology and Pharmacology,* 21**,** 296-306.

BARRY, P. & MOSSMAN, D. 1970. Lead concentrations in human tissues. *British Journal of Industrial Medicine,* 27**,** 339-351.

BEERMAN, K. A. & DITTUS, K. 1993. Sources of error associated with self-repots of food intake. *Nutrition Research,* 13**,** 765-770.

BERGDAHL, I. A., GERHARDSSON, L., SCHÜTZ, A., DESNICK, R. J., WETMUR, J. G. & SKERFVING, S. 1997a. Delta-aminolevulinic acid dehydratase polymorphism: influence on lead levels and kidney function in humans. *Archives of Environmental Health: An International Journal,* 52**,** 91-96.

BERGDAHL, I. A., GRUBB, A., SCHÜTZ, A., DESNICK, R. J., WETMUR, J. G., SASSA, S. & SKERFVING, S. 1997b. Lead Binding to δ-Aminolevulinic Acid Dehydratase (ALAD) in Human Erythrocytes. *Pharmacology & toxicology,* 81**,** 153-158.

BERGDAHL, I. A., SCHÜTZ, A., GERHARDSSON, L., JENSEN, A. & SKERFVING, S. 1997c. Lead concentrations in human plasma, urine and whole blood. *Scandinavian journal of work, environment & health***,** 359-363.

BERNARD, A. M., VYSKOCIL, A., ROELS, H., KRIZ, J., KODL, M. & LAUWERYS, R. 1995. Renal Effects in Children Living in the Vicinity of a Lead Smelter. *Environmental Research,* 68**,** 91-95.

BETTS, K. S. 2012. cdc Updates Guidelines for children's lead exposure. *Environmental Health Perspectives,* 120**,** a268.

BIHAQI, S. W., HUANG, H., WU, J. & ZAWIA, N. H. 2011. Infant exposure to lead (Pb) and epigenetic modifications in the aging primate brain: implications for Alzheimer's disease. *Journal of Alzheimer's Disease,* 27**,** 819-833.

BINNS, H. J., LEBAILLY, S. A., FINGAR, A. R. & SAUNDERS, S. 1999. Evaluation of risk assessment questions used to target blood lead screening in Illinois. *Pediatrics,* 103**,** 100-106.

BLAKE, K. C. H. & MANN, M. 1983. Effect of calcium and phosphorus on the gastrointestinal absorption of 203Pb in man. *Environmental Research,* 30**,** 188-194.

BLAND, J. M. & ALTMAN, D. G. 1996. Transformations, means, and confidence intervals. *BMJ: British Medical Journal,* 312**,** 1079.
BLAND, J. M. & ALTMAN, D. G. 2003. Applying the right statistics: analyses of measurement studies. *Ultrasound in obstetrics & gynecology,* 22**,** 85-93.
BLAND, J. M. & ALTMAN, D. G. 2010. Statistical methods for assessing agreement between two methods of clinical measurement. *International Journal of Nursing Studies,* 47**,** 931-936.
BOCCA, B., PINO, A., ALIMONTI, A. & FORTE, G. 2014. Toxic metals contained in cosmetics: A status report. *Regulatory Toxicology and Pharmacology,* 68**,** 447-467.
BOLIN, C. M., BASHA, R., COX, D., ZAWIA, N. H., MALONEY, B., LAHIRI, D. K. & CARDOZO-PELAEZ, F. 2006. Exposure to lead and the developmental origin of oxidative DNA damage in the aging brain. *The FASEB Journal,* 20**,** 788-790.
BORJA-ABURTO, V. H., HERTZ-PICCIOTTO, I., LOPEZ, M. R., FARIAS, P., RIOS, C. & BLANCO, J. 1999. Blood lead levels measured prospectively and risk of spontaneous abortion. *American Journal of Epidemiology,* 150**,** 590-597.
BOSGRA, S., BOS, P. M. J., VERMEIRE, T. G., LUIT, R. J. & SLOB, W. 2005. Probabilistic risk characterization: An example with di(2-ethylhexyl) phthalate. *Regulatory Toxicology and Pharmacology* 43**,** 104–113.
BRANDAO, J., OKONKWO, O., SEHKULA, M. & RASELEKA, R. 2012. Concentrations of lead in cosmetics commonly used in South Africa. *Toxicological & Environmental Chemistry,* 94**,** 70-77.
BRAUN, J. M., KAHN, R. S., FROEHLICH, T., AUINGER, P. & LANPHEAR, B. P. 2006. Exposures to environmental toxicants and attention deficit hyperactivity disorder in US children. *Environmental Health Perspectives***,** 1904-1909.
BREMMER, H. J. & VEEN, M. P. V. 2002. Children's toys fact sheet: To assess the risks for the consumer.
BRUNING, J. L. & KINTZ, B. 1987. *Computational handbook of statistics,* Scott, Foresman & Co.
BUDTZ, J., ESBEN, BELLINGER, D., LANPHEAR, B. & GRANDJEAN, P. 2013. An international pooled analysis for obtaining a benchmark dose for environmental lead exposure in children. *Risk Analysis,* 33**,** 450-461.
BURSTYN, I. & TESCHKE, K. 1999. Studying the determinants of exposure: a review of methods. *American Industrial Hygiene Association Journal,* 60**,** 57-72.
BUSHNELL, P. J., BOWMAN, R. E. & MARLAR, R. 1977. Scotopic vision deficits in young monkeys exposed to lead. *Science,* 196**,** 333-335.
CALABRESE, E. J., BARNES, R., STANEK III, E. J., PASTIDES, H., GILBERT, C. E., VENEMAN, P., WANG, X., LASZTITY, A. & KOSTECKI, P. T. 1989. How much soil do young children ingest: An epidemiologic study. *Regulatory Toxicology and Pharmacology,* 10**,** 123-137.
CALABRESE, E. J., STANEK III, E. J., PEKOW, P. & BARNES, R. M. 1997. Soil Ingestion Estimates for Children Residing on a Superfund Site. *Ecotoxicology and Environmental Safety,* 36**,** 258-268.
CAMPBELL, B., BEATTIE, A., MOORE, M., GOLDBERG, A. & REID, A. 1977. Renal insufficiency associated with excessive lead exposure. *BMJ,* 1**,** 482-485.
CAMPBELL, J. R. & AUINGER, P. 2007. The association between blood lead levels and osteoporosis among adults: Results from the Third National Health and Nutrition Examination Survey (NHANES III). *Environmental Health Perspectives***,** 1018-1022.

CARAVANOS, J., CLARK, E., FULLER, R. & LAMBERTSON, C. 2011. Assessing worker and environmental chemical exposure risks at an e-waste recycling and disposal site in Accra, Ghana. *Journal of health and pollution,* 1**,** 16-25.
CARAVANOS, J., DOWLING, R., TÉLLEZ-ROJO, M. M., CANTORAL, A., KOBROSLY, R., ESTRADA, D., ORJUELA, M., GUALTERO, S., ERICSON, B. & RIVERA, A. 2014. Blood Lead Levels in Mexico and Pediatric Burden of Disease Implications. *Annals of global health,* 80**,** 269-277.
CARMOUCHE, J. J., PUZAS, J. E., ZHANG, X., TIYAPATANAPUTI, P., CORY-SLECHTA, D. A., GELEIN, R., ZUSCIK, M., ROSIER, R. N., BOYCE, B. F. & O'KEEFE, R. J. 2005. Lead exposure inhibits fracture healing and is associated with increased chondrogenesis, delay in cartilage mineralization, and a decrease in osteoprogenitor frequency. *Environmental Health Perspectives***,** 749-755.
CARRIZALES, L., RAZO, I., TÉLLEZ-HERNÁNDEZ, J. I., TORRES-NERIO, R., TORRES, A., BATRES, L. E., CUBILLAS, A.-C. & DÍAZ-BARRIGA, F. 2006. Exposure to arsenic and lead of children living near a copper-smelter in San Luis Potosi, Mexico: Importance of soil contamination for exposure of children. *Environmental Research,* 101**,** 1-10.
CDC. 1991. *Preventing Lead Poisoning in Young Children. A Statement by the Centers for Disease Control* [Online]. ERIC Clearinghouse. Available: http://www.cdc.gov/nceh/lead/publications/books/plpyc/contents.htm [Accessed 06/02/2014].
CDC 2001. Blood Lead Levels and Risk Factors for Lead Poisoning Among Children in Torreón, Coahuila, Mexico. Citeseer.
CDC. 2015. *Sources of lead: Lead jewerly* [Online]. Available: http://www.cdc.gov/nceh/lead/tips/jewelry.htm [Accessed 02/03/2016].
CDTSC. 2007. *LeadSpread 7* [Online]. Available: http://www.dtsc.ca.gov/AssessingRisk/leadspread7.cfm [Accessed 01/07/2013].
CHANDRAMOULI, K., STEER, C. D., ELLIS, M. & EMOND, A. M. 2009. Effects of early childhood lead exposure on academic performance and behaviour of school age children. *Archives of Disease in Childhood,* 94**,** 844-848.
CHANEY, R. L., MIELKE, H. W. & STERRETT, S. B. 1989. Speciation, mobility and bioavailability of soil lead. *Environ. Geochem. Health,* 11**,** 105-129.
CHATHAM-STEPHENS, K., CARAVANOS, J., ERICSON, B., LANDRIGAN, P. & FULLER, R. 2014. The pediatric burden of disease from lead exposure at toxic waste sites in low and middle income countries. *Environmental Research,* 132**,** 379-383.
CHEN, L., XU, Z., LIU, M., HUANG, Y., FAN, R., SU, Y., HU, G., PENG, X. & PENG, X. 2012. Lead exposure assessment from study near a lead-acid battery factory in China. *Science of The Total Environment,* 429**,** 191-198.
CHIA, K. S., JEYARATNAM, J., TAN, C., ONG, H. Y., ONG, C. N. & LEE, E. 1995. Glomerular function of lead-exposed workers. *Toxicology Letters,* 77**,** 319-328.
CHOI, Y.-H., HU, H., MUKHERJEE, B., MILLER, J. & PARK, S. K. 2012. Environmental cadmium and lead exposures and hearing loss in US adults: the National Health and Nutrition Examination Survey, 1999 to 2004. *Environmental Health Perspectives,* 120**,** 1544.
CLARK, C. S., RAMPAL, K. G., THUPPIL, V., CHEN, C. K., CLARK, R. & RODA, S. 2006. The lead content of currently available new residential paint in several Asian countries. *Environmental Research,* 102**,** 9-12.
CLARK, C. S., RAMPAL, K. G., THUPPIL, V., RODA, S. M., SUCCOP, P., MENRATH, W., CHEN, C. K., ADEBAMOWO, E. O., AGBEDE, O. A.,

SRIDHAR, M. K. C., ADEBAMOWO, C. A., ZAKARIA, Y., EL-SAFTY, A., SHINDE, R. M. & YU, J. 2009. Lead levels in new enamel household paints from Asia, Africa and South America. *Environmental Research,* 109**,** 930-936.
CLARK, M. T. 2000. *Paint and pigments* [Online]. Available: http://nzic.org.nz/ChemProcesses/polymers/10D.pdf [Accessed 09/04/2015].
COCCO, P., DOSEMECI, M. & HEINEMAN, E. F. 1998. Brain cancer and occupational exposure to lead. *Journal of Occupational and Environmental Medicine,* 40**,** 937-942.
CORNELIS, C., BERGHMANS, P., SPRUNDEL, M. & AUWERA, J. 2006. The use of the IEUBK model for determination of exposure routes in view of site remediation. *Human and Ecological Risk Assessment,* 12**,** 963 - 982.
COSTA DE ALMEIDA, G. R., DE FREITAS TAVARES, C. F., DE SOUZA, A. M., SAMPAIO DE SOUSA, T., RODRIGUES FUNAYAMA, C. A., BARBOSA JR, F., TANUS-SANTOS, J. E. & GERLACH, R. F. 2010. Whole blood, serum, and saliva lead concentrations in 6- to 8-year-old children. *Science of The Total Environment,* 408**,** 1551-1556.
CPSC. 2008. *Total lead content* [Online]. Available: http://www.cpsc.gov/en/Business--Manufacturing/Business-Education/Lead/Total-Lead-Content/ [Accessed 15/09/2014].
CPSC. 2011. Standard Operating Procedure for Determining Lead (Pb) in Paint and Other Similar Surface Coatings. Available: http://www.cpsc.gov/about/cpsia/CPSC-CH-E1003-09_1.pdf [Accessed 29/1/2012].
CRUMP, K., ALLEN, B. & FAUSTMAN, E. 1995. The Use of the Benchmark dose approach in health risk assessment.
CUADRADO, C., KUMPULAINEN, J., CARBAJAL, A. & MOREIRAS, O. 2000. Cereals Contribution to the Total Dietary Intake of Heavy Metals in Madrid, Spain. *Journal of Food Composition and Analysis,* 13**,** 495-503.
CUS 2004. Playing With Poison Lead Poisoning Hazards of Children's Product Recalls 1990 - 2004.
DANIEL, W. W. 1995. Biostatistics: a foundation for analysis in the health sciences.
DARTEY, E., ADIMADO, A. & AGYARKO, K. 2010. Evaluation of airborne lead levels in storage battery workshops and some welding environments in Kumasi metropolis in Ghana. *Environmental Monitoring and Assessment,* 164**,** 1-8.
DAVIES, B. E. & WHITE, H. M. 1981. Environmental pollution by wind blown lead mine waste: A case study in wales, U.K. *Science of The Total Environment,* 20**,** 57-74.
DE ALMEIDA, G. R. C., DE FREITAS, C. U., BARBOSA, F., TANUS-SANTOS, J. E. & GERLACH, R. F. 2009. Lead in saliva from lead-exposed and unexposed children. *Science of The Total Environment,* 407**,** 1547-1550.
DE FREITAS, C. U., DE CAPITANI, E. M., GOUVEIA, N., SIMONETTI, M. H., DE PAULA E SILVA, M. R., KIRA, C. S., SAKUMA, A. M., DE FÁTIMA HENRIQUES CARVALHO, M., DURAN, M. C., TIGLEA, P. & DE ABREU, M. H. 2007. Lead exposure in an urban community: Investigation of risk factors and assessment of the impact of lead abatement measures. *Environmental Research,* 103**,** 338-344.
DE KORT, W., VERSCHOOR, M., WIBOWO, A. & VAN HEMMEN, J. 1987. Occupational exposure to lead and blood pressure: a study in 105 workers. *American Journal of Industrial Medicine,* 11**,** 145-156.
DE MEJÍA, E. G. & CRAIGMILL, A. 1996. Transfer of lead from lead-glazed ceramics to food. *Archives of Environmental Contamination and Toxicology,* 31**,** 581-584.

