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Association between lead levels and adverse health effects: findings from the birth to twenty cohort

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ASSOCIATION BETWEEN LEAD LEVELS AND ADVERSE HEALTH EFFECTS: FINDINGS FROM THE BIRTH TO TWENTY COHORT

PALESA MANTHABISENG NKOMO

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

September, 2018

DECLARATION

I, Palesa Manthabiseng Nkomo declare that this thesis is my work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at this or any other University.



Signature

on the 4th **day of** October **2018**

DEDICATION

This PhD is dedicated to my maternal great grandfather Joseph Zwelibanzi Mbebe (1881 – 1967) and great grandmother Jostina Nomkhungu Mbebe (1890 – 1964). My great grandparents were blessed with 10 children, 8 of whom were daughters including my grandmother Mrs Isabella Stofile (1912 – 2006). My deep respect, love and admiration for my great grandfather Zwelibanzi stems mostly from how he valued all his children irrespective of their gender. He understood the value of educating a girl child. He personally paid for the education of each and every one of his daughters. His first born child, my grandmother was my first grade teacher and from a very young age she instilled in me a sense of belief in myself that I can achieve anything that I put my mind to. To my great grandfather Zwelibanzi - Enkosi Rhadebe! Hlubi! Mthimkhulu! Ndlebentle'zombini!

PUBLICATIONS RELATED TO THIS THESIS

1. **Nkomo, P**; Naicker, N; Mathee, A; Galpin, J; Richter, LM and Norris SA. The association between environmental lead exposure with aggressive behaviour, and dimensionality of direct and indirect aggression during mid-adolescence: Birth to Twenty Plus cohort. *Science of the Total Environment.* 2018 Jan 15; 612:472-9.

   How each author contributed to the journal article:

   - **Nkomo, P.** Conceptualization and design of the paper; data management and analysis; primary write-up, finalization and submission of the paper.
   - **Norris, SA; Mathee, A and Naicker N.** Supervision of conceptualizing of the manuscript components.
   - **Richter, LM.** Advisory role in manuscript write up.
   - **Galpin, J.** Advisory role with regard to statistical analyses.

2. **Nkomo, P**; Mathee, A; Naicker, N; Galpin, J; Richter, LM and Norris, SA. The association between elevated blood lead levels and violent behaviour during late adolescence: The South African Birth to Twenty Plus cohort. *Environment International.* 2017 Dec 1; 109:136-45.

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3. **Nkomo, P**; Richter, LM; Kagura, J; Mathee, A; Naicker, N and Norris, SA. Environmental lead exposure and pubertal trajectory classes in South African adolescent males and females. *Science of the Total Environment.* 1 July 2018, 628-629:1437–1445.

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- **Richter, LM.** Advisory role in the manuscript write up.
- **Kagura, J.** Advisory role with regard to statistical analyses.

NOTABLE ACHIEVEMENTS

1. **Nkomo, P**; Mathee, A; Naicker, N; Galpin, J; Richter, LM and Norris, SA. The association between elevated blood lead levels and violent behaviour during late adolescence: The South African Birth to Twenty Plus cohort. *Environment international.* 2017 Dec 1;109:136-45.

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DEFINITION OF TERMS

Blood Lead Levels at Birth (Exposure variable)

Cord blood lead levels were measures from blood samples collected just after birth.

Blood Lead Levels at Age 13 Years (Exposure variable)

Adolescent blood lead levels were measured using blood samples collected at age 13 years.

Aggressive Behaviour (outcome variable)

Data were collected for aggressive behaviour at ages 11/12 and 14/15 years old. The aggressive behaviour items are listed below in Box A. There were four response options for each item as follows: not true, sometimes true, true and very true.

Box A: Aggressive Behaviour Items	
1) I argue a lot	10) I scream a lot
2) I am mean to others	11) I am stubborn
3) I try to get a lot of attention	12) My moods and feeling change suddenly
4) I destroy my own things	13) I am suspicious
5) I destroy things belonging to others	14) I tease others a lot
6) I disobey my parents	15) I have a hot temper
7) I disobey at school	16) I threaten to hurt people
8) I get into many fights	17) I am louder than other kids
9) I physically attack people	

Violent Behaviour (outcome variables)

Data for violent behaviour were collected at ages 14/15 years. The violent behaviour items are listed below in Box B. The items related to perpetration of violent behaviour at school

and outside of school. There were four response options for each violent behaviour item as follows: never, once or twice, a few times and many times.

Box B: Violent Behaviour Items	
At school, how often have you done these things?	**Outside of school, how often have you done these things?**
1) Hit or kicked someone	1) Hit or kicked someone
2) Pushed or shoved someone when you are angry	2) Pushed or shoved someone when you are angry
3) Badly beaten up someone	3) Badly beaten up someone
4) Threatened someone with a knife or sharp weapon	4) Threatened someone with a knife or sharp weapon
5) Attacked someone with a knife or sharp weapon	5) Attacked someone with a knife or sharp weapon
6) Threatened someone with a gun	6) Threatened someone with a gun
7) Verbally or emotionally abused someone, that is, being called names or having things said to you that make you feel bad about yourself or afraid	7) Verbally or emotionally abused someone, that is, being called names or having things said to you that make you feel bad about yourself or afraid
8) Sexually harassed someone	8) Sexually harassed someone
9) Robbed someone	9) Robbed someone
10) Been suspended from school	10) Gotten into fight after drinking or getting high
11) Gotten into fight after drinking or getting high	

Youth Self Report (YSR) Questionnaire

In the Birth to Twenty Plus study data for aggressive behaviour and violent behaviour were collected using a YSR questionnaire. YSR questionnaire is a self-report measure made up of questions pertaining to perpetration of aggressive behaviour and violent behaviour during adolescence, among other variables.

It is one of Achenbach System of Empirical Based Assessment (ASEBA) instruments (Achenbach and Rescorla, 2002). The main purpose of the ASEBA-school aged instruments is to identify young people with behavioural, emotional and social adjustment problems. YSR is a screening tool used to assess behavioural and emotional problems in adolescents ages 11 to 18 years. It is comprised of 112 problem items; and has empirically based syndrome scales based on factor analyses. These include; "anxious/depressed • withdrawn/depressed • somatic complaints • social problems • thought problems • attention problems • rule-breaking behaviour • aggressive behaviour". Its validation and validation in South Africa is described in the Materials and Methods section in Chapter 3 of this thesis.

Data Collection Time Points

Data collection time points for variables relevant to this study are listed in Table A.

Table A: Data collection time points relevant to this thesis

Factors	Data collection tools	Description of Factors	Data collection time points
Socio-demographic	Standardized questionnaire	Gender, maternal education, maternal marital status, maternal age, hospital of birth, residential area.	Birth
Household items	Standardized questionnaire	Type of home, access to water inside the dwelling, sole use of water, access to flush toilet, sole use of toilet, ownership of households appliances (television, motor car, refrigerator, washing machine and telephone).	Birth
Anthropometric measures		Body weight	Birth 8 years
		Height	8 years
		BMI	8 years
Environmental	EDTA-containing tubes	Blood Lead	Birth 13 years
Social adjustment	Youth Self Report (YSR) questionnaire	Aggressive behaviour items	11/12 years 14/15 years
	YSR questionnaire	Violent behaviour items	15/16 years
Physiological measures	Tanner-stage development questionnaire (administered by trained medical doctors)	Pubertal development (boys and girls)	9 to 10 years
	Validated self reporting Tanner-stage development questionnaire		11 to 16 years

ABSTRACT

Introduction

Environmental lead exposure continues to be a health hazard particularly in children. Globally, populations in low or middle income countries are generally the most exposed to environmental lead, thus increasing their risk of lead related detrimental health effects (Abadin et al., 2007a). However, such exposure in these populations remains under-researched. To try and address this deficit in evidence from developing countries like South Africa, the aim of this thesis was to examine possible environmental lead toxicological contributions to specific types of aggressive behaviour, violent behaviour and altered pubertal progression in adolescents.

Methods

The Birth to Twenty Plus (BT20+) cohort is the "largest and longest running" longitudinal birth cohort in the whole of Africa (Richter et al., 2004). At the beginning the study was called the Birth to Ten (BT10) cohort (1990-2000). The main aim of BT10 was to study children's health and development; including "growth, well-being and education" in the first 10 years of their lives. After 10 years the study was extended for another 10 years and the cohort subsequently renamed to Birth to Twenty (BT20) cohort (2001-2010). The BT20 cohort continued with the examination of children's health and development but also included another component. It sought to answer targeted questions related to risks associated with "life-style including sexual and reproductive disorders, cardiovascular diseases and diabetes" (Richter et al., 2004, Richter et al., 2007). Post 2011, the cohort is now referred to as Birth to Twenty Plus (BT20+).

The cohort is comprised of all singleton births to women residing in Soweto, Johannesburg area, South Africa over a specified 7-week period at the public health facilities. Children born from the 23rd of April to the 8th of June 1989 were recruited into the study. Of the 5449 births during this period, 3273 fulfilled the inclusion criteria and were included in the study. Inclusion criteria included that mother and child were to reside in Soweto, Johannesburg area for at least six months after birth. This was required in order to exclude mothers from outside Johannesburg who had only come to give birth in the city because of perceived better access to healthcare than where they actually lived or other family reasons.

Prior to the commencement of the BT10 study, pilot studies had shown great resistance from the private healthcare sector in participating in the study. As such, only mothers who gave birth in the public health facilities were included in the study. This resulted in great under-representation of White study participants in the cohort because under *Apartheid,* private healthcare was mainly reserved for Whites. In order to compensate for this discrepancy, after 10 years 120 White study participants born during the same period of study enrolment but not the same area were recruited into the study (Richter et al., 2004, Richter et al., 2007). For additional information regarding the cohort please see Richter et al., 2004 & 2007.

For the current study, blood lead analyses were performed using blood samples collected at birth and at age 13 years. Aggressive and violent behaviour were assessed using data collected using YSR questionnaires at ages 14/15 years and 15/16 years, respectively. Data for sex; socio-demographic factors at birth such as maternal education, maternal marital status, hospital of birth, place of birth, maternal age; house hold items; and anthropometric measurements at birth and 8 years old were collected using structured questionnaires.

Principal Component Analysis (PCA) was used for 1) data reduction 2) to determine the specific type(s) of aggressive behaviour associated with lead exposure during mid-adolescence and 3) to determine the specific type(s) of violent behaviour associated with lead exposure in late adolescence. Aggressive behaviour and violent behaviour are multi-faceted variables, as such, in order to understand the structure of aggressive behaviour and violent behaviour items PCA was selected to reduce these data sets. PCA is a data reducing statistical procedure used to assess if a number of measures really describe a single variable. That is, to examine if different variables actually reflect a single underlying variable (Field, 2009). As such, to measure the dimensionality of aggressive behaviour and violent behaviour we used principal components derived from PCA.

For this study PCA was chosen over Exploratory Factor Analysis (FA) because unlike FA which is more suitable for latent variables which cannot be directly measured; the individual aggressive behaviour items and violent behaviour items examined in this thesis can be directly measured. PCA is a linear combination of variables which reduces data while retaining as much of the variance of the observed variances as possible (Conway and Huffcutt, 2003, Field, 2009). Therefore, PCA was a more appropriate method to use to address the aims and objectives of this thesis. The PCA derived components were used to distinguish the dimensionality of direct and indirect aggressive behaviour.

Additionally, pubertal progression was assessed using pubertal data collected at age 9 through to 16 years and measured with the Tanner Sexual Maturation Scale. Data for breast and pubic hair development in girls and genital and pubic hair development were collected.

Using Mplus, Latent Class Growth Analysis (LCGA) was carried out to group study participants into specific classes based on a "common developmental trajectory for the Tanner Sexual Maturation Scale indicators of pubertal stage" (Lundeen et al., 2016).

Results

First, in the study to examine the link between lead exposure and aggressive behaviour the BT20+ participants with aggressive behaviour data at mid-adolescence (n = 1086) had mean blood lead levels of 5.6 ± 2.3 μg/dL and geometric mean blood lead concentration of 5.1 μg/dL at age 13 years. Fifty nine percent of study participants had blood lead levels ≥5 μg/dl. Blood lead levels ≥5 μg/dL were positively associated with direct aggression (p=0.02). A higher proportion of males compared to females displayed a propensity towards direct aggression while females were shown to be more likely to perpetrate indirect aggression during mid-adolescence. Consequently, there was a positive association between male sex and direct aggression but a negative association with indirect aggression ($p<0.05$).

Second, in the study to examine the association between lead exposure and violent behaviour during late adolescence, study participants with data for violent behaviour during late-adolescence (n = 1332) had mean blood lead levels of 5.8 ± 2.6 μg/dl. There was a statistically significant association between blood lead category ≥ 5 μg/dL and physical violence and fighting. Blood lead levels at age 13 years were only associated with physical violence during late adolescence ($p<0.0001$). Furthermore, being male was associated with violence using a weapon, physical violence, fighting and robbing others ($p < 0.05$).

Third, in the BT20+ sub-sample with data for pubertal growth trajectory classes for public hair development and breast development in girls (n=732) and pubic hair development and genital development in boys (n=684), 49.5% of females and 75.0% of males had blood lead levels ≥5 μg/dL at age 13 years. The mean blood lead levels were 6.55 ±2.6 μg/dL and 4.97 ± 1.9 μg/dL for boys and girls, respectively. In girls blood lead levels ≥ 5 μg/dL relative to blood lead levels <5 μg/dL were associated slower pubertal transition for pubic hair development ($p<0.0001$) and breast development ($p<0.05$) from age 9 through to 16 years. When data were analyzed for the association between cord blood lead levels and pubertal progression, in boys elevated cord blood lead levels (≥ 5 μg/dl) were associated with a 72.0% decreased risk of being in trajectory class 3 compared to trajectory class 1 for pubic hair development ($p< 0.05$).

Conclusion

The statistically significant association between blood lead levels ≥5 μg/dL and direct aggressive behaviour in mid-adolescence; and physical violence and fighting in late-adolescence should be of great concern considering the reported high rates of violent behaviour among adolescents in South African schools in particular. Lead exposure was also associated with altered pubertal progression in both girls and boys. Healthy pubertal development is pivotal for young people. These study findings speak to how environmental lead exposure may significantly change the trajectory and future of young people; and the need for nationally driven public health policies to monitor blood lead levels in children from high risk communities in particular.

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ABBREVIATIONS

ACCLPP	Advisory Committee on Childhood Lead Poisoning Prevention
ACTH	Adrenocortical Tropic Hormone
AD	Axial Diffusivity
1st century AD	Anno Domini
ALA	δ aminolevulinic acid
APD	Antisocial Personality Disorder
APP	Amyloid Precursor Protein
ASEBA	Achenbach System of Empirically Based Assessment
BC	Before Christ
Aβ	Beta Ameloid
BBB	Blood Brain Barrier
BLLs	Blood Lead Levels
BT20+	Birth to Twenty plus
BMI	Body Mass Index
BMIZ	BMI-for-age z-score
CBCL	Child Behaviour Checklist
CDC	Centers for Disease Control and Prevention
CEP	Cumulative Exposure Project
CI	Confidence Interval
CLS	Cincinnati Lead Study
CNS	Central Nervous System
CRH	Adrenocorticotropin Releasing Hormone
CRH	Corticotrophin-Releasing Hormone
DHEA	Dehydroepiandrosterone
DALYs	Disability-adjusted life years
DNA	Deoxyribonucleic acid

DNMT1	Deoxyribonucleic acid methylation regulators
DTI	Diffusion Tensor Imaging
EBMT	East Boston Memory Test
EDCs	Endocrine Disrupting Compounds
ECFs	Executive Cognitive Functions
FA	Fractional Anisotropy
FDR	False Discovery Rate
FSH	Follicle Stimulating Hormone
GABA	*gamma*-Aminobutyric acid
GH	Growth Hormone
GIT	Gastrointestinal Tract
GnRH	Gonadotropin Release Hormone
HAZ	Height-for-age z-score
HPA	Hypothalamic-Pituitary Adrenal
HPG	Hypothalamic-Pituitary-Gonadal
IGF-I	Insulin-Like Growth Factor-1
ILPPW	International Lead Poisoning Prevention Week
IQ	Intelligence Quotient
KMO	Kaiser-Meyer-Olkin
LCGA	Latent Class Growth Analysis
LH	Gonadotropins Luteinizing Hormone
LTP	Long Term Potential
MD	Mean Diffusivity
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MRS	Magnetic Resonance Spectroscopy

NHAHES	National Health and Nutrition Examination Survey
NGRI	Not Guilty by Reason of Insanity
NHS	Nursery's Health Study
OECD	Organization for Economic Cooperation and Development
PCA	Principal Component Analysis
PET	Positron Emission Tomography
PM	Particulate Matter
PVN	Periventricular Nucleus
RAVLT	REY Auditory Verbal Learning Test
RBCs	Red Blood Cells
RD	Radial Diffusivity
ROIs	Regions of Interest
RRR	Relative Risk Ratio
SA	South Africa
SCALT	Spatial Conditional Association Learning Task
SE	Standard Error
SES	Socio-Economic Status
SMS	Sexual Maturation Scale
TAP	Taylor Aggression Paradigm
TEL	Tetraethyl Lead
TICS	Telephone Interview for Cognitive Status
TIMMS	Third International Math and Science Stud
TRIP	Thyroid hormone Receptor Interactor
UPSIT	University of Pennsylvania Smell Identification Test
WAIS-R	Wechsler Adult Intelligence Scale-Revised
WMS-R	Wechsler Memory Scale–Revised
WHO	World Health Organization

XRF	X-ray Fluorescence
YSR	Youth Self Report

PREFACE

The adverse health effects of lead exposure have been documented as early as the 15th Century (Hernberg, 2000) and contemporary studies informed by new developments in technology have demonstrated further its detrimental health effects even at concentration levels previously deemed safe for children (Bellinger et al., 1992, Canfield et al., 2003, Needleman and Bellinger, 1991, Bellinger, 2008, Cecil et al., 2011). Of great concern is that the damage caused by exposure to lead may be irreversible (Cecil et al., 2008); thus affecting the livelihood of the affected individuals and potentially that of their future generations. In developing countries, including South Africa, there is paucity of empirical data regarding the possible contribution of environmental lead to neuro-behavioural and pubertal transition in young people.

There is dearth of information regarding the effects of environmental lead exposure on the development of children and adolescents from low and middle income countries including South Africa. A few studies have been conducted to evaluate the link between lead exposure and socio-behavioural adjustments and delayed onset of puberty in South African adolescents (Naicker et al., 2012, Naicker et al., 2010a). Beyond this, there is a lack of information regarding the dimensions of aggressive behaviour in relation to lead exposure in young people. To our knowledge, no study has been conducted to examine the association between lead exposure and violent behaviour among South African adolescents; or its effects on pubertal progression in girls and boys.

The aim of this thesis is to answer the following questions in a series of journal articles.

- What is the nature of neuro-developmental effects associated with lead exposure, in South African adolescents?
- Are there sex-differences in neuro-behavioural effects associated with lead exposure in children from lower middle income communities?
- What are the effects of lead exposure on the tempo of pubertal progression in South African boys and girls?

The thesis consists of three parts. Part 1 (Chapter 1) describes the literature review which informs this thesis. Part 2 includes journal articles in chapters 2, 3 and 4. The study findings of this thesis are amalgamated in Part 3. All sources of information were acknowledged using the Vancouver referencing system.

Chapter 1 gives a detailed literature review which informed the conceptual framework outlined at the beginning of this thesis. It seeks to examine the link between environmental lead exposure and biological pathways in relation to adolescent health in South Africa. It is followed by published journal articles as represented in chapters 2, 3 and 4, which sought to address the identified gaps in the literature, especially pertaining to low and/ or middle income countries.

Chapter 2 examines the association between exposure to lead at age 13 years and aggressive behaviour in mid-adolescence. Using Principal Component Analysis (PCA) composite variables were derived from the aggressive behaviour data to determine the dimensionality of direct and indirect aggression.

Chapter 3 is the second journal article and it assesses the relationship between lead exposure at age 13 years and violent behaviour during late-adolescence using PCA derived components from the violent behaviour data.

Chapter 4 is the third journal article and it examines the association between blood lead levels at age 13 years and latent classes of pubertal progression in girls and boys. Furthermore, it included a subset of the data to assess if there is an association between cord blood lead levels and pubertal transition in girls and boys.

Chapter 5 combines and discusses findings of the various journal articles in relation to the conceptual framework, background information and research subject matter as evidenced throughout the thesis.

In conclusion, Chapter 6 highlights the significance and implications of this study.

PART 1

RELEVANT BACKGROUND LITERATURE

CHAPTER 1: INTRODUCTION

Chapter 1 describes the underlying concepts that inform the present study as outlined in the conceptual framework (Figure 1). The aims and objectives of the study are to analyse the nature of the impact of childhood lead exposure on the developing central nervous system. Furthermore, the study will examine subsequent and attendant health implications on the neuro-endocrine system and gene-expression. Finally, gaps in research and the significance of the study will be discussed.

1.1 CONCEPTUAL FRAMEWORK

This study builds on existing evidence regarding the effects of lead exposure on the development of children, especially from low and middle income countries. It takes cognisance of the new developments in literature and how these may address further what we already know. It is important to not only understand what the ramifications of lead exposure are, but also the possible extent of its damage on the health and wellbeing of young people.

Depicted below in Figure 1 is a conceptual framework of environmental lead exposure, its absorption, biochemical and physiological effects on the body, effects on the central nervous system (CNS), gene expression and the endocrine system. The framework also depicts the related health effects on the development and social behaviour of young people.

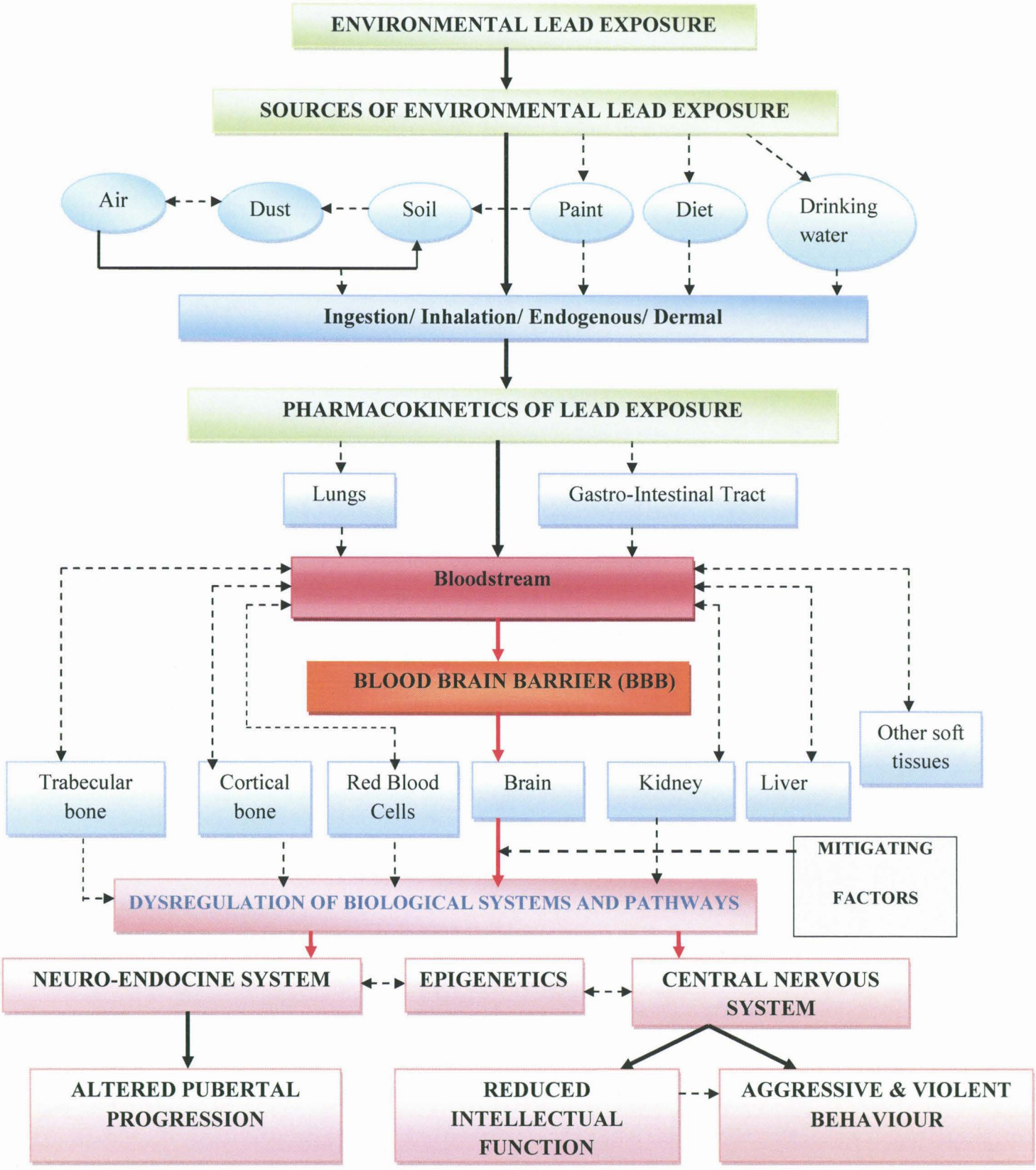


Figure 1.1 Conceptual Framework of Exposure to Environmental Lead Exposure and its detrimental health effects based on literature review. Adapted from: (White et al., 1998, Needleman, 1999, Needleman, 2004, Bellinger et al., 1992, Dietrich et al., 2001, Stretesky and Lynch, 2001, Cecil et al., 2008, Zawatski and Lee, 2013, Pilsner et al., 2009, Sen et al., 2015a)

1.2 BACKGROUND LITERATURE

1.2.1 FORMS OF LEAD

1.2.1.1 Elemental lead

Elemental lead is the form of lead in its natural occurring state. Its chemical symbol is Pb and has an atomic number of 82 in the periodic table. It is a bluish grey colour metal with a density of 11.34 g/cm^3 and a melting point of 327.46° C. The naturally occurring lead ores cover 0.002% of the crust of the earth (Tarragó and Brown, 2017). In its natural occurring state lead is harmless to humans (World Health Organization, 2010b).

1.2.1.2 Inorganic lead

Inorganic lead is the form of lead found in leaded paint, soil, dust, water and various consumer products. The most common forms of inorganic lead are "white lead (a lead carbonate compound), yellow lead (lead chromate, lead monoxide), or red lead (lead tetraoxide)" (Tarragó and Brown, 2017). Lead acetate also referred to as 'sugar of lead' is the most soluble salt of lead. It is a white crystalline chemical compound with a sweetish taste commonly used in hair dyes and historically it was used as a sweetener (Marzulli et al., 1978, Patnaik, 2003, Needleman, 2004, Tarragó and Brown, 2017). When inhaled it is absorbed through the lungs into the gastrointestinal tract (White et al., 1998).

1.2.1.3 Organic lead

Organic lead is generally associated with occupational lead exposure (Patnaik, 2003). An example of organic lead is tetraethyl lead (TEL) which is an antiknock agent that is added to petrol to increase octane rating and enhance its performance in motor cars (Gibbs, 1990,

Patnaik, 2003, Bolla and Cadet, 2007). It was first introduced into the market as an additive in petrol in 1923 (Gibbs, 1990, Douglas et al., 2015). Its combustion causes the release of lead into the air. It is highly toxic, much more than inorganic lead and is absorbed through the skin, lungs and gastrointestinal tract. Once in the circulatory system it is converted to triethyl lead which is another form of lead that is thought to be responsible for its toxicity. TEL is extremely lipophylic and thus able to pass through the blood brain barrier effortlessly (Patnaik, 2003, Bolla and Cadet, 2007). Use of lead in petrol is banned in most countries, including South Africa.

1.2.2 BRIEF HISTORICAL BACKGROUND OF ENVIRONMENTAL LEAD EXPOSURE

Lead is a xenobiotic element (White et al., 2007) and has no known nutritional health benefits in humans (White et al., 1998). Humans have been exposed to lead for centuries. It is estimated that lead was mined in Turkey around 6,500 BC even before bronze and iron mining (Needleman, 1999). In the 4th century BC Hippocrates defined lead as colic. Nicander is said to be the first person to define lead palsy in the 2nd century BC. It was in the 1st century AD that Dioscorides and Pliny were able to make some connection between lead exposure and its toxic health outcomes (Hernberg, 2000) in humans.

According to Hernberg (2000), the Romans were probably one of the highest consumers of lead, producing 60,000 tonnes of lead on average annually for over a period of about 400 years. Use of lead in wine-making and access to upper-class luxuries at the time such as (leaded) plumbing impacted greatly on the health and wellbeing of the Roman aristocracy. It is believed that their widespread use of lead as evidenced in the archaeological bones of

Romans, may have contributed to their reduced fertility and brain damage inevitably leading to the demise of the Roman Empire (Hernberg, 2000, Needleman, 2004, Gilfillan, 1965). Considering that as early the 13th and 14th centuries use of lead in wine was punishable by death in Germany (Hernberg, 2000), it is astonishing that as late as the 21th century it is still used in some products like pottery, traditional medicines and household paint.

The upsurge of industrialization, introduction of ethyl lead into petrol and leaded paint brought lead poisoning to epidemic proportions. In the early 20th century some scientists were discredited for any research findings that showed the harmful effects of lead in humans (Needleman, 2000, Rosner and Markowitz, 2005); and the industry got away with it for many years.

TEL was first manufactured by Ethyl Gasoline Company in the United States in 1923 and was first marketed in 1924 (Needleman, 2000). Evidence of its harmful effects was apparent shortly thereafter, as some workers died and many more became "floridly psychotic". The plants were closed temporarily in order to investigate the safe use of TEL. They were given a green light by the Surgeon General in 1926 after meeting with scientists and relevant officials in the industry. Initial study findings by select pro-industry scientists were flawed in design and interpretation, but were accepted for years because of paucity of reliable data from dissenting scientists. For example, one scientist regarded as 'an opinion leader' in the topic at the time concluded that "lead was naturally present in everyone" and therefore its presence in study participants did not necessarily suggest lead poisoning. These findings were later disputed and proved to be flawed; however, for decades the industry had the monopoly of empirical data with regard to lead poisoning.

In 1959 new epidemiologic findings by a geochemist Claire Patterson showed that lead exposure does not always result in "florid disease of workers" but can be a "silent danger". Armed with these new findings from public health, civil rights groups were able to mobilize for action against lead poisoning. In 1970 the Surgeon General called for an investigation of "undue" lead exposure in children (Needleman, 2000). Even though the politically charged term lead "poisoning" was not used it was according to Needleman (2000) a step in the right direction. Prior to this no research funds from the federal government had been allocated for studies on the ramifications of lead exposure in children before. It is important to note that at the time, lead poisoning was defined as blood lead levels $\geq$ 60 µg/dL (Needleman, 2000).

In the 1990s, with evidence mounting in support of the harmful health effects of lead exposure, particularly in children - various international bodies such as the Summit of the Americas, World Bank, Third "Environment for Europe", The United Nations, Organization for Economic Cooperation and Development (OECD) and senior government officials across the globe called for the reduction and eventual phasing out of TEL in gasoline UNITEