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Lead exposure and its impact on the health of adolescents: The birth to twenty cohort

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LEAD EXPOSURE AND ITS IMPACT ON THE HEALTH OF ADOLESCENTS: THE BIRTH TO TWENTY COHORT

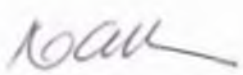
NISHA NAICKER

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2012

DECLARATION

I, Nisha Naicker declare that this thesis is my 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.



Signature

22nd day of February, 2012.

DEDICATION

This PhD is dedicated to my wonderful children, Inia and Karthik. You are my inspiration.

PUBLISHED THESIS MATERIAL

Current publications and manuscripts related to this thesis

1. Naicker, N., Norris, S., Mathee, A., von Schirnding, Y.E., Richter, L. 2010. Prenatal and adolescent blood lead levels in South Africa: Child, maternal and household risk factors in the Birth to Twenty cohort. **Environmental Research**. 110 (4): 355-362.

Contributions to the paper by each author:

- **Naicker N**: Conceptualisation of the paper, data management, data analysis and primary write up and submission of the paper.
- **Norris S and Mathee A**: Supervision of conceptualising of the study components.
- **von Schirnding YE and Richter L**: Advisory role in write up of the paper.

2. Naicker, N., Norris, S., Mathee, A., Becker, P., Richter, L. 2010. 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.

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- **Norris S and Mathee A**: Supervision of conceptualising of the study components.
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- **Richter L**: Advisory role in write up of the paper.

3. Naicker, N., Richter, L., Mathee, A., Becker, P., Norris, S. 2012. Association between environmental lead exposure and socio- behavioural adjustment: Findings from the Birth to Twenty Cohort. **Science of the Total Environment.** 414: 120-125.

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- **Becker P**: Advisory role with regards to the statistical analyses
- **Richter** L: Advisory role in write up of the paper

Conference proceedings (Presentations by N Naicker)

1. Naicker, N., Norris, S., Mathee, A., von Schirnding, Y.E., Richter, L. Prenatal and adolescent blood lead levels in South Africa: Child, maternal and household risk factors in the Birth to Twenty cohort. School of Public Health

Research Day, University of Witwatersrand, Johannesburg, South Africa (2008).

2. Naicker, N., Norris, S., Mathee, A., Becker, P., Richter, L. Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: Findings from the Birth to Twenty cohort. 5th Annual Public Health Association of South Africa (PHASA) conference in Durban, South Africa (2009); the annual International Society for Environmental Epidemiology (ISEE) Conference in Seoul, Korea (2010) and the Faculty of Health Sciences Research Day (2010).

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2. Ford Foundation Fellowship Grant (2009, 2010, 2011).

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4. Young Researchers Prize from the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

ABSTRACT

Introduction

Lead exposure continues to be a major public health issue in South Africa, and other low and middle income countries. Environmental lead exposure has been associated with detrimental health effects in children. The aim of this thesis was to assess the prevalence of lead exposure and its association with various risk factors, its effects on puberty and socio-behavioural adjustment in adolescents.

Methods

The Birth to Twenty (Bt20) cohort study started in 1990, and is a long-term prospective follow-up study of children's health and well-being. Mothers were recruited from antenatal clinics in the Johannesburg-Soweto metropolitan area between April and June 1990 (n=3273). Lead levels were analysed in samples of cord blood collected at birth (n=618) and whole venous blood collected at 13 years of age (n=1546). Data on selected child, maternal and household factors were collected using a structured questionnaire in the third trimester of pregnancy and at 13 years of age. Additional data on puberty (attainment of menarche and self-reported Tanner staging for breast and pubic development) and behaviour using the Youth Self Report was obtained at 13 years of age.

Results

In the Bt20 cohort the mean blood lead level at birth was 5.9 µg/dl, and at 13 years of age it was 5.7 µg/dl. The majority of children had blood lead levels above 5.0 µg/dl (52% at birth and 56% at 13 years). At birth, being a teenage mother and having low educational status were strong predictors for elevated cord blood lead levels. Being a male child, having an elevated cord blood level, and lack of household ownership of a phone were significant risk factors for high blood lead levels at 13 years.

In 13 year old females with pubertal data (n= 682) the mean blood lead level was 4.9 µg/dl. Fifty percent had blood lead levels < 5.0 µg/dl, 49 % were ≥5.0 µg/dl and 1% was > 10.0 µg/dl. The average age of menarche was 12.7 years. At 13 years, 4% and 7% had reached Tanner stage 5 for pubic hair and breast development, respectively. Analyses showed that higher blood lead levels were significantly associated with delays in all measures of puberty ($p < 0.001$).

In the 13 year old sample with data on the Youth Self Report (n= 1041), the geometric mean blood lead level was significantly (P value<0.001) higher in boys (6.0 µg/dl) compared to girls (4.5 µg/dl). The bivariate analyses stratified by gender showed that boys' blood lead levels were significantly associated with four types of aggressive behaviour. There were no significant associations found in girls. A multivariate analysis was conducted in the sample of boys and after

adjusting for socio-economic factors "Attacking People" remained significantly associated with blood lead levels.

Conclusion

Significant associations found in the study point to the low socio-economic status of lead exposed children. These poor circumstances frequently persist into adolescence resulting in continued high lead levels. Higher blood lead levels were associated with a delay in the onset of puberty in girls, and with anti-social behaviour among boys in early adolescence. Lead exposure in low and middle countries is generally higher compared to high income countries, and thus the effects of high blood levels are much greater and have larger personal and public health significance.

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NOMENCLATURE

AACAP	American Academy of Child and Adolescent Psychiatry
ADHD	Attention Deficit Hyperactivity Disorder
ALAD	Aminolevulinic acid dehydratase
ALA	Aminolevulinic acid
ATSDR	Agency for Toxic Substances and Disease Registry
BLL	Blood lead levels
Bt20	Birth to Twenty
Cd	Cadmium
Ca EDTA	Calcium disodium ethylenediame tetra acetic acid
CDC	Centers for Disease Control
DALYS	Disability adjusted life years
Db	Decibels
DSM	Diagnostic and Statistical Manual of Mental Disorders
E_2	Estradiol
GABA	Gamma-aminobutyric acid
GFAAS	Graphite furnace atomic absorption spectrometry
GIT	Gastrointestinal
HUD	Housing and Urban Development
ICP-MS	Inductively coupled plasma mass spectrometry
IGF	Insulin like growth factor
Pb	Lead
LH	Luteinising hormone
LHRH	Luteinising hormone releasing factor

MRI	Magnetic Resonance Imaging
µg/dl	Micrograms per decilitre
NHANES	National Health and Nutritional Examination Survey
ppm	parts per million
PGE_2	Prostaglandin E_2
RBC	Red Blood Cell
SD	Standard deviation
TEL	Tetra ethyl lead
USA	United States of America
VIF	Variance Inflation Factor
WHO	World Health Organization
XRF	X-ray fluorescence
YSR	Youth Self Report

PREFACE

Lead is a ubiquitous heavy metal that has contaminated the earth mainly because of human activities (Hernberg, 2000; Tong et al., 2000). The potential dangers of lead exposure have been known for centuries, and numerous international studies, mainly conducted in resource-rich countries, have demonstrated the detrimental health effects of lead exposure. Lead exposure in these countries has dropped substantially following measures to routinely monitor and actively prevent lead exposure (Koller et al., 2004; Harper et al., 2003; Campbell and Osterhoudt, 2000). However, in South Africa, as well as in other low and middle income countries, lead exposure continues to be high and affects mainly children.

After reviewing the literature on lead exposure and the health effects caused by environmental lead, there are several gaps in the literature mainly in low and middle income countries such as South Africa. The prevalence of lead exposure and poisoning has been determined only in a few cross-sectional studies (Mathee et al., 2006; 2004; 2002; von Schirnding et al., 2001; 1991b). There has been a particular dearth of studies looking at the health effects of lead exposure in children in South Africa.

Thus this thesis aims to answer the following questions in a series of published manuscripts:

- What is the prevalence of lead exposure at birth and in early adolescence in a cohort of children in urban South Africa?
- What are the risk factors associated with lead exposure at birth and at 13 years of age, including longitudinally?
- What are the effects of lead exposure on biological systems of children in adolescence?
- What are the effects of lead on behaviour in early adolescence?

The thesis has been divided into three parts. Part one (Chapter 1) describes the relevant scientific literature. Part 2 comprises the published papers in chapters 2, 3 and 4. Part 3 of the thesis integrates the findings of the study. All references have been standardised using the Harvard referencing system.

Chapter 1 describes the conceptual framework on which this thesis was based. An extensive review of the literature on lead exposure levels nationally and internationally and the evidence for detrimental health effects in children is discussed. This background information was used to identify the relevant gaps in the literature and provide the foundation for the published papers of the thesis.

Chapter 2 is the first manuscript and assesses the degree of lead exposure at birth and at the 13 years of age. Maternal, child and household factors have been described and their associations with blood lead levels were determined. This

paper included a longitudinal analysis of blood lead levels and risk factors from birth to 13 years of age.

Chapter 3 details the biological effects of lead exposure. This was measured by looking at the effect of lead on puberty in female adolescents. Three measures of puberty (age of attainment of menarche, self-reported Tanner staging for pubic hair and breast development) were assessed in relation to blood lead levels at 13 years of age.

Chapter 4 assessed the effect of lead exposure on socio-behavioural adjustment at 13 years of age in the total sample as well as when the sample was stratified by gender.

Chapter 5 integrates the above four chapters and discusses the findings with respect to the conceptual and contextual frameworks as well as the research theme that emerges throughout this thesis.

Chapter 6 concludes the thesis by highlighting the particular relevance of this study.

PART 1

RELEVANT BACKGROUND LITERATURE

CHAPTER 1: INTRODUCTION

Chapter 1 describes the conceptual framework on which this research was based. The literature review will outline the prevalence of lead exposure and the health implications for children. The motivation for the study and research gaps in the current literature is followed by the study aims and objectives.

1.1 Conceptual framework

This study was based on the international body of evidence describing the health effects of lead exposure on a particularly vulnerable group in our society i.e. children. This study will contribute to existing knowledge by describing and analysing the prevalence and health effects of lead exposure in a low and middle income setting. Good health is a global goal; however there are environmental and social factors that put the health of the individual, the community and ultimately societies/populations at risk.

The conceptual framework illustrated in Figure 1.1 is based on a public health model and describes the main factors and exposures and their relationships that contribute to the health of the individual and the population (Bellinger, 2008; Fewtrell et al., 2004; Selevan et al, 2003; Tong et al., 2000). Exposures (lead exposure) leads to health effects and these health effects can be mitigated or enhanced by social, economic and genetic factors.

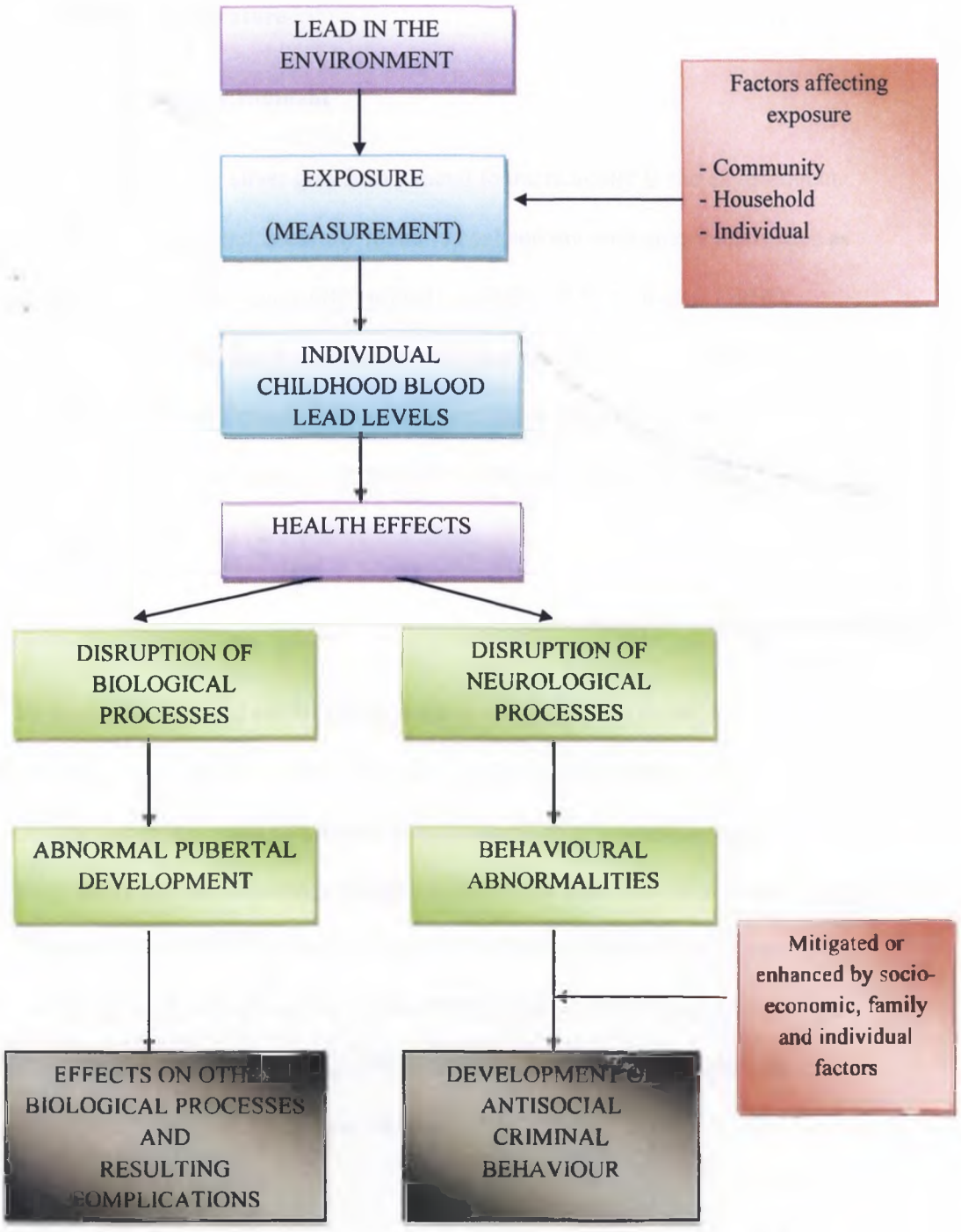

Figure 1.1: Conceptual Framework- Lead exposure and its impact on biological processes

(Bellinger, 2008; Fewtrell et al., 2004; Selevan et al., 2003; Tong et al., 2000)

1.2 Background literature

1.2.1 Lead in the environment

Lead (Pb) is a blue or silver grey heavy metal found naturally in the environment. Naturally occurring lead is usually found in coal and ore with other metals such as zinc, silver and copper and is often mined together with these metals (WHO, 2001). It is estimated that three hundred million tonnes of lead have been released into the environment through the processing of these ores over the last five hundred centuries especially in the past five centuries (Tong et al., 2000; Hernberg, 2000).

The properties that lead exhibits have made it a highly useful metal. Lead is soft and highly malleable. It is a relatively poor conductor of electricity but is very resistant to corrosion and exhibits excellent absorption of sounds and other vibrations as well as radiation, making it useful in the manufacture of x-ray shields (http://science.jrank.org). Lead has a melting point of 327.5 degrees Celsius making it highly resistant to fire (WHO, 2001). Stable organic compounds of lead are formed by combining with carbon atoms. These compounds were the basis of anti- knocking agents used in petrol (WHO, 2001).

1.2.1.1 Sources of lead

Lead is released into the environment mainly from human activities (Budd et al., 2004). Currently over 2.5 million tons of lead are mined and used annually around

the world (Keate et al., 1983). There are about 120 occupations that use and result in exposure to lead, including lead smelting, ship building, ship repairing, battery manufacturing and building demolition (Keate et al, 1983) as well as the production of copper, steel and iron and the combustion of coal, since many minerals, rocks and sediments contain lead (WHO, 2001). Thus the use of lead in formal and informal occupational settings has resulted in widespread environmental contamination.

In the pre-industrialization era, lead exposures were relatively low, but with industrialization and increased mining activities lead exposure has increased dramatically. Lead exposure can result through the ingestion of food contaminated with lead from cooking utensils and pottery. Acidic food and liquids such as salad dressings, fruit juices and wine cause lead to dissolve out of the glaze in certain ceramics (Tong, et al., 2000; Hernberg, 2000). Artificial softening of water from the 1960's resulted in lead dissolving out of lead containing pipes (Hernberg, 2000).

Environments surrounding lead smelters and other industries utilising lead are usually contaminated due to the emissions from such industries. In low and middle income countries this is a major public health problem because of poor monitoring and regulatory systems. There is evidence of workers in these types of industries going home with lead contaminated dust on clothing, vehicles and even on skin and hair due to poor occupational hygiene practices (Gulson et al., 1994).

Cottage industries using lead solders, discarded car batteries for distilling whiskey or dismantling old car batteries are another source of lead in the environment (Tong et al., 2000; Hernberg, 2000). Cosmetics and traditional medicines containing lead continue to be a problem especially in low and middle income countries (Hernberg, 2000). In addition, studies have shown that cheap jewellery (plastic and metal) made from recycled lead battery waste may be another source of lead exposure (Yost and Weidenhamer, 2008; Weidenhamer and Clement, 2007; CDC, 2006).

The most significant contributing factor to the contamination of the environment was leaded petrol that first made its appearance in the 1920's. Tetraethyl lead (TEL) was added to petrol as an anti-knocking agent and contributed to increasing the performance of the engine. The use of leaded petrol caused global widespread lead pollution due to lead emissions from cars' exhaust pipes. It was only in the 1990's that the United States of America (USA) stopped the use of leaded petrol, with a similar position subsequently adopted in Europe (Hernberg, 2000). In South Africa the use of leaded petrol was stopped only in 2006. In a declining number of some low income countries the use of leaded petrol continues unabated (United Nations Environmental Protection, 2008).

Lead in paint is another major source of lead exposure. Many manufacturers have decreased or stopped adding lead to paint; however some continue to produce leaded paints. Recent studies showed that paint containing lead is still being produced and sold in many countries especially in low and middle income

countries (Mathee et al., 2007; Clark et al., 2009). In countries that have discontinued the use of leaded paints, old housing stock with peeling paint, renovations and demolition contributes to elevated levels of lead in soil, dust and air, with the potential for public lead exposure (Rabito et al., 2007; Farfel et al., 2005; Jacobs et al., 2002; Lanphear et al., 1998). In addition to homes and schools, lead-based paint is known to have been applied to toys and playground equipment (Mathee et al., 2009).

Lead from all sources contaminates air, water, food, soil and household dust. The commonest route for lead exposure is ingestion – the hand to mouth route. Lead exposure through the hand to mouth pathway is a major contributor to the particular vulnerability of children to lead exposure. Young children normally explore their surroundings with hand to mouth type of behaviour (Bellinger, 2004). Thus a child who resides in an area with high traffic flow e.g. in inner city areas or houses with old peeling paint or near industries such as lead smelters, tend to have higher levels of lead exposure due to contaminated soil and dust in the home. Figure 1.2 summarises the sources and routes of exposures that influence the body burden of lead.

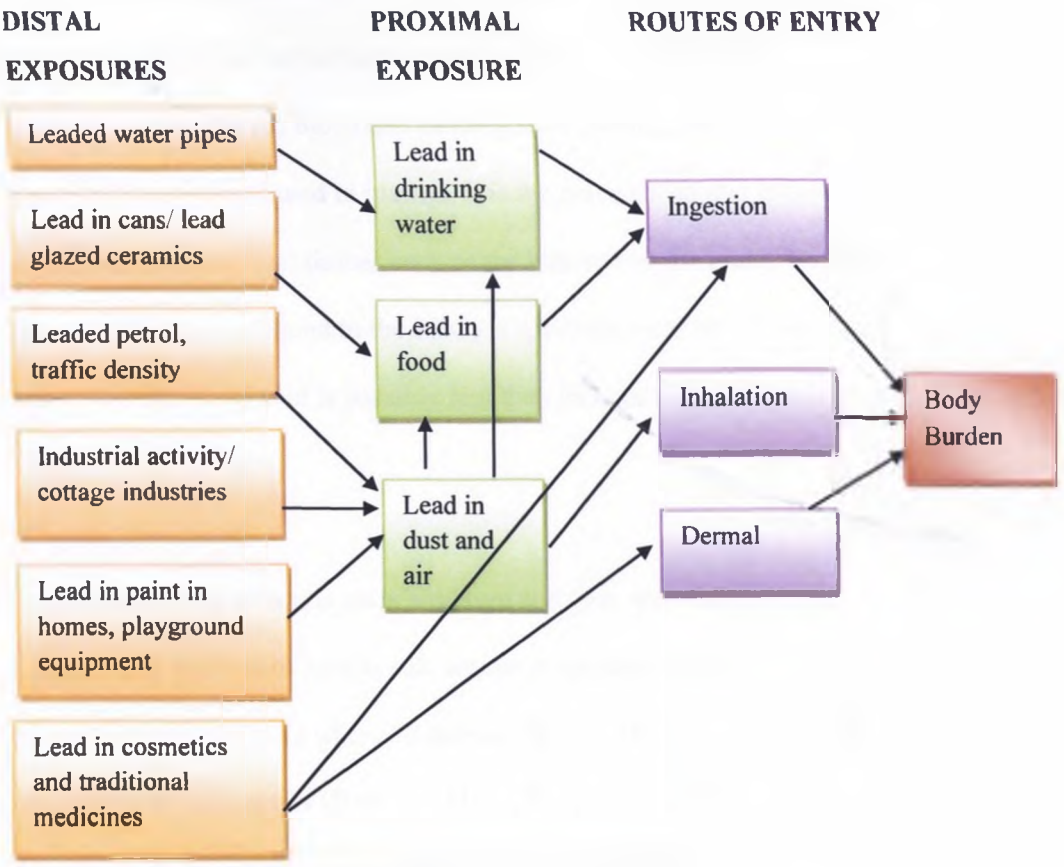

Figure 1.2: Sources and Routes of lead exposure
(Adapted from Fewtrell et al., 2004; 2003)

1.2.1.2 Metabolism of lead

Once one is exposed to lead either via the lungs or gastrointestinal tract, lead is absorbed. The amount of lead absorbed depends on its particle size and its solubility. Other factors affecting its absorption are based on individual characteristics such as age, sex and nutritional status (Barbosa et al., 2005). Lead enters the blood stream and this usually constitutes 1-5% of the total body lead. At

low lead concentrations 95-99% of blood lead is bound to the red blood cells (RBC) and 1% is found in the ionized form in plasma. At higher lead concentrations, when the red blood cell binding sites are saturated, a larger percentage of lead is distributed in plasma. It is the plasma lead that is easily exchanged into bone and soft tissues such as the kidney and the brain. Thus the half-life of lead in blood (bound to the RBC) is approximately 36 ±5 days, while in plasma, the half-life of lead is possibly less than an hour (Sakai, 2000).