DELVES, H. 1996. Blood collection for screening children for exposure to lead. *Clinical chemistry,* 42**,** 983-985.
DEMAIO, T. J. 1980. Refusals: Who, where and why. *Public Opinion Quarterly,* 44**,** 223-233.
DIETERT, R. & PIEPENBRINK, M. 2006. Lead and Immune Function. *Critical Reviews in Toxicology,* 36**,** 359–385.
DIETERT, R. R., LEE, J.-E., HUSSAIN, I. & PIEPENBRINK, M. 2004. Developmental immunotoxicology of lead. *Toxicology and Applied Pharmacology,* 198**,** 86-94.
DIXON, S. L., GAITENS, J. M., JACOBS, D. E., STRAUSS, W., NAGARAJA, J., PIVETZ, T., WILSON, J. W. & ASHLEY, P. J. 2008. Exposure of U.S. Children to Residential Dust Lead, 1999–2004: II. The Contribution of Lead-Contaminated Dust to Children's Blood Lead Levels. *Environ Health Perspect,* 117.
DOOYEMA, C. A., NERI, A., LO, Y.-C., DURANT, J., DARGAN, P. I., SWARTHOUT, T., BIYA, O., GIDADO, S. O., HALADU, S. & SANI-GWARZO, N. 2012. Outbreak of fatal childhood lead poisoning related to artisanal gold mining in northwestern Nigeria, 2010. *Environmental Health Perspectives,* 120**,** 601.
DORMAN, D. 2012. National Research Council Report: Potential Health Risks to DoD Firing-Range Personnel from Recurrent Lead Exposure. The National Academies Press, Washington, DC.
DORSEY, C. D., LEE, B.-K., BOLLA, K. I., WEAVER, V. M., LEE, S.-S., LEE, G.-S., TODD, A. C., SHI, W. & SCHWARTZ, B. S. 2006. Comparison of patella lead with blood lead and tibia lead and their associations with neurobehavioral test scores. *Journal of Occupational and Environmental Medicine,* 48**,** 489-496.
EDWARDS, M., TRIANTAFYLLIDOU, S. & BEST, D. 2009. Elevated Blood Lead in Young Children Due to Lead-Contaminated Drinking Water: Washington, DC, 2001−2004. *Environmental Science & Technology,* 43**,** 1618-1623.
EFSA 2010. Scientific Opinion on Lead in Food. *EFSA Journal* 8(4):1570**,** 1570 -
ELWOOD, P. C. 1986. The sources of lead in blood: A critical review. *Science of The Total Environment,* 52**,** 1-23.
EPA. 2011. Risk Characterization. Available: http://www.epa.gov/region8/r8risk/hh_risk.html [Accessed 28/10/2011].
ERCAL, N., GURER-ORHAN, H. & AYKIN-BURNS, N. 2001. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current topics in medicinal chemistry,* 1**,** 529-539.
ESTEBAN, E. & RUBIN, C. H. 1999. Hair and Blood as Substrates for Screening Children for Lead Poisoning. *Archives of Environmental Health,* 54**,** 436.
FAO/WHO 2005. Dietary exposure Assessment Of chemicals In food.
FARFEL, M. R., LEES, P. S. J., ROHDE, C. A., LIM, B. S., BANNON, D. & CHISOLM, J. J. 1994. Comparison of a Wipe and a Vacuum Collection Method for the Determination of Lead in Residential Dusts. *Environmental Research,* 65**,** 291-301.
FELS, L. M., WÜNSCH, M., BARANOWSKI, J., NORSKA-BORÓWKA, I., PRICE, R. G., TAYLOR, S. A., PATEL, S., DE BROE, M., ELSEVIER, M. M. & LAUWERYS, R. 1998. Adverse effects of chronic low level lead exposure on kidney function--a risk group study in children. *Nephrology Dialysis Transplantation,* 13**,** 2248-2256.
FERGUSSON, J. E. 1986. Lead: Petrol lead in the environment and its contribution to human blood lead levels. *Science of The Total Environment,* 50**,** 1-54.
FERTMANN, R., HENTSCHEL, S., DENGLER, D., JANßEN, U. & LOMMEL, A. 2004. Lead exposure by drinking water: an epidemiologial study in Hamburg, Germany. *International Journal of Hygiene and Environmental Health,* 207**,** 235-244.

FEWTRELL, L., PRÜSS-ÜSTÜN, A., LANDRIGAN, P. & AYUSO-MATEOS, J. 2004. Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. *Environmental Research,* 94**,** 120-133.

FILIPPELLI, G. M. & LAIDLAW, M. A. 2010. The elephant in the playground: Confronting lead-contaminated soils as an important source of lead burdens to urban populations. *Perspectives in biology and medicine,* 53**,** 31-45.

FILIPSON, A. F., SAND, S., NILSSON, J. & VICTORIN, K. 2003. The Benchmark Dose Method -- Review of Available Models, and Recommendations for Application in Health Risk Assessment. *Critical Reviews in Toxicology,* 33**,** 505-542.

FORST, L. S., FREELS, S. & PERSKY, V. 1997. Occupational lead exposure and hearing loss. *Journal of Occupational and Environmental Medicine,* 39**,** 658-660.

FOSTER, W. G. 1992. Reproductive toxicity of chronic lead exposure in the female cynomolgus monkey. *Reproductive Toxicology,* 6**,** 123-131.

FREEMAN, G., JOHNSON, J., KILLINGER, J., LIAO, S., FEDER, P., DAVIS, A., RUBY, M., CHANEY, R., LOVRE, S. & BERGSTROM, P. 1992. Relative bioavailability of lead from mining waste soil in rats. *Fundamental and Applied Toxicology,* 19**,** 388-398.

FRIEDMAN, L. S., LUKYANOVA, O. M., KUNDIEV, Y. I., SHKIRYAK-NIZHNYK, Z. A., CHISLOVSKA, N. V., MUCHA, A., ZVINCHUK, A. V., OLIYNYK, I. & HRYHORCZUK, D. 2005. Predictors of elevated blood lead levels among 3-year-old Ukrainian children: A nested case-control study. *Environmental Research,* 99**,** 235-242.

FRYER, M., COLLINS, C. D., FERRIER, H., COLVILE, R. N. & NIEUWENHUIJSEN, M. J. 2006. Human exposure modelling for chemical risk assessment: a review of current approaches and research and policy implications. *Environmental Science & Policy,* 9**,** 261-274.

FU, H. & BOFFETTA, P. 1995. Cancer and occupational exposure to inorganic lead compounds: a meta-analysis of published data. *Occupational and Environmental Medicine,* 52**,** 73-81.

GARCÍA-RICO, L., LEYVA-PEREZ, J. & JARA-MARINI, M. E. 2007. Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico. *Food and Chemical Toxicology,* 45**,** 1599-1605.

GAYLOR, D. W. 1992. Incidence of developmental defects at the no observed adverse effect level (NOAEL). *Regulatory Toxicology and Pharmacology,* 15, Part 2**,** 151-160.

GEMMEL, A., TAVARES, M., ALPERIN, S., SONCINI, J., DANIEL, D., DUNN, J., CRAWFORD, S., BRAVEMAN, N., CLARKSON, T. W. & MCKINLAY, S. 2002. Blood lead level and dental caries in school-age children. *Environmental Health Perspectives,* 110**,** A625.

GEPHART, L. A., SALMINEN, W. F., NICOLICH, M. J. & PELEKIS, M. 2001. Evaluation of Subchronic Toxicity Data Using the Benchmark Dose Approach. *Regulatory Toxicology and Pharmacology,* 33**,** 37-59.

GERBER, G. B., LÉONARD, A. & JACQUET, P. 1980. Toxicity, mutagenicity and teratogenicity of lead. *Mutation Research/Reviews in Genetic Toxicology,* 76**,** 115-141.

GERSBERG, R. M., GAYNOR, K., TENCZAR, D., BARTZEN, M., GINSBERG, M., GRESHAM, L. S. & MOLGAARD, C. 1997. Quantitative modeling of lead exposure from glazed ceramic pottery in childhood lead poisoning cases. *International Journal of Environmental Health Research,* 7**,** 193-202.

GILBERT, S. G. & WEISS, B. 2006. A rationale for lowering the blood lead action level from 10 to 2 μg/dL. *NeuroToxicology,* 27**,** 693-701.
GODWIN, H. A. 2001. The biological chemistry of lead. *Current opinion in chemical biology,* 5**,** 223-227.
GOERING, P. 1992. Lead-protein interactions as a basis for lead toxicity. *NeuroToxicology,* 14**,** 45-60.
GORELL, J., JOHNSON, C., RYBICKI, B., PETERSON, E., KORTSHA, G., BROWN, G. & RICHARDSON, R. 1997. Occupational exposures to metals as risk factors for Parkinson's disease. *Neurology,* 48**,** 650-658.
GOTTESFELD, P., KUEPOUO, G., TETSOPGANG, S. & DURAND, K. 2013. Lead concentrations and labeling of new paint in Cameroon. *Journal of occupational and environmental hygiene,* 10**,** 243-249.
GOYER, R. 1989. Mechanisms of lead and cadmium nephrotoxicity. *Toxicology Letters,* 46**,** 153-162.
GOYER, R. A. 1990. Lead toxicity: from overt to subclinical to subtle health effects. *Environmental Health Perspectives,* 86**,** 177.
GOYER, R. A. 1993. Lead toxicity: current concerns. *Environmental Health Perspectives,* 100**,** 177.
GRABER, L. K., ASHER, D., ANANDARAJA, N., BOPP, R. F., MERRILL, K., CULLEN, M. R., LUBOGA, S. & TRASANDE, L. 2010. Childhood lead exposure after the phaseout of leaded gasoline: an ecological study of school-age children in Kampala, Uganda. *Environmental Health Perspectives,* 118**,** 884.
GRAVES, A., VAN DUIJN, C., CHANDRA, V., FRATIGLIONI, L., HEYMAN, A., JORM, A., KOKMEN, E., KONDO, K., MORTIMER, J. & ROCCA, W. A. 1991. Occupational exposures to solvents and lead as risk factors for Alzheimer's disease: a collaborative re-analysis of case-control studies. *International Journal of Epidemiology,* 20**,** S58-S61.
GREENWAY, J. A. & GERSTENBERGER, S. 2010. An evaluation of lead contamination in plastic toys collected from day care centers in the Las Vegas Valley, Nevada, USA. *Bulletin of Environmental Contamination and Toxicology,* 85**,** 363-366.
GULSON, B. L. 1996. Nails: concern over their use in lead exposure assessment. *Science of The Total Environment,* 177**,** 323-327.
GULSON, B. L. & DAVIS, J. J. 1994. Lead Bioavailability in the Environment of Children: Blood Lead Levels in Children Can Be Elevated in a Mining Community. *Archives of Environmental Health,* 49**,** 326-331.
GULSON, B. L., DAVIS, J. J. & BAWDEN-SMITH, J. 1995. Paint as a source of recontamination of houses in urban environments and its role in maintaining elevated blood leads in children. *Science of The Total Environment,* 164**,** 221-235.
GULSON, B. L., JAMES, M., GIBLIN, A. M., SHEEHAN, A. & MITCHELL, P. 1997. Maintenance of elevated lead levels in drinking water from occasional use and potential impact on blood leads in children. *Science of The Total Environment,* 205**,** 271-275.
GULSON, B. L., MAHAFFEY, K. R., JAMESON, C. W., MIZON, K. J., KORSCH, M. J., CAMERON, M. A. & EISMAN, J. A. 1998. Mobilization of lead from the skeleton during the postnatal period is larger than during pregnancy. *Journal of Laboratory and Clinical Medicine,* 131**,** 324-329.
GULSON, B. L., MIZON, K. J., DAVIS, J. D., PALMER, J. M. & VIMPANI, G. 2004. Identification of sources of lead in children in a primary zinc-lead smelter environment. *Environmental Health Perspectives,* 112**,** 52.

GUNEY, M. & ZAGURY, G. J. 2014. Bioaccessibility of As, Cd, Cu, Ni, Pb, and Sb in toys and low-cost jewelry. *Environmental Science & Technology,* 48**,** 1238-1246.
HA, M., KWON, H.-J., LIM, M.-H., JEE, Y.-K., HONG, Y.-C., LEEM, J.-H., SAKONG, J., BAE, J.-M., HONG, S.-J. & ROH, Y.-M. 2009. Low blood levels of lead and mercury and symptoms of attention deficit hyperactivity in children: a report of the children's health and environment research (CHEER). *NeuroToxicology,* 30**,** 31-36.
HAIDER, T., HAIDER, M., WRUSS, W., SOMMER, R. & KUNDI, M. 2002. Lead in drinking water of Vienna in comparison to other European countries and accordance with recent guidelines. *International Journal of Hygiene and Environmental Health,* 205**,** 399-403.
HALLÉN, I. P. & OSKARSSON, A. 1995. Bioavailability of lead from various milk diets studied in a suckling rat model. *Biometals,* 8**,** 231-236.
HAMMAD, T. A., SEXTON, M. & LANGENBERG, P. 1996. Relationship between blood lead and dietary iron intake in preschool children: A cross-sectional study. *Annals of Epidemiology,* 6**,** 30-33.
HAYES, E. B. M. M. D. 1994. Long-term trends in blood lead levels among children in Chicago: Relationship to air lead levels. *Pediatrics,* 93**,** 195.
HE, L., POBLENZ, A. T., MEDRANO, C. J. & FOX, D. A. 2000. Lead and calcium produce rod photoreceptor cell apoptosis by opening the mitochondrial permeability transition pore. *Journal of Biological Chemistry,* 275**,** 12175-12184.
HEARD, M. J., CHAMBERLAIN, A. C. & SHERLOCK, J. C. 1983. Uptake of lead by humans and effect of minerals and food. *Science of The Total Environment,* 30**,** 245-253.
HERBERT, N. 2009. Low Level Lead Exposure: History and Discovery. *Annals of Epidemiology,* 19**,** 235-238.
HERNBERG, S., NURMINEN, M. & HASAN, J. 1967. Nonrandom shortening of red cell survival times in men exposed to lead. *Environmental Research,* 1**,** 247-261.
HERRMAN, J. L. & YOUNES, M. 1999. Background to the ADI/TDI/PTWI. *Regulatory Toxicology and Pharmacology,* 30**,** S109-S113.
HODGES, D. & SKELDING, D. 1981. Determination of lead in urine by atomic-absorption spectroscopy with electrothermal atomisation. *Analyst,* 106**,** 299-304.
HU, H., ARO, A., PAYTON, M., KORRICK, S., SPARROW, D., WEISS, S. T. & ROTNITZKY, A. 1996. The relationship of bone and blood lead to hypertension: the Normative Aging Study. *Jama,* 275**,** 1171-1176.
IAVICOLI, I., CARELLI, G., STANEK, E., CASTELLINO, N. & CALABRESE, E. 2003. Effects of low doses of dietary lead on red blood cell production in male and female mice. *Toxicology Letters,* 137**,** 193-199.
IDPH. 2011. Lead Screening and Case Follow-up Guidelines for Local Health Departments. Available: http://www.idph.state.il.us/envhealth/Lead_ScreeningGuidelinesForLHDs.pdf.
IHEDIOHA, J. & OKOYE, C. 2013. Dietary intake and health risk assessment of lead and cadmium via consumption of cow meat for an urban population in Enugu State, Nigeria. *Ecotoxicology and Environmental Safety*.
IPCS. 1977. *Environmental Health Criteria 3: Lead* [Online]. Available: http://www.inchem.org/documents/ehc/ehc/ehc003.htm [Accessed 03/03/2016].
IPCS. 1995. INORGANIC LEAD. *ENVIRONMENTAL HEALTH CRITERIA* [Online], 165. Available: http://www.bvsde.ops-oms.org/bvsacg/e/cd-cagua/guias/b.parametos/4.BasTox/IPCS/076b.pdf [Accessed 6/11/2011].

ISIDRA, H.-S. M., ROSALBA, R.-M., CARLOS, G.-G., HULME, J. M. & GUSTAVO, O.-F. 2003. Factors associated with lead exposure in Oaxaca, Mexico. *Journal of Exposure Science and Environmental Epidemiology,* 13**,** 341-347.

JAAKO-MOVITS, K., ZHARKOVSKY, T., ROMANTCHIK, O., JURGENSON, M., MERISALU, E., HEIDMETS, L.-T. & ZHARKOVSKY, A. 2005. Developmental lead exposure impairs contextual fear conditioning and reduces adult hippocampal neurogenesis in the rat brain. *International journal of developmental neuroscience,* 23**,** 627-635.

JAIN, N. B., LADEN, F., GULLER, U., SHANKAR, A., KAZANI, S. & GARSHICK, E. 2005. Relation between Blood Lead Levels and Childhood Anemia in India. *American Journal of Epidemiology,* 161**,** 968-973.

JANG, J.-Y., JO, S.-N., KIM, S.-J., MYUNG, H.-N. & KIM, C.-I. 2014. Food Ingestion Factors of the Korean Exposure Factors Handbook. *Journal of Preventive Medicine and Public Health,* 47**,** 18.

JAROSIŃSKA, D., BIESIADA, M. & MUSZYŃSKA-GRACA, M. 2006. Environmental burden of disease due to lead in urban children from Silesia, Poland. *Science of The Total Environment,* 367**,** 71-79.

JECFA 2004. Codex Alimentarius: Code of practice for the prevention and reduction of lead contamination in foods.

JECFTA. 1972. EVALUATION OF MERCURY, LEAD, CADMIUM AND THE FOOD ADDITIVES AMARANTH, DIETHYLPYROCARBONATE, AND OCTYL GALLATE. Available: whqlibdoc.who.int/trs/WHO_TRS_505.pdf [Accessed 7/11/2011].

KALAČ, P. & STAŠKOVÁ, I. 1991. Concentrations of lead, cadmium, mercury and copper in mushrooms in the vicinity of a lead smelter. *Science of The Total Environment,* 105**,** 109-119.

KAPKA, L., BAUMGARTNER, A., SIWIŃSKA, E., KNUDSEN, L. E., ANDERSON, D. & MIELŻYŃSKA, D. 2007. Environmental lead exposure increases micronuclei in children. *Mutagenesis,* 22**,** 201-207.

KAUFMAN, A. S. 2001. Do low levels of lead produce IQ loss in children? A careful examination of the literature. *Archives of Clinical Neuropsychology,* 16**,** 303-341.

KEETER, S., MILLER, C., KOHUT, A., GROVES, R. M. & PRESSER, S. 2000. Consequences of reducing nonresponse in a national telephone survey. *Public Opinion Quarterly,* 64**,** 125-148.

KHOURY, G. A. & DIAMOND, G. L. 2003. Risks to children from exposure to lead in air during remedial or removal activities at Superfund sites: A case study of the RSR lead smelter Superfund site†. *Journal of Exposure Science and Environmental Epidemiology,* 13**,** 51-65.

KIRKBY, H. & GYNTELBERG, F. 1985. Blood pressure and other cardiovascular risk factors of long-term exposure to lead. *Scandinavian journal of work, environment & health***,** 15-19.

KOSTIAL, K. & KELLO, D. 1979. Bioavailability of lead in rats fed "human" diets. *Bulletin of Environmental Contamination and Toxicology,* 21**,** 312-314.

KOSTIAL, K., SIMONOVIC, I. & PISONIC, M. 1971. Lead absorption from the intestine in newborn rats. *Nature,* 233**,** 564.

KRANZ, B. D., SIMON, D. L. & LEONARDI, B. G. 2004. The behavior and routes of lead exposure in pregrasping infants. *Journal of Exposure Science and Environmental Epidemiology,* 14**,** 300-311.

KRAUSE, P., BOYLE, D. & BÄSE, F. 2005. Comparison of different efficiency criteria for hydrological model assessment. *Advances in Geosciences,* 5**,** 89-97.

KROES, R., MÜLLER, D., LAMBE, J., LÖWIK, M. R. H., VAN KLAVEREN, J., KLEINER, J., MASSEY, R., MAYER, S., URIETA, I., VERGER, P. & VISCONTI, A. 2002. Assessment of intake from the diet. *Food and Chemical Toxicology,* 40**,** 327-385.

KUMAR, A. & PASTORE, P. 2007. Lead and cadmium in soft plastic toys. *CURRENT SCIENCE,* 93**,** 818 -.

KUMAR, A. & SCOTT CLARK, C. 2009. Lead loadings in household dust in Delhi, India. *Indoor Air,* 19**,** 414-420.

KWON, S. O., HWANG, H., AN, H. & OH, S. 2012. Dietary exposure of lead, cadmium and mercury in Korean adults. *The FASEB Journal,* 26**,** lb409.

KWONG, W. T., FRIELLO, P. & SEMBA, R. D. 2004. Interactions between iron deficiency and lead poisoning: epidemiology and pathogenesis. *Science of The Total Environment,* 330**,** 21-37.

LACEY, R. F., MOORE, M. R. & RICHARDS, W. N. 1985. Lead in water, infant diet and blood: The Glasgow duplicate diet study. *Science of The Total Environment,* 41**,** 235-257.

LAKIND, J. S. 1998. Comparison of three models for predicting blood lead levels in children: episodic exposures to lead. *Journal of exposure analysis and environmental epidemiology,* 8**,** 399.

LAMOLA, A. A. & YAMANE, T. 1975. Zinc protoporphyrin (ZPP): A simple, sensitive, fluorometric screening test for lead poisioning. *Clinical chemistry,* 21**,** 93-97.

LANDRIGAN, P., NORDBERG, M., LUCCHINI, R., NORDBERG, G., GRANDJEAN, P., IREGREN, A. & ALESSIO, L. 2007. The Declaration of Brescia on prevention of the neurotoxicity of metals. *American Journal of Industrial Medicine,* 50**,** 709.

LANDRIGAN, P. J. 1989. Toxicity of Lead at Low Dose. *British Journal of Industrial Medicine,* 46**,** 593-596.

LANDRIGAN, P. J., BOFFETTA, P. & APOSTOLI, P. 2000. The reproductive toxicity and carcinogenicity of lead: a critical review. *American Journal of Industrial Medicine,* 38**,** 231-243.

LANDRIGAN, P. J., SCHECHTER, C. B., LIPTON, J. M., FAHS, M. C. & SCHWARTZ, J. 2002. Environmental pollutants and disease in American children: estimates of morbidity, mortality, and costs for lead poisoning, asthma, cancer, and developmental disabilities. *Environmental Health Perspectives,* 110**,** 721.

LANDRIGAN, P. J., SONAWANE, B., BUTLER, R. N., TRASANDE, L., CALLAN, R. & DROLLER, D. 2005. Early environmental origins of neurodegenerative disease in later life. *Environmental Health Perspectives***,** 1230-1233.

LANDRIGAN, P. J. & TODD, A. C. 1994. Lead poisoning. *Western Journal of Medicine,* 161**,** 153.

LANPHEAR, B. P., MATTE, T. D., ROGERS, J., CLICKNER, R. P., DIETZ, B., BORNSCHEIN, R. L., SUCCOP, P., MAHAFFEY, K. R., DIXON, S., GALKE, W., RABINOWITZ, M., FARFEL, M., ROHDE, C., SCHWARTZ, J., ASHLEY, P. & JACOBS, D. E. 1998. The Contribution of Lead-Contaminated House Dust and Residential Soil to Children's Blood Lead Levels: A Pooled Analysis of 12 Epidemiologic Studies. *Environmental Research,* 79**,** 51-68.

LANPHEAR, B. P. & ROGHMANN, K. J. 1997. Pathways of Lead Exposure in Urban Children. *Environmental Research,* 74**,** 67-73.

LEE, H.-S., CHO, Y.-H., PARK, S.-O., KYE, S.-H., KIM, B.-H., HAHM, T.-S., KIM, M., OK LEE, J. & KIM, C.-I. 2006. Dietary exposure of the Korean population to arsenic, cadmium, lead and mercury. *Journal of Food Composition and Analysis,* 19, Supplement**,** S31-S37.

LEWIS, R., FORTMANN, R. & CAMANN, D. 1994. Evaluation of methods for monitoring the potential exposure of small children to pesticides in the residential environment. *Archives of Environmental Contamination and Toxicology,* 26**,** 37-46.
LEZAK, M. D. 2004. *Neuropsychological assessment*, Oxford university press.
LI, T., DAI, Y.-H., XIE, X.-H., TAN, Z.-W., ZHANG, S.-M. & ZHU, Z.-H. 2014a. Surveillance of childhood blood lead levels in 11 cities of China. *World Journal of Pediatrics,* 10**,** 29-37.
LI, Y., HU, J., WU, W., LIU, S., LI, M., YAO, N., CHEN, J., YE, L., WANG, Q. & ZHOU, Y. 2016. Application of IEUBK model in lead risk assessment of children aged 61–84months old in central China. *Science of The Total Environment,* 541**,** 673-682.
LI, Y., WU, S., XIANG, Y. & LIANG, X. 2014b. An Investigation of Outpatient Children's Blood Lead Level in Wuhan China. *PloS one,* 9**,** e95284.
LIANG, F., ZHANG, G., TAN, M., YAN, C., LI, X., LI, Y., LI, Y., ZHANG, Y. & SHAN, Z. 2010. Lead in children's blood is mainly caused by coal-fired ash after phasing out of leaded gasoline in Shanghai. *Environmental Science & Technology,* 44**,** 4760-4765.
LILIENTHAL, H., KOHLER, K., TURFELD, M. & WINNEKE, G. 1994. Persistent Increases in Scotopic B-wave Amplitudes After Lead Exposure in Monkeys. *Experimental Eye Research,* 59**,** 203-209.
LIN, J.-L., TAN, D.-T., HSU, K.-H. & YU, C.-C. 2001. Environmental lead exposure and progressive renal insufficiency. *Archives of Internal Medicine,* 161**,** 264-271.
LIU, D. 1994. Review of mathematical models for health risk assessment: VII. chemical dose. *Environmental Software,* 9**,** 153-160.
LO, Y.-C., DOOYEMA, C. A., NERI, A., DURANT, J., JEFFERIES, T., MEDINA-MARINO, A., DE RAVELLO, L., THOROUGHMAN, D., DAVIS, L. & DANKOLI, R. S. 2012. Childhood lead poisoning associated with gold ore processing: a village-level investigation—Zamfara State, Nigeria, October–November 2010. *Environmental Health Perspectives,* 120**,** 1450.
LOCKITCH, G. 1993. Perspectives on lead toxicity. *Clinical Biochemistry,* 26**,** 371-381.
LOWRY, J. A. 2010. Oral chelation therapy for patients with lead poisoning. *Am Acad Pediatr,* 116**,** 1036-1046.
LU, F. C. & SIELKEN JR, R. L. 1991. Assessment of safety/risk of chemicals: inception and evolution of the ADI and dose-response modeling procedures. *Toxicology Letters,* 59**,** 5-40.
LUCONI, M. O., OLSINA, R. A., FERNÁNDEZ, L. P. & SILVA, M. F. 2006. Determination of lead in human saliva by combined cloud point extraction–capillary zone electrophoresis with indirect UV detection. *Journal of Hazardous Materials,* 128**,** 240-246.
LYNCH, R. A., BOATRIGHT, D. T. & MOSS, S. K. 2000. Lead-Contaminated Imported Tamarind Candy and Children s Blood Lead Levels. *PUBLIC HEALTH REPORTS* 115**,** 537-543.
MADISE, N. J. & MPOMA, M. 1997. Child malnutrition and feeding practices in Malawi. *FOOD AND NUTRITION BULLETIN-UNITED NATIONS UNIVERSITY-*, 18**,** 190-201.
MADUABUCHI, J.-M., NZEGWU, C., ADIGBA, E., ALOKE, R., EZOMIKE, C., OKOCHA, C., OBI, E. & ORISAKWE, O. 2006. Lead and cadmium exposures from canned and non-canned beverages in Nigeria: a public health concern. *Science of The Total Environment,* 366**,** 621-626.

MAHAFFEY, K. R. 1998. Predicting Blood Lead Concentrations from Lead in Environmental Media. *Environmental Health Perspectives* 106**,** 1485 - 1493.
MAHAFFEY, K. R., GARTSIDE, P. S. & GLUECK, C. J. 1986. Blood lead levels and dietary calcium intake in 1-to 11-year-old children: the Second National Health and Nutrition Examination Survey, 1976 to 1980. *Pediatrics,* 78**,** 257-262.
MAHESWARAN, R., GILL, J. S. & BEEVERS, D. G. 1993. Blood pressure and industrial lead exposure. *American Journal of Epidemiology,* 137**,** 645-653.
MAKOKHA, A. O., MGHWENO, L. R., MAGOHA, H. S., NAKAJUGO, A. & WEKESA, J. M. 2008. Environmental lead pollution and contamination in food around Lake Victoria, Kisumu, Kenya. *African Journal of Environmental Science and Technology,* 2**,** 349-353.
MALCOE, L. H., LYNCH, R. A., KEGLER, M. C. & SKAGGS, V. J. 2002. Lead Sources, Behaviors, and Socioeconomic Factors in Relation to Blood Lead of Native American and White Children: A Community-Based Assessment of a Former Mining Area. *Environ Health Perspect,* 110.
MARJORIE, S. 1985. Intellectual and behavioural consequences of low level lead exposure: A review of recent studies. *Clinics in Endocrinology and Metabolism,* 14**,** 657-680.
MASON, L. H., HARP, J. P. & HAN, D. Y. 2014. Pb neurotoxicity: neuropsychological effects of lead toxicity. *BioMed research international,* 2014.
MATHEE, A., KHAN, T., NAICKER, N., KOOTBODIEN, T., NAIDOO, S. & BECKER, P. 2013. Lead exposure in young school children in South African subsistence fishing communities. *Environmental Research,* 126**,** 179-183.
MATHEE, A., RÖLLIN, H., VON SCHIRNDING, Y., LEVIN, J. & NAIK, I. 2006. Reductions in blood lead levels among school children following the introduction of unleaded petrol in South Africa. *Environmental Research,* 100**,** 319-322.
MATHEE, A., SINGH, E., MOGOTSI, M., TIMOTHY, G., MADUKA, B. & OLIVIER, J. 2009. Lead-based paint on playground equipment in public children's parks in Johannesburg, Tshwane and Ekurhuleni. *SAMJ: South African Medical Journal,* 99**,** 819-821.
MATHEE, A., VON SCHIRNDING, Y. E. R., LEVIN, J., ISMAIL, A., HUNTLEY, R. & CANTRELL, A. 2002. A survey of blood lead levels among young Johannesburg school children. *Environmental Research,* 90**,** 181-184.
MATHERS, C. & WOODWARD, A. 2003. Introduction and methods: Assessing the environmental burden of disease at national and local levels.
MBONGWE, B., BARNES, B., TSHABANG, J., ZHAI, M., RAJARAM, S., MPUCHANE, S. & MATHEE, A. 2005. Exposure to lead among children aged 1-6 years in the city of Gaberone. *Journal of Environmental health Research,* 10**,** 17-26.
MDCH 2004. Paint Chip Sampling Procedure for Lead.
MENEZES-FILHO, J., VIANA, G. & PAES, C. 2011. Determinants of lead exposure in children on the outskirts of Salvador, Brazil. *Environmental Monitoring and Assessment***,** 1-11.
MIRANDA, M. L., KIM, D., GALEANO, M. A., PAUL, C., HULL, A. & MORGAN, S. 2007. The relationship between early childhood blood lead levels and performance on end-of-grade tests. *Environ Health Perspect.,* 115**,** 1242-7.
MIRANDA, M. L., KIM, D., REITER, J., OVERSTREET GALEANO, M. A. & MAXSON, P. 2009. Environmental contributors to the achievement gap. *NeuroToxicology,* 30**,** 1019-1024.

MONNA, F., POUJOL, M., LOSNO, R., DOMINIK, J., ANNEGARN, H. & COETZEE, H. 2006. Origin of atmospheric lead in Johannesburg, South Africa. *Atmospheric Environment,* 40**,** 6554-6566.
MORIASI, D. N., ARNOLD, J. G., VAN LIEW, M. W., BINGNER, R. L., HARMEL, R. D. & VEITH, T. L. 2007. Model evaluation guidelines for systematic quantification of accuracy in watershed simulations. *Transactions of the ASABE,* 50**,** 885-900.
MOSS, M. E., LANPHEAR, B. P. & AUINGER, P. 1999. Association of dental caries and blood lead levels. *Jama,* 281**,** 2294-2298.
MROSS, K. G., ADAM, G. E., NOORT, D. & ZIMMER, J. 2007. Human Exposure Biomarkers-Permethrin as a Militarily-Relevant Model. DTIC Document.
MUCHUWETI, M., BIRKETT, J., CHINYANGA, E., ZVAUYA, R., SCRIMSHAW, M. D. & LESTER, J. 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agriculture, Ecosystems & Environment,* 112**,** 41-48.
MULWARE, S. J. 2013. Trace elements and carcinogenicity: a subject in review. *3 Biotech,* 3**,** 85-96.
MUÑOZ, O., BASTIAS, J. M., ARAYA, M., MORALES, A., ORELLANA, C., REBOLLEDO, R. & VELEZ, D. 2005. Estimation of the dietary intake of cadmium, lead, mercury, and arsenic by the population of Santiago (Chile) using a Total Diet Study. *Food and Chemical Toxicology,* 43**,** 1647-1655.
MUNTNER, P., HE, J., VUPPUTURI, S., CORESH, J. & BATUMAN, V. 2003. Blood lead and chronic kidney disease in the general United States population: results from NHANES III. *Kidney international,* 63**,** 1044-1050.
MURATA, K., IWATA, T., DAKEISHI, M. & KARITA, K. 2009. Lead toxicity: does the critical level of lead resulting in adverse effects differ between adults and children? *Journal of occupational health,* 51**,** 1-12.
MURRAY, C. J. 1994. Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bulletin of the World Health Organization,* 72**,** 429.
MYKKA, H. & WASSERMAN, R. 1981. Gastrointestinal Absorption of Lead (-" Pb) in Chicks: Influence of Lead, Calcium, and Age. *J. Nutr,* 101**,** 1757-1765.
NABULO, G., ORYEM-ORIGA, H. & DIAMOND, M. 2006. Assessment of lead, cadmium, and zinc contamination of roadside soils, surface films, and vegetables in Kampala City, Uganda. *Environmental Research,* 101**,** 42-52.
NAICKER, N., NORRIS, S. A., MATHEE, A., BECKER, P. & RICHTER, L. 2010a. Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: Findings from the Birth to Twenty cohort. *Science of The Total Environment,* 408**,** 4949-4954.
NAICKER, N., NORRIS, S. A., MATHEE, A., VON SCHIRNDING, Y. E. & RICHTER, L. 2010b. Prenatal and adolescent blood lead levels in South Africa: Child, maternal and household risk factors in the Birth to Twenty cohort. *Environmental Research,* 110**,** 355-362.
NAING, L., WINN, T. & RUSLI, B. 2006. Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences,* 1**,** 9-14.
NATION, J. R. & GLEAVES, D. H. 2001. Low-level lead exposure and intelligence in children. *Archives of Clinical Neuropsychology,* 16**,** 375-388.
NAVAS-ACIEN, A., GUALLAR, E., SILBERGELD, E. K. & ROTHENBERG, S. J. 2007. Lead exposure and cardiovascular disease—a systematic review. *Environmental Health Perspectives,* 115**,** 472.

NDUKA, J., ORISAKWE, O. & MADUAWGUNA, C. 2008. Lead levels in paint flakes from buildings in Nigeria: a preliminary study. *Toxicology and industrial health,* 24**,** 539-542.
NEEDLEMAN, H. 2004. Lead poisoning. *Annu. Rev. Med.,* 55**,** 209-222.
NEEDLEMAN, H. L., MCFARLAND, C., NESS, R. B., FIENBERG, S. E. & TOBIN, M. J. 2002. Bone lead levels in adjudicated delinquents: A case control study. *Neurotoxicology and Teratology,* 24**,** 711-717.
NEEDLEMAN, H. L., RIESS, J. A., TOBIN, M. J., BIESECKER, G. E. & GREENHOUSE, J. B. 1996. Bone lead levels and delinquent behavior. *Jama,* 275**,** 363-369.
NEVIN, R. 2000. How lead exposure relates to temporal changes in IQ, violent crime, and unwed pregnancy. *Environmental Research,* 83**,** 1-22.
NEVIN, R. 2007. Understanding international crime trends: the legacy of preschool lead exposure. *Environmental Research,* 104**,** 315-336.
NIELSEN, E., OSTERGAARD, G. & LARSEN, J. C. 2008. *Toxicological risk assessment of chemicals: A practical guide,* CRC Press.
NORMAN, R., MATHEE, A., BARNES, B., VAN DER MERWE, L., BRADSHAW, D. & COLLABORATION, S. A. C. R. A. 2007. Estimating the burden of disease attributable to lead exposure in South Africa in 2000. *South African Medical Journal,* 97**,** 773-780.
NOWACK, R., WIECEK, A., EXNER, B., GRETZ, N. & RITZ, E. 1993. Chronic lead exposure in rats: effects on blood pressure. *European journal of clinical investigation,* 23**,** 433-443.
NRC 1983. Risk Assessment in the Federal Government: Managing the Process.
NRC 1993. Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations.
NRIAGU, J., JINABHAI, C., NAIDOO, R. & COUTSOUDIS, A. 1996. Atmospheric lead pollution in KwaZulu/Natal, South Africa. *Science of The Total Environment,* 191**,** 69-76.
NRIAGU, J., JINABHAI, C. C., NAIDOO, R. & COUTSOUDIS, A. 1997a. Lead poisoning of children in Africa, II. Kwazulu/Natal, South Africa. *Science of The Total Environment,* 197**,** 1-11.
NRIAGU, J., OLERU, N. T., CUDJOE, C. & CHINE, A. 1997b. Lead poisoning of children in Africa, III. Kaduna, Nigeria. *Science of The Total Environment,* 197**,** 13-19.
NSO 2004. Second Integrated Household Survey, 2004: Household characteristics, income and expenditure questionnaire.
NSO 2008. 2008 Population and Housing Census. Zomba: National statistical Office.
NSO 2013. Living Standards measurement study: Note on Conversion Factors for Food Item-Non-standard Measurement Unit Combinations In the Malawi Third Integrated Household Survey (IHS3) 2010/11 Data.
OBI, E., AKUNYILI, D. N., EKPO, B. & ORISAKWE, O. E. 2006. Heavy metal hazards of Nigerian herbal remedies. *Science of The Total Environment,* 369**,** 35-41.
OFLAHERTY, E. J. 1993. Physiologically Based Models for Bone-Seeking Elements: IV. Kinetics of Lead Disposition in Humans. *Toxicology and Applied Pharmacology,* 118**,** 16-29.
OKONKWO, J. & MARIBE, F. 2004. Assessment of Lead Exposure in Thohoyandou, South Africa. *The Environmentalist,* 24**,** 171-178.
OLAIZ, G. & FORTOUL, T. I. 1996. Risk factors for high levels of lead in blood of schoolchildren in Mexico City. *Archives of Environmental Health,* 51**,** 122.

OLIVIER, J., JOHNSON, W. D. & MARSHALL, G. D. 2008. The logarithmic transformation and the geometric mean in reporting experimental IgE results: what are they and when and why to use them? *Annals of Allergy, Asthma & Immunology,* 100**,** 333-337.

OMOLAOYE, J., UZAIRU, A. & GIMBA, C. 2010. Heavy metal assessment of some soft plastic toys imported into Nigeria from China. *Journal of Environmental Chemistry and Ecotoxicology,* 2**,** 126-130.

ONALAJA, A. O. & CLAUDIO, L. 2000. Genetic susceptibility to lead poisoning. *Environmental Health Perspectives,* 108**,** 23.

ORISAKWE, O. E. & NDUKA, J. K. 2009. Lead and cadmium levels of commonly administered pediatric syrups in Nigeria: A public health concern? *Science of The Total Environment,* 407**,** 5993-5996.

ORISAKWE, O. E. & OTARAKU, J. O. 2013. Metal concentrations in cosmetics commonly used in Nigeria. *The Scientific World Journal,* 2013.

OSMAN, K., PAWLAS, K., SCHÜTZ, A., GAZDZIK, M., SOKAL, J. A. & VAHTER, M. 1999. Lead Exposure and Hearing Effects in Children in Katowice, Poland. *Environmental Research,* 80**,** 1-8.

PAPANIKOLAOU, N. C., HATZIDAKI, E. G., BELIVANIS, S., TZANAKAKIS, G. N. & TSATSAKIS, A. M. 2005. Lead toxicity update. A brief review. *Medical science monitor,* 11**,** RA329-RA336.

PARK, S. K., SCHWARTZ, J., WEISSKOPF, M., SPARROW, D., VOKONAS, P. S., WRIGHT, R. O., COULL, B., NIE, H. & HU, H. 2006. Low-level lead exposure, metabolic syndrome, and heart rate variability: the VA Normative Aging Study. *Environmental Health Perspectives***,** 1718-1724.

PARKINSON, D., HODGSON, M., BROMET, E. J., DEW, M. A. & CONNELL, M. M. 1987. Occupational lead exposure and blood pressure. *British Journal of Industrial Medicine,* 44**,** 744-748.

PARSONS, P. & CHISOLM, J. 1997. The Lead laboratory.

PATRICK, L. 2006. Lead Toxicity, A Review of the Literature. Part 1: Exposure, Evaluation, and Treatment. *Alternative Medicine Review,* 11**,** 2-23.

PAUSTENBACH, D. J. 2010. The practice of exposure assessment: a state-of-the-art review. *Journal of Toxicology and Environmental Health, Part B,* 3**,** 179-291.

PEIJNENBURG, W. & JAGER, T. 2003. Monitoring approaches to assess bioaccessibility and bioavailability of metals: matrix issues. *Ecotoxicology and Environmental Safety,* 56**,** 63-77.

PIRKLE, J. L., SCHWARTZ, J., LANDIS, J. R. & HARLAN, W. R. 1985. The relationship between blood lead levels and blood pressure and its cardiovascular risk implications. *American Journal of Epidemiology,* 121**,** 246-258.

POCOCK, S., SHAPER, A., WALKER, M., WALE, C., CLAYTON, B., DELVES, T., LACEY, R., PACKHAM, R. & POWELL, P. 1983. Effects of tap water lead, water hardness, alcohol, and cigarettes on blood lead concentrations. *Journal of epidemiology and community health,* 37**,** 1-7.

POHL, H. R. & ABADIN, H. G. 1995. Utilizing uncertainty factors in minimal risk levels derivation. *Regulatory Toxicology and Pharmacology,* 22**,** 180-188.

PRINCE, M. 1998. Is Chronic Low-Level Lead Exposure in Early Life an Etiologic Factor in Alzheimer's Disease? *Epidemiology,* 9**,** 618-621.

PRÜSS-ÜSTÜN, A., FEWTRELL, L., LANDRIGAN, P. J. & AYUSO-MATEOS, J. L. 2006. Lead exposure. *In:* EZZATI M, AD, L., RODGERS A & CJL, M. (eds.) *Comparative Quantification of Health Risks.*

PRÜSS-ÜSTÜN, A., MATHERS, C., CORVALÁN, C. & WOODWARD, A. 2003a. Assessing the environmental burden of diseaseat national and local levels. *WHO Environmental Burden of Disease Series, No. 1.* Geneva.
PRÜSS-ÜSTÜN, A., MATHERS, C., CORVALÁN, C. & WOODWARD, A. 2003b. Quantifying environmental health impacts. *The Global Burden of Disease concept.* Geneva: World Health Organization.
RABINOWITZ, M. B., KOPPLE, J. D. & WETHERILL, G. W. 1980. Effect of food intake and fasting on gastrointestinal lead absorption in humans. *The American Journal of Clinical Nutrition,* 33**,** 1784-1788.
RABINOWITZ, M. B., WETHERILL, G. W. & KOPPLE, J. D. 1976. Kinetic analysis of lead metabolism in healthy humans. *Journal of Clinical Investigation,* 58**,** 260.
RAHBAR, M. H., WHITE, F., AGBOATWALLA, M., HOZHABRI, S. & LUBY, S. 2002. Factors associated with elevated blood lead concentrations in children in Karachi, Pakistan. *Bulletin of the World Health Organization,* 80**,** 769-775.
RAO, K. S., BALAJI, T., RAO, T. P., BABU, Y. & NAIDU, G. 2002. Determination of iron, cobalt, nickel, manganese, zinc, copper, cadmium and lead in human hair by inductively coupled plasma-atomic emission spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy,* 57**,** 1333-1338.
RAPPAPORT, S. 2011. Implications of the exposome for exposure science. *Journal of Exposure Science and Environmental Epidemiology,* 21**,** 5-9.
RASMUSON, J. O., RASMUSON, E., OLSEN, R. L., HALL, D., STRODE, R., LARSON, D. M. & KORCHEVSKIY, A. 2012. Application of a Bio-Kinetic Model (IEUBK) to Estimate the Effectiveness of Different Soil Remediation Scenarios for Lead Contamination in Shymkent, Kazakhstan. *ХАБАРШЫСЫ* 4.
RASTOGI, S. 2008. Renal effects of environmental and occupational lead exposure. *Indian journal of occupational and environmental medicine,* 12**,** 103.
RASTOGI, S. C. 1992. Cadmium, chromium, lead, and mercury residues in finger-paints and make-up paints. *Bulletin of Environmental Contamination and Toxicology,* 48**,** 289-294.
RENNER, R. 2009. Out of plumb: when water treatment causes lead contamination. *Environ Health Perspect,* 117**,** A542-A547.
REZENDE, V. B., BARBOSA JR, F., MONTENEGRO, M. F., SANDRIM, V. C., GERLACH, R. F. & TANUS-SANTOS, J. E. 2008. Haplotypes of vitamin D receptor modulate the circulating levels of lead in exposed subjects. *Archives of toxicology,* 82**,** 29-36.
ROBERTS, J. R., ROBERTS, J., REIGART, J. R., EBELING, M. & HULSEY, T. C. 2001. Time required for blood lead levels to decline in nonchelated children. *Journal of Toxicology: Clinical Toxicology,* 39**,** 153-160.
RODUSHKIN, I. & AXELSSON, M. D. 2000. Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part II. A study of the inhabitants of northern Sweden. *Science of The Total Environment,* 262**,** 21-36.
ROELS, H. A., BUCHET, J.-P., LAUWERYS, R. R., BRUAUX, P., CLAEYS-THOREAU, F., LAFONTAINE, A. & VERDUYN, G. 1980. Exposure to lead by the oral and the pulmonary routes of children living in the vicinity of a primary lead smelter. *Environmental Research,* 22**,** 81-94.
RONIS, M. J. J., BADGER, T. M., SHEMA, S. J., ROBERSON, P. K. & SHAIKH, F. 1996. Reproductive Toxicity and Growth Effects in Rats Exposed to Lead at

Different Periods during Development. *Toxicology and Applied Pharmacology,* 136**,** 361-371.
RSC. 2007. *Why use lead in paint?* [Online]. Available: http://www.rsc.org/chemistryworld/News/2007/August/21080701.asp [Accessed 09/04/2015].
RUBIO, C., GONZALEZ-IGLESIAS, T., REVERT, C., REGUERA, J. I., GUTIERREZ, A. J. & HARDISSON, A. 2005. Lead Dietary Intake in a Spanish Population (Canary Islands). *Journal of Agricultural and Food Chemistry,* 53**,** 6543-6549.
SAGVOLDEN, T., RUSSELL, V. A., AASE, H., JOHANSEN, E. B. & FARSHBAF, M. 2005. Rodent models of attention-deficit/hyperactivity disorder. *Biological psychiatry,* 57**,** 1239-1247.
SAKAI, T. 2000. Biomarkers of lead exposure. *Industrial health,* 38**,** 127-142.
SANDERS, T., LIU, Y., BUCHNER, V. & TCHOUNWOU, P. B. 2009. Neurotoxic effects and biomarkers of lead exposure: a review. *Reviews on environmental health,* 24**,** 15-46.
SATHYANARAYANA, S., BEAUDET, N., OMRI, K. & KARR, C. 2006. Predicting Children's Blood Lead Levels From Exposure to School Drinking Water in Seattle, Washington, USA. *Ambulatory Pediatrics,* 6**,** 288-292.
SCHAUMBERG, D. A., MENDES, F., BALARAM, M., DANA, M. R., SPARROW, D. & HU, H. 2004. Accumulated lead exposure and risk of age-related cataract in men. *Jama,* 292**,** 2750-2754.
SCHREY, P., WITTSIEPE, J., BUDDE, U., HEINZOW, B., IDEL, H. & WILHELM, M. 2000. Dietary intake of lead, cadmium, copper and zinc by children from the German North Sea island Amrum. *International Journal of Hygiene and Environmental Health,* 203**,** 1-9.
SCHUHMACHER, M., BOSQUE, M. A., DOMINGO, J. L. & CORBELLA, J. 1991. Dietary intake of lead and cadmium from foods in Tarragons Province, Spain. *Bulletin of Environmental Contamination and Toxicology,* 46**,** 320-328.
SCHWARTZ, B. S., LEE, B.-K., BANDEEN-ROCHE, K., STEWART, W., BOLLA, K., LINKS, J., WEAVER, V. & TODD, A. 2005. Occupational lead exposure and longitudinal decline in neurobehavioral test scores. *Epidemiology,* 16**,** 106-113.
SCHWARTZ, B. S., LEE, B.-K., LEE, G.-S., STEWART, W. F., SIMON, D., KELSEY, K. & TODD, A. C. 2000. Associations of blood lead, dimercaptosuccinic acid-chelatable lead, and tibia lead with polymorphisms in the vitamin D receptor and [delta]-aminolevulinic acid dehydratase genes. *Environmental Health Perspectives,* 108**,** 949.
SCHWARTZ, J. 1994. Low-Level Lead Exposure and Children′s IQ: A Metaanalysis and Search for a Threshold. *Environmental Research,* 65**,** 42-55.
SCHWARTZ, J., LANDRIGAN, P. J., BAKER JR, E. L., ORENSTEIN, W. A. & VON LINDERN, I. 1990. Lead-induced anemia: dose-response relationships and evidence for a threshold. *American Journal of Public Health,* 80**,** 165-168.
SCHWARTZ, J. & LEVIN, R. 1991. The risk of lead toxicity in homes with lead paint hazard. *Environmental Research,* 54**,** 1-7.
SCORZA JÚNIOR, R. & BOESTEN, J. 2005. Simulation of pesticide leaching in a cracking clay soil with the PEARL model, Pestic. *Management Sci*.
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**,** 1527-1536.
SENEKAL, M. & STEYN, N. P. 2004. *The food photo manual*.

SHERLOCK, J. 1987. Lead in food and the diet. *Environmental Geochemistry and Health,* 9**,** 43-47.
SHIH, R., GLASS, T., BANDEEN-ROCHE, K., CARLSON, M., BOLLA, K., TODD, A. & SCHWARTZ, B. 2006. Environmental lead exposure and cognitive function in community-dwelling older adults. *Neurology,* 67**,** 1556-1562.
SHUTTLER, I. L. & DELVES, H. T. 1986. Determination of lead in blood by atomic absorption spectrometry with electrothermal atomisation. *Analyst,* 111**,** 651-656.
SILBERGELD, E. K., WAALKES, M. & RICE, J. M. 2000. Lead as a carcinogen: experimental evidence and mechanisms of action. *American Journal of Industrial Medicine,* 38**,** 316-323.
SINDIKU, O. & OSIBANJO, O. 2011. Some priority heavy metals in children toys imported to Nigeria. *Journal of Toxicology and Environmental Health Sciences,* 3**,** 109-115.
SMITH, P., SMITH, J., POWLSON, D., MCGILL, W., ARAH, J., CHERTOV, O., COLEMAN, K., FRANKO, U., FROLKING, S. & JENKINSON, D. 1997. A comparison of the performance of nine soil organic matter models using datasets from seven long-term experiments. *Geoderma,* 81**,** 153-225.
STAESSEN, J. A., LAUWERYS, R. R., BULPITT, C. J., FAGARD, R., LIJNEN, P., ROELS, H., THIJS, L. & AMERY, A. 1994. Is a positive association between lead exposure and blood pressure supported by animal experiments? *Current opinion in nephrology and hypertension,* 3**,** 257-263.
STAESSEN, J. A., ROELS, H., FAGARD, R., AMERY, A., BIENIASZEWSKI, L., BULPITT, C. J., CELIS, H., DOLENC, P., LIJNEN, P. & PETROV, V. 1996. Lead exposure and conventional and ambulatory blood pressure: a prospective population study. *Jama,* 275**,** 1563-1570.
STANEK, K., MANTON, W., ANGLE, C., ESKRIDGE, K., KUEHNEMAN, A. & HANSON, C. 1998. Lead Consumption of 18- to 36-Month-Old Children as Determined from Duplicate Diet Collections: Nutrient Intakes, Blood Lead Levels, and Effects on Growth. *Journal of the American Dietetic Association,* 98**,** 155-158.
STERLING, D. A., ROEGNER, K. C., LEWIS, R. D., LUKE, D. A., WILDER, L. C. & BURCHETTE, S. M. 1999. Evaluation of Four Sampling Methods for Determining Exposure of Children to Lead-Contaminated Household Dust. *Environmental Research,* 81**,** 130-141.
STOKES, L., LETZ, R., GERR, F., KOLCZAK, M., MCNEILL, F. E., CHETTLE, D. R. & KAYE, W. E. 1998. Neurotoxicity in young adults 20 years after childhood exposure to lead: the Bunker Hill experience. *Occupational and Environmental Medicine,* 55**,** 507-516.
STURGES, W. T. & HARRISON, R. M. 1985. An assessment of the contribution from paint flakes to the lead content of some street and household dusts. *Science of The Total Environment,* 44**,** 225-234.
SUN, Y., ZHUANG, G., ZHANG, W., WANG, Y. & ZHUANG, Y. 2006. Characteristics and sources of lead pollution after phasing out leaded gasoline in Beijing. *Atmospheric Environment,* 40**,** 2973-2985.
TAYLOR, E. 1998. Clinical foundations of hyperactivity research. *Behavioural brain research,* 94**,** 11-24.
TELIŠMAN, S., ČOLAK, B., PIZENT, A., JURASOVIĆ, J. & CVITKOVIĆ, P. 2007. Reproductive toxicity of low-level lead exposure in men. *Environmental Research,* 105**,** 256-266.

THOMAS, V. M., SOCOLOW, R. H., FANELLI, J. J. & SPIRO, T. G. 1999. Effects of Reducing Lead in Gasoline: An Analysis of the International Experience. *Environmental Science & Technology,* 33**,** 3942-3948.
THOMPSON, F. E. & BYERS, T. 1994. Dietary Assessment Resource Manual. *The Journal of Nutrition,* 124**,** 2245s-2317s.
TIMBRELL, J. A. 1998. Biomarkers in toxicology. *Toxicology,* 129**,** 1-12.
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* [Online], 78. Available: http://www.who.int/bulletin/archives/78(9)1068.pdf.
UNEP 2013. Lead in enamel decorative paints.
USEPA 1986. Lead effects on cardiovascular function, early development, and stature.
USEPA 1994a. IEUBK manual.
USEPA 1994b. Technical support document: Parameters and equations used in theintegrated exposure uptake biokineticmodel for lead in children (v0.99d). Washington.
USEPA 1995a. Guidance for risk characterisation.
USEPA. 1995b. THE USE OF THE BENCHMARK DOSE APPROACH IN HEALTH RISK ASSESSMENT. Available: www.epa.gov/raf/publications/pdfs/BENCHMARK.PDF [Accessed 8/11/2011].
USEPA 1997. Exposure Factors Handbook
USEPA 1999. Short sheet: IEUBK model soil/dust ingestion rates. Washington, DC 20460.
USEPA 2000. Exposure and human health reassessment of 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Part III. Integrated summary and risk characterization for 2,3,7,8-TCDD and related compounds.
USEPA 2002. Child-Specific Exposure Factors Handbook.
USEPA. 2004. *Integrated Risk Information System: Lead and compounds (inorganic) (CASRN 7439-92-1)* [Online]. Available: http://www.epa.gov/iris/subst/0277.htm [Accessed 22/04/2014].
USEPA 2005. Guidelines for Carcinogen Risk Assessment.
USEPA. 2006. *Air quality criteria for lead* [Online]. Available: pub.epa.gov/ncea/risk/recordisplay.cfm?deid=158823 [Accessed 17/02/2016].
USEPA. 2012. *All-Ages Lead Model (AALM) Version 1.05 (External Draft Report)* [Online]. Available: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=139314 [Accessed 05/02/2014].
USHUD 1997. Guidelines for the Evaluation and Control of Lead-Based Paint Hazards in Housing.
UVERSKY, V. N., LI, J. & FINK, A. L. 2001. Metal-triggered Structural Transformations, Aggregation, and Fibrillation of Human α-Synuclein A POSSIBLE MOLECULAR LINK BETWEEN PARKINSON′ S DISEASE AND HEAVY METAL EXPOSURE. *Journal of Biological Chemistry,* 276**,** 44284-44296.
VAZIRI, N. D. 2008. Mechanisms of lead-induced hypertension and cardiovascular disease. *American Journal of Physiology-Heart and Circulatory Physiology,* 295**,** H454-H465.
VERMEIRE, T. G., POEP, P. V. D., VANDELAAR, R. T. H. & OELFZEMA, H. R. 1993. Estimation of consumer exposure to chemicals: application of simple models. *The Science of the Total Environment,* 136**,** 155-176.
VON LINDERN, I., SPALINGER, S., PETROYSAN, V. & VON BRAUN, M. 2003. Assessing remedial effectiveness through the blood lead:soil/dust lead relationship

at the Bunker Hill Superfund Site in the Silver Valley of Idaho. *Science of The Total Environment,* 303**,** 139-170.
VON SCHIRNDING, Y., BRADSHAW, D., FUGGLE, R. & STOKOL, M. 1991. Blood lead levels in South African inner-city children. *Environmental Health Perspectives,* 94**,** 125.
VRIJHEID, M., RICHARDSON, L., ARMSTRONG, B. K., AUVINEN, A., BERG, G., CARROLL, M., CHETRIT, A., DELTOUR, I., FEYCHTING, M. & HOURS, M. 2009. Quantifying the impact of selection bias caused by nonparticipation in a case–control study of mobile phone use. *Annals of Epidemiology,* 19**,** 33-41. e1.
WANG, B., SHAO, D., XIANG, Z., YE, H., NI, W., YANG, S., WU, C., LI, P. & FU, H. 2011. Contribution of environmental lead exposure to blood lead level among infants based on IEUBK model. *Wei sheng yan jiu= Journal of hygiene research,* 40**,** 478-480.
WANG, H.-L., CHEN, X.-T., YANG, B., MA, F.-L., WANG, S., TANG, M.-L., HAO, M.-G. & RUAN, D.-Y. 2008. Case-control study of blood lead levels and attention deficit hyperactivity disorder in Chinese children. *Environmental Health Perspectives,* 116**,** 1401.
WANG, T. Y., THORNTON, I. & FARAGO, M. 1997. Changes in lead concentrations in the home environment in Birmingham, England over the period 1984-1996. *The Scieme of the Total Environment* 207**,** 149-156.
WANG, X., WANG, M., DONG, W., LI, Y., ZHENG, X., PIAO, F. & LI, S. 2013. Subchronic exposure to lead acetate inhibits spermatogenesis and downregulates the expression of Ddx3y in testis of mice. *Reproductive Toxicology,* 42**,** 242-250.
WEAVER, V. M., GRISWOLD, M., TODD, A. C., JAAR, B. G., AHN, K.-D., THOMPSON, C. B. & LEE, B.-K. 2009. Longitudinal associations between lead dose and renal function in lead workers. *Environmental Research,* 109**,** 101-107.
WEISS, B. 1988. Quantitative perspectives on behavioral toxicology. *Toxicology Letters,* 43**,** 285-293.
WETMUR, J. G., LEHNERT, G. & DESNICK, R. J. 1991. The δ-aminolevulinate dehydratase polymorphism: Higher blood lead levels in lead workers and environmentally exposed children with the 1–2 and 2-2 isozymes. *Environmental Research,* 56**,** 109-119.
WHO 1985. Guidelines for the study dietary intakes of chemical contaminants.
WHO 1997. Guidelines for predicting dietary intake of pesticide residues.
WHO. 2003a. *Lead: Assessing the environmental burden of disease at national and local levels* [Online]. Available: http://www.who.int/quantifying_ehimpacts/publications/en/leadebd2.pdf [Accessed 08/03/2016].
WHO. 2003b. *Lead: Assessing the environmental burden of disease* [Online]. Available: http://www.who.int/quantifying_ehimpacts/publications/9241546107/en/ [Accessed 07/02/2016].
WHO 2010a. WHO guidelines on drawing blood: Best practices in phlebotomy.
WHO 2010b. WHO human health risk assessment toolkit: chemical hazards.
WHO. 2011. *Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA): LEAD* [Online]. Available: http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3511 [Accessed 22/04/2014].
WHO. 2015. *Metrics: Disability-Adjusted Life Year (DALY)* [Online]. Available: http://www.who.int/healthinfo/global_burden_disease/metrics_daly/en/ [Accessed 06/05/2015].

WHO. 2016. *Risk factors* [Online]. Available: http://www.who.int/topics/risk_factors/en/ [Accessed].
WHO/FAO 2009. Dose-Response Assessment And Derivation Of Health-Based Guidance Values. *In:* SHEFFER, M. (ed.) *Principles And Methods For The Risk Assessment Of Chemicals In Food*
WHO/IPCS. 1994. *Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits.* [Online]. Available: http:==www.inchem.org=documents=ehc=ehc=ehc170.htm [Accessed 01/03/2016].
WILHEIM, M., WITTSIEPE, J., SCHREY, P., FELDMANN, C. & IDEL, H. 2003. Dietary intake of lead by children and adults in germany measured by the duplicate method. *International Journal of Hygiene and Environmental Health,* 206**,** 93-503.
WILHELM, M., HEINZOW, B., ANGERER, J. & SCHULZ, C. 2010. Reassessment of critical lead effects by the German Human Biomonitoring Commission results in suspension of the human biomonitoring values (HBM I and HBM II) for lead in blood of children and adults. *International Journal of Hygiene and Environmental Health,* 213**,** 265-269.
WILLIAMS, P. R. D. & PAUSTENBACH, D. J. 2002. RISK CHARACTERIZATION: PRINCIPLES AND PRACTICE. *Journal of Toxicology and Environmental Health, Part B,* 5**,** 337-406.
WRIGHT, R. O., TSAIH, S.-W., SCHWARTZ, J., WRIGHT, R. J. & HU, H. 2003. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *The Journal of Pediatrics,* 142**,** 9-14.
WU, J., BASHA, M. R., BROCK, B., COX, D. P., CARDOZO-PELAEZ, F., MCPHERSON, C. A., HARRY, J., RICE, D. C., MALONEY, B. & CHEN, D. 2008. Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD. *The Journal of Neuroscience,* 28**,** 3-9.
XU, J., JI, L.-D. & XU, L.-H. 2006. Lead-induced apoptosis in PC 12 cells: involvement of p53, Bcl-2 family and caspase-3. *Toxicology Letters,* 166**,** 160-167.
XU, J., LIAN, L.-J., WU, C., WANG, X.-F., FU, W.-Y. & XU, L.-H. 2008. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. *Food and Chemical Toxicology,* 46**,** 1488-1494.
YÁÑEZ, L., GARCíA-NIETO, E., ROJAS, E., CARRIZALES, L., MEJíA, J., CALDERÓN, J., RAZO, I. & DíAZ-BARRIGA, F. 2003. DNA damage in blood cells from children exposed to arsenic and lead in a mining area. *Environmental Research,* 93**,** 231-240.
YU, C.-C., LIN, J.-L. & LIN-TAN, D.-T. 2004. Environmental exposure to lead and progression of chronic renal diseases: a four-year prospective longitudinal study. *Journal of the American Society of Nephrology,* 15**,** 1016-1022.
ZELIKOFF, J., LI, J., HARTWIG, A., WANG, X., COSTA, M. & ROSSMAN, T. 1988. Genetic toxicology of lead compounds. *Carcinogenesis,* 9**,** 1727-1732.
ZHANG, A., HU, H., SÁNCHEZ, B. N., ETTINGER, A. S., PARK, S. K., CANTONWINE, D., SCHNAAS, L., WRIGHT, R. O., LAMADRID-FIGUEROA, H. & TELLEZ-ROJO, M. M. 2012. Association between prenatal lead exposure and blood pressure in children. *Environmental Health Perspectives,* 120**,** 445.
ZHENG, J., HUYNH, T., GASPARON, M., NG, J. & NOLLER, B. 2013. Human health risk assessment of lead from mining activities at semi-arid locations in the context

of total lead exposure. *Environmental Science and Pollution Research,* 20**,** 8404-8416.
ZIEGLER, E. E., EDWARDS, B. B., JENSEN, R. L., MAHAFFEY, K. R. & FOMON, S. J. 1978. Absorption and retention of lead by infants. *Pediatric research,* 12**,** 29-34.
ZIELHUIS, R. 1975. Dose-response relationships for inorganic lead. *International Archives of Occupational and Environmental Health,* 35**,** 19-35.
ŻUKOWSKA, J. & BIZIUK, M. 2008. Methodological Evaluation of Method for Dietary Heavy Metal Intake. *Journal of Food Science,* 73**,** R21-R29.

7.0 Appendices

7.1 Appendix 1: Tool and aids used in the risk assessment

The lead risk assessment questionnaire

Date:

Child's code number:

1 How long has the child stayed at this address?

less than 6 months More than 1 year More than 2 years more than 3 years

More than 4 years more than 4 years more than 5 years

2 What is the previous address?

3a Does the child spend time at:

Day care Baby sitter Preschool Primary school?

b What is the name of the school, day care etc?

c How many hours does he spend at school?

3 hrs 4 hrs 5 hrs 6 hrs 7 hrs 8 rs other

4 Please list the parents' occupation and hobbies

Mother

Father

5 Is the house painted? Yes No

If the house is painted, what are the colours?

6 If you have lived in another house in the past 6 months, was the house painted?

Yes No

If yes, what were the colours?

7 How far is the house from the main road where cars pass frequently?

100 m 500m 1000m more than 1000m Other

8 Has the child been observed eating/ mouthing non-food items?

Yes No

9 If the answer to question 8 is yes, what does the child put in his mouth?

Hands Toys Dirt Paint chips others

10 If the answer to question 8 is yes, how often does the child put items to his/her mouth?

Rarely Sometimes Often

11 Does the child use a pacifier? Yes No

12 Is there loose paint on the walls inside the house or on the ceiling? Yes No

13 Do you use pottery for preparing food or eating? Yes No

14 In the house, where does your child usually play?

- In the bed room
- In the sitting room
- In the kitchen
- Other

15 Outside of the house, where does your child usually play?

16 Does the child play more in the house or outside?

- More in the house
- More outside
- Equally in the house as outside

17 Does the child wear jewelry? Yes No

18 Does the child eat imported candy? Yes No

19 Does the child consume canned foods and drinks?

Often Rarely Never

20 How often does the family use candles?

Very often Often Rarely Never

Food frequency questionnaire (ffq)

Date: Child code number

This questionnaire will give us information about your child's eating habits. There are no "right" or "wrong" answers. We want to know how often the child ate certain foods. For each of the foods listed, please indicate how many servings per week the child usually ate in the past month. (If you ate a food less than once a week, write a "0" in the space provided. Where indicated, check whether your servings are large, small, or about average in size.

1 Which meals does the child skip almost on a daily basis?

Breakfast	1
Lunch	2
Evening meal	3
None	4

2 Does the school that the child attends have a school feeding programme?

Yes No

3 If the answer to question 2 is yes, what foods are given to your child?

Does the child ever eat outside the home e.g. at fast food shops such as Nandos etc?

YES	1
NO	0

5 If the answer to question 4 is YES, in an average month how often do you eat at the following places?

	Frequency of visits		
	Times/week	Times/month	Rarely/never
Nandos			
Ali Baba			
KFC			
Kips			
Other restaurants/takeaways			
(Quarters from tuck shop)			

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Cereals, grains, cereal products											
Nsima ya*ufa mgaiwa* (normal flour)			101								
Nsima ya*ufa* refined (fine flour)			102								
Nsima ya*ufa madeya* (bran flour)			103								
Nsima ya mawere (millet)			104								
Nsima ya mapira (sorghum)			105								
Green maize			106								
Rice			107								
Pearl millet (*mchewere)*			108								
Cake			109								
Bread			110								
Buns, scones			111								
Biscuits			112								
Spaghetti, macaroni, pasta			113								
Breakfast cereal			114								
Infant feeding cereals			115								
Mandazi , doughnut			116								
Samosa			117								
Maize porridge stiff			118								
Maize porridge soft			119								
Oats			120								
Popcorn			121								

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Kamba puffs											
Other (specify)			123								
Roots and tubers, plantain											
Cassava tubers			201								
Msima ya condole (Cassava flour)			202								
White sweet potato			203								
Orange sweet potato			204								
Irish potato (baked)			205								
Irish potato (boiled)			206								
Irish potato (Mashed)			207								
Irish potato (Roasted)			208								
Chips			209								
Potato crisps			210								
Plantain, cooking banana			211								
Cocoyam (*masimbi*)			212								
Other (specify)			213								

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Pulses											
Bean, white			301								
Bean, brown			302								
Pigeon pea (*nandolo*)			303								
Groundnut			304								
Groundnut flour (nsinjilo)			305								
Soya bean flour			306								
Ground bean (*nzama)*			307								
Cowpea (*khobwe*)			308								
Other (specify)			309								
Vegetables											
Cabbage			401								
Tanaposi			402								
Rape											
Nkhwani			403								
Chinese cabbage			404								
Other cultivated green leafy vegetables			405								
Gathered wild green leaves			406								
Tomato			407								
Cucumber			408								
Pumpkin			409								

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Okra / *Therere*			410								
Carrots			411								
Tinned vegetables (specify):			412								
Mushrooms			413								
Other vegetables (specify):			414								
Meat, Fish, and Animal products											
Eggs fried			501								
Eggs Boiled/poached			502								
Eggs Scrambled			503								
Eggs omelet			504								
Dried fish			505								
Matemba											
Micheni											
Chambo											
kapenta											
usipa											
Fresh fish			506								
Matemba											
Micheni											
Chambo											
kapenta											

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
usipa											
Beef			507								
Goat meat			508								
Pork			509								
Chicken			510								
Mutton			511								
Sausage rolls			512								
Other poultry - guinea fowl, doves, etc.			513								
Small animal – rabbit, mice, etc.			514								
Tinned meat or fish			515								
Other (specify):											
Fruits											
Mango			601								
Banana			602								
Citrus – naartje, orange, etc.			603								
Pineapple			604								
Papaya			605								
Guava			606								
Avocado			607								
Watermelons											
Wild fruit (*masau, mlambe, etc.*)			608								
Apple			609								
Other fruits (specify)			610								

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Milk and Milk Products											
Fresh milk			701								
Powdered milk			702								
Chambiko - soured milk			703								
Yoghurt (sweetened)			704								
Yoghurt (fruit)			705								
Yoghurt plain			708								
Cheese			709								
Infant feeding formula (for bottle)			710								
Ice cream			711								
Ice lollies			712								
Other (specify)			713								
Sugar, Fats, and Oil											
Sugar			801								
Sugar Cane			802								
Cooking oil			803								
Butter			804								

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Margarine			805								
Honey			806								
Syrup			807								
Jam			808								
Sweets			809								
Chocolates			810								
Other (specify)			811								
Beverages											
Tea with milk			901								
Tea without milk			902								
Coffee with milk			903								
Coffee without milk			904								
Cocoa with milk			905								
Cocoa without milk			906								
Squash (Sobo drink concentrate)			907								
Fruit juice (specify)			908								
Freezes (flavoured ice)			909								
Soft drinks (Coca-cola, Fanta, Sprite, etc.)			910								
Local sweet beer (*thobwa*)			911								
Other (specify)			912								
Water											

A. Food items (with FPM numbers	**B**. Description of food item	**Tick for yes**	**C item code**	**D**. Amount usually eaten No of spoons, cups etc	Serving size [1]Lg Av Sm	**E**. Times Eaten every day	**F**. Times Eaten every week	. Eaten Occasionally	**H.** Never eaten	**Grams (g) /day**	**Comment**
Tap water			1001								
Bottled water			1002								
Well water			1003								
Miscellaneous											

[1]Lg = Large; Av: Average; Sm = Small

7.2 Appendix II: Concentrations of lead in various samples

Table 11: Measured blood lead

Sample no	Age	Sex	BPb/ µg/dL(±14.5%)
MC1	6	F	2
MC2	6	M	2
MC3	3	F	3
MC5	4	F	10
MC6	4	F	18
MC7	1	F	3
MC8	3	M	3
MC9	3	F	5
MC10	3	F	4
MC11	2	M	20
MC12	2	F	4
MC13	6	M	5
MC14	3	M	4
MC15	6	M	8
MC16	3	M	2
MC 17	4	M	7
MC 19	4	F	5
MC20	3	M	9
MC21	6	M	2
MC22	2	M	4
MC23	5	F	10
MC29	4.9	M	8
MC30	3.8	F	37
MC38	6	M	10
MC39	6	M	11
MC24	3.8	M	6
MC25	3	F	9
MC26	4	M	10
MC27	3	M	8
MC28	5	M	10
MC31	2	F	7
MC 32	5	F	6
MC35	4	M	6
MC36	6	M	8
MC37	2	M	7

Sample no	Age	Sex	BPb/ µg/dL(±14.5%)
MC40	4	F	3
MC41	6	F	3
MC42	2	F	3
MC43	4	M	22
ZW 01	2	M	12
Zw 02	2	M	10
ZW 03	6	F	9
ZW 04	6	M	8
ZW 05	2	M	9
ZW06	6	M	8
ZW07	5	M	7
ZW09	2	F	8
ZW10	5	M	4
ZW11	5	M	5
ZW12	3	F	12
ZW13	2	F	8
ZW14	5	F	5
ZW15	6	F	4
ZW 16	2	F	4
ZW17	2	F	7
ZW 18	4	M	4
ZW19	5	F	5
ZW20	2	F	6
ZW21	2	F	2
ZW22	4	F	4
ZW23	6	F	6
ZW24	6	F	5
ZW25	5	F	19
ZW26	1.8	F	7
ZW27	3	F	5
ZW28	6	M	4
ZW29	3	M	6
ZW30	6	F	6
ZW 31	3	M	5
ZW32	4	F	52
ND01	6	M	3
ND02	2	M	4

Sample no	Age	Sex	BPb/ µg/dL(±14.5%)
ND04	6	M	5
ND05	4	M	5
ND08	6	M	5
ND07	3	M	5
ND20	4	F	4
ND28	6	M	3
ND09	2	M	6
ND10	4	F	7
ND11	3	F	6
ND12	2	F	8
ND13	3	M	5
ND14	3	M	4
ND15	6	F	11.6
ND16	5	M	4
ND17	5	M	4
ND18	5	M	4
ND21	3	F	8
ND22	2	F	6
ND23	2	F	5
ND24	5	M	5
ND25	5	M	8
ND26	2	F	8
ND30	2	M	8
ND31	6	M	7
ND32	5	M	5
ND33	6	F	3
LM03	3	M	6
LM04	3	F	4
LM05	4	F	3
LM06	6	F	6
LM07	6	F	6
LM08	3	M	5
BN1	3	F	3
BN2	6	F	15
BN3	6	M	6
BN4	1.7	M	10
BN5	1.5	M	4

Sample no	Age	Sex	BPb/ µg/dL(±14.5%)
BN6	5	M	13
BN7	2.5	M	8
BN8	6	M	6
BN9	3	M	4
BN10	5	M	4
BN12	2	F	6
BN13	2.3	M	4
BN14	4	F	6
BN16	3	M	7
BN17	2	M	2
BN18	6	M	7
BN19	2.4	F	7
CH 01	6	F	3
CH02	2	M	5
CH03	3	M	9
CH04	4	M	4
CH05	6	F	5
CH07	6	F	6
CH08	6	M	7
CH09	6	F	5
CH10	6	M	7
CH11	4	F	8
CH12	6	F	6
CH13	3.9	M	10
CH14	5	M	4
CH15	5	M	7
CH16	3	F	7
CH17	3.5	F	6
CH18	2	F	8
CH19	3.9	M	7
CH20	6	M	12
CH21	4	F	8
CH22	2	M	4
CH23	3	M	7
CH25	6	M	7
CH26	6	M	8
CH27	5	F	7

Sample no	Age	Sex	BPb/ µg/dL(±14.5%)
CH28	5	F	13
CH29	1.5	M	9
CH30	6	F	8

Table 12: Lead in fresh domestic paint samples

Paint Samples	Lead (mg/kg) ± 5%
Plascon cream	170
Plascon white	46
Nuroc Bermuda blue	233
Nuroc white	341
Nuroc soft white	6372
Nuroc black	1320
Dulux gloss enamel red	7139
Dulux black	1.1
Dulux cream	2.0
Rainbow high gloss black	2.7
Rainbow high gloss white	6.0
Olympic brilliant green	15
Olympic white	9.3
Olympic glossen cream	1996
Olympic golden yellow	1074
Olympic glossen cornflower	646

Table 13: Concentrations of lead in paint chips

Sample no	Lead concentration (mg/kg)
ZW 1	3.5
ZW 2	2.0
ZW 12	161
ZW16	6458
ZW 17	44
LM 05	89
MC 9/10	2.4
MC 10	2.4
CH 8	2.3
CH 9	71
CH 21	1.0
CH 23	22
ND 3	2.9
ND 6	4.0
NdD7	2.4
ND 8	1.7
ND 17	0.86
ND 21	0.65
ND 22	2.0
ND 23	0.62
ND24/25	4.5
ND 30/31	4.5

Table 14: Concentrations of lead in toys

Sample no	Concentration of lead (mg/kg)
Toy 1	0.29
Toy 2	0.47
Toy 3	0.12
Toy 4	0.90
Toy 5	0.092
Toy 6	0.091
Toy 7	0.29
Toy 8	0.10
Toy 9	0.39
Toy 10	5.1
Toy 11	3.8
Toy 12	3.4
Toy 13	0.26
Toy 14	0.16
Toy 15	0.82
Toy 16	0.91
Toy 17	0.48
Toy 18	4.4
Toy 19	2.2
Toy 20	9.0

Table 15: Lead in various composite samples of food

Food Sample	Lead (mg/kg) ±5%
Beans	0.076
Green Maize	1.1
Eggs	0.21
Ground Nuts	0.059
Popcorn	0.49
Bread	0.40
Irish potatoes	0.14
Chips	3.3
Dough nut	0.090
Pap	0.65
Mgaiwa porridge	0.040
Buns	0.19
Jiggles	0.27
Kamba puffs	0.35
Sobo Squash	0.019
Thobwa	0.014
Micheni	0.055
Beef	0.15
Rice	0.046
Chambo	0.21
Usipa	0.18
Chinese cabbage	0.27
Mpiru	0.35
Mkhwani	0.49
Rape	0.15
Mangoes	0.058
Sweet potatoes	0.12
Bananas	0.047
Chicken	0.010
Cabbage	0.054
Soya	0.16

Table 16: Lead in dust and soil

Sample No	House dust (mg/kg) ± 5 %	Yard dust (mg/kg) ±5%
MC 1	2.5	6.6
MC2	2.3	5.0
MC3	3.4	13
MC 5/6	4.6	482
MC7	11	11
MC 8	2.7	11
MC 11	3.0	20
MC12	4.4	63
MC13	26	13
MC 14	5.3	6.9
MC15	3.4	3.7
MC 16	11	11
MC17	4.7	7.9
MC 18	-	5.8
MC 19	-	5.8
MC 20	10	5.7
MC 21	-	4.4
MC 22	-	4.4
MC 23	10	4.9
MC 24	3.4	9.4
MC 25	3.4	5.2
MC29	5.3	3.5
MC38	3.4	3.7
MC 26	22	28
MC27	-	5.6
MC 28	16	-
MC 31	8.5	2.7
MC 32	8.5	2.7
MC35	2.7	4.3
MC 36	2.7	4.3
MC37	2.7	4.3
MC 39	16	3.7
MC 40	2.7	4.3
MC 30	-	193
MC 43	4.7	73
MC 44	7.6	2.8
BN4	4.0	
BN 12	2.5	1.5
BN 13	2.7	2.0
BN 14	4.1	2.7
BN 16	21	11
BN 17	6.8	8.0
BN 18	5.6	5.0
BN 19	14	5.5

CH05	8.8	8.1
CH08	3.6	6.0
CH04	-	6.1
CH10	-	5.3
CH11	3.4	5.2
CH12	3.7	4.3
CH13	27	6.5
CH20	37	4.9
CH24	-	11
CH25	-	6.4
CH01	4.9	5.5
CH02	15	23
CH03	48	16
CH05	14	10
CH07	6.5	5.8
CH09	8.6	7.3
CH14	7.4	6.6
CH15	7.5	11
CH16	2.6	4.6
CH22	9.8	7.0
CH24	-	29
CH26	16	20
Chilomoni Primary	12	-
ND01	3.2	12
ND02	3.2	12
ND06	6.0	16
ND08	7.6	6.5
ND09	9.0	9.9
ND10	9.0	-
ND11	14	-
ND13	7.0	9.6
ND14	8.6	38
ND15	-	7.9
ND16	3.2	-
ND17	-	34
ND18	8.5	5.9
ND19	23	4.7
ND20	7.7	8.5
ND21	8.9	10
ND22	11	19
ND23	6.5	12
ND24	14	36
ND25	-	36
ND26	10	19
ND28	23	8.8
ND29	8.5	11
ND30	22	12

ND31	22	12
ND32	17	19
ND33	48	5.4
ND34	-	15
ND35	-	4.7
Makata Primary	14	-
LM01	33	9.1
LM02	15	9.1
LM03	17	113
LM04	8.8	10.1
LM05	6.9	8.5
LM06	99	6.5
LM07	17179	39
LM08	8.9	16
ZW01	25	20
ZW02	18	20
ZW03	17	17
ZW04	9.5	16
ZW05	-	17
ZW06	-	81
ZW07	14	165
ZW09	15	12
ZW10	265	16
ZW11	-	11
ZW12	36	10
ZW13	4.9	9.1
ZW14	4.9	9.1
ZW15	-	8.9
ZW16	44	25
ZW17	16	11
ZW18	14	10
ZW19	15	10
ZW20	10	10
ZW21	-	10
ZW23	13	10
ZW22	12	-
ZW24	17	10
ZW26	20	10
ZW28	15	13
ZW30	110	-
ZW31	15	11
ZW32	20	11
ZW Primary school	5.4	3.8
Naotcha Primary school	8.3	4.3

Table 17: Average food consumption data for children aged 1 to 6 years

Food Item	Average intake /week	Average intake/day	90th percentile/ week	90th percentile/ day	25th percentile/week	25th percentile/ day	% consuming
Msima	3642	520.3	5754	822.0	1940	277.1	100
Green maize	243.75	34.82	455.0	65.00	65	9.286	14.5
Rice porridge	377.4	53.91	1257	179.6	0	0	23.6
Rice	739.4	105.6	1267	181.0	387.3	55.32	32.7
Bread	219.4	31.35	702.6	100.4	0	0	65.5
Buns	80.5	11.50	189.0	27.00	0	0	34.5
Biscuits	7.735	1.105	28.00	4.000	0	0	29.1
Spaghetti	28.36	4.052	75.00	10.71	0	0	12.7
Mandasi	81.93	11.70	192.5	27.50	0	0	21.8
Samousa	40.91	5.844	50.00	7.142	0	0	14.5
Soya poridge	850.9	121.6	1775	253.6	0	0	47.3
Porridge	765.9	109.4	2359	337.0	0	0	56.4
Pop corn	80.98	11.56	280.0	40.00	0	0	32.7
Kamba puffs/jiggies	132.6	18.94	470.0	67.14	0	0	30.9
Sweet potatoes	163.9	23.41	400.0	57.15	0	0	50.9
Irish potatoes	168.3	24.04	456.0	65.15	0	0	36.4
Chips	269.5	38.50	800.1	114.3	0	0	49
Beans	216.2	30.88	797.4	113.9	0	0	43.6
Cabbage	119.9	17.14	288.0	41.14	0	0	41.8
Turnips	192.2	27.46	547.2	78.17	0	0	63.6
Rape	130.2	18.60	441.6	63.09	0	0	49.1
Pumpkin leaves	156.9	22.42	312.0	44.57	0	0	58.2
Chinese cabbage	47.91	6.844	197.6	28.23	0	0	27.3
Eggs	115.6	16.51	342.0	48.86	0	0	56.4
Matemba	120.8	17.25	415.44	59.35	0	0	34.6
Micheni	67.16	9.594	240.0	34.29	0	0	32.2
Chambo	24.43	3.490	131.3	18.76	0	0	25.5
Kapenta	58.06	8.295	202.8	28.97	0	0	30.9
Usipa	103.9	14.86	320.0	45.71	0	0	41.8
Beef	51.13	7.305	126.4	18.05	0	0	32.7
Goat meat	35.28	5.040	68.00	9.714	0	0	23.6
Pork	22.39	3.198	88.00	12.57	0	0	16.4
Chicken	466.6	66.65	836.0	119.4	209	29.86	45.5
Sausage	8.333	1.190	31.50	4.5	0	0	10.9

Food Item	Average intake /week	Average intake/day	90th percentile/ week	90th percentile/ day	25th percentile/week	25th percentile/ day	% consuming
Mango	607.5	86.78	1470	210	0	0	70.9
Banana	75.00	10.71	230.0	32.86	0	0	34.5
Fresh milk	147.7	21.10	500.0	71.43	0	0	14.5
Tea with milk	352.3	50.32	1400	200	0	0	29.1
Tea w/o mik	1653	236.2	3000	428.6	750	107.1	76.6
Sobo	673.8	96.25	2310	330	0	0	29.1
Freezes	481.9	68.83	1365	195	0	0	61.8
Fanta/coke	111.0	15.85	660	94.29	0	0	20
Thobwa	218.1	31.16	500	71.43	0	0	36.4
Water	8120	1160	9016	1288	7560	1080	100

Table 18: Predicted BPb from all sources

Sample no	Age	Measured BPb (µg/dL)	BPb at 31% bioavailability for food lead (µg/dL)	BPb at 50% bioavailability for food lead (µg/dL)
MC1	6	2	8	12.2
MC2	6	2	8	12.2
MC3	3	3	7.9	11.8
MC5	4	10	9	12
MC6	4	18	9	12
MC7	1	3	10.6	17.3
MC8	3	3	7.9	11.8
MC11	2	20	10	14.4
MC12	2	4	10	14.5
MC13	6	5	8.1	12.3
MC14	3	4	7.8	11.9
MC15	6	8	8	12.2
MC16	3	2	7.9	11.9
MC 17	4	7	6.9	10.5
MC20	3	9	7.9	11.9
MC23	5	10	7.9	12.2
MC29	4.9	8	6.8	10.5
MC38	6	10	8	12.2
MC39	6	11	8	12.2
MC24	3.8	6	7.8	10.5
MC25	3	9	7.8	11.8
MC26	4	10	7	10.7
MC28	5	10	7.9	12
MC31	2	7	9.7	14.3
MC 32	5	6	7.8	12
MC35	4	6	6.8	10.5
MC36	6	8	8	12.2
MC37	2	7	9.7	14.3
MC40	4	3	6.8	10.5
MC43	4	22	7.2	10.7
ZW 01	2	12	9.9	14.5
Zw 02	2	10	9.8	14.4
ZW 03	6	9	8.1	12.3
ZW 04	6	8	8.4	12.2

Sample no	Age	Measured BPb (µg/dL)	BPb at 31% bioavailability for food lead (µg/dL)	BPb at 50% bioavailability for food lead (µg/dL)
ZW07	5	7	8.4	12.4
ZW09	2	8	9.8	14.4
ZW10	5	4	8.6	12.8
ZW12	3	12	7.9	12
ZW13	2	8	9.7	14.3
ZW14	5	5	7.9	12
ZW 16	2	4	10	14.6
ZW17	2	7	9.8	14.3
ZW 18	4	4	6.9	10.6
ZW19	5	5	7.9	12
ZW20	2	6	9.7	14.4
ZW22	4	4	6.9	10.6
ZW23	6	6	6.9	12.2
ZW24	6	5	8	12.3
ZW26	1.8	7	8	15.6
ZW28	6	4	8	12.3
ZW30	6	6	8.6	12.7
ZW 31	3	5	7.9	11.9
ND01	6	3	8	12.2
ND02	2	4	9.7	14.3
ND08	6	5	8	12.2
ND09	2	6	9.7	14.4
ND13	3	5	7.9	11.9
ND14	3	4	8	12
ND16	5	4	7.8	12
ND18	5	4	7.9	12
ND21	3	8	7.9	11.9
ND22	2	6	9.8	14.4
ND23	2	5	9.7	14.4
ND24	5	5	8	12.1
ND26	2	8	9.8	14.4
ND30	2	8	9.8	14.4
ND31	6	7	8.1	12.3
ND32	5	5	7.9	12
ND33	6	3	8.1	12.3
LM03	3	6	8.5	12.3

Sample no	Age	Measured BPb (µg/dL)	BPb at 31% bioavailability for food lead (µg/dL)	BPb at 50% bioavailability for food lead (µg/dL)
LM04	3	4	7.9	11.9
LM05	4	3	6.9	10.5
LM06	6	6	8.2	12.5
LM08	3	5	7.9	11.9
BN4	1.7	10	10.6	15.5
BN12	2	6	9.7	14.3
BN13	2.3	4	9.7	14.3
BN14	4	6	6.8	10.5
BN16	3	7	7.9	11.9
BN17	2	2	9.7	14.3
BN18	6	7	7.8	12.2
BN19	2.4	7	9.7	14.4
CH 01	6	3	8	12.2
CH02	2	5	9.8	14.4
CH03	3	9	8.1	12.1
CH05	6	5	8	12.2
CH07	6	6	8	12.4
CH08	6	7	8	12.2
CH09	6	5	8	12.2
CH11	4	8	6.8	10.5
CH12	6	6	8	12.2
CH13	3.9	10	7.9	11.9
CH14	5	4	7.9	12
CH15	5	7	7.9	12
CH16	3	7	7.8	11.8
CH20	6	12	8.1	12.3
CH22	2	4	9.7	14.4
CH25	6	7	8	12.2
CH26	6	8	8.1	12.3

Table 19: Estimation of contribution of lead from food to measured BPb

Sample no	Measured BPb (µg/dL)	BPb using 50 % bioavailability	% contribution to BPb for 50% bioavailability	BPb using 31 % bioavailability	% contribution to measured BPb for 31% bioavailability	50% contribution/31 % contribution
MC1	2	12.2	610	8.2	410	1.49
MC2	2	12.2	610	8.2	410	1.49
MC3	3	11.8	393	8	266	1.48
MC5	10	10.5	105	7	70	1.50
MC6	18	10.5	58	7	38	1.50
MC7	3	12.4	413	8.7	290	1.43
MC8	3	11.8	393	8	266	1.48
MC11	20	14.2	71	9.9	49	1.43
MC12	4	14.2	355	9.9	247	1.43
MC13	5	12.2	244	8.2	164	1.49
MC14	4	11.8	295	8	200	1.48
MC15	8	12.2	152.5	8.2	102	1.49
MC16	2	11.8	590	8	400	1.48
MC 17	7	10.5	150	7	100	1.50
MC20	9	11.8	131	8	88	1.48
MC23	10	11.9	119	8	80	1.49
MC29	8	10.5	131	7	87	1.50
MC38	10	12.2	122	8.2	82	1.49
MC39	11	12.2	110	8.2	74	1.49
MC24	6	11.8	197	8	133	1.48
MC25	9	11.8	131	8	88	1.48
MC26	10	10.5	105	7	70	1.50
MC28	10	11.9	119	8	80	1.49
MC31	7	14.2	202	9.9	141	1.43
MC 32	6	11.9	198	8	133	1.49
MC35	6	10.5	175	7	116	1.50
MC36	8	12.2	152.	8.2	102	1.49
MC37	7	14.2	202	9.9	141	1.49
MC40	3	11.8	393	7	233	1.69
MC43	22	11.8	53	7	31	1.69
ZW 01	12	14.2	118	9.9	82	1.43
ZW 02	10	14.2	142	9.9	99	1.43
ZW 03	9	12.2	135	8.2	91	1.49

Sample no	Measured BPb (µg/dL)	BPb using 50 % bioavailability	% contribution to BPb for 50% bioavailability	BPb using 31 % bioavailability	% contribution to measured BPb for 31% bioavailability	50% contribution/31 % contribution
ZW 04	8	12.2	152	8.2	102	1.49
ZW07	7	11.9	170	8	114	1.49
ZW09	8	14.2	177	9.9	123	1.43
ZW10	4	11.9	297	8	200	1.49
ZW12	12	11.8	9	8	66	1.48
ZW13	8	14.2	177	9.9	123	1.44
ZW14	5	11.9	238	8	160	1.49
ZW 16	4	11.4	285	7.9	197.5	1.44
ZW17	7	14.2	202	9.9	141	1.43
ZW 18	4	7.5	187.5	5.0	125	1.50
ZW19	5	11.9	238	8	160	1.49
ZW20	6	14.2	236	9.9	165	1.43
ZW22	4	10.5	262	7	175	1.50
ZW23	6	12.2	203	8.2	136	1.49
ZW24	5	12.2	244	8.2	164	1.49
ZW26	7	12.4	177	8.7	124	1.43
ZW28	4	14.2	355	8.2	205	1.73
ZW30	6	14.2	236	8.2	136	1.73
ZW 31	5	11.8	236	8	160	1.48
ND01	3	12.2	406	8.2	273	1.49
ND02	4	14.2	355	9.9	247	1.43
ND08	5	12.2	244	8.2	164	1.49
ND09	6	14.2	236	9.9	165	1.43
ND13	5	11.8	236	8	160	1.48
ND14	4	11.8	295	8	200	1.48
ND16	4	11.9	297	8	200	1.49
ND18	4	11.9	297	8	200	1.49
ND21	8	11.8	147	8	100	1.48
ND22	6	14.2	236	9.9	165	1.43
ND23	5	14.2	284	9.9	198	1.43
ND24	5	11.9	238	8	160	1.49
ND26	8	14.2	177	9.9	123	1.43
ND30	8	14.2	177	9.9	123	1.43
ND31	7	12.2	174	8.2	117	1.49
ND32	5	11.9	238	8	160	1.49

Sample no	Measured BPb (µg/dL)	BPb using 50 % bioavailability	% contribution to BPb for 50% bioavailability	BPb using 31 % bioavailability	% contribution to measured BPb for 31% bioavailability	50% contribution/31 % contribution
ND33	3	12.2	406	8.2	273	1.49
LM03	6	11.8	196	8	133	1.48
LM04	4	11.8	295	8	200	1.48
LM05	3	10.5	350	7	233	1.50
LM06	6	12.2	203	8.2	136	1.49
LM08	5	11.8	236	8	160	1.48
BN4	10	12.4	124	8.7	87	1.43
BN12	6	14.2	236	9.9	165	1.43
BN13	4	14.2	355	9.9	247	1.43
BN14	6	10.5	175	7	116	1.50
BN16	7	11.8	168	8	114	1.48
BN17	2	14.2	710	9.9	495	1.43
BN18	7	12.2	174	8.2	117	1.49
BN19	7	14.2	202	9.9	141	1.43
CH 01	3	12.2	406	8.2	273	1.49
CH02	5	14.2	284	9.9	198	1.43
CH03	9	11.8	131	8	88	1.48
CH05	5	12.2	244	8.2	164	1.49
CH07	6	12.2	203	8.2	136	1.49
CH08	7	12.2	174	8.2	117	1.49
CH09	5	12.2	244	8.2	164	1.49
CH11	8	10.5	131	7	87	1.50
CH12	6	12.2	203	8.2	136	1.49
CH13	10	11.8	118	8	80	1.48
CH14	4	11.9	297	8	200	1.49
CH15	7	11.9	170	8	114	1.49
CH16	7	11.8	168	8	114	1.48
CH20	12	12.2	101	8	66	1.53
CH22	4	14.2	355	9.9	247	1.43
CH25	7	12.2	174	8.2	117	1.49
CH26	8	12.2	152	8.2	102	1.49

Table 20: Estimation of contribution of lead from soil to measured BPb

Sample no	Age	Measured BPb (µg/dL)	Predicted BPb for soil (µg/dL)	% contribution of soil lead to measured BPb
MC3	3	3	0.1	3.33
MC5	4	10	2.3	23.0
MC6	4	18	2.3	12.8
MC7	1	3	0.2	6.67
MC8	3	3	0.1	3.33
MC11	2	20	0.2	1.00
MC12	2	4	0.5	12.5
MC13	6	5	0.2	4.00
MC14	3	4	0.1	2.50
MC16	3	2	0.2	10.0
MC 17	4	7	0.1	1.43
MC20	3	9	0.1	1.11
MC23	5	10	0.1	1.00
MC29	4	8	0.1	1.25
MC39	6	11	0.1	0.909
MC24	3	6	0.1	1.67
MC25	3	9	0.1	1.11
MC26	4	10	0.3	3.00
MC28	5	10	0.1	1.00
MC31	2	7	0.1	1.43
MC 32	5	6	0.1	1.67
MC36	6	8	0.1	1.25
MC43	4	22	0.4	1.82
ZW 01	2	12	0.3	2.50
Zw 02	2	10	0.3	3.00
ZW 03	6	9	0.1	1.11
ZW 04	6	8	0.1	1.25
ZW07	5	7	0.7	10.0
ZW09	2	8	0.2	2.50
ZW10	5	4	1.1	27.5
ZW12	3	12	0.3	2.50
ZW13	2	8	0.1	1.25
ZW14	5	5	0.1	2.00
ZW 16	2	4	0.5	12.5
ZW17	2	7	0.2	2.86

Sample no	Age	Measured BPb (µg/dL)	Predicted BPb for soil (µg/dL)	% contribution of soil lead to measured BPb
ZW 18	4	4	0.1	2.50
ZW19	5	5	0.1	2.00
ZW20	2	6	0.2	3.33
ZW22	4	4	0.1	2.50
ZW23	6	6	0.1	1.67
ZW24	6	5	0.1	2.00
ZW26	1	7	0.2	2.86
ZW28	6	4	0.1	2.50
ZW30	6	6	0.8	13.3
ZW 31	3	5	0.2	4.00
ND01	6	3	0.1	3.33
ND02	2	4	0.1	2.50
ND08	6	5	0.1	2.00
ND09	2	6	0.1	1.67
ND13	3	5	0.1	2.00
ND14	3	4	0.3	7.50
ND18	5	4	0.1	2.50
ND21	3	8	0.1	1.25
ND22	2	6	0.2	3.33
ND23	2	5	0.1	2.00
ND24	5	5	0.2	4.00
ND26	2	8	0.2	2.50
ND30	2	8	0.3	3.75
ND31	6	7	0.1	1.43
ND32	5	5	0.2	4.00
ND33	6	3	0.2	6.67
LM03	3	6	0.8	13.3
LM04	3	4	0.1	2.50
LM05	4	3	0.1	3.33
LM06	6	6	0.4	6.67
LM08	3	5	0.2	4.00
BN4	1	10	0.1	1.00
BN16	3	7	0.2	2.86
BN17	2	2	0.1	5.00
BN19	2	7	0.2	2.86
CH02	2	5	0.3	6.00
CH03	3	9	0.5	5.56

Sample no	Age	Measured BPb (µg/dL)	Predicted BPb for soil (µg/dL)	% contribution of soil lead to measured BPb
CH05	6	5	0.1	2.00
CH09	6	5	0.1	2.00
CH13	3	10	0.3	3.00
CH14	5	4	0.1	2.50
CH15	5	7	0.1	1.42
CH16	3	7	0.1	1.43
CH20	6	12	0.2	1.67
CH22	2	4	0.1	2.50
CH26	6	8	0.1	1.25

7.3 Appendix 3

Ethics Approval from Malawi

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.09/12/1282 – Health Risk Assessment Exposure to Children in Blantyre, Malawi by Mr. Wells Utembe

On 4th October

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC as indicated on the next page

Dr. G. Kalanda - Chairperson (COMREC)

Approved by College of Medicine
04 OCT 2012
(COMREC)
Research and Ethics Committee

04 October 2012
Date

Ethics approve from University of Witwatersrand

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Mr Wells R Utembe

CLEARANCE CERTIFICATE	**M120662**
PROJECT	Health Risk Assessment of Lead Exposure to Children in Blantyre, Malawai
INVESTIGATORS	Mr Wells R Utembe.
DEPARTMENT	School of Public Health
DATE CONSIDERED	29/06/2012
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 01/03/2013 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof Mary Gulmain

DECLARATION OF INVESTIGATOR(S)
To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...