In the soft tissues lead affects protein structure and gene translation, leading to its toxic effects. The half-life of lead in soft tissues is approximately 40 days. In bone it replaces calcium and this is where 70-80% of lead is stored in the body in children and 90-95% in adults (Barry, 1981; Ambrose et al., 2000). Lead in bone is stored for years and continues to accumulate throughout an individual's lifetime. Rabinowitz (1991) estimated the bone lead half-life to range from 10 to 30 years, reflecting chronic or lifetime exposure (Rabinowitz, 1991; Hu, 1998). Bone also acts as an endogenous source of lead. During certain times of increased turnover, lead from bone is released into the blood and then transferred into tissues where it exerts its toxic effects. This is especially important in pregnancy and lactation where increased release into the blood stream leads to placental transfer of lead into the foetus (Rollin et al., 2009; Ambrose et al., 2000). Maternal calcium and iron deficiencies increase the risk and degree of placental lead transfer via passive diffusion (Goyer, 1990) during pregnancy and during breast feeding (Gulson et al., 2004; Bradman et al., 2001). Studies have also

shown that release of lead from bone stores is high during the postpartum period, leading to increased levels of exposure in the breastfed neonate (Rothenberg et al., 2000; Gulson et al., 1998; Hernandez-Avila et al., 1996). Calcium supplementation decreases the risk of lead release from bone during lactation (Ettinger et al., 2006; Ettinger et al., 2007; Hernandez- Avila et al, 2003). Growth in childhood results in increased turnover of bone due to continuous remodelling of bone. Thus lead is constantly released into the blood stream of children (O' Flaherty, 1995). Different types of bone have different turnover times. Trabecular bone such as the patella has a shorter turnover time than cortical bone found for example in the tibia (Barbosa et al., 2005).

Some of the lead in plasma is excreted mainly through faeces and urine. Very small amounts are excreted in saliva and sweat. The excretion process is very inefficient, and most lead remains stored in the body (Sakai, 2000; Ambrose et al., 2000). Excretion also depends on the body burden and kidney function in the case of urinary excretion. The urinary excretion will reflect current/recent exposure since it increases as the plasma lead increases (Sakai, 2000). Figure 1.3 illustrates the metabolic pathway of lead in the body.

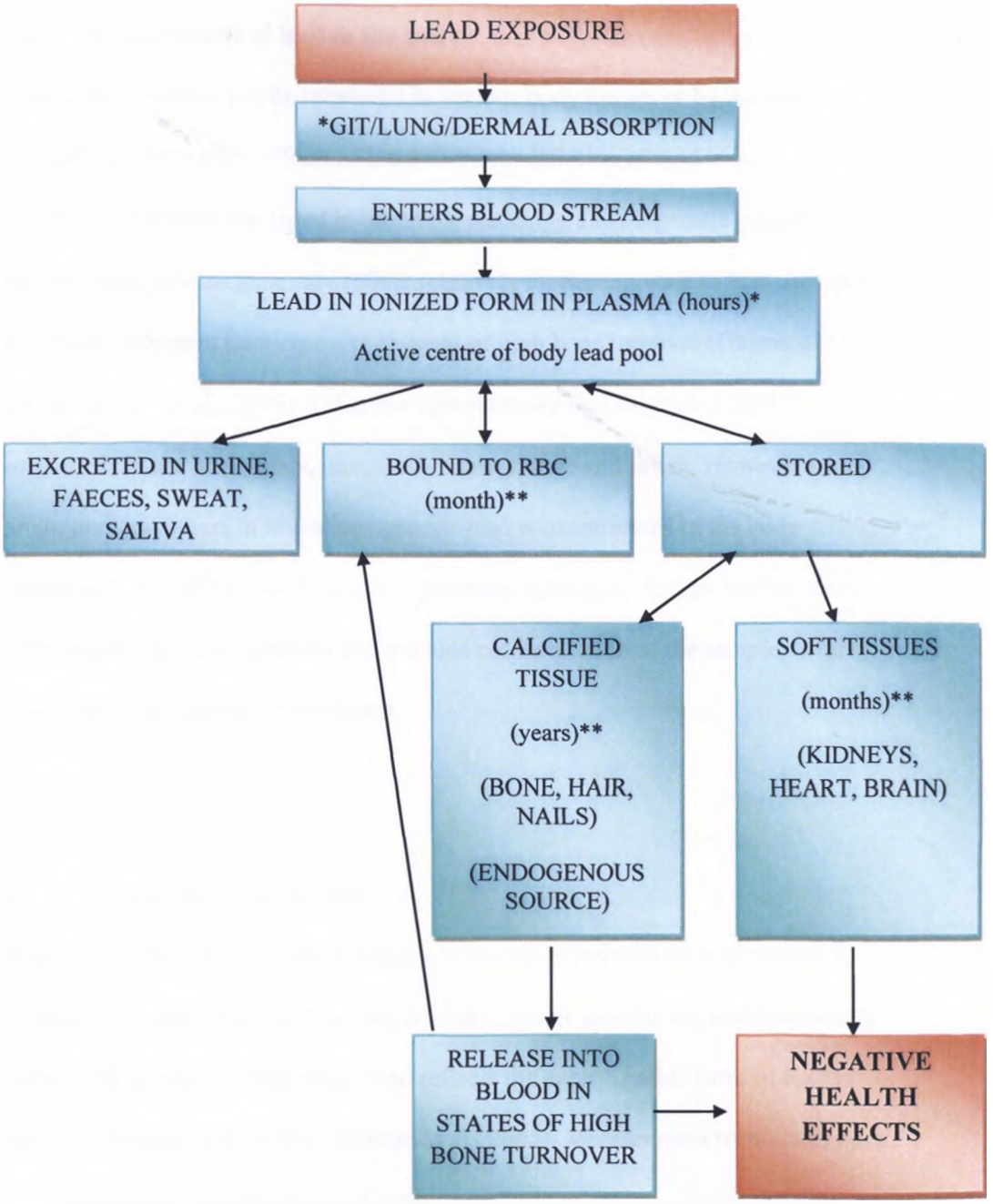

Figure 1.3: Lead metabolism pathway

*GIT Gastrointestinal

** Indicates total time that lead is present in the various body tissues

1.2.2 Measurement of lead in the body

Lead concentrations can be measured in various body tissues or by measuring the biomarkers of the toxic effects of lead. The concentration of lead in one's body has most often been measured in blood for screening and diagnostic purposes. Blood measurements generally reflect relatively recent exposure to lead, however it can also represent past exposure in cases of high bone turnover (Gulson et al., 1996; Barbosa et al., 2005). Other biological tissues that have been used to measure lead are bone, nails, hair, teeth, urine, faeces and saliva. However some are better than others in revealing accurate lead concentrations in the body (Barbosa et al., 2005). In all samples, specimen collection, storage and processing must be meticulously performed to exclude contamination of the samples with lead in the surrounding environment.

1.2.3.1 Lead in blood and plasma

Lead can be measured in whole blood and plasma to indicate current or recent exposure. Plasma blood lead is a better lead exposure monitoring tool than whole blood lead because plasma blood lead reflects the exchangeable form of lead in the body (Schütz et al., 1996; Barbosa et al., 2005). Measurement techniques have been improved over the years and instruments are now able to detect very low levels of lead. The latest techniques are the inductively coupled plasma mass spectrometry (ICP-MS) and the graphite furnace atomic absorption spectrometry (GFAAS). The GFAAS method has been widely used, however the ICP-MS is a newer technique that is also highly sensitive and can measure plasma lead levels

of < 1.0 µg/dl (Schütz et al., 1996; Sakai, 2000; Barbosa et al., 2005). In the studies presented in chapter 2, whole blood was used to determine blood lead levels.

1.2.3.2 Lead in urine, faeces and saliva

Lead in urine indicates lead that has been filtered through the kidneys. Urine lead is one of the ways of long term bio-monitoring since it is non-invasive. However measuring the urine once only is not a reliable reflection of lead exposure since it can be affected by kidney function. The GFAAS and ICP-MS techniques may be used to measure urine lead (Sakai, 2000).

Another method of measuring lead in urine is to orally administer a chelating agent such as calcium disodium ethylenediame tetraacetic acid (CaEDTA) and measuring the lead in urine 24 hours post administration (Sakai, 2000). The plasma lead can be measured two hours after the administration and correlates with the lead in urine that is collected after 24 hours (Sakai et al., 1998; Sakai, 2000).

Faecal lead measurement is not the most feasible method as collection of samples needs to occur over several days. Lead in faeces needs to be used together with other tissue measurements in order to get a true picture (Barbosa et al., 2005).

Saliva has also been shown to be an unreliable source for measuring lead. There are wide variations in saliva flow, no consistent association between plasma and

saliva lead measurements and there are no standardized reference values (Barbosa et al., 2005). However a study by Costa de Almeida et al (2010) showed a weak correlation between plasma lead levels and parotid saliva. The whole blood and saliva levels did not have a significant correlation (Costa de Almeida et al., 2010).

1.2.3.3. Lead in bone

Bone lead accounts for 94% and 70% of the body lead burden in adults and children respectively. Bone lead is measured using x-ray fluorescence (XRF) techniques. Fluorescing photons remove an inner-shell electron from a lead atom, leaving it in an excited state. The result is emissions of x-ray photons that are characteristic of lead. There are four types of XRF: two types use the florescence of the K-shell electrons of lead (K-XRF) and two use the L-shell electrons (L-XRF) (Barbosa et al., 2005; Todd et al., 2002). K-XRF used with the 109 Cd (cadmium) isotope has been shown to have a better detection limit and lower radiation dose than the L-XRF (Todd and Cheatle, 1994). The radiation exposure is less than that obtained from a routine chest x-ray. However, the equipment used to measure lead in bone is not routinely available in most countries including South Africa. Thus use of this equipment has generally been restricted to research institutions in high income countries.

1.2.3.4. Lead in nails and hair

Nail and hair samples are easy to collect and lead measurements are non-invasive. Nail lead reflects long term exposure (Takagi et al., 1988) but the concentrations of lead found varies according to age of the individual and the variability in

results obtained from the same fingernails means that fingernails are not reliable indicators of body lead burdens (Gulson, 1996; Nowak and Chmielnicka, 2000).

Hair, like nails, has limited use because one cannot distinguish between endogenous lead (lead derived from the body) and exogenous lead deposits from the surrounding environment (Barbosa et al., 2005). Even after washing the hair, studies have shown that not all exogenous lead is removed (Mortan et al., 2002). Lead concentrations in hair also vary according "age, sex, hair colour, length of hair, smoking, geographic factors as well as ethnicity" (Barbosa et al., 2005). Thus hair is not useful in measurement of the body burden of lead.

1.2.3.5 Lead in teeth

Teeth reflect long term exposure to lead, similar to bone. The loss of lead from teeth is much slower than the loss from bone and this might make teeth a better indicator of lifetime lead exposure. Most studies published have used deciduous teeth to measure lead but these studies have not determined the lead references in different types of teeth, there has been no distinction between the dentin and enamel parts of the tooth, and variations in age or sex were not always considered (Barbosa et al., 2005). Further, children less than six years who still have "milk" teeth cannot be assessed (Barbosa et al., 2005). Thus lead in teeth cannot be routinely analysed to assess body lead burden.

1.2.3.6 Biomarkers of lead toxicity

Lead disrupts various enzymatic processes such as those responsible for haeme synthesis. Levels of the resultant metabolites can be measured in urine and blood, and used to indicate lead toxicity. However at low levels of lead exposure, which are still harmful, the metabolite levels may not be abnormal, thus measurement of these biomarkers may not always be useful.

1.2.3 Evidence of lead exposure (historical and current)

Evidence of toxicity due to lead exposure was first documented in 370 BC (Tong et al., 2000). References to possible toxicity from lead exposure was found in Egyptian papyrus scrolls; however the link between lead exposure and detrimental effects on health was only made around 1AD. Dioscorides made the association between lead exposure and toxicity and Pliny reported that toxicity from lead was common in people who built ships. In ancient Rome lead was used in pipes, glazing pottery, cooking utensils, pots used to boil and condense grape juice and to sweeten wine (Hernberg, 2000). Thus lead poisoning was common in the ancient Roman Empire, especially among the Roman elite (Needleman, 2004; Woolley, 1984). Higher concentrations of lead were found in bones in the patrician tombs than in the plebeian graves (Gilfillan, 1965; Hernberg, 2000). Table 1.1 illustrates the historical evidence of lead toxicity.

Table 1.1: Historical evidence of health effects due to lead exposure

DATE OF DISCOVERY	PERSON RESPONSIBLE FOR THE DISCOVERY	DOCUMENTED NEGATIVE HEALTH EFFECTS	HEALTH EFFECT ATTRIBUTED TO LEAD
370 BC	Hippocrates	Colic	No
2 BC	Nicander	Lead palsy	No
1 AD	Dioscorides	Clinical manifestations of lead poisoning	Yes
	Pliny	Stated lead poisoning common in ship builders	Yes
1498 & 1577	Germans	Epidemics of lead toxicity	Yes, due to lead used in wine and ciders
16 century	Paracelsus	"Miners disease"	Yes
18 century	Ramazzini	Paralysis, lethargy, cachetic, toothless potters	Yes
1767	Sir George Baker	Devonshire colic:- palsy, encephalopathy, pallor and abdominal cramps	Yes, due to lead weights crushing apples to make cider
1831	Laennec	Anaemia	Yes
1832	Thackrah	Plumbism	Yes
1839	Tanquerel des Planches	Abdominal, neurological and arthritic signs of lead poisoning.	Yes, based findings on 1200 cases
1854	Garrod	Lead gout	Yes

1899	Behrend	- Basophilic stippling of erythrocytes (RBC) - Counting of these stippled cells used in health surveillance in lead workers from first half of the 20th century until 1960's.	Yes
1898	Garrod	Increased excretion of porphyrins in urine	Yes
Beginning of 20th century	Australia	Childhood lead poisoning	Yes
Early 20th century	Nye	Renal effects like Fanconi syndrome in adults who had lead poisoning in childhood.	Yes
Late 19th and early 20th century		-Abortions and stillbirths in women working in lead industry. -Higher mortality of children born to these women. -Infertility in male workers Also used to induce illegal abortions	Yes
1934	Vigliani and Angerleri	Showed that incorporation of iron into heame was disrupted, thus protophorphyrin accumulated in the RBC	Yes
1951	Sheets	Lead decreased lifespan of RBC which also contributes to the anaemia. Introduction of chelating agents which was	Yes

		sometimes used in symptomless workers as prophylaxis	
1960	Haeger-Aronsen	Excretion of aminolevulinic acid (ALA) in urine of lead workers	Yes
1960's	Bonsignore and colleagues	-Lead inhibited enzyme delta-aminolevulinic acid dehydratase which explained the increased aminolevulinic acid excretion. - By late 1960's new methods for measuring lead were now developed – dithizone method and then later atomic absorbtion spectrophotometry which improved medical surveillance of workers	Yes
1970's	Globally	Subclinical manifestations in adults and children were noted.	Yes
1970-2010	Globally	Biological disruption by low level lead exposure continues.	Yes

(Data compiled from: Needleman, 2004; Hernberg, 2000; Gilfillan, 1965)

Currently there is evidence for continued exposure to lead during the prenatal and postnatal period. The risk of foetal exposure and subsequent neonatal blood lead levels is dependent on maternal blood lead levels. Maternal blood lead levels are from either current or past exposure where the lead is released from endogenous sources such as bone during pregnancy and lactation (Ambrose et al., 2000). In high income countries such France, Poland and Belgium, the average cord blood

levels were 1.2 µg/ dl, 1.2 µg/ dl, 2.3 µg/ dl respectively between 2001 and 2006 (Yazbeck et al., 2007; Jedrychowsky et al., 2009; Koppen et al., 2009). Prior to 2000, in 1993 mean cord blood levels in similar European resource rich countries, such as Italy, were around 4.9 µg/dl (Carbone et al., 1998).

In low and middle income countries the situation is different. Here cord blood lead levels continue to be high. In China and India during the 1990's the geometric mean lead concentrations in cord blood were 9.2 µg/dl and 5.1 µg/dl respectively (Shen et al., 1998; Ragunath et al., 2000). In 2005 in Pakistan, the mean cord blood lead level was 10.8 µg/dl (Janjua et al., 2008). In South Africa the situation is similar. A pilot study in 1993 that assessed cord blood from 21 newborns showed that the mean cord blood level was 15.5 µg/dl and 95% had blood lead levels > 10.0 µg/dl (Chetty et al., 1993). No other studies of newborn exposure to lead have been reported in South Africa.

As with the cord blood lead levels, blood levels in children beyond the neonatal period in resource rich settings has declined dramatically over the past few years. In the USA median blood lead levels have dropped substantially from 12.8 - 15.0 µg/dl in the 1970's to 2.8 µg/dl by the late 1980's (Bellinger and Bellinger, 2006; Pirkle et al., 1994; Nriagu et al., 1997). In the 1970's over 88% of children had blood lead levels greater than 10.0 µg/dl. Currently only 2% of American children have blood lead levels > 10.0 µg/dl which is the Centre for Disease Controls' action limit (Bellinger and Bellinger, 2006, CDC, 1991). In Sweden by 2001, blood lead levels averaged around 2.0 µg/dl compared to 7.4 µg/dl in 1978

(Strömberg et al., 2003). Similar decreases in blood lead levels have been seen in Belgium, Germany, New Zealand, Australia and the United Kingdom. However in some high income countries, such as the USA, there are areas where children continue to have high exposures to lead. These are generally in areas of poverty, such as inner cities, and affect mainly children from minority population groups living in poor dilapidated housing (Bellinger, 2004; Tong et al., 2000).

In the Western Cape, South Africa cross sectional studies conducted in 1991, found that inner city school children aged 6 to 8 years had mean blood lead levels ranging from 14 to 16 µg/dl (von Schirnding et al., 1991a; 2001; Mathee et al., 2002; 2006). Over 90% had blood lead levels greater than10.0 µg/dl. In 1995 in a sample of Johannesburg school children aged 6-7 years old, it was found that 78% had blood lead levels >10.0 µg/dl and mean sample level of 11.9 µg/dl (Mathee et al., 2002). By 2002 the study was repeated in the same Johannesburg schools and the mean blood lead level dropped to 9.1 µg/dl, and 10 % had blood lead levels > 10.0 µg/dl. This change was attributed to the introduction of unleaded petrol in 1996 (Mathee et al., 2004).

In low and middle income countries such as Nigeria prior to 2004, the mean blood lead was 11.2 µg/dl in the 0-5 year age group and in the 13 to 20 age group the mean was 6.6 µg/dl. (Wright et al., 2005). Earlier studies showed that in urban regions of Nigeria 15-30% of children had blood lead levels > 25 µg/dl (Nriagru, 1992; Wright et al., 2005). In a cross sectional study in four urban areas of

Thailand the average blood lead level was 5.7 µg/dl with 12.5% of the children aged 6 months to 4 years having a blood lead level > 10 µg/dl (Chomchai et al., 2005). In Bangladesh in 2000 the mean blood lead levels of children between 4 and 12 years was 15 µg/dl and 87.4% had a blood lead level > 10 µg/dl (Kaiser et al., 2001). In China, children living near industrial (64.9% to 99.5%) and non-industrial areas (>50%) had blood lead levels >10 µg/dl (Tong et al, 2000). In other low and middle income countries in Africa and Asia, where rapid urbanisation and industrialisation is taking place, children are still exposed to high levels of lead (Tong et al., 2000).

The blood lead levels differ between countries and within countries. Most importantly the blood lead levels in low and middle income countries is much higher than that of more high income countries and the number of children with blood lead levels > 10.0 µg/dl is still too high, impacting negatively on the health of these children.

1.2.4 Health effects of lead exposure

During the course of the centuries evidence of abnormal physical signs and symptoms and abnormal laboratory findings related to lead toxicity was described. This was illustrated in Table 1.1. Although the detrimental effect of lead exposure was known, lead continued to be mined and used. To date lead continues to be a major public health hazard.

Environmental, social and biological factors affect the degree and impact of lead exposure on the health of an individual. There are also differences in the health effects of lead exposure between children and adults. Children are more vulnerable to the effects of lead exposure due to their immature organ systems and different metabolism of lead. The absorption of lead by the gastrointestinal tract in infants and children is greater than in adults. Nutritional deficiencies of iron and calcium which are more common in children increase the absorption of lead (Bellinger, 2004; Wright et al., 1999; Bradman et al., 2001). The half-life of lead in blood is also greater in children than in adults (Bellinger, 2004; Manton et al., 2000).

Exposure to lead causes the disruption of multiple organ systems in children. Table 1.2 shows the effect of lead at different concentrations. Acute high concentrations of 70.0 µg/dl up to 100.0 µg/dl of lead can produce severe renal abnormalities such as in the absorption and secretion functions of the proximal tubule of the renal nephron. Initially this may be reversible but if lead exposure continues then an irreversible chronic interstitial nephritis results. In children if treatment occurs within two months of exposure there is usually good recovery (Chisolm et al., 1968). Encephalopathy and eventually death can occur at these very high blood lead concentrations close to a 100.0 µg/dl and above. At blood levels of ≥40.0 µg/dl lead affects the activity of vital enzymes in the haeme pathway (WHO, 1991; ATSDR, 2007) leading to anaemia. Lead also decreases the red blood cell life span which contributes to the development of anaemia (ADSTR, 2007). Haemolytic anaemia occurs when one is exposed to high

concentrations of lead over a short period of time. A blood lead level of ≤30.0 µg/dl affects Vitamin D metabolism resulting in abnormal calcium homeostasis and this subsequently impedes normal growth and bone development.

At levels of ≤10.0 µg/dl lead exposure impairs neurological (Lanphear et al., 2005; Bellinger 2004; Canfield et al., 2003; Bernard 2003) and physical (Kaji and Nishi, 2006; Vivoli et al., 1993; Frisancho and Ryan, 1991) development in childhood. It affects the auditory system by disrupting conduction in the distal auditory nerve and the auditory pathway in the brainstem. Hearing loss of 2Db(A) resulted when blood lead levels ranged from 7-18 µg/dl (WHO, 2001; Swartz and Otto, 1987, 1991).

Table 1.2: Clinical effects at specific blood lead concentrations in children

Clinical effects in children	Blood lead in µg/dl
Death	**>100**
Severe Brain damage (Encephalopathy) Kidney damage Severe anaemia	**100**
Severe stomach cramps	**50**
Damage to Heamopoiesis (decreased haemoglobin synthesis)	**40**
Reduced Vitamin D metabolism Increased risk of hypertension in adults	**30**
Impaired nerve function- increased nerve conduction velocity	**20**
Increased level of erythrocyte protoporhyrin Decreased Vitamin D metabolism Decreased calcium homeostasis	**20-10**
Development toxicity Reduced IQ, Behaviour problems Hearing impairment Decreased growth (including puberty) Impaired peripheral nerve function Transplacental transfer	**≤10**

Adapted from ATSDR, Toxicological profile for Lead, 2007 and Bellinger and Bellinger, 2006

1.2.4.1 Effects on puberty

In this thesis the effects on puberty in girls are discussed in detail. Although boys may be affected as well, this has not been discussed due to the lack of sufficient data on male pubertal development in the Birth to Twenty Cohort for analysis. Thus the literature review is focused on female puberty.

a. Normal female pubertal development

Puberty is the biological process that allows one to be able to reproduce. It normally follows the same sequence in everyone, however the timing and speed at which it occurs varies between individuals.

In a female adolescent the evidence of puberty is with telarche (breast formation) and pubarche (appearance of hair over the mons pubis). These changes usually occur between 8 and 13 years of age. Breast development from Tanner stage 2 to 5 (adult stage) can take approximately four years, but may be as short as 18 months or as long as 9 years. The progression of pubarche Tanner stage 2 to 5 can take 2.5 years on average, and ranges from 1.5-3.5 years. During this early stage i.e. Tanner stage 3 of telarche there is an increase in height, usually about a year after the start of telarche. This growth spurt precedes the onset of menarche (menstruation). It usually commences 6 months after the growth spurt. There is very little growth after menarche, usually 2.5 to 5cm. Tables 1.3 and 1.4 reflects the Tanner staging of girls for telarche and pubarche respectively. The described physical appearances of puberty are initiated by hormonal changes that occur in the body (Behrman and Kliegman, 2002).

Table 1.3: Tanner staging for Telarche

Stage	Appearance
1	Pre-adolescent: Elevation of papilla only
2	Breast bud: Elevation of breast and papilla forming a small mound. Areolar diameter enlarges
3	Further enlargement of breast and areolar with no separation of the contours
4	There is a projection of the areolar and papilla to form a second mound above the level of the breast
5	The breasts resemble those of a mature female

Source: Behrman and Kliegman, 2002.

Table 1.4: Tanner staging for Pubarche

Stage	Appearance
1	Pre-adolescent: no pubic hair
2	Sparse growth of long, slightly pigmented, downy hair usually straight along the labia
3	The hair is darker, coarser and curlier, spreads over the junction of the pubis
4	Hair is now like that of an adult, but covers a smaller area and does not extend to thighs
5	Adult in type and quantity

Source: Behrman and Kliegman, 2002.

b. Effects of lead on pubertal development

Animal models have shown that lead affects the endocrine system through disruption of the hypothalamic or pituitary function, as well as direct action at peripheral sites such as the gonads (Winder, 1989; Klein et al., 1994; Wiebe et al. 1982; Huseman et al., 1992; Camoratto et al., 1993). Thus there are probably different pathways through which lead affects puberty (Ronis et al., 1996).

The following studies describe the hormonal changes in animals that resulted in delayed puberty because of exposure to lead (Ronis et al., 1996, 1998; Dearth et al., 2002). Lead caused decreases in levels of Insulin like Growth Factor (IGF-1), Luteinizing Hormone (LH) and Estradiol (E_2) (Dearth et al., 2002; 2004), the endocrine hormones that are responsible for the onset of puberty. Release of IGF-1 leads to the secretion of LHRH from the brain. The presence of LHRH results in the release of LH which leads to the commencement of puberty. Thus if IGF-1 levels are decreased, then LH and estradiol levels are decreased and puberty is delayed. Dearth et al hypothesised several ways in which lead may affect IGF-1 i.e. through an alteration in translation or ineffective IGF- 1 peptide production or an alteration of IGF-1 receptors in the brain (Dearth et al, 2002). Lead also decreases prostaglandin E2 (PGE2) levels. PGE2 stimulates LRHR production by increasing IGF-1 levels (Dearth et al., 2002).

A few studies conducted in children assessed physical appearance or the start of menarche to determine the onset of puberty. There are not many studies that have

looked at hormonal changes in children. Gollenberg et al (2010) assessed inhibin B levels in pre-pubertal girls in the National Health and Nutrition Examination Survey (NHANES) cohort. Inhibin B is produced in the ovary and is a marker for ovarian follicular development in pre-pubertal stage. This event occurs at the same time as the LH release (Buck Louis et al., 2008). The study found that girls with higher levels of lead exposure > 1.0 µg/dl, had lower levels of inhibin B. This effect was greater if there was concurrent iron deficiency. This is especially important in low income countries with high levels of iron deficiency (Tripathi et al., 2001).

A few cross sectional epidemiological studies in girls have been conducted mainly in the USA. In 2003 using data from the NHANES III Selevan et al showed a delay in the onset of puberty in girls with blood lead levels of 3 µg/dl or higher even after adjusting for possible confounding factors (Selevan et al., 2003; Wang et al., 2005; Kaji and Nishi, 2006). Delays in the onset of puberty i.e. telarche, pubarche and the onset of menarche were significant in African-American girls. However in girls of Mexican origin lead exposure was significantly associated with delays in breast and pubic hair development only. In White girls although the trend analyses showed delays in association with lead exposure, the trend was not significant. Another study by Wu et al, that also used the NHANES III data, showed that there was a significant association between higher blood lead levels and a delay in attainment of menarche and pubic hair development overall amongst all the girls in the cohort (Wu et al., 2003; Wang et al., 2005; Kaji and Nishi, 2006). Another study based in the USA looking at the effect of lead on the

timing of menarche in Akwesasne Mohawk Girls showed that in those with higher blood lead levels the attainment of menarche was delayed (Denham et al., 2005). All the above studies focused on girls; however a study in Russia showed that boys had a 43% increased risk of delayed puberty if their blood lead levels were ≥5.0 μg/dl (Hauser et al., 2008).

Therefore lead appears to delay puberty in boys and girls, but the degree of delays may not be similar in all population groups (Selevan et al., 2003; Wu et al., 2003).

c. Consequences of a delay in puberty

Delays in puberty have been shown to cause physical and psychological problems in adolescents and these issues may progress into adulthood. In girls with a delay in puberty there may be an increase of fractures due to decreased bone strength (Chevalley et al., 2009). Another consequence of a delay in puberty might be short stature (Albanese and Stanhope, 1993; Crowne et al., 1991). Short stature may in turn be a precursor for obesity later in life (Komlos, 2010). However not all studies show the same effect (Himes, 2006; Ong et al., 2006). Psychological abnormalities such as depression may occur (Crowne et al., 1991; Herva et al., 2004). It is postulated that lower levels of oestrogen in those with delays in puberty contributes to the onset of depression (Herva et al., 2004). Boys are at risk for similar psychological effects (Michaud et al., 2006).

1.2.4.2 Neurological effects

At high and low levels lead can cause detrimental neurological and behavioural changes especially in children (Bellinger, 2008). Lead easily crosses the blood brain barrier. The half-life of lead in brain tissue is approximately 2 years.

a. Neuropathology due to lead exposure

Evidence from studies has shown that structural changes occur in the brain of adults and children exposed to lead (Bellinger, 2008; Cecil et al., 2008; Stewart et al., 2006). Lead exposure causes neuropathology via several different routes- "cell death due to apoptosis, excitotoxicity, influences on neurotransmitter storage and release process, mitochondria, second messengers, cerebrovascular endothelial cells, astroglia and oligodendroglia" (Lidsky and Schneider, 2003). There is evidence from human and animal studies for possible pathophysiological mechanisms of lead neurotoxicity; however the mechanisms are not completely understood (Toscana and Guilarte, 2005). Lead has been shown to directly and indirectly affect the brain.

It directly affects the neurological system and causes neurotoxicity through the displacement of calcium and zinc ions (Lidsky and Schneider, 2003). First, lead is able to pass through the blood brain barrier via Ca-ATPase pumps and this has been demonstrated in *in vitro* studies of brain capillary endothelial cells that make up the blood brain barrier (Kerper and Hinkle, 1997). Lead can then lead to neuronal death or apoptosis by disrupting mitochondrial function. This is caused by the intracellular accumulation of lead leading to disruption of calcium

homeostasis. Lead exposure can lead to oxidative stress due to lipid peroxidation. Lead disrupts energy metabolism in adult nerve endings. Due to its substitution of calcium it also affects the activity of the second messenger system. Neurotransmitter storage, release and alteration of the neurotramsmitter receptors such as the glutamate receptor are affected. It leads to demyelination and hypomyelination of astroglia and oligodendroglia. The astrocytes sequester and accumulate lead possibly as a protective mechanism for surrounding cells but it also serves as a store for continuous lead exposure in the brain. The younger or more immature astroglia have a greater degree of sequestration and retention of lead, thus the consequences of lead exposure in the prenatal and childhood period are more significant. The astrocytes adjust synaptic activity and excitotoxicity by taking up glutamate after its release and converting it to glutamine catalysed by enzyme glutamine synthetase. Even at low levels, lead reduces the activity of this enzyme (Lidsky and Schneider, 2003). Structural damage to the brain due to childhood lead exposure has been shown in Magnetic Resonance Imaging (MRI) scans of adult brains. Brain volumes were decreased in those with higher levels of childhood lead exposure (Cecil et al., 2008). In this study the mean childhood blood lead level was 13.3 µg/dl (range 4.65 µg/dl - 37.2 µg/dl).

Indirect effects of lead on the brain are via its effects on haeme synthesis. Lead disrupts enzymes 5-aminolevulinic acid dehydratase (ALAD) and ferrochelatase resulting in the accumulation of 5-aminolevulinic acid (ALA). The increased ALA inhibits gamma-aminobutyric acid (GABA) release and competes for GABA receptors thereby decreasing neurotransmission. Olympio et al (2010)

showed that binding of ALA to the GABA receptors leads to receptor damage. Another indirect effect on the brain is via decreased thyroid hormone transport in the brain. Lead decreases transthyretin levels. Thyroid hormones are essential to the normal development of the brain. A review by Finkelstein et al has shown that there is no threshold for lead induced neurotoxicity (Finkelstein et al., 1998).

b. Neurological and behavioural consequences of lead exposure

These structural and neurotransmitter abnormalities result in several clinical neurological effects. At very high lead concentrations of > 80 µg/dl, lead encephalopathy results (Perlstein and Attala, 1966). Lead induced encephalopathy is usually fatal; however if survival occurs there are usually irreversible neurological consequences. At lower blood lead concentrations studies have shown cognitive impairment, decreasing IQ, behavioural abnormalities including aggressive antisocial behaviour, decreased impulse control and increased risk of developing attention deficit hyperactivity disorder (Lanphear et al., 2005; Canfield et al., 2003, 2004; Bellinger et a., 1994; Braun et al., 2006; Needleman et al., 2002; Nevin, 2000).

Behavioural problems in particular include characteristics of Conduct Disorders and Oppositional Defiant Disorders that are described in the Diagnostic and Statistical Manual of Mental Disorders –Fourth Edition (DSM IV) and have been shown to be raised among children exposed to lead. This type of behaviour includes temper tantrums, argumentativeness, active defiance and refusal to

comply with adult requests and rules, deliberate attempts to annoy and upset people, frequent anger and resentment, mood instability, substance abuse, aggression towards people and animals, destruction of property, and deceitfulness, lying or stealing (AACAP; DSM IV, 2000).

Epidemiological studies conducted mainly in resource rich countries have shown detrimental effects of low level lead exposure (< 10.0 µg/dl) on behaviour of young children and adolescents. The longitudinal Cincinnati Lead Study showed that in children aged between 15 and 17 years there was a significant association between lead exposure and antisocial behaviour (Dietrich et al., 2001). The longitudinal Port Pirie Cohort study found similar results (Burns et al., 1999). Cross sectional studies have shown similar effects of delinquency and aggressive behaviour in children that have been exposed to low levels of environmental lead (Bellinger et al., 1994; Needleman et al., 1996). To date there have been no studies in South Africa that have researched the behavioural response to lead exposure.

Possible long term consequences of the neurological effects of lead exposure are overt criminal behaviours. Studies have shown that antisocial or aggressive behaviour in childhood can result in criminal activity as the individual gets older (Liu and Wuerker, 2005). Evidence from ecological and cross sectional studies conducted in the USA suggests that lead exposure and criminal activities are

significantly associated with each other (Wright et al., 2008; Nevin, 2000; 2007; Stretesky and Lynch, 2001).

1.3 Summary of literature review

The reviewed literature highlighted the following:

- The extent of the detrimental health effects in children and the prevalence of the problem globally and specifically in South Africa. The Centers for Disease Control has recommended that the blood lead level of 10 µg/dl is the acceptable action limit and only at levels above this need any action to stop further exposure be taken. However studies have shown that there is no safe threshold for lead exposure. Blood lead levels as low as 2.0 µg/dl are associated with adverse health outcomes. Thus the WHO has commenced a process to review the action level of 10.0 µg/dl.
- Children are at higher risk for exposure due to their physiological processes, immature organ systems and their exploratory behaviour.
- In South Africa lead exposure is still a problem especially in inner city areas or in areas close to where lead is mined and used in formal and informal industries. Blood lead levels have decreased since the removal of leaded petrol in 2006, but large numbers of children continue to be at risk of exposure. In children aged 6-8 years old blood lead levels decreased from 12.0 µg/dl in 1995 to 9.0 µg/dl in 2002. This situation is the similar in many other African countries such as Nigeria and Asian countries like China and India. These low and middle income countries like South Africa

are undergoing rapid urbanisation and industrialisation and are thus faced with multiple sources and pathways of lead exposure from formal and informal industries, at home and at school. In high income countries however the prevalence of high exposures has decreased substantially since the 1970's and the median blood lead level in children and adults is between 2.0 and 3.0 µg/dl.

- Environmental lead exposure affects multiple organ systems in the body.
- Low levels of exposure are associated with delays in the onset of puberty in boys and girls. This is supported by evidence in animal and human hormonal studies as well as a few epidemiological studies conducted in high income countries.
- Behavioural changes such as antisocial and aggressive behaviour occur in children exposed to low levels of lead.

1.4 Gaps in the Literature

Despite the higher burden of lead exposure in South Africa and other middle and low income countries there have been major gaps in the knowledge of lead exposure and its impact on children in South Africa and in the rest of Africa. Routine surveillance and screening programmes for lead exposure in children or adults does not exist and thus knowledge about the prevalence of lead exposure is only obtained from a limited number of cross sectional studies conducted in some

communities. There is also a scarcity of information regarding risk factors in South Africa.

Similarly the health effects of lead exposure has been researched in high income countries such the USA, however in countries like South Africa where the prevalence of lead exposure is much higher, the effects on biological functions and behaviour are not known.

1.5 Relevance of the study

This study hopes to bridge the gap in the evidence and will look at longitudinal data to determine the contribution of lead exposure to prenatal and adolescent health in a low and middle income country. Lead exposure prenatally and in later childhood can potentially result in serious public health challenges.

Lead exposure is preventable. Thus if one can show an association between lead exposure either prenatally, or at adolescence, and negative behaviour as well as biological effects, the findings may be used to lobby for policy change and interventions to decrease exposure and the risk of detrimental health effects.

1.6 Aims and objectives

1.6.1 Aim:

The prevalence of elevated blood lead levels in South Africa is more prevalent than in many high income countries. Lead exposure still continues due to use in formal and informal industries. Thus the purpose of this study was to assess the impact of lead exposure in children in Johannesburg, South Africa. This was achieved by addressing the objectives listed below.

1.6.2 Objectives:

1. The prevalence of lead exposure in the Birth to Twenty cohort[1]
2. Risk factors affecting lead exposure.
3. The impact of lead exposure on puberty in adolescent females in the Birth to Twenty cohort.
4. To determine the effect of lead exposure on behaviour in adolescence in the Birth to Twenty cohort.

[1] The Birth to Twenty cohort is a longitudinal study conducted by the University of Witwatersrand that aimed to assess child health and development. It commenced in 1990, when mothers were recruited between April and June from the Soweto-Johannesburg metropolitan public clinics. This area was approximately 200 square kilometres and consisted of 3.5 million people. Only public clinics were accessed, thus the population sampled were mainly Black families of a lower socioeconomic status (Richter et al, 2004).

PART 2

PUBLISHED PAPERS

CHAPTER 2:

Prenatal and adolescent blood lead levels in South Africa: Child, maternal and household risk factors in the Birth to Twenty cohort.

Published in the journal: Environmental Research. 110:355-362, 2010.

This paper, published in May 2010, aims to answer objectives 1 and 2 of the thesis. It describes the blood lead levels at birth and at 13 years of age in the Birth to Twenty cohort. Risk factors at birth and at 13 years are analysed and include longitudinal analyses for a subset of the cohort.

2.1 Introduction

Lead is a versatile, but highly toxic blue-grey metal found naturally in the earth's crust. Human activities such as burning of fossil fuels, the use of lead in paint, petrol, water pipes, smelting, and applications in the manufacturing industry and informal sector (for example repairs to electrical appliances using lead solder, making of jewellery and stained glass and lead recycling in cottage industries), have resulted in increased lead exposure in the environment, including in homes, schools and the workplace (Mathee et al., 2004; Tong et al., 2000). The harmful effects of lead at levels greater than 10.0 µg/dl, especially in children, are well established (Hernberg, 2000; Needleman, 2004). A blood lead level above 10.0 µg/dl is widely considered the value at which action should be taken to prevent further exposure to lead (CDC, 1991). However recent studies have shown that levels <10.0 µg/dl in children can lead to substantial neuro-behavioural problems, such as a reduction in IQ, decreased math and reading levels and problem behaviours (Bellinger, 2004; Canfield et al., 2003; Bernard, 2003). Some studies have also pointed to an association between bone lead levels of greater than 25 ppm and impulsive, aggressive or delinquent behaviour in children (Needleman et al., 2002; 1996). To date there is no evidence of a safe level for lead exposure at which detrimental effects are negligible (Binns et al., 2007; Gilbert and Weiss, 2006; Koller et al., 2004; Bernard, 2003; Canfield et al., 2003).

In 2000, around 40% of all children globally were estimated to have blood lead concentrations greater than 5.0 µg/dl, and 20% greater than 10.0 µg/dl. Based on

the limited data available, Fewtrell et al showed that over 97% of exposed children are estimated to live in low- and middle-income countries (Fewtrell et al., 2004). In resource-rich countries, such as the United States of America (USA), the United Kingdom and parts of Europe (for example, Sweden), the level of lead exposure in the general population has decreased to a current mean level of approximately 3.0 µg/dl (Koller et al., 2004; Harper et al., 2003), although there are pockets of urban poor children that still have levels greater than 10.0 µg/dl (Breysse et al., 2004). In poor countries lead exposure remains a problem (Fewtrell et al., 2004; Falk, 2003). South Africa and most other countries in Africa do not have routine lead screening systems. In an epidemiological survey conducted in South Africa, the mean cord lead level in the early 1990's was 15.5 µg/dl in Durban (Chetty et al., 1993). In 1995 Johannesburg school children aged 6 to 7 years were shown to have a mean blood lead level of 12.0 µg/dl. By 2002, the mean blood lead level, in a repeat study involving the same schools, had declined to 9.1 µg/dl (Mathee et al., 2004). In South Africa following epidemiological studies showing substantially elevated lead levels in young urban Cape children (von Schirnding et al., 1991a), lead-free gasoline was introduced in 1996 and eventually in 2006 all leaded gasoline was eliminated. It is, however, still used in certain paint formulations and applications and in the informal and formal industrial sectors. Thus although blood lead levels are decreasing in South Africa due to the discontinuation of leaded gasoline, lead exposure remains a significant local public health problem (Mathee et al., 2006).

Lead exposure and poisoning is preventable, and it is therefore important to determine the most significant predictors of blood lead levels at birth and in later childhood so that appropriate action can continue to be taken. Longitudinal studies examining the potential effects of lead exposure have mainly been conducted in resource rich countries (Canfield et al., 2003; Bellinger and Needleman, 2003). In African countries on the other hand, there is a paucity of research information available on lead exposure at birth and during childhood/adolescence, including in respect of risk factors and health outcomes.

The primary aim of this study was to determine child, maternal, and household factors associated with blood lead levels at birth, and at the age of 13 years among urban South African children.

2.2 Materials and methods

2.2.1 Study design and sampling

The study used sub-samples from The Birth to Twenty (Bt20) cohort at birth (cord blood sub-sample) and at 13 years of age (13 year sub-sample). Birth to Twenty (Bt20) is a longitudinal birth cohort study in the metropolitan area of Johannesburg-Soweto, South Africa which aims to assess the environmental, economic, psychosocial and biological determinants of health, development and well-being amongst the cohort from birth to 20 years of age. The study commenced in 1990.

Participants were included in the Bt20 cohort if they were born between April and June 1990, and the mother resided in the Johannesburg-Soweto area for at least 6 months following delivery (n=3273). Enrolment into the study began prior to delivery when women were interviewed during their third trimester of pregnancy while attending public antenatal clinics. Attrition from the cohort occurred because the participants moved away from the study area, moved to an unknown address or the mother or child died. The cohort enrolment and attrition is well described in several publications and 72% of the original cohort were being successfully followed up at age 16 (Yach et al., 1990; Richter et al., 2004; Norris et al., 2007; Richter et al., 2007).

Cord blood samples were collected from 862 Bt20 participants who were enrolled for a lead exposure study in the antenatal phase. However, only 618 cases (cord blood sub-sample) fulfilled the enrolment criterion of remaining within the study area for at least six months after delivery: 244 participants moved out of the study area during this period, the mother or child died or the blood sample could not be used for analysis due to clotting (Norris et al., 2007). In 2003, when the children were 13 years of age, 1546 (47%) cohort participants (13 year old sub-sample) had their blood lead levels analysed. The remaining participants (n=1727) could not be enrolled due to blood sampling errors such as clotting of the blood sample or the participant could not be located during the time period for data collection. Of the 1546 13 year olds, 312 adolescents (13 year with cord blood sub-sample) also had cord blood lead data. Figure 2.1 illustrates the sampling scheme of the two lead assessments conducted at birth and at 13 years of age.

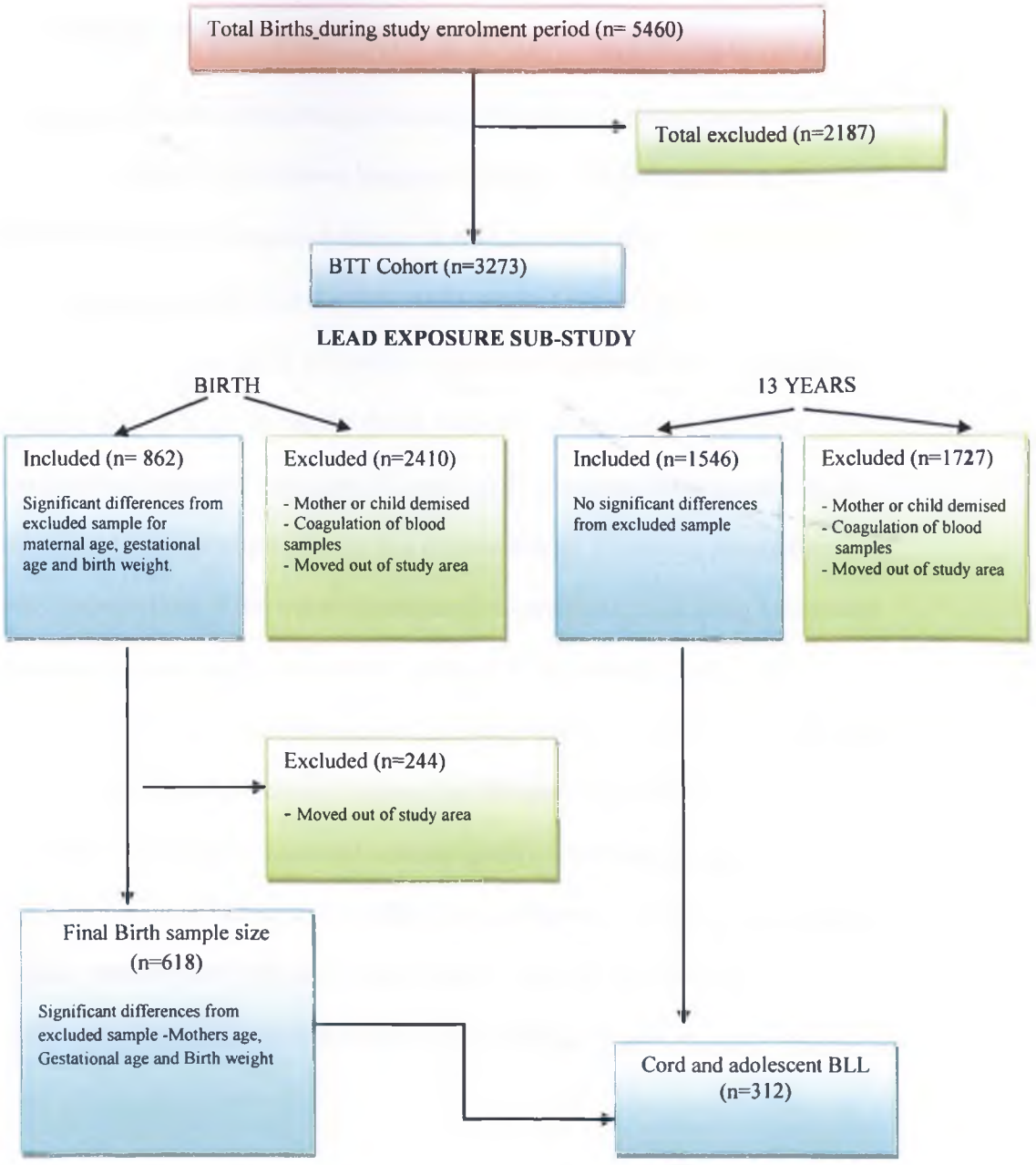

Figure 2.1 Sampling scheme

2.2.2 Procedures/ Data collection

A detailed structured questionnaire was administered to pregnant women by a trained interviewer in their home language to collect data on birth history, maternal factors and household factors such as access to water and electricity, type of dwelling, ownership of assets. At 13 years of age trained interviewers collected data on household factors from caregivers and participants. Cord blood samples were obtained during the fourth stage of delivery and whole venous blood was collected when children were 13 years old. Blood samples were collected in heparinised tubes determined to be free of trace metals. Following preparation, lead concentrations in the whole blood samples were determined using a flameless atomic absorption spectrophotometer equipped with a graphite furnace. Blood lead measurements were performed by the South African Centre for Occupational Health (now called the National Institute for Occupational Health), which participates in an international and national quality control programme for blood lead determinations (Röllin et al., 1988). The coefficient of variation in blood lead samples was 5.8% in 1990. In 1990 the limit of detection of lead in blood was 1.0 µg/dl and in 2003 the limit of detection equalled 0.1µg/dl.

2.2.3 Data analysis

Associations between blood lead levels and independent variables in the two sub-samples were tested in a bivariate analysis using the student t test, spearman rho, X^2 and analysis of variance (ANOVA) as appropriate. Multiple regression analyses were used to identify associated risk factors. VIF and tolerance tests in

STATA were conducted to identify multicolinearity among the independent variables. The results show that the mean VIF was less than 10 and the tolerance (1/VIF) was also > 0.1 in all three sub- samples. Thus there is no strong evidence of multicolinearity among the independent variables. Independent variables found to have significant relationships with blood lead levels were then used in a logistic regression model. In this model, blood lead levels were dichotomised at 5.0 µg/dl (≤5.0 µg/dl vs. > 5.0 µg/dl). The level of 5.0 µg/dl was chosen because it is close to the mean blood lead level in the sample and was also based on the current global discussions around lowering the action level to 5 µg/dl (Gilbert and Weiss, 2006). Blood lead levels were also logarithmically transformed to correct skewed distribution. However analyses using the natural log transformed blood lead levels did not significantly alter the results. Statistical significance was determined at a level of $p<0.05$. All analyses were conducted using STATA 9.

2.2.4 Ethics

Ethical approval was obtained from the Human Research Ethics Committee of the University of the Witwatersrand in Johannesburg. Confidentiality was maintained by assigning participant identification numbers that were known only to the data management team, and stored separately from the questionnaires. Prior to the commencement of the study, written informed consent was obtained from each participant's parent or guardian, and at 13 years of age, assent for participation was also obtained from the young participants. As part of the informed consent

process, it was explained that participation was voluntary and could be withdrawn at any time without any repercussions.

2.3 Results

2.3.1 Sample characteristics

The total Bt20 cohort consisted of 3273 live singleton births. The demographic characteristics of the cord blood sub-sample (n=618) did not differ significantly from the rest of cohort (n=2655), except for maternal age ($p<0.001$), birth weight ($p=0.003$) and gestational age ($p<0.001$). There were a higher percentage of teenage mothers in the cord blood sub-sample (19.6% compared to 14.8% in the total Bt20 cohort). The cord blood sub-sample had a lower percentage (6.5%) of babies with low birth weights (< 2500 grams), compared to the total Bt20 cohort, in which 10.7% of babies had low birth weight. There were no babies with a gestational age of less than 28 weeks in the cord blood sub-sample; however approximately 1% of the total Bt20 cohort had been born at <28 weeks gestation. The 13 year sub-sample (n=1546) did not differ significantly from the remaining subjects in the remaining Bt20 cohort (n=1727) with respect to any of the demographic characteristics. The sub-sample at 13 years with cord blood (13 year with cord blood sub-sample, n=312) did not differ significantly from the rest of the 13 year sub-sample.

2.3.2 Blood lead levels

The mean group blood lead levels at birth (cord blood sub-sample) and at 13 years of age (13 year sub-sample) were 5.9 µg/dl and 5.7 µg/dl respectively. The 13 year with cord blood sub-sample had the same mean blood lead level of 5.9 µg/dl at birth and 5.7 µg/dl at 13 years of age. The blood lead distribution ranged from 2.0 to 17.0 µg/dl in the cord blood sub-sample, and 1.0 to 28.0 µg/dl in the 13-year sub-sample. Even though few newborns (4%) and adolescents (3%) had blood lead levels over the WHO action level of 10.0 µg/dl, a large number of participants had lead levels above 5 µg/dl (50% in the cord blood sub-sample and 53% in the 13 year sub-sample respectively). As can be seen from Figure 2.2, the data were positively skewed.

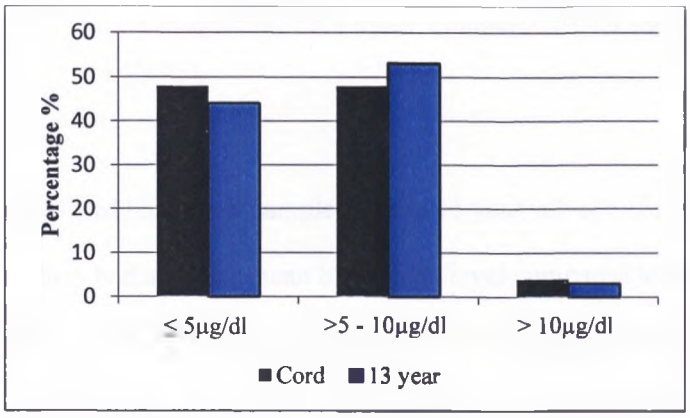

Figure 2.2: Blood lead levels at birth and at 13 years of age.

Tracking the blood lead levels for individual subjects between birth and 13 years (n=312) showed that blood lead levels increased in 42.3%, decreased in 56.4% and stayed the same in 1.3% of the children followed up. Longitudinal regression

analysis over 13 years showed that the change in blood lead levels over time was not significant (p=0.1). However boys at 13 years had significantly higher mean blood lead levels than girls (p <0.0001).

A description of the study sample with the mean blood lead levels in the cord blood sub-sample and in the 13 year sub-sample is given in Table 2.1. The majority of the population was Black (99.3% at birth and 92.1% at 13 years of age). The mean blood lead levels were highest in the Black population groups at birth, as well as at 13 years of age when compared to the other ethnic groups; however the sample sizes for the other ethnic groups were very small. Being male was associated with higher mean blood lead levels in the 13 year sub-sample with a mean blood lead level of 6.5 µg/dl, compared to 4.9 µg/dl in females.

In the cord blood sub-sample and the 13 year sub-sample, children of teenage mothers had a higher mean blood lead level compared to mothers of 20 years and older, and lower educational attainment was associated with higher blood lead levels. In the 13 year with cord blood sub-sample 25% of the teenage moms had children with both cord and 13 year blood lead levels of > 5.0 µg/dl. However the mean blood lead levels of children in the 13 year with cord blood sub-sample was higher in the adult mothers compared to children of the teenage mothers. Table 2.2 illustrates the characteristics of the 13 year with cord blood sub-sample.

Table 2.1: Characteristics of the participants in the cohort at birth and at 13 years.

	At Birth (n=618)	Mean BLL at Birth (µg/dl)	P value	13 years (n=1546)	Mean BLL at 13 years (µg/dl)	P value
INDIVIDUAL FACTORS						
Gestational age						
≤28 weeks	0	-		10 (0.6%)	6.68	
≥29 weeks	617 (100%)	5.80	-	1424 (92%)	5.71	0.29
Sex						
Male	312 (50.5%)	5.89		713 (46.1%)	6.51	
Female	306 (49.5%)	5.81	0.61	751 (48.6%)	4.96	<0.01*
Birth Weight						
≤2500g	40 (6.5%)	6.12		169 (10.9%)	5.87	
≥2500g	577 (93.4%)	5.83	5.83	1295 (83.8%)	5.69	0.53
Population group						
Asian	1 (0.2%)	-		14 (0.9%)	5.27	
Black	630 (99.3%)	5.89		1424 (92.1%)	5.80	
White	3 (0.5%)	4.67	0.33	26 (1.7%)	4.69	0.12
MATERNAL FACTORS						
Age in years						
Mean	24.8			26.05		
Range	14-43			14-46		
Teenage Pregnancy	121(19.6%)	5.96		247 (16.0%)	5.76	
Adult (>19 years)	497 (80.4%)	5.40	0.02*	1215 (83.0%)	5.53	0.79
Education						
None	9 (1.5%)	8.73		12 (0.8%)	6.80	
Primary	87 (14.1%)	6.33		156 (10.1%)	5.76	
Secondary	452 (73.1%)	5.81		1069 (69.1%)	5.69	
Tertiary	55 (8.9%)	5.32	<0.01*	123 (7.9%)	5.68	0.45
Marital Status						
Married	177 (28.6%)	5.80		531 (34.3%)	5.51	
Single	439 (71.1%)	5.87	0.72	932 (59.7%)	5.84	0.01*
Parity						
Mean	2			2		
Range	1-8			1-9		
≤3	543 (87.7%)	5.83		1216 (83.1%)	5.70	
> 3	75 (12.1%)	5.97	0.01*	248 (16.9%)	5.79	0.29
Type of home						
Formal	520 (84.2%)	5.80		1238 (82.4%)	5.72	
Informal	67 (10.9%)	5.85	0.85	94 (6.1%)	5.91	0.45
Ownership of home						
Owned	126 (20.4%)	5.67		328 (21.2%)	5.65	
Rented	459 (74.3%)	5.82	0.42	998 (64.6%)	5.75	0.49
Access to water						
Indoor access	241 (39%)	5.75		609 (39.4%)	5.71	
Access outside house	319 (51.6%)	5.75	0.99	507 (32.8%)	5.64	0.62
Access to electricity						
Yes	545 (88.2%)	5.78		1243 (80.4%)	5.49	
No	51 (8.2%)	6.07	0.31	62 (4.0%)	5.73	0.46

Assets owned						
TV						
Yes	375 (60.7%)	5.76		929 (60.1%)	5.77	
No	180 (29.1%)	5.93	0.33	284 (18.4%)	5.74	0.83
Car						
Yes	119 (19.3%)	5.82		335 (21.7%)	5.85	
No	435 (70.4%)	5.82	0.99	879 (56.9%)	5.44	0.01*
Fridge						
Yes	370 (59.9%)	5.76		891 (57.6%)	5.86	
No	186 (30.1%)	5.95	0.27	322 (20.8%)	5.70	0.32
Washing Machine						
Yes	52 (8.4%)	5.77		180 (11.6%)	5.79	
No	503 (81.4%)	5.83	0.81	1034 (66.9%)	5.51	0.18
Phone						
Yes	267 (43.2%)	5.63		663 (42.9%)	5.94	
No	287 (46.4%)	6.02	0.02*	550 (35.6%)	5.59	0.01*

*Indicates significant bivariate associations

Table 2.2 Characteristics of the 13 year cord blood sub-sample (the longitudinal sample) n=312

	%	Mean BLL at Birth (µg/dl)	P value	Mean BLL at 13 years (µg/dl)	P value
INDIVIDUAL FACTORS					
Gestational age					
≤28 weeks	0 (0%)				
≥29 weeks	312 (100%)	5.91		5.67	
Sex					
Male	153 (49.0%)	5.89		6.56	
Female	159 (51.0%)	5.94	0.83	4.81	<0.001*
Population group					
Asian	1 (0.3%)	9		3.33	
Black	311 (99.7%)	5.90		5.68	
White	0 (0%)	-		-	
MATERNAL FACTORS					
Age in years					
Mean	24.9				
Range	14-43				
Teenage Pregnancy	64 (20.5%)	5.55		5.28	
Adult (>19 years)	248 (79.5%)	6.01	0.67	5.78	0.13
Education					
None	3 (1%)	6.33		7.50	
Primary	33 (10.6%)	6.03		5.73	
Secondary	237 (76%)	5.91		5.56	
Tertiary	34 (10.9%)	5.14	0.02*	6.19	0.02*
Marital Status					
Married	81 (26%)	5.99		5.39	
Single	230 (73.7%)	5.88	0.68	5.77	0.21
Parity					
Mean	2				
Range	1-8				
≤3	274 (87.8%)	5.89		5.65	
> 3	38 (12.2%)	6.05	0.65	5.82	0.68
Type of home					
Formal	281 (90.1%)	5.87		5.72	
Informal	23 (7.4%)	5.78	0.84	5.24	0.36
Ownership of home					
Owned	70 (22.4%)	5.64		5.65	
Rented	233 (74.7%)	5.91	0.31	5.70	0.89
Access to water					
Indoor access	135 (43.3%)	5.87		5.76	
Access outside house	153 (49.0%)	5.74	0.55	5.67	0.73
Access to electricity					
Yes	292 (93.6%)	5.80		5.71	
No	15 (4.8%)	6.80	0.05*	5.01	0.27
Assets owned					
TV					
Yes	215 (68.9%)	5.76		5.72	
No	81 (26%)	6.12	0.15	5.52	0.53

Car					
Yes	68 (21.8%)	5.84		5.19	
No	228 (73.1%)	5.87	0.91	5.81	0.06
Fridge					
Yes	220 (70.5%)	5.75		5.75	
No	77 (24.7%)	6.23	0.06	5.47	0.39
Washing Machine					
Yes	30 (9.6%)	6.27		5.38	
No	267 (85.6%)	5.83	0.24	5.71	0.47
Phone					
Yes	159 (51%)	5.66		5.49	
No	137 (43.9%)	6.14	0.03*	5.87	0.19

* Indicates significant results

2.3.3 Factors associated with blood lead levels

Maternal, child and household factors were illustrated in Table 2.1 and Table 2. 2. The analysis of household factors showed that most participants lived in formal, rented housing, and only a small proportion of households owned relatively high cost items such as washing machines and cars. Access to commodities such as a telephone was also low. The homes of the majority of participants were served with electricity, but only 39% of participants lived in homes with indoor access to water.

With respect of the cord blood sub-sample lead levels, bivariate analyses showed that lower maternal age at birth ($p=0.02$) and lower maternal education were significantly ($p <0.01$) associated with higher cord blood lead levels. Higher parity levels ($p=0.01$) and not owning a phone ($p=0.02$) was also significantly associated with higher cord blood lead levels. In the 13 year sub-sample, male gender of the child ($p <0.001$), the mother being a single parent ($p=0.01$) and lack

of ownership of a car (p=0.01) or telephone (p=0.01) were significantly associated with higher blood lead levels. Bivariate analysis conducted with respect to the 13 year with cord blood sub-sample (n=312) showed that the cord blood lead levels and blood lead levels at 13 years were significantly associated with each other (p=0.02).

Table 2.3 illustrates the multiple regression analyses conducted with respect to the cord and 13-year blood lead levels. As can be seen, lower maternal age and low maternal education as well as access to electricity were strong predictors of elevated blood lead levels at birth. In the 13 year sub-sample, the sex of the child, lack of home ownership and lack of telephone ownership were strong predictors for elevated blood lead levels. In the longitudinal analysis of the 13 year with cord blood sub-sample the regression analysis showed that sex of the child and cord lead levels were strong predictors of elevated blood lead levels at 13 years. Log transformation of the blood lead distributions did not alter the observed associations between lead levels and the risk factor variables.

Table 2.3: Multiple regression analysis

	At Birth (n=618)			At 13 years (n=1546)			13 year cord sub-sample (n=312)		
	β	SE	P Value	β	SE	P Value	β	SE	P Value
Individual factors									
Gestational age	0.04	0.03	0.16	-0.01	0.01	0.24	0.17	0.12	0.16
Sex	-0.03	0.17	0.85	-1.53	0.14	<0.001*	-1.71	0.28	<0.01*
Birth weight	0.00	0.00	0.94	-0.00	0.00	0.31	-0.00	0.00	0.45
Race	-0.03	0.35	0.93	-0.02	0.13	0.89	-0.61	0.59	0.29
Maternal Factors									
Parity	-0.11	0.99	0.28	0.10	0.08	0.19	-0.06	0.17	0.70
Age	0.05	0.02	0.01*	-0.01	0.15	0.54	0.02	0.03	0.52
Education	-0.47	0.25	0.05*	-0.22	0.16	0.16	0.02	0.29	0.95
Marital Status	0.08	0.21	0.69	0.15	0.18	0.38	0.26	0.37	0.47
Household factors									
Formal Housing	0.30	0.33	0.34	-0.33	0.31	0.28	-0.34	0.58	0.56
Lack of home ownership	0.19	0.21	0.37	0.37	0.17	0.03*	0.05	0.34	0.87
Access to indoor running water	0.00	0.18	0.96	-0.14	0.16	0.36	-0.23	0.30	0.44
Access to electricity	0.19	0.36	0.01*	0.03	0.38	0.92	0.75	0.71	0.29
TV Ownership	0.01	0.21	0.98	0.19	0.18	0.28	0.53	0.34	0.13
Fridge ownership	0.11	0.21	0.60	0.03	0.19	0.87	0.45	0.36	0.21
Washing machine ownership	0.12	0.31	0.69	-0.14	0.23	0.55	-0.22	0.50	0.66
Car ownership	-0.34	0.22	0.11	-0.31	0.18	0.08	-0.48	0.37	0.21
Phone ownership	0.41	0.19	0.03*	-0.32	0.16	0.05*	-0.54	0.32	0.09
Cord lead							0.16	0.07	0.03*

* $P \leq 0.05$ for association between blood lead and potential risk factor

A logistic regression model was created using significant independent variables at birth and at 13 years of age. Table 2.4 shows that in the cord blood sub-sample, maternal age and maternal education were the most important predictors of cord blood lead levels. At 13 years, gender and cord lead levels, as well as lack of ownership of a telephone were the strongest predictors of blood lead levels as illustrated in Table 2.5.

Table 2.4: Logistic Regression model examining the predictors of high (>5 µg/dl) cord blood lead levels.

VARIABLE	OR	CI	P VALUE
Maternal factors			
Teenage mothers	1.85	1.19- 2.88	<0.01*
Low educational level	1.75	1.04-2.97	0.03*

* P≤0.05 for association between blood lead and potential risk factor

Table 2.5: Logistic Regression model examining the predictors of high (>5 µg/dl) blood lead levels at 13 years of age.

VARIABLE	OR	CI	P VALUE
Individual characteristics			
Male gender	3.01	2.41 – 3.77	<0.001*
#Cord lead level	1.91	1.10 – 3.32	0.02*
Socio-economic factors			
Lack of ownership of a phone	1.25	0.99 - 1.58	0.05*

* P≤0.05 for association between blood lead and potential risk factor
# Sample size = 312 participants with cord lead and adolescent lead levels

2.4 Discussion

This is the first study to explore lead exposure among children in South Africa longitudinally. The study findings demonstrated that the majority of newborns, followed up to 13 years of age and reassessed, had blood lead levels below the CDC action level of 10.0 µg/dl; however, over 50% had levels above 5.0 µg/dl at both ages. The mean blood lead level in the cord blood sub-sample was 5.9 µg/dl, and in the 13 year sub-sample it was 5.7 µg/dl. The blood lead levels are similar at birth and at thirteen years possibly because the level of environmental exposure

remained the same. While there are well known problems associated with inter-country and inter-laboratory comparisons, the cord blood lead distribution obtained for this study appeared to be similar to that determined in other urban settings such as the USA in the 1980s and 1990s (Bellinger et al., 1994; Ernhart et al., 1986). The blood lead levels of young Bt20 adolescents, however, were much higher than those found in urban settings in developed resource rich countries (Koller et al., 2004). In the USA the mean blood lead level in the 1-5 year age group was 2.0 µg/dl (Koller et al., 2004). This mean blood level was also found in Sweden (Strömberg et al., 2003). Thus the reason for the higher levels found South Africa could be due to the continued exposure to environmental lead, amongst others, through the continued use of lead-based paint (Mathee et al., 2007), increased urbanization, the growing informal sector (cottage industries) using lead and leaded gasoline in South Africa during the period of the study. The use of leaded petrol was only phased out in South Africa in 2006. Studies conducted around the world have shown that blood lead levels have declined following the phase out of leaded gasoline (Mathee et al., 2006; Thomas et al., 1999). However blood lead distributions in low- and middle-income countries usually still exceed that of the West (Koller et al., 2004; Falk 2003).

At birth, lower maternal age, specifically having a teenage mother, was significantly associated with higher cord lead levels ($p<0.01$). Teenage mothers were 1.85 times more likely to have babies with higher cord blood lead levels than their counterparts over the age of 20 years. Teenage pregnancies have been associated with higher lead levels amongst mothers in the USA (Lane et al., 2008;

Nevin, 2000). In the study conducted by Lane et al, 74.6% of the sample of teenage mothers had a blood lead level <20.0 µg/dl (Lane et al., 2008). The higher the blood lead level is, the greater the risk of teenage pregnancy. It is postulated that lead exposure may decrease "cognitive and judgement capacity", leading in turn to an increased risk of teenage pregnancy (Lane et al., 2008). It has been reported that more than 30% of South African 19 year olds have given birth at least once (Kaufman et al.,, 2001). However the majority of the Bt20 sample had blood lead levels <10.0 µg/dl, thus lead exposure may not contribute significantly to elevated levels of teenage pregnancy in the country.

Lower maternal education levels have been widely associated with an increased risk for lead exposure. For example, the education level of the caregiver was found to be a strong risk factor in a Mexican study of children aged 1-6 years old (Albalak et al., 2003). Lower maternal educational status is an indirect measure of poorer socio-economic status. Another indicator of socio-economic status is asset ownership. In this study the lack of ownership of a telephone was significantly related to higher blood lead levels at 13 years of age (p=0.05). Poorer communities have been shown to be at much higher risk for adverse environmental exposures, including lead exposure in South Africa and in other low and middle income countries (Von Schirnding et al., 1991b , Wright et al., 2005; Mathee et al., 2002; Rahbar et al., 2002; Tong et al., 2000). Poorer communities usually have lower quality housing, live near busy roads, have jobs in dirtier occupations such as lead mining and vehicle repair workshops, and conduct informal industries in their homes that may use lead products (Von

Schirnding et al., 1991b, Tong et al., 2000; Nriagu et al., 1996; Nriagu et al., 1997).

At 13 years of age, male gender was a very strong risk factor for higher blood lead levels. This has not been found in some previous studies of preschool-aged children (Guttinger et al., 2008; Albalak et al., 2003; Melman et al., 1998). However, in the US National Health and Nutrition Examination Survey III (NHANES), of all children tested from 5 to 18 years of age, male children had higher blood lead levels (Fox and Cole, 2004). The reason for this effect was not postulated in the above NHANES study. This effect may be due to the activities of male children such as greater outdoor activities, thus exposed to higher levels of environmental lead exposure and also increase participation in cottage industries using lead.

Maternal factors such as maternal age and education level did not play a significant role at 13 years of age. In the current study, cord blood lead levels were significantly related to blood lead levels at 13 years of age. Children with high lead levels at birth were 1.9 times more likely to have higher levels later in life. The results from this study suggest that in this setting, the risk factors for high blood levels at birth probably persist through later childhood and adolescence, with new risk factors emerging during adolescence. Home visits paid to two of the adolescents with the highest blood lead concentrations revealed their

use of lead solder to repair electrical appliances, television sets and music equipment for the purpose of income generation.

Even though the study was prospective, one limitation was that it was not designed to look at all risk factors for lead exposure, thus information regarding other potential risk factors, such as paternal history, parental occupational exposure to lead, passive smoking and the physical state of the homes such as peeling paint was not available for analysis. However peeling paint and very dilapidated homes was not a major factor of importance in Soweto at the time of the study. Attrition in the sample due to death or moving away from the area could be related to lead exposure and thus the possibility of participation bias must also be noted. The cord blood sub-sample had some significant demographic differences from the rest of the Bt20 cohort with a higher percentage of teenage mothers and a lower percentage of preterm and low birth weight babies. This may have resulted in sample bias, and the findings from this period may not be representative of the entire cohort. Improvements in detecting low levels of blood lead over the 13-year period of the study may have resulted in the recording of lower blood lead levels at 13 years i.e.<1.0µg/dl.

2.5 Conclusion

This study has highlighted several risk factors for elevated lead exposure at birth and at 13 years of age in the Johannesburg-Soweto area that requires attention. At birth, maternal education and age were found to be strong risk factors for high

cord blood lead levels, potentially reflecting the effects of maternal risk exposure in childhood and continued exposure during adulthood. At 13 years of age the lack of asset ownership (telephones), having had an elevated cord blood level and male sex were the strongest risk factors for lead exposure. A range of significant associations found in the study point to the low socio-economic status of affected mothers and children. The fact that elevated cord blood levels predict higher lead levels at adolescence among a sub-group of children demonstrates that the poor circumstances in which some children reside persist over time. Thus screening and treatment of children for lead exposure needs to be conducted alongside socio-environmental upliftment programmes. This study also highlights the need for interventions, such as educational campaigns and health promotion initiatives, targeted at high risk communities. Most importantly measures to alleviate poverty would impact positively on blood lead levels in these vulnerable children. Further research is necessary to look at the impact of elevated blood lead levels on long-term health, behaviour and life achievements of children in the Birth to Twenty Cohort.

CHAPTER 3

Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: Findings from the Birth to Twenty cohort.

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This is the second paper published in October 2010. This paper answers the third objective of the thesis: Is low level lead exposure associated with biological dysfunction in adolescents?

3.1 Introduction

Blood lead levels have been decreasing across the world, especially in resource-rich countries where blood lead levels now average around 3.0 µg/dl or lower (Koller et al., 2004; Harper et al., 2003). However, in low and middle income countries elevated blood lead distributions continue to be widespread because of the ongoing use of lead in the informal and formal sectors (Fewtrell et al., 2004; Falk, 2003; Tong et al., 2000). Poor children are amongst the worst affected. In 1995, Johannesburg school children aged 6 to 7 years were found to have a mean blood lead level of 12.0 µg/dl (Mathee et al., 2004). By 2002, the mean blood lead level in a repeat study involving children from the same schools had declined somewhat to 9.1 µg/dl (Mathee et al., 2004), but were still decidedly above international levels. Lead exposure in South Africa thus continues to be a significant public health problem.

The detrimental health effects of lead at high and even relatively low levels are well established in children both in terms of physical development and mental health. Elevated lead levels have been associated with impaired neurological development and behavioural problems such as hyperactivity and delinquent behaviour as well as renal and haematological abnormalities (Bellinger et al., 1994; Dietrich et al., 2001; Needleman et al., 2002; Lin et al., 2003; Cheng et al., 2001). At blood lead levels as low as 3.0 µg/dl significant detrimental health effects have also been found, including intellectual deficits and behavioural

abnormalities (Lanphear et al., 2005; Bellinger 2004; Canfield et al., 2003; Bernard, 2003).

One of the suggested effects of lead exposure is a delay in the onset of puberty. Animal models have shown that lead affects the endocrine system, possibly through disrupting hypothalamic or pituitary function, and/or direct action at peripheral sites such as the gonads (Winder, 1989; Klein et al., 1994; Wiebe et al., 1982; Huseman et al., 1992; Comoratto et al., 1993). Animal studies have also shown an association between blood lead and delayed puberty (Ronis et al., 1998; Dearth et al., 2002). However, only a few human studies have looked at the impact of lead on pubertal development (Wu et al., 2003; Selevan et al., 2003; Denham et al., 2005). Nonetheless, all studies report an association between low level lead exposure and pubertal delay. To our knowledge, all of the published studies have been conducted in resource-rich countries.

There is a dearth of information on the impacts of lead levels in resource-poor countries, including South Africa. The objective of this study was to determine the association between lead exposure and pubertal development in female adolescents living in Johannesburg, South Africa.

3.2 Materials and methods

3.2.1 Study design and sampling

The Birth to Twenty (Bt20) study is a longitudinal birth cohort study. The study commenced in 1990 in the metropolitan area of Johannesburg/Soweto, South Africa. Inclusion into the study occurred if participants were born between April and June 1990, and if their mother lived in the Johannesburg/Soweto area for at least 6 months after delivery (n=3273). The cohort enrolment and attrition is well described in several publications (Yach et al., 1990; Richter et al., 2004; Norris et al., 2007, Richter et al., 2007). The study aimed to assess the socio-economic, environmental, development, health and overall well-being of the participants.

The Bt20 has a total of 1682 female participants. Of these, 1529 were Black and Mixed ancestry adolescent females. White and Indian females were excluded because of their small numbers. Of these, 725 had venous blood collected for lead analyses at the 13 year data collection wave; 804 participants did not have blood lead levels for a variety of reasons, including not providing consent for a blood draw, a small number of blood samples were not suitable for lead concentration analyses, some participants still in contact with the study did not present at the data collection site, and participants lost due to study attrition. Of the725 participants with blood lead data, 712 had data on the onset of menarche, 684 had pubic hair Tanner staging data and 682 had breast staging data. The sample included in the current analysis is illustrated in Figure 3.1; 682 cases were available for the analysis.

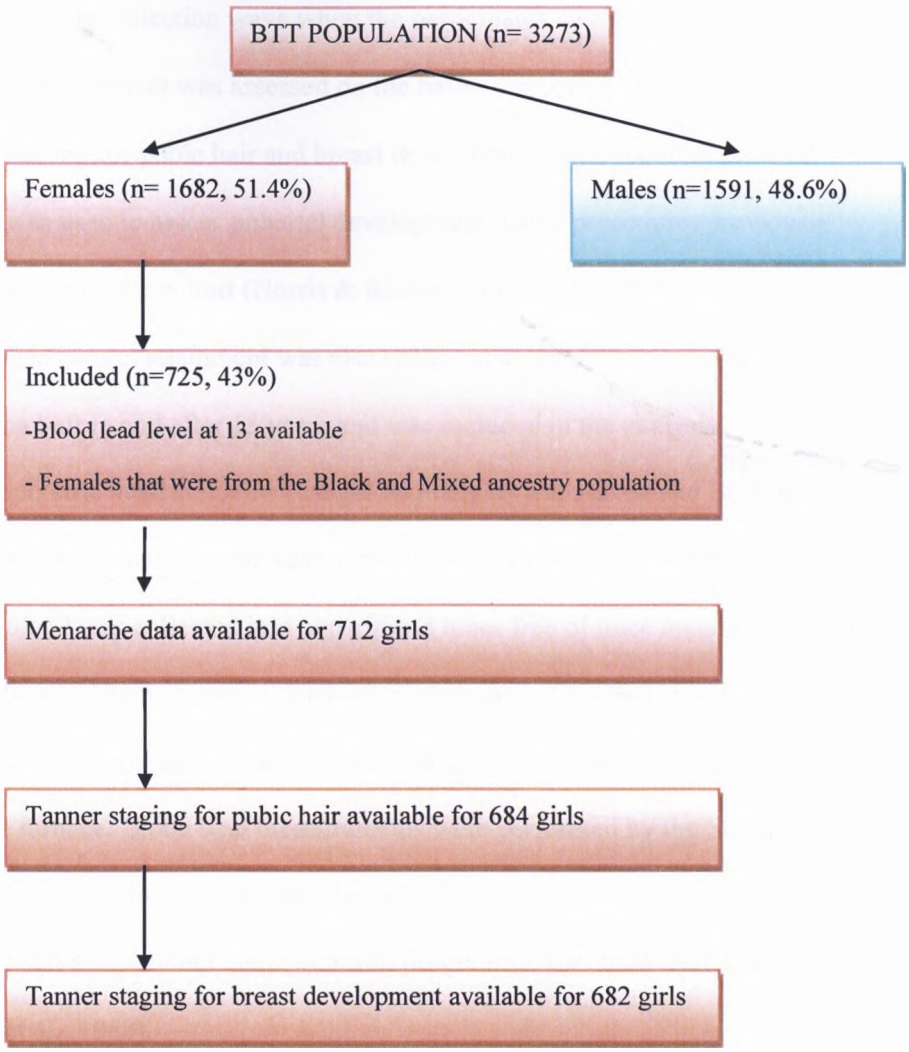

Figure 3.1: Sample included in current analysis

3.2.2 Procedures/Data collection

During the data collection wave when the participants were 13 years of age, pubertal development was assessed on the basis of reported age of menarche and Tanner staging for pubic hair and breast development. Self-reported pubertal staging was used to assess pubertal development, using procedures previously validated among the cohort (Norris & Richter, 2005; Norris & Richter, 2008). Age of menarcheal attainment was also collected at annual waves of data collection before and after 13 years and was included in the analysis. Anthropometric measurements (weight and height) were taken and Body Mass Index (BMI) calculated in the year of menarcheal attainment. In addition, whole venous blood was collected into heparinised tubes free of trace metals. Following preparation and centrifugation, lead concentrations in the whole blood samples were determined using an atomic absorption spectrophotometer equipped with a graphite furnace. Blood lead measurements were performed by the South African National Institute for Occupational Health. The laboratory is part of an international and national quality control programme for blood lead analyses (Röllin et al., 1988).

3.2.3 Data Analysis

Demographic data, maternal education, and social-economic status collected on the cohort was utilised in the analyses.

The blood lead level was the exposure variable. Blood lead levels ranged from 1.0 to 16.3 µg/dl. Lead levels were not normally distributed; however log transformation did not influence the results. There were no differences between Black females and those of mixed ancestry. The mean blood lead level for Black females was 4.9 µg/dl and for Mixed Ancestry females it was 4.7 µg/dl.

Sexual maturation as measured by attainment of menarche and Tanner staging of pubic hair and breast development were the outcome variables. Tanner staging for breast development and pubic hair was classified into five stages with stage one being the most immature and stage five equalling pubertal attainment. The trends in sexual maturation did not differ between Black and Mixed Ancestry participants.

Means and proportions were used to describe the study population. Trend analyses for mean lead levels and pubertal factors were conducted. For logistic regression analyses, blood lead levels were dichotomised at 5.0 µg/dl: 4.9 µg/dl and below and 5.0 µg/d and above. The level of 5.0 µg/dl was used because it was close to the mean blood lead level of 4.9 µg/dl and was also based on international discussions regarding changing of the action level for blood lead from 10.0 µg/dl to 5.0 µg/dl (Gilbert, 2006). The attainment of menarche and attained Tanner stage across the dichotomised blood lead categories was adjusted for socio-economic status and anthropometric measures. The onset of menarche and Tanner stage served as dependent variables, and blood lead levels were fitted into logistic

regression models to test their associations with them. All statistical tests were conducted using the STATA 9 statistical package. Statistical significance was determined at a level of $p<0.05$.

3.2.4 Ethics

Ethical approval for the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand in Johannesburg. Confidentiality was maintained by assigning participant identification numbers that were known only to the data management team. Prior to the commencement of the study, written informed consent was obtained from each participant's parent or guardian and assent was provided by all participants. As part of the informed consent process, it was explained that participation was voluntary and could be withdrawn at any time without any adverse repercussions.

3. 3. Results

3.3.1 Demographic profile

The analytical study sample consisted of 682 Black females on whom we had complete data on lead levels, age of menarche and Tanner staging data. The demographic profile of the participants is presented in Table 3.1. Over 80% of the girls lived in formal housing with access to electricity. However, only 21% of the family of the participants owned their homes. Indoor water access was available

to 40% of participants. High cost items such as a washing machine or a car was owned by 12% and 24% of the sample, respectively.

Table 3.1: Demographic profile of the analytical sample in the Bt20 cohort

Characteristic	Analytical study sample (n=682)	
	No.	%
Birth Weight (grams)		
Mean	3026.52	
Range Highest weight	4920	
Lowest weight	1070	
Parity		
Mean	2	
Range	1-9	
≤3	574	(84)
>3	107	(16)
#Maternal Education		
None	7	1
Primary	77	11
Secondary	509	75
Tertiary	53	8
#Type of home		
Formal	595	87
Informal	35	5
#Ownership of house		
Owned	141	21
Rented	436	64
#Access to water		
Indoor access	270	40
Access outside the house	253	37
Access to electricity	588	86
Assets owned by family		
TV	452	66
Car	165	24
Fridge	429	62
Washing machine	79	12
Phone	316	46
Growth at 13 years		
Weight in kilograms		
Mean	47.6 (sd* 11.3)	
Range	28-117	
Height in centimetres		
Mean	155.4 (sd* 7.5)	
Range	115.1-177.4	
Body Mass Index (BMI)		
Mean	19.6 (sd* 4.0)	
Range	12.6-44.9	

 # % not totalling a 100% due to missing information
***sd Standard deviation**

3.3.2 Blood lead levels

The mean blood lead level in the study sample was 4.9 μg/dl with a range of 1.0-16.3 μg/dl. Fifty percent of the sample had blood lead levels < 5.0 μg/dl and 49 % had blood lead levels ≥ 5.0 μg/dl. Only one percent (1%) had blood lead levels > 10.0 μg/dl. The blood lead levels are given in Table 3.2.

Table 3.2. Blood lead levels at 13 years of age (μg/dl)

13 year lead level	**N=682**
Low risk (< 5.0 μg/dl)	343 (50%)
Intermediate risk (5.0-10.0 μg/dl)	334 (49%)
Very high risk (>10.0 μg/dl)	5 (1%)
Mean	4.9 μg/dl
Standard Deviation	1.9 μg/dl
Range	1.0-16.3 μg/dl

3.3.3 Pubertal measures at 13 years

The average age of menarche was 12.7 years with a range of 9-16 years. At 13 years of age 76% had attained menarche. The mean Tanner staging for pubic hair and breast development was 3. At 13 years, 4% and 7% had reached Tanner stage 5 for pubic hair and breast development, respectively. Fifteen percent (15%) and 14% had not reached the Tanner stage 3 for pubic hair and breast development,

respectively. Figure 3.2 illustrates the distribution of Tanner staging for pubic hair and Figure 3.3 breast development at 13 years of age.

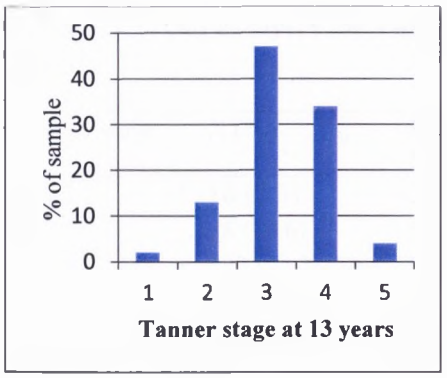

Figure 3.2 Tanner stage for pubic hair (13 years)

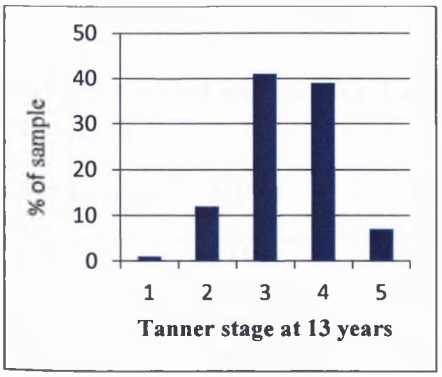

Figure 3.3 Tanner stage for breast development (13years)

3.3.4 The association between blood lead levels and pubertal development

3.3.4.1 Trends in pubertal development in association with blood lead levels at 13 years of age

Lead levels are significantly associated with pubertal stage at 13 years of age ($p<0.001$). Tables 3.3 and 3.4 showed that younger ages of Tanner stage for breast

and pubic hair development were associated with increased mean levels of blood lead.

Table 3.3 Trend analyses for Tanner pubic hair growth and mean blood lead levels†

Tanner stage	N (%)	Mean Lead Level	SD	P value
1	16 (2.3)	5.5	1.67	<0.001**
2	86 (12.6)	5.4	1.87	
3	322 (47.2)	5.0	2.04	
4	230 (33.7)	4.7	1.59	
5	28 (4.1)	4.6	2.18	

†Adjusted for BMI

**significant P value

Table 3.4 Trend analyses for Tanner breast development and mean blood lead levels †

Tanner stage	N (%)	Mean Lead Level	SD	P value
1	9 (1.3)	7.3	2.47	<0.001**
2	82 (12.0)	5.6	1.74	
3	283 (41.5)	5.0	1.83	
4	262 (38.4)	4.8	1.92	
5	46 (6.7)	4.5	1.69	

†Adjusted for BMI

** Significant P value

Table 3.5 shows that increased mean blood lead levels were significantly associated with an older age of menarche ($p<0.001$)

Table 3.5. Trend analysis for attainment of menarche and mean blood lead levels (n=682)†

Age	N (%)	Mean Lead Level	SD	P value
9	5 (0.7)	3.8	0.87	<0.001**
10	15 (2.2)	4.2	1.31	
11	91 (13.3)	4.9	2.04	
12	186 (27.3)	4.8	2.01	
13	220 (32.3)	4.8	1.75	
14	115 (16.9)	5.5	1.66	
15	33 (4.8)	5.6	2.04	
16	9 (1.3)	5.9	2.73	

†Adjusted for BMI

** Significant P value

The association between blood lead levels and pubertal staging and age of attainment of menarche was analysed, taking account of potential confounding factors (socio-economic data and anthropometric measures). However, there were also no significant associations between the confounding variables and the pubertal measurement variables.

3.3.4.2 Effect of blood lead ≥5.0 µg on pubertal development.

Results from the logistic regression analysis are shown in Table 3.6. The blood lead level was dichotomised at ≥5.0 µg/dl and < 5.0 µg/dl for the analysis. Breast development and pubic hair staging was dichotomised at 3. The study by Cameron and Wright in 1990 showed that the average Black female in South Africa would start puberty by 10.42 years to 11.69 years (Cameron and Wright, 1990). A study in a sub sample of Bt20 girls showed that there was an average decrease of 0.5 years per decade in the age of menarcheal attainment, which was 12.4 in 2004 (Jones et al, 2009). Thus by 13 years of age the majority of females

should have at least achieved stage 2 of the Tanner staging and those that have not started puberty or achieved Tanner stage 2 were considered delayed and those ≥3 were considered to be progressing at 13 years of age.

The results shown in Table 3.6 showed that for blood lead levels ≥5.0 µg/dl, the odds ratio for a delay in breast development was 2.05. The odds of a delay in pubic hair development was 1.62 if blood lead levels were ≥5.0 µg/dl and 2.04 for a delay in the attainment of menarche. After adjusting for BMI the odd ratios remained significant.

Table 3.6. The effect of blood lead concentrations of ≥5 µg/dl as compared with < 5 µg/dl on measures of pubertal development.

	Crude OR	95% CI	P value	Adjusted OR†	95% CI	P value
Beast development at 13 years	2.05	1.29-3.24	0.002**	2.34	1.45-3.79	0.001**
Pubic hair development at 13 years	1.62	1.05-2.48	0.03*	1.81	1.15-2.84	0.01*
Attainment of menarche at 13 years	2.04	1.42-2.96	<0.001**	2.01	1.38-2.94	<0.001**

† adjusted for Body Mass Index

* / ** Significant level

3.4 Discussion

This study has found, in an African setting, that elevated blood lead levels are associated with a delay in the onset of puberty among adolescent girls. In those with elevated blood lead levels, delays were observed in breast development, pubic hair growth and in the age of attainment of menarche. The association was significant even after adjusting for socio-economic factors and anthropometric measurements. The findings of this study among Black and Mixed ancestry South African girls living in Johannesburg/Soweto are consistent with those of other studies that have looked at the effect of blood lead on pubertal development (Wu et al., 2003; Selevan et al., 2003). For example, in 2003 Selevan et al used the United States of America (USA) National Health and Nutrition Examination Survey (NHANES III) to show a delay in the onset of puberty in girls with blood lead levels of 3.0 µg/dl or higher. The association remained significant after adjusting for possible confounding factors (Selevan et al., 2003; Wang et al., 2005; Kaji and Nishi, 2006). Delays in the onset of puberty were significant for all three of the same measures of puberty used in this study among African-American girls, and for breast and pubic hair development among Mexican-American girls. In White girls the trend was similar but not significant. Wu et al, using the same NHANES III study showed that there was a significant association between higher blood lead levels and a delay in attainment of menarche and pubic hair development overall, amongst all the girls in the cohort (Wu et al., 2003; Wang et al., 2005; Kaji and Nishi, 2006).

Animal experiments have shown that lead exposure alters pubertal hormones and thus leads to the delay in pubertal attainment (Iavicoli et al. 2004; Dearth et al. 2004; 2002; Ronis et al. 1998). Exposure to lead causes decreases in levels of Insulin like Growth Factor (IGF-1), Luteinizing Hormone (LH) and Estradiol (E_2) (Dearth et al., 2002). IGF-1 results in the secretion of LHRH from the brain, which induces the release of LH and the start of puberty. Thus if IGF-1 is suppressed then LH and estradiol levels are decreased and puberty is delayed. Dearth et al hypothesised that the possible mechanism by which lead effects IGF-1 may be due to an alteration in translation or ineffective IGF- 1 peptide production as well as altering IGF-1 receptors in the brain (Dearth et al., 2002). Lead also decreases prostaglandin E2 (PGE2) which facilitates LRHR production via IGF-1 (Dearth et al. 2002). Thus lead exposure decreases the brains response to IGF-1 and peripherally decreases its production as well.

In this study, and others, the effect of blood lead levels on pubertal development has been shown to occur at lower blood lead levels, adding to the substantial body of evidence demonstrating health effects at even what are considered to be relatively low levels of exposure. In many African countries, for example South Africa and Nigeria, blood lead distributions continue to be elevated compared with resource-rich countries (Mathee et al., 2004; Wright et al., 2005), and the potential public health significance of lead exposure on pubertal development could therefore be considerable. The urban poor are amongst those at greatest risk due to past lead deposition from the use of leaded petrol, the ongoing use of lead in paint (Mathee et al., 2007 ;2004), as well as the use of lead in the informal

sector and cottage industries (Mathee et al., 2007; Wright et al., 2005; Tong et al., 2000).

In this study socio-economic status and BMI measurements was not significantly associated with any of the measures of puberty. Lower socio-economic status is usually associated with nutritional deficiencies which can result in a delay in puberty. Although the socio-economic status of the Birth to Twenty Cohort is generally low, there was no evidence of overt nutritional deficiencies in the study sample as evidenced by the BMI. This finding was also found by Denham et al in a study looking the effect of lead on the timing of menarche in Akwesasne Mohawk Girls. The study showed that body size and socio-economic status had minimal impact on the association between lead and delayed menarche (Denham et al, 2005).

One of the limitations of the study that has to be considered is that lead levels were taken at 13 years and not at the time of menarche attainment, variable as it was for each girl in the sample. Not all the girls had attained menarche at 13 years of age. Thus at the actual attainment of menarche blood lead levels may have been higher or lower, which could possibly have affected the results. An assessment of the effect on male puberty was not examined in this study, but may be of interest. A study by Hauser et al showed that Russian boys with blood lead levels of ≥ 5 µg/dl had a 43% risk of delayed puberty (Hauser et al., 2008). Genetic factors may be responsible for delays in puberty. Unfortunately in this study we were not

able to assess maternal age of menarche. Race or ethnic differences were not assessed as well due to the low numbers of Asian and White groups in the study population.

This study has highlighted the association of relatively low and high level environmental lead exposure with delayed pubertal attainment in girls in a developing country. One of the potential correlates of a delay in puberty is short stature. Studies have shown that potential adult height as assessed by mid-parental heights was not attained in those with pubertal delay as compared to those girls with normal pubertal development (Albanese and Stanhope 1993; Crowne et al., 1991). In the long term this short stature may lead to obesity. A study using the NHANES data showed that African American females with short stature were more likely to be obese or overweight (Komlos, 2010). In South Africa the incidence of obesity among Black females is high (Poane et al., 2002; Case and Menendez, 2009). Therefore lead exposure which is still high in South Africa may be one of the factors that contribute to short stature in the short term and obesity in the long term. However the effect of the timing of puberty in relation to the development of obesity in other American and European studies is still inconclusive as shown in recent reviews conducted in the US and Europe (Himes, 2006; Ong et al., 2006). Further research is needed to assess how do delays in puberty impact on later body composition, and its possible contribution to the development of obesity.

Other potential effects of a delayed puberty were shown in the study by Crowne et al (1991). In this study 80% of the female participants who had a delayed onset of puberty also felt that their progress at school, at work and their social interactions may have been negatively affected (Crowne et al., 1991). A 31 year prospective study in Northern Finland showed that a delay in attainment of menarche in adolescents resulted in an increased risk of depression at the age of 31years (Herva et al., 2004). It was proposed that the cause was because of the lower levels of oestrogen in those with delayed menarche (Herva et al., 2004). Thus both physical and psychological sequelae of delays in puberty have been documented.

3.5 Conclusion

The findings of this study suggest that lead exposure, even at relatively low levels of 5 µg/dl, is associated with delayed pubertal development in girls in South Africa. Delays in pubertal maturation have been documented to have physical effects such as short stature and possibly obesity as well as psychological consequences later in life. The degree of lead exposure is higher in Africa and other resource-poor countries than in those countries in which most of the research has been conducted to date. This means that the public health implications of lead exposure on health and social well-being are likely to be far greater compared to resource-rich countries. Lead exposure, especially in low and middle income countries has to be substantially decreased in order to prevent negative health effects in future generations.

CHAPTER 4

Environmental lead exposure and socio-behavioural adjustment in the early teens: the Birth to Twenty cohort

Published in the journal: Science of the Total Environment, 414: 120-125.

The above paper describes the association between low level lead exposure and problem behaviours in 13 year old children. The effects of lead exposure are assessed in boys and girls.

4.1 Introduction

Blood lead levels in South Africa continue to be high compared to resource-rich countries where the mean blood lead levels in children are approximately 3.0 µg/dl (Koller et al., 2004). There are no routine screenings and monitoring processes for lead exposure in South Africa, thus evidence for environmental lead exposure has been determined from several cross-sectional epidemiological studies. In the Western Cape inner city school children aged 6 to 8 years had mean blood lead levels ranging from 14.0 to 16.0 µg/dl in 1991 (von Schirnding et al., 1991, 2001). Over 90% had blood lead levels greater than 10.0 µg/dl. In Johannesburg it was found that in 1995, 78% of children aged 6 to 8 years had blood lead levels >10.0 µg/dl with a mean blood lead level of 11.9 µg/dl (Mathee et al., 2002). By 2002, a follow up study in the same schools showed that the mean blood lead level dropped to 9.1 µg/dl, but 10 % of children had blood lead levels > 10.0 µg/dl (Mathee et al,. 2004). This change was attributed to the introduction of unleaded petrol in 1996 (Mathee et al., 2006). Thus despite some reductions, the detrimental health effects due to lead exposure remains a major public health challenge in South Africa.

One of the potential negative health outcomes of lead exposure is neurotoxicity and its effect on behaviour. Studies have shown an association between lead exposure and behavioural problems in children. These problems includes characteristics of Conduct Disorders and Oppositional Defiant Disorders that are described in the Diagnostic and Statistical Manual of Mental Disorders –Fourth Edition (DSM IV, 2000) and has been shown to be raised among children

exposed to lead. This type of behaviour includes temper tantrums, argumentativeness, active defiance and refusal to comply with adult requests and rules, deliberate attempts to annoy and upset people, frequent anger and resentment, mood instability, substance abuse, aggression towards people and animals, destruction of property, and deceitfulness, lying or; stealing (DSM IV,2000; AACAP).

In 1994 Bellinger et al. used the Teacher Report Form of the Child Behaviour Profile to assess childhood behavioural problems and its association with blood and dentine lead levels (Bellinger et al., 1994). This study found that tooth lead levels, used as an indicator of postnatal lead exposure in 8-year-olds, were associated with higher behavioural problem scores (Bellinger et al., 1994). Needleman in 1996 showed that in 11-year-old boys there was a significant association between higher bone lead levels and behavioural problems, specifically aggression, attention and delinquency as assessed on the Child Behaviour Checklist (Needleman et al., 1996). These retrospective cross-sectional studies have been cited to suggest a possible causal association between lead exposure and problem behaviour. The Cincinnati Lead Study, a longitudinal study, showed that in children aged between 15 and 17 years there was a significant association between lead exposure and antisocial behaviour as determined by the Self Report and Parental Report of Delinquent Behaviour (Dietrich et al., 2001). The longitudinal Port Pirie Cohort study found similar results using the Child Behaviour Checklist (Burns et al., 1999). These latter

studies indicate that behavioural problems may be manifest at blood levels lower than 10.0 μg/dl.

Socio- behaviour problems in adolescents is often both an outcome of earlier developmental difficulties and a precursor for anti-social and/or criminal behaviour later in life (Liu and Wuerker, 2005). Studies have reported that violent criminals were more likely to have higher childhood or lifetime blood lead concentrations (Nevin, 2000, 2007; Stretesky and Lynch, 2001; Wright et al., 2008). Besides lead exposure, risk factors for socio-behaviour problems include socio-economic status of an individual and other adversities in the home and social environment (Webster-Stratton and Taylor, 2001; Needleman et al., 2002), all of which act as possible confounders in exploring the relationships between lead and socio-behavioral adjustment. For example, children from lower socio-economic strata frequently have an increased risk of exposure to lead, violence and social problems as well as decreased resources (financial or compensatory experiences) to cope with the effects of exposure (Bellinger, 2008; Tong et al., 2000). An earlier study on the Bt20 cohort showed that at 13 years of age that low socio-economic status and having a high cord blood lead level increased the risk of lead exposure (Naicker et al., 2010). There is evidence that behavioural problems due to lead exposure may be attenuated by higher socio-economic status and better social conditions (Bellinger, 2008). In South Africa where poverty is pervasive, lead exposure may contribute to socio-behavioural abnormalities.

There are no other known studies that have assessed the impact of lead exposure on behaviour in adolescents in South Africa or in any other African countries. Consequently this study aims to explore the association between lead exposure and socio-behavioural adjustment among young adolescents in Johannesburg, South Africa.

4.2 Materials and methods

4.2.1 Study design and sampling

The Birth to Twenty (Bt20) cohort is a uniquely South African cohort study that started in 1989 with pilot studies to test the feasibility of a long-term follow-up study of children's health and wellbeing in the Greater Johannesburg area (Yach et al., 1991). Women were enrolled in their second and third trimester of pregnancy through public health facilities and were interviewed regarding their health and social history and current circumstances. Singleton children (n=3 273) born between April and June 1990 and resident for at least 6 months after birth in the municipal area of Soweto-Johannesburg were enrolled into the birth cohort and have been followed up 16 times between birth and 20 years of age (Richter et al., 2004, 2007). Attrition over two decades has been comparatively low (30%), mostly occurring during infancy and early childhood, and approximately 2 300 children and their families remain in contact with the study (Norris et al., 2007). The sample closely resembles the demographic parameters of South Africa with equal numbers of male and female participants. Assessments across multiple domains have been made of children, families, households, schools and

communities during the course of the study, including growth, development, psychological adjustment, physiological functioning, genetics, school performance, and sexual and reproductive health. The Bt20 research programme, including all waves of data collection, has received clearance by the Ethics Committee on Human Subjects at the University of the Witwatersrand (M010556). The Federal Wise Assurance registration number of the Committee is FWA00000715.

4.2.2 Analytical study sample

Blood lead data are available for 1546 young people. No information on lead was available for the rest of the cohort due to lack of opportunities for or problems with blood sampling and sample attrition. Of these 1546 adolescents, 1041 also completed the Youth Self Report (YSR), a socio-behavioural adjustment measurement tool, at 13 years of age, 487 boys and 554 girls.

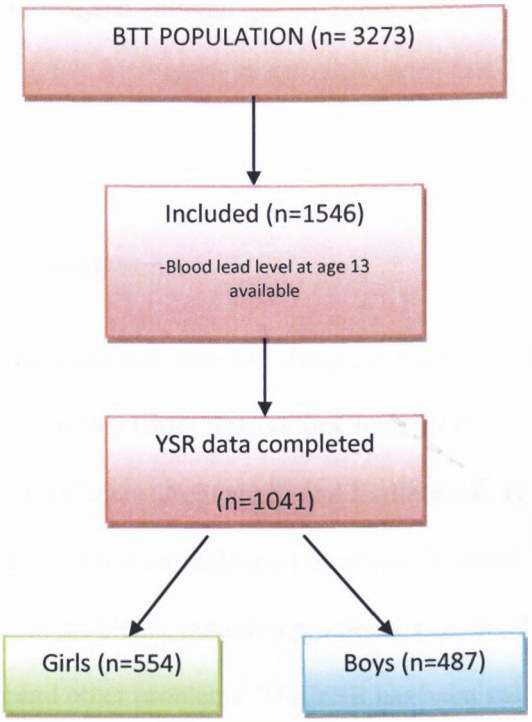

Figure 4.1: Flow chart of the analytical sample

4.2.3 Procedures/Data collection

4.2.3.1 Blood lead

Whole venous blood was collected into heparinised tubes determined to be free of trace metals at 13 years of age. Following preparation and centrifugation, lead concentrations in the whole blood samples were determined using an atomic absorption spectrophotometer equipped with a graphite furnace. Blood lead measurements were performed by the South African Centre for Occupational Health (now called the National Institute for Occupational Health), which

participates in international and national quality control programmes for blood lead determinations.

4.2.3.2 Socio-behavioural adjustment

Socio-behavioural adjustment was assessed using the Youth Self Report (YSR), an adolescent adaptation of the Child Behaviour Checklist (CBCL), designed to assess children aged 11-18 years (Achenbach and Endlebrock, 1991). It consists of 112 items covering questions around mood disorders, somatic complaints, social problems, thought problems, attention problems, rule-breaking behaviour, aggressive behaviour and other problems. The YSR has been validated in several countries with cultural and language differences (Verhulst, 2003), but not in South Africa. However it has been used in other South African studies to assess emotional and behavioural problems (Sabet et al., 2009).

4.2.4 Data Analysis

Historical data (demographic, maternal education, & social-economic status) collected on the cohort was utilised in the analyses. The blood lead level was the exposure variable. The average blood lead levels in the Bt20 cohort at 13 years was 5.7 µg/dl. Three percent (3%) had blood lead levels >10.0 µg/dl at 13 years. Blood lead levels were not normally distributed, and log transformed values were analysed.

In this study the questions that address rule–breaking and aggressive behaviour were analysed to assess the relationship between lead levels and socio-behavioural abnormalities. The questions consisted of 32 items (15 addressing the rule-breaking behaviour and 17 addressing aggressive behaviour). The individual items/ questions were analysed for the total sample, as well as stratified for boys and girls, given well-established gender differences on these dimensions (Bongers et al., 2003). Each question required a response in one of four categories (never, sometimes, true and very true). True and very true were combined into one category and regarded as a positive response to the question. Medians, geometric means, 95% confidence intervals (95% CI) and proportions were used to describe the study population. Differences between boys and girls were tested for using the chi square test. Testing was set at the 0.05 level of significance.

Bivariate analyses were conducted to assess for associations between blood lead levels and socio-behavioural variables using the Student's t-test. Those behavioural variables that were associated with blood lead levels at the 0.05 level using the Student's t-test were considered significant and were included in a multiple regression analyses. Socio-economic status was shown to be a significantly associated with lead exposure in the Bt20 cohort (Naicker et al., 2010). Thus a socio-economic index was created and was added to the multivariate analysis model. The socio-economic index was a composite score derived from 10 items that included asset ownership and access to electricity, flush toilets and indoor water. Other confounding factors such as nutritional status

or factors within the family or school environment were not available for analyses.

Further analyses would be considered if the multiple regression analyses produced significant associations. All statistical tests were conducted using the STATA 9 statistical package.

4.2.5 Ethical Issues

Ethical approval for the study was obtained from the Human Research Ethics Committee of the University of the Witwatersrand in Johannesburg. Confidentiality was maintained by assigning participant identification numbers that were only known to the data management team. Prior to the commencement of the study, written informed consent was obtained from each participant's parent or guardian, and as part of the informed consent process, it was explained that participation was voluntary and could be withdrawn at any time without any repercussions. Assent was obtained from the teenage participants as well.

4.3 Results

4.3.1 Analytical sample characteristics

The total analytical sample size was 1041 (487 males and 544 females). The socio-demographic details of the sample are shown in Table 4.1. There were no significant differences between males and females. Birth weights ranged from

1125 grams to 4800 grams. Unfortunately accurate data on apgar scores were not available. Maternal history showed that 76% of mothers had at least secondary school education, and 63% were single at the time of birth. Economic aspects were described in relation to housing and asset ownership. While only 21% of the sample owned their homes, 84% lived in formal housing with access to electricity. Indoor water access was available to 40% of participants. High cost items such as a washing machine or a car was owned by 11% and 21% of the sample, respectively.

Table 4.1: Socio-demographic profile of the analytical sample in the Bt20 cohort

Characteristic	Analytical study sample		
	TOTAL (n=1041)	BOYS (n=487)	GIRLS (n=554)
Birth Weight (grams)			
Mean (SD)	3070.42 (502.93)	3124.19 (512.32)	3023.24 (490.16)
Range Highest weight	4800	4800	4560
Lowest weight	1125	1125	1200
Parity			
Mean	2	2	2
Median	2	2	2
Range	1-9	1-8	1-9
≤2	681 (65%)	309 (63%)	372 (67%)
>2	359 (35%)	178 (37%)	181(33%)
Mother's age at birth			
<20 years	176 (17%)	77(16%)	99 (18%)
≥20 years	865 (83%)	410 (84%)	455 (82%)
Marital status at birth			
Married/together	380 (37%)	185 (38%)	195 (35%)
Single/divorced	661 (63%)	302 (62%)	359 (65%)
Maternal Education*			
None	8 (1%)	2 (0.4%)	6 (1%)
Primary	101 (10%)	47 (10%)	54 (10%)
Secondary	786 (76%)	364 (75%)	422 (76%)
Tertiary	70 (7%)	35 (7%)	35 (6%)
Type of home*			
Formal	871 (84%)	399 (81%)	427 (77%)
Informal	72 (7%)	40(8%)	32(6%)
Ownership of house*			
Owned	215 (21%)	118 (24%)	97 (18%)
Rented	725 (70%)	318 (65%)	407 (73%)
Access to water*			
Indoor access	415 (40%)	204(42%)	211 (38%)
Access outside the house	354 (34%)	155(32%)	199 (36%)
Access to electricity	876 (84%)	408 (84%)	468 (84%)
Assets owned by family			
TV	643 (62%)	294 (60%)	349 (63%)
Car	222 (21%)	98(20%)	124(22%)
Fridge	623 (60%)	285 (59%)	338 (61%)
Washing machine	113 (11%)	53(11%)	60(11%)
Phone	462 (44%)	215 (44%)	247 (45%)

***Values not totalling 100% indicate unknown data**

4.3.2 Blood lead levels

Descriptive statistics for blood lead levels by sex is listed in Table 4.2. The geometric mean blood lead levels for boys was significantly higher compared to girls (P<0.001, 6.0 μg/dl vs. 4.5 μg/dl)*. Sixty seven percent of boys and 48% of girls had blood lead levels between 5 and 10 μg/dl. Six percent of boys and 1% of girls had blood lead levels above 10 μg/dl.

Table 4.2. Blood lead levels at 13 years of age (μg/dl)

13 year lead level	Total (N=1041)	Boys (N=487)	Girls(N=554)
Low risk (< 5.0 μg/dl)	417 (40%)	132 (27%)	285 (51%)
Intermediate risk (5.0-10.0 μg/dl)	548 (57%)	327 (67%)	265 (48%)
Very high risk (>10.0 μg/dl)	32 (3%)	28 (6%)	4 (1%)
Mean	5.4	6.3	4.8
Range	1.0-28.1	1.3-28.1	1.0-15.4
Geometric mean	5.2	6.0	4.5
95% CI	5.05-5.32	5.83-6.24	4.38-4.71

*With respect to blood lead levels boys and girls differed significantly (student t test for logarithmic transformed data)

4.3.3 Socio-behavioural problems

Problems were indexed into two categories from the Youth Self Report (YSR): rule-breaking and aggressive behaviour. Individual adolescent positive responses i.e. if answered very true or true for individual items of two categories, ranged

from 1 to19 types. However the total percentage of positive responses to each individual item in the two behavioural categories were low. Table 4.3 and 4.4 reflect the prevalence of rule-breaking and aggressive behaviour in boys and girls.

4.3.3.1 Rule-breaking behaviour

One or more types of rule-breaking behaviour were found in 96% of the analytical sample. High percentages were found to questions regarding "Breaking rules" (84%) and "Using tobacco" (75%), although positive responses to the other questions were 25% or less. Girls and boys differed significantly on a number of behavioural responses, including "Breaks rules", "Associating with bad friends", "Preferring older kids", "Lying and cheating", "Setting fires", "Swearing", and "Thinking too much about sex". However the actual percentage differences between boys and girls was not high except for telling lies and cheating. These findings are illustrated in Table 4.3.

Table 4.3 Number of children, n (%), with rule breaking behaviour by sex.

Behaviour type	Total (%) N= 1041	Boys (%) N=487	Girls (%) N=554	P value*
Rule breaking behaviour	**995 (96%)**	**471(97%)**	**524 (95%)**	**0.09**
Drinks alcohol	237 (23%)	103 (21%)	134(24%)	0.25
Lacks guilt	54 (5%)	30 (6%)	24 (4%)	0.18
Breaks rules	877 (84%)	429 (88%)	488 (81%)	0.001**
Bad friends	64 (6%)	42 (9%)	22 (4%)	0.002**
Lies/ Cheats	107 (10%)	80 (16%)	27 (5%)	<0.001**
Prefers older kids	161 (16%)	96 (20%)	65 (12%)	0.004**
Runs away	8 (1%)	4 (1%)	4 (1%)	0.84
Sets fires	14 (1%)	11 (2%)	3 (1%)	0.02*
Steals at home	11 (1%)	8 (2%)	3 (1%)	0.08
Steals outside home	6 (1%)	6 (1%)	0	-
Swearing	39 (4%)	27 (6%)	12 (2%)	0.004**
Thinks of sex too much	7 (1%)	6 (1%)	1 (0.2%)	0.03*
Uses tobacco	779 (75%)	368 (76%)	411 (74%)	0.61
Truant	6 (1%)	5 (1%)	1 (0.2%)	0.07
Uses drugs	5 (1%)	3 (1%)	2 (0.4%)	0.55

*Chi square test used to determine significant differences between boys and girls

4.3.3.2 Aggressive behaviour

One or more types of aggressive behaviour were indicated by 63% of the total sample. Boys had significantly increased proportions of the following items compared to girls: "Teasing a lot" and "Threatening others". "Being loud" was significantly higher in girls (12% vs. 8%) (Table 4.4).

Table 4.4 Number of children, n (%), with aggressive behaviour by sex.

Behaviour type	Total (%) N= 1041	Boys (%) N=487	Girls (%) N=554	P value*
Aggressive behaviour total	**654 (63%)**	**319 (66%)**	**335 (60%)**	**0.09**
Argues a lot	238 (23%)	109 (22%)	129 (23%)	0.73
Mean	64 (6%)	28 (6%)	36 (7%)	0.62
Demands attention	210 (20%)	108 (22%)	102 (18%)	0.13
Destroys own things	13 (1%)	10 (2%)	3 (1%)	0.03
Destroys others things	9 (1%)	6 (1%)	3 (1%)	0.23
Disobedient at home	12 (1%)	6 (1%)	6 (1%)	0.82
Disobedient at school	9(1%)	5 (1%)	4 (1%)	0.59
Gets in fights	33 (3%)	18 (4%)	15 (3%)	0.36
Attacks people	15 (1%)	7 (1%)	8 (1%)	0.99
Screams a lot	124 (12%)	60 (12%)	64 (12%)	0.70
Stubborn, sullen	96 (9%)	48 (10%)	48 (9%)	0.51
Mood changes	132 (13%)	55 (11%)	77 (14%)	0.21
Suspicious	117 (11%)	47 (10%)	70 (13%)	0.13
Teases a lot	129 (12%)	87 (18%)	42 (8%)	<0.001**
Temper	61 (6%)	30 (6%)	31 (6%)	0.69
Threatens others	8 (1%)	7 (1%)	1 (0.2%)	0.02*
Loud	102 (10%)	37 (8%)	64 (12%)	0.03*

*Chi square test used to determine significant differences between boys and girls

4.3.4 The association between blood lead levels and behaviour

In the bivariate analyses described in Table 4.5 and 4.6, four individual items in the total analytical sample were significantly associated with the geometric mean blood lead levels.

The bivariate analyses for the association between rule-breaking behaviour and blood lead levels showed that "Running away" and "Stealing outside the home" was significantly associated with higher blood lead levels in the total sample but

after stratifying by sex, the association was no longer significant in boys or girls. However in boys the geometric mean blood lead level for those that reported "Running away" was 8.8 µg/dl compared to 6.0µg/dl in those that did not run away. The higher geometric mean blood lead levels were also higher for those that reported stealing outside the home. The mean blood lead levels for the other individual items in the rule-breaking category were similar for the negative and positive responses. (Table 4.5).

Table 4.5: Comparison of outcomes of individual rule breaking behaviour (pos/neg) with respect to the geometric mean BLL and significant levels for all children and sexes separately.

Behaviour type	Total (%) N= 1041			Boys (%) N=487			Girls (%) N=554		
	Mean BLL		P value	Mean BLL		P value	Mean BLL		P value
	Pos	Neg		Pos	Neg		Pos	Neg	
Rule breaking behaviour	5.2	5.1	0.66	6.0	5.4	0.27	4.5	4.9	0.41
Drinks alcohol	5.1	5.2	0.49	5.8	6.1	0.25	4.6	4.5	0.61
Lacks guilt	5.4	5.2	0.47	6.7	6.0	0.13	4.1	4.6	0.29
Breaks rules	5.2	5.0	0.31	6.1	5.7	0.23	4.5	4.7	0.39
Bad friends	5.4	5.2	0.45	6.1	6.0	0.90	4.3	4.6	0.55
Lies/ Cheats	5.5	5.2	0.13	5.9	6.1	0.69	4.4	4.6	0.69
Prefers older kids	5.4	5.2	0.30	6.0	6.0	0.82	4.6	4.6	0.95
Runs away	7.3	5.2	0.02*	8.7	6.0	0.06	6.2	4.5	0.14
Sets fires	6.4	5.2	0.06	6.5	6.0	0.51	6.2	4.5	0.22
Steals at home	6.4	5.2	0.11	6.7	6.0	0.47	5.7	4.5	0.35
Steals outside home	7.6	5.2	0.03*	7.6	6.0	0.15	-	4.5	-#
Swearing	5.2	5.2	0.94	5.6	6.1	0.35	4.2	4.6	0.56
Thinks of sex too much	5.1	5.2	0.94	5.6	6.0	0.66	2.9	4.5	-#
Uses tobacco	5.2	5.2	0.78	6.0	6.1	0.69	4.5	4.6	0.79
Truant	6.2	5.2	0.32	6.4	6.0	0.73	5.2	4.5	-#
Uses drugs	5.8	5.2	0.59	6.7	6.0	0.66	4.6	4.5	0.95

*** Significant associations if p<0.05**

 # P value not available because of very low positive response ≤ 1

Pos Positive

Neg Negative

The analyses for the association between aggressive behaviour and blood lead levels showed that in this category there were two items that were significantly associated i.e " Destroys own things" and "Threatens others". When the sample was stratified by sex, four individual items that were significantly associated with blood lead levels in boys i.e. "Argues a lot", "Destroys own things", "Attacks people", and being "Loud". For all these items except for "Argues a lot" the mean blood lead levels were higher in those boys that gave a positive answer compared to those with negative responses. In girls there were no significant associations found. This might reflect the lower blood lead levels found in girls compared to boys. The findings are reflected in Table 4.6.

Table 4.6: Comparison of outcomes of individual aggressive behaviour (pos/neg) with respect to the geometric mean BLL and significant levels for all children and sexes separately

Behaviour type	Total (%) N= 1041			Boys (%) N=487			Girls (%) N=554		
	Mean BLL		P value	Mean BLL		P value	Mean BLL		P value
	Pos	Neg		Pos	Neg		Pos	Neg	
Aggressive behaviour total	**5.2**	**5.1**	**0.47**	**6.0**	**6.1**	**0.66**	**4.6**	**4.5**	**0.54**
Argues a lot	5.1	5.2	0.71	5.6	6.2	0.03*	4.7	4.5	0.16
Mean	4.9	5.2	0.22	5.7	6.1	0.47	4.3	4.6	0.39
Demands attention	5.2	5.2	0.95	6.0	6.1	0.79	4.5	4.6	0.69
Destroys own things	7.7	5.2	<0.001*	8.2	6.0	0.01*	6.3	4.5	0.18
Destroys others things	6.2	5.2	0.21	7.9	6.0	0.09	3.9	4.5	0.49
Disobedient at home	5.3	5.2	0.91	6.8	6.0	0.42	4.0	4.6	0.51
Disobedient at school	6.0	5.2	0.86	5.7	6.0	0.75	4.3	4.5	0.84
Gets in fights	5.3	5.2	0.85	6.4	6.0	0.51	4.2	4.6	0.41
Attacks people	6.5	5.2	0.04	9.2	6.0	0.03*	4.7	4.5	0.80
Screams a lot	5.2	5.2	0.95	6.2	6.0	0.60	4.4	4.6	0.48
Stubborn, sullen	5.2	5.2	0.75	6.1	6.0	0.82	4.5	4.5	0.95
Mood changes	5.3	5.2	0.43	6.3	6.0	0.47	4.8	4.5	0.29
Suspicious	5.4	5.2	0.23	6.5	6.0	0.16	4.8	4.5	0.25
Teases a lot	5.5	5.2	0.17	6.1	6.0	0.66	4.3	4.6	0.33
Temper	5.3	5.2	0.64	5.9	6.0	0.82	4.8	4.5	0.49
Threatens others	6.9	5.2	0.05*	7.4	6.0	0.15	4.4	4.5	-#
Loud	5.4	5.2	0.24	7.0	6.0	0.02*	4.7	4.5	0.46

*** Significant associations**
 # P value not available because of very low positive response ≤ 1
Pos Positive
Neg Negative

The four items remained significant in the multivariate analysis for boys shown in Table 4.7. Although "Argues a lot" was significantly associated with blood lead levels it showed that those boys with lower blood lead levels had higher levels of "Argues a lot". The reason for this could be due to the personality traits of the

cohort or because of the low level of responses to this question. There were no significant results for girls in the bivariate analysis, thus a multivariate analysis was not conducted for girls.

Table 4.7: Multivariate regression analysis for boys

Variable	β	P value	95% Confidence Level
Argues a lot	-0.12	0.01	-0.20 - -0.04
Destroys own things	0.25	0.05	-0.00 - -0.49
Attacks people	0.34	0.02	0.46 - 0.63
Loud	0.14	0.04	0.01 - 0.27

R squared= 0.05

After adjustment with the socio-economic index " Argues a lot" **(β= -0.13; 95% CI= -0.23- to 0.02)** and "Attacking people" **(β=0.54; 95% CI= 0.09-0.98)** remained significant, however "Argues a lot" had a negative correlation. Thus "Attacking people" is a type of aggressive behavioural characteristic that was significantly associated with blood lead levels.

4.4 Discussion

Blood lead levels in this adolescent cohort were generally high compared to blood lead levels in many countries. The geometric mean blood lead level in this study was 5.2 µg/dl. In the United States (US) the current mean blood lead level in the population is approximately 3.0 µg/dl (Koller et al., 2004; Harper et al., 2003). US children > 10 years of age were shown to have blood lead levels of 4.4 µg/dl

or lower (Soldin et al., 2003). The study by Soldin et al (2003) also found that boys had higher blood lead concentrations than girls This study confirms this finding i.e. boys had a geometric mean blood lead level of 6.0 µg/dl compared to 4.5 µg/dl in girls The reasons for this maybe because of higher risk of exposure because of the behavioural characteristics of boys. That is boys generally have more outdoor activities where risk of exposure is higher.

Gender difference was also found in the assessment of behaviour. Boys generally had a higher percentage than girls of rule-breaking and aggressive behaviour. Studies using the YSR have noted differences in behaviour among boys and girls. Boys are more likely to display rule breaking and aggressive behaviour compared to girls in childhood and early adolescence (Flannery, 1994; Keily et al., 2000; Broberg et al., 2001; Calvete and Cardeñoso, 2005).

The bivariate analyses showed significant associations between the lead exposure and abnormal behaviour in the total analytical sample and among boys. In the multivariate regression analyses conducted in the boys sample "Attacking people" remained significant after adjusting for socio-economic factors. Numerous studies have found associations between lead exposure and adverse behavioural outcomes in childhood as measured by different behavioural tests (Bellinger et al., 1994; Needleman et al., 1996; Mendelsohn et al., 1998; Burns et al., 1999). The ages of assessment vary from very young 1-3 years old to 17 years of age. In the Port Pirie cohort study, a study conducted in a lead mining town, behaviour was

assessed among 11-13 year olds (Burns et al., 1999). The geometric mean lifetime blood lead levels were higher (boys 14.3 μg/dl and girls 13.9 μg/dl) compared to the geometric means in the Bt20 cohort , probably due to higher levels of exposure in Port Pirie. A study looking at dental lead levels and antisocial behaviour in Brazilian adolescents showed that higher dental levels were associated with antisocial/ maladjusted behaviour when the child behaviour checklist was used, however when the Self Reported Delinquency Scale was used in the same sample no significant associations were found (Olympio et al., 2010). Thus it is possible that the different measures used in the determination of socio-behavioural behaviour could produce different results as well as the role of potential confounders.

In the Bt20 study girls' blood lead levels were not significantly associated with behavioural characteristics. This maybe because of lower mean blood lead levels in girls. Rutter (2005) states that psychological responses to environmental factors are modulated by genetic factors. Thus the way boys and girls manifest behavioural changes in response to lead exposure may be different. However this is in contrast to the Port Pirie cohort study and the study by Bellinger et al, who found that there were no differences in behavioural abnormalities between boys and girls in relation to lead levels (Burns et al., 1999; Bellinger et al., 1994).

The majority of participants in this study were from a lower socio-economic group as indicated by low ownership of high cost items. In a previous study in the

Bt20 cohort, it was found that having a low socio-economic status was a significant risk factor for lead exposure (Naicker et al., 2010). Thus socio-economic status may have an effect on the degree of lead exposure (Tong et al., 2000; Nriagru et al., 1996) and subsequently influence the manifestation of socio-behavioural problems in those with higher blood lead levels (Bellinger, 2008).

There are several limitations in this study. This is a cross-sectional analysis and thus it measured blood lead and behaviour at one point in time. Blood lead indicates current exposure (1 month), thus past exposure and lifetime exposure may be different and this may influence behaviour patterns at adolescence. Although in this cohort there was no significant change in blood lead level at birth and 13 years of age (Naicker et al., 2010). Children at 13 years of age may not have been old enough as yet to display overt abnormal behaviour patterns. This age group was chosen because at this age socio-behavioural problems may begin to present itself, but long term follow up of these adolescent children will need to be conducted to assess the association between lead exposure and socio-behavioural problems in a later age group or even in early adulthood. Other factors affecting behaviour such as parental psychopathology, home and school environment were not known and thus could not be added to the analytical model.

4.5 Conclusion

The results from this study have shown significant associations between blood lead and socio-behavioural problems namely "Attacking People". The study also

highlights the issue of continued lead exposure and thus higher blood lead levels in South Africa. Another important finding is that boys have a higher degree of lead exposure and socio-behavioural problems compared to girls. Behavioural abnormalities, which can be influenced by low level lead exposure and socio-economic factors, is a known precursor to overt antisocial and criminal behaviour. This has major public health and economic implications for a country. Interventions need to be aimed at preventing lead exposure as well as improving social and economic conditions for communities.

PART 3

INTEGRATION OF RESULTS

CHAPTER 5: DISCUSSION

The discussion chapter summarises the results of the study, highlighting the key points and research theme of the study. The theoretical (conceptual) and contextual relevance of findings will be discussed in depth. The limitations of the study as well as the possibilities for future research will be summarised.

5.1 Summary of findings

The broad aim of the study was to determine the impact of lead exposure on children in living in urban South Africa. In order to accomplish these aims there were four objectives listed in Table 5.1. The three papers, published or submitted to international, peer reviewed journals, answered the objectives. The summary of the results and in which of the three papers the evidence can be found is described in Table 5.1.

Table 5.1 Consolidated findings

Objectives	Papers	Evidence
1. The prevalence of lead exposure in the Birth to Twenty Cohort	1, 2, 3	The mean blood lead levels at birth and at 13 years of age are higher than levels in resource rich countries.
2. Risk factors for lead exposure at birth and at 13 years	1	At birth having a teenage mother and low maternal educational status were significant risk factors. At 13 years of age being male, having a high cord blood lead level and lack of ownership of a phone were significant risk factors
3. The impact of lead exposure on puberty in adolescent females in the Birth to Twenty Cohort	2	Environmental lead exposure was associated in delays in all three measurements of puberty: menarcheal attainment, breast and pubic hair development.
4. To determine the effect of lead exposure on socio-behavioural adjustment in adolescence in the Birth to Twenty Cohort.	3	Overall the levels of socio-behavioural problems were low. Boys had greater levels of aggressive and rule breaking behaviour than girls. Significant associations were found between aggressive behaviour in boys and blood lead levels at 13 years of age.

5.2. Key findings from the research

This study has highlighted five important points:

- The prevalence of high blood lead levels
- Effects of low socio-economic status on lead exposure and its health effects
- Higher risk of lead exposure in boys
- Biological disruptions due to lead exposure as evidenced by the associated delay in puberty
- Lead exposure is associated with aggressive behaviour in boys

- **Blood lead levels**

At birth the cord blood levels ranged from 2.0 to 17.0 µg/dl, with a mean of 5.9 µg/dl. Four (4%) percent had a blood lead above 10.0 µg/dl. Thirteen years later the blood lead levels ranged from1.0 to 28.0 µg/dl with a mean of 5.7 µg/dl. Three percent (3%) had blood lead levels above 10.0 µg/dl. A blood level of > 10.0 µg/dl is considered the level at which action might be taken to reduce lead exposure (CDC, 1991). However, numerous international studies have shown that even blood lead levels at and below 10.0 µg/dl can lead to detrimental health effects (Binns et al., 2007; Canfield et al., 2003; Selevan et al., 2003; Lanphear et al., 2000; Swartz et al., 1986). In the Birth to Twenty cohort 50% and 53% of the birth and 13 year cohort respectively had blood lead levels greater than 5 µg/dl.

Blood lead levels in the Bt20 cohort have thus remained practically unchanged over the 13 years.

These blood lead levels are low relative to those found in other studies in South Africa and other African and low and middle income countries. However when compared to resource rich countries the Bt20 blood lead levels are relatively high. This is probably due to resource rich countries taking more active measures to reduce the risk of exposure in children and adults. These measures were put in place much earlier when compared to South Africa and other low and middle income countries. Resource rich countries have multiple measures in place to prevent and manage lead exposure in children such as safe removal of hazardous building material including paint, removal of lead from petrol, educational awareness, better nutrition and nutritional supplementation when needed as well as routine screening at ages 1 or 2 years or targeted screening in high risk communities (Campbell and Osterhoudt, 2000).

- **Socio-economic status and its effect on lead exposure**

The difference in risk factors could explain why the Bt20 cohort had lower levels of lead compared to children in other parts of South Africa. In the Bt20 cohort the majority of the children lived in Soweto, an area on the outer southern part of Johannesburg. Inner city children in the Western Cape and Johannesburg however were exposed to high traffic volumes, the children also lived in old dwellings with

flaking paint (Von Schirnding et al., 1991b). Another point is that the Bt20 cohort is much older than the children that participated in the other South African studies.

In the Bt20 cohort the risk factors associated with high blood lead levels at birth were low maternal education and having teenage mothers. At 13 years of age the significant risk factors were having a high cord blood level, male sex and poor socio-economic status. These findings are similar to other studies in South Africa and elsewhere in the world (von Schirnding et al., 1991b). In the USA where the majority of the children have blood lead levels < 3 µg/dl, certain pockets of children continue to have high BLL (Bellinger, 2008). Screening of children aged 10 to 72 months in an inner city paediatric clinic that serviced mainly Black and Hispanic economically poor patients, showed that > 68% had BLL > 10 µg/dl (Melman et al., 1998). In 2004 in the USA 17% of low income children had BLL > 10 µg/dl (Breysse et al., 2004).

Thus overall the most important risk factor for an elevated blood lead level is socio-economic status. Poorer communities have increased risk because of living in older, more dilapidated housing painted with lead containing paints, live near high traffic volume areas, have poor access to education and employment and participate in cottage industries resulting in higher levels of lead exposure.

- **Gender differences in the risk of lead exposure.**

Male gender was significantly associated with a higher risk of lead exposure in adolescents in the Bt20 cohort ($p<0.001$). In the US National Health and Nutrition Examination Survey III (NHANES), of all children tested from 5 to 18 years of age, male children had higher blood lead levels (Fox and Cole, 2004). The reason for this effect was not discussed in the NHANES study. This effect may be due to the activities of male children such as more time spent undertaking outdoor activities, and thus higher levels of environmental lead exposure (Costa de Almeida et al., 2010) and also increased participation in cottage industries using lead (Mathee et al, 2006). Other studies have shown similar findings when comparing lead levels in boys and girls. Roels et al (1980) conducted a study in 1978 and noted that 11 year old boys had higher blood lead levels than girls and that lead measured on hands were higher in boys. More recent studies have also shown this difference between boys and girls at different age groups (Trepka et al., 1997; Paoliello et al., 2002).

- **Biological disruption associated with lead exposure**

This study showed that elevated blood lead levels were associated with a delay in the onset of puberty among adolescent girls. In those with elevated blood lead levels, delays were observed in breast development, pubic hair growth and in the age of attainment of menarche. The association was significant even after adjusting for socio-economic factors and anthropometric measurements. The findings of this study among Black and mixed ancestry South African girls living

in Johannesburg/Soweto are consistent with those of other studies that have looked at the effect of blood lead on pubertal development (Denham et al., 2005; Selevan et al., 2003; Wu et al., 2003).

This finding highlights the biological disruption that can occur as a consequence of exposure to lead. The finding is significant because the effects have occurred at low levels of exposure. In this study only one percent of adolescent females had blood lead levels above 10.0 µg/dl. The logistic regression analysis showed that a blood lead level of ≥5.0 µg/dl had an increased risk of delay of 2.05 times for breast development, 2.04 for attainment of menarche and 1.62 times for a delay in pubic hair development. The effects of low level lead exposure is similar to the studies conducted in the USA where levels as low as 3 µg/dl have been associated with pubertal delays in girls, an indication of biological disruption (Selevan et al., 2003; Wu et al., 2003).

- **Lead exposure associated with higher levels of aggressive behaviour in boys.**

In the third paper of the thesis blood lead levels in boys were significantly associated with aggressive behaviour specifically "attacking people". Girls did not have a significant association between blood lead levels and aggressive or rule breaking behaviour. However this finding has not been found in all studies assessing lead exposure and behavioural changes. In the Port Pirie cohort study by

Burns et al (1999)and the study by Bellinger et al (1994) there were no differences in behavioural abnormalities between boys and girls in relation to lead levels.

In this study the fact that boys had significantly higher blood lead levels compared to girls may contribute to the above result. There is extensive evidence that boys and girls differ in behaviour responses. Girls usually have more internalizing behaviours such as mood disorders of depression and anxiety, can be withdrawn and have more somatic complaints. Boys on the other hand exhibit more externalizing behavioural characteristics such as aggression and rule breaking (Hoffmann et al., 2004; Allgood-Merten et al., 1990; Horwitz and White, 1987; Huselid and Cooper, 1984). These gender differences usually begin in adolescence and may continue into adulthood (Hoffman et al., 2004; Nolen-Hoeksema and Girgus, 1994).

Studies specifically using the YSR have noted differences in behaviour among boys and girls. Boys are more likely to display rule breaking and aggressive behaviour compared to girls in childhood and early adolescence (Flannery, 1994; Keily et al., 2000; Broberg et al., 2001; Calvete and Cardeñoso, 2005). Thus there is a possibility of gender affecting the degree or type of detrimental health effect and further research is necessary in this regard.

5.3 Emerging research theme

Collectively all the papers show that lead exposure and the subsequent health effects contributes to public health challenges. It follows a classic public health model of exposure related to specific outcomes that can be enhanced or mitigated by other external factors as reflected in Figure 5.1.

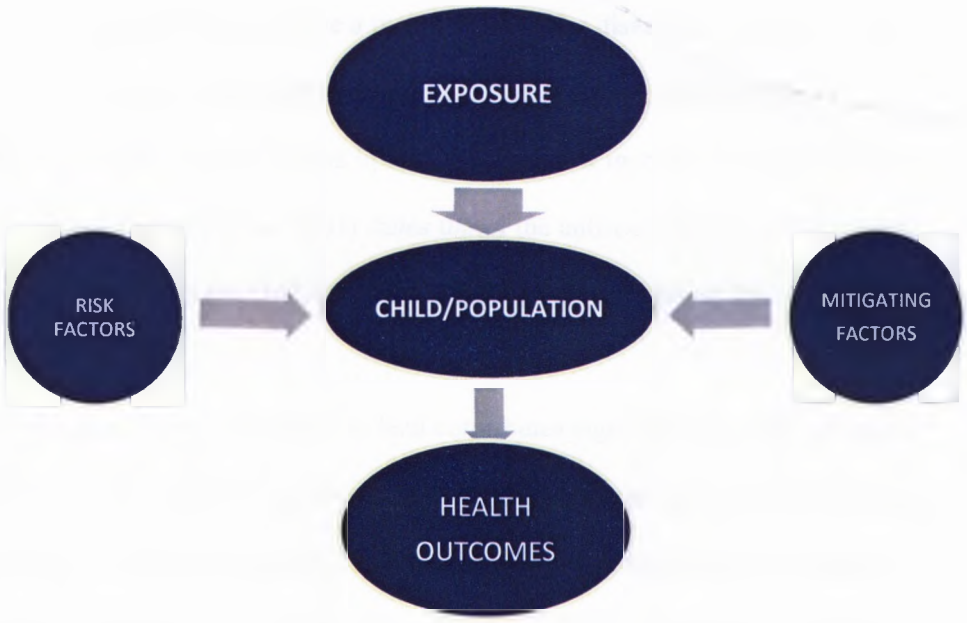

Figure 5.1: Public health model of exposure and health outcomes

The consequences of lead exposure constitute a public health challenge of global relevance. Even in countries with active measures to prevent and control lead exposure, the legacy of past exposures continues to affect the population. In poor,

deprived communities worldwide the effects of lead exposure are even more extreme.

In an individual, exposure to low level environmental lead either prenatally or in childhood can produce decreased cognitive function (mild mental retardation) and adverse behaviour changes (aggression, attention deficits, hyperactivity etc). These problems potentially escalate as the child gets older. At a population level these individual challenges have a major impact. The disease burden due to mild mental retardation because of lead exposure was 1% at a global level i.e. 12.9 million DALYS and 90% occurs in children from low income countries (Fewtrell et al., 2004). Fewtrell et al (2004) states that if the antisocial behaviour described above is included in the DALYS, the disease burden will exceed 1%.

The burden of disease attributed to lead contributes significantly to the health and education costs and subsequent economic burden on societies. Decreased IQ leads to poor educational attainment, decreased chances of employment and decreased productivity (Grosse et al., 2002; Fewtrell et al., 2004). Delinquent antisocial behaviour in childhood can eventually lead to violent criminal behaviour in adulthood. This places additional costs on the justice and criminal systems as well as on other societal processes. In the USA the estimated cost in 2006 for lead hazard control in households ranged from $1.2 to $11.0 billion or between $1.200- $10800 per household effected (Gould, 2009; Korfmacher, 2003). However the estimated cost of problems related to lead exposure totals $192-$270 billion. This value includes costs from medical treatments, loss of earnings,

income tax loss, special education needs, treatment for ADHD associated with lead and costs due to criminal activity (Gould, 2009). There was a saving of $17 to $221 for every dollar spent on controlling lead exposure. This can be compared to routine immunization programs that show a return of $5.30 to $16.50 for every dollar spent (Zhou et al, 2005; Gould, 2009). Thus lead hazard control and prevention of lead exposure is cost effective and has enormous savings for countries. This is essential in low and middle income settings where financial and human resources, as well as infrastructure, are limited.

Thus evidence-based public health policies need to be created to reduce and ultimately eliminate childhood lead exposure. This includes routine screening and surveillance in high risk communities, educational awareness of the hazards of lead exposure and controlling and monitoring the use of lead in formal and informal industries. In conjunction with these measures, risk factors for lead exposure need to be addressed. Low socio-economic status is largely related to other risk factors found in this and other studies such as low maternal educational status, teenage mothers and poor nutrition.

Lead exposure is a major concern at an individual and population level. If it is allowed to continue there will be enormous health and economic costs globally, especially for the most vulnerable.

5.4 Conceptual (Theoretical) relevance

This study has contributed to the environmental health field of research by highlighting the prevalence of lead exposure in a low to middle income country and the detrimental effect of lead on health of adolescents. The findings from this thesis have been added to the conceptual framework presented in Chapter 1. Thus although the findings are not unique, it corroborates the findings from other studies and adds insight into the impact of lead exposure in the South African context. Figure 5.2 illustrates how the results from this study contributed to the overall conceptual framework.

Factors such as maternal education, teenage mothers, socio-economic status and gender of the child or adolescent have been shown to be significant risk factors for lead exposure. These factors are associated with higher blood lead levels and the resultant effects of this have been detrimental changes in the biological processes particularly pubertal delays in girls and aggressive behaviour in boys.

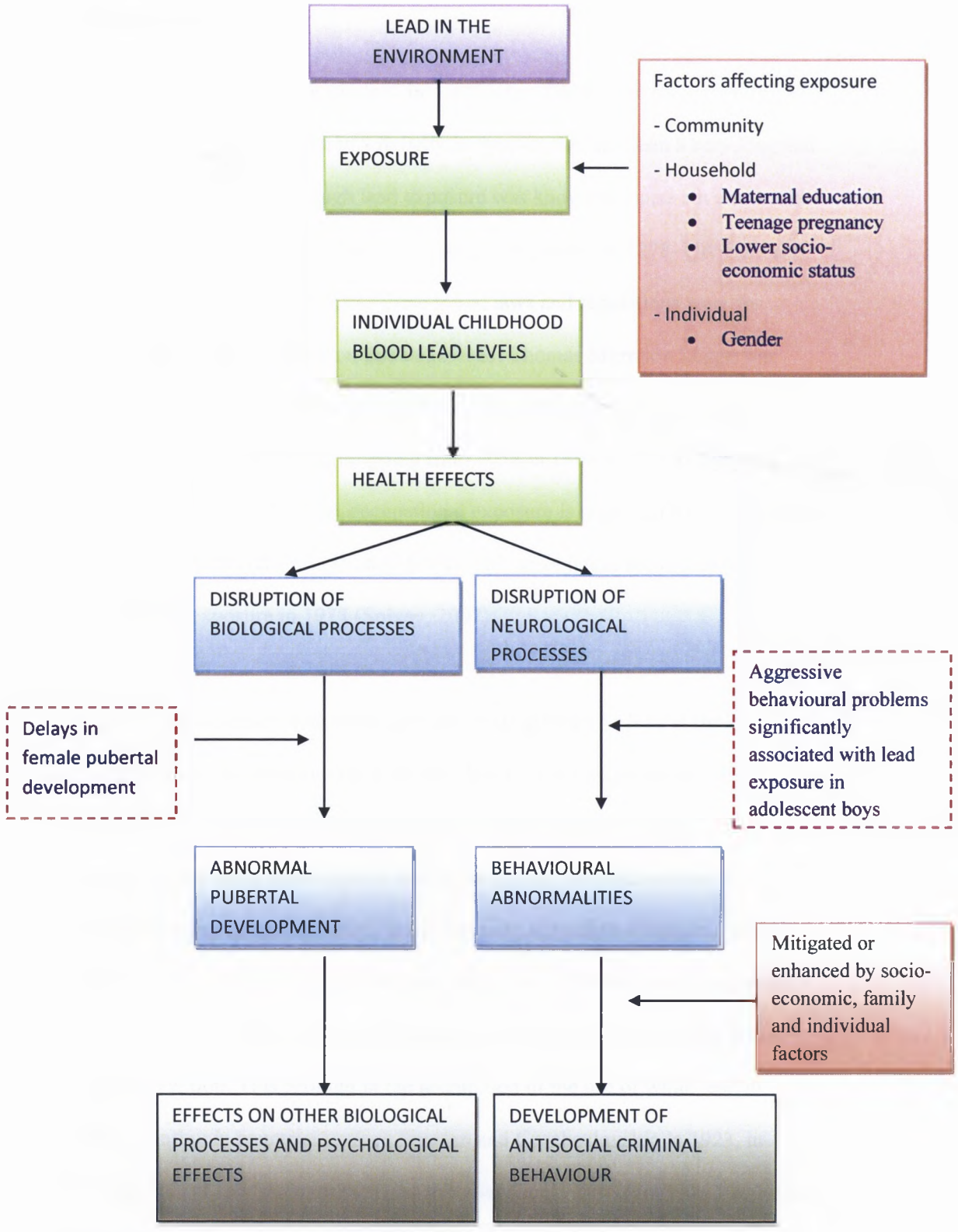

Figure 5.2 Theoretical relevance of the study.

5.5 Contextual relevance

The results of this study should feed into the policy framework for preventing lead exposure in adults and children. Globally in the past this has been a very long and protracted process even though lead exposure was known to cause adverse health effects. The first laws regulating lead exposure was noted in 1878- UK Factories and Workshop Act of 1878. Thus these initial laws and regulations were for occupational settings. Based on this in 1898 Sir Thomas Morrison Legge was appointed the first medical inspector of factories. This resulted in the number of lead toxicity cases in the UK dropping from 1058 in 1900 to only 41 by 1944 (Hernberg, 2000). In the USA occupational exposure is regulated by Occupational Safety and Health Administration (OSHA). This association set standards for occupational exposure in 1978 (Spivey, 2007).

Occupational exposure is an important contributing factor to lead in the neighbourhood environment and in homes, however the major causes of environmental exposure i.e. lead in paint and lead in gasoline/ petrol were regulated much later. Lead in paint leading to toxicity was first noticed in Australia among children in 1892, but it was only in 1920 in Australia that the first law (Lead paint prevention act) banning the use of leaded paint was passed. In 1921 the International Labour Conference in Geneva put in place the White Lead Convention. This resulted in the prohibition of the use of white lead in indoor painting in countries such as Sweden and Czechoslovakia in 1923, in Austria, Poland and Spain in 1924 and in Finland and Norway in 1929. However this agreement was not ratified by the USA and UK. The UK, France, Belgium,

Cuba, Yugoslavia, Tunisia and Greece soon approved such legislation (Markowitz et al., 2000; Belinger and Bellinger, 2006). It was only in 1977 that the USA eventually regulated the use of leaded paint in residential settings (Bellinger and Bellinger, 2006). However to date there continues to be evidence that lead is still used in paint in many countries (Clark et al., 2009; Mathee et al., 2007; 2009). In 1975 the US Environmental Protection Agency issued regulations for the gradual phasing out of leaded petrol. In 1996 it was completely banned. In Germany the phase out of lead in petrol started much earlier in 1972 and other European countries followed in the 1980's. However in some parts of South America, Asia, Eastern Europe, Africa and the Middle East, leaded petrol is still used (United Nations Environment Protection, 2008; Landrigan et al., 2000).

Measures to control and prevent lead exposure have been a priority in the more developed parts of the world. These include international conventions to protect the rights of children which include protecting them from lead exposure and laws and regulations controlling lead usage such as eliminating lead from paint and petrol (Tong et al., 2000). In the USA there are programmes specifically aimed at reducing lead exposure such as the US Department of Housing and Urban Development (HUD) Lead-Based Paint Hazard Control Grant Program. Six years post commencing this intervention an evaluation showed that dust lead levels decreased by 75% in homes (Wilson et al., 2006). Screening, intervention and evaluation was conducted country wide until average blood lead levels dropped and now only high risk populations are routinely screened. National educational awareness initiatives are in place by government and other agencies.

In South Africa lead exposure in industries is regulated by the Occupational Health and Safety Act (OHS Act) of 1993 (Department of Health, South Africa). The Lead Regulations were amended in 2002. Currently the action level is 60 µg/dl of lead in the blood of an adult worker. This level is much higher than in Europe and the USA where the occupational action level is 40 µg/dl.

Regulations were promulgated in terms of the Hazardous Substances Act (Act no. 15 of 1973) in July 2009 (and came into effect in July 2010), to include lead as a Group 1 category A hazardous substance (Acts Online, 2012). Prior to this leaded paint had been used in homes, children's playground equipment and toys and evidence of this is still found (Mathee et al., 2009; 2007). South Africa commenced the phasing out of lead in petrol in 1996 when the first lead free petrol was introduced. However it was only completely stopped in 2006. Thus both these sources of lead still continue to cause harmful effects in children and adults.

Despite these laws and regulations the most vulnerable and deprived populations are still exposed to high amounts. Monitoring the implementation of laws pertaining to lead usage is slack or non- existent. This is especially the case in cottage industries in South Africa. In this and other low and middle income settings lead exposure has not been seen as an issue to be prioritised in light of the many other social and economic problems that are experienced. Thus there are no screening programs and high risk populations cannot be identified and lead

exposure continues unabated. The legacy of past use of lead based paint in homes and schools also contributes to the problem of ongoing exposure to lead.

The findings from the papers presented in this thesis provide scientific evidence that can be used by policy makers and those responsible for monitoring and implementing regulations around lead exposure in South Africa.

Paper 1 described the continued high prevalence of lead exposure and the main risk factors for lead exposure i.e. maternal education and related to it, teenage pregnancy, low socio-economic status and male gender. These are important findings that can indicate where policy makers should focus educational, screening and surveillance programmes. In the USA all children used to be screened for lead exposure. Since 1997 this screening and surveillance system was only conducted in high risk populations (Bellinger and Bellinger 2006). This change occurred because of the studies and initial national screening programmes that have shown improvements in lead exposure following interventions such as improved housing, the lead based paint hazard control programme that was implemented in 1992 where hazardous paint was removed from homes, and the discontinuation of the use of leaded petrol. Thus in South Africa school children in low socio-economic urban areas and children living near lead industries maybe targeted initially for screening programmes.

Paper 2 and paper 3 highlighted the biological disruption and negative behavioural consequences due to low level lead exposure. The findings contribute

to the international body of evidence that blood lead levels as low as 2.0 µg/dl can cause adverse health effects that can have significant long term effects for the individual and population. Studies looking at the association between lead exposure and clinical effects in children have provided substantial evidence for policy makers to ban exposures to lead in paint and petrol. This has also contributed to the understanding that no amount of lead exposure is acceptable. The CDC has over the years amended lead action levels. In the 1960's the action limit was 60 µg/dl; by 1985 it was decreased to 25 µg/dl; and eventually since 1991 to present it is 10 µg/dl. In the past few years there has been increasing evidence as discussed in paper 2 and international studies that 10 µg/dl is still too high and there is possibly no safe level of lead exposure (Gilbert and Weiss, 2006; Bellinger and Bellinger, 2006; Binns et al., 2007; Barnard, 2003; Rogan and Ware, 2003).

5.6 Limitations of this work

- Even though the study was prospective, it was not designed to look at all risk factors for lead exposure, thus information regarding other potential risk factors, such as paternal history, parental occupational exposure to lead, passive smoking and the physical state of the homes such as peeling paint was not available for analysis.

- The cord blood sub-sample had some significant demographic differences from the rest of the BTT cohort with a higher percentage of

teenage mothers and a lower percentage of preterm and low birth weight babies. This may have resulted in sample bias, and the findings from this period may not be representative of the entire cohort.

- Improvements in detecting low levels of blood lead over the 13-year period of the study may have resulted in the recording of lower blood lead levels at 13 years i.e.<2μg/dl. This may have affected the results in the lower ranges.

- Although male puberty was assessed in the Birth to Twenty Cohort it was not examined in this study, but may be of interest.

- Paper 3 was a cross-sectional analysis and thus it measured blood lead and behaviour at one point in time. Blood lead indicates current exposure (1 month), thus past exposure and lifetime or cumulative exposure may be different and this may influence behaviour patterns at adolescence.

- Children at 13 years of age may not have been old enough to display overt abnormal behaviour patterns. This age group was chosen because at this age socio-behavioural problems may begin to manifest, but long term follow up of these adolescent children will need to be conducted to assess the association between lead exposure and socio-behavioural problems in a later age group or even in early adulthood.

5.7 Research gaps and future research

- Blood lead levels were used in this study, but it will only indicate current exposure. Measures of life-time or cumulative lead exposure such as bone lead measurements will provide a more accurate picture of the body burden of lead. In order to assess cumulative lead exposure one requires specialised bone x-ray fluorescence equipment to assess lead in bone. Cumulative lead measurements will provide a more accurate picture of lead associated pathology. Future research will need to include this aspect to determine associations with delinquent behaviours in childhood and violent criminal behaviour in adulthood.

- Long term consequences of biological changes due to low level lead exposure need to be established. How does the delay in puberty contribute to body composition? Does it result in obesity due to the delay in puberty? Does it cause psychological disturbances in the long term?

- Significant socio-behavioural adjustment problems due to lead exposure were not found in this adolescent population. However this age group might be too young to present with overt behavioural problems. Long term follow-up in this population at 18 years or older is necessary.

CHAPTER 6: CONCLUSION

Low levels lead exposure is prevalent in urban communities in South Africa. The exposure to lead is associated with significant detrimental health effects in particular pubertal delays in girls and aggressive behaviour in boys

Action to prevent lead exposure has taken a long time, even though the detrimental health effects have been known for centuries. Currently it is still not seen as a priority for many countries especially in low and middle income countries where the prevalence of lead exposure is high. This is a major public health problem. Health care, schooling, social welfare and criminal justice systems will be faced with the higher costs and require added resources to deal with the adverse effects of lead exposure. This places a significant burden on resource poor societies that are faced with multiple challenges.

Effective screening and surveillance systems need to be targeted at vulnerable high risk populations. Regular monitoring and evaluation is needed. This has to be done in conjunction with poverty alleviation and improved education which are the major risk factors for increased environmental lead exposure.

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APPENDIX

1. Ethics clearance certificate

2. Copy of a part of the Youth Self Report (Achenbach, 2001)

The YSR is protected by copyright and thus only the section that was used in this thesis is represented here.



VII. RULE-BREAKING BEHAVIOR

___ 2. Drinks alcohol
___ 26. Lacks guilt
___ 28. Breaks rules
___ 39. Bad friends
___ 43. Lies, cheats
___ 63. Prefers older kids
___ 67. Runs away
___ 72. Sets fires
___ 81. Steals at home
___ 82. Steals outside home
___ 90. Swearing
___ 96. Thinks of sex too much
___ 98. Uses tobacco
___101. Truant
___105. Uses drugs
___ Total

VIII. AGGRESSIVE BEHAVIOR

___ 1. Argues a lot
___ 16. Mean
___ 19. Demands attention
___ 20. Destroys own things
___ 21. Destroys others' things
___ 22. Disobedient at home
___ 23. Disobedient at school
___ 37. Gets in fights
___ 57. Attacks people
___ 86. Screams a lot
___ 86. Stubborn, sullen
___ 87. Mood changes
___ 88. Suspicious
___ 94. Teases a lot
___ 95. Temper
___ 97. Threatens others
___ 104. Loud
___ Total

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Naicker

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M080702**
PROJECT	Association between Lead Exposure and Maladjusted Behaviour in Adolescence and Young Adulthood: The Birth to Twnety Cohort
INVESTIGATORS	Dr N Naicker
DEPARTMENT	Birth to Twenty (Baragwanath)
DATE CONSIDERED	08.07.25
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 08.08.13 **CHAIRPERSON** .....
(Professor P E Cleaton Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof A Mathee

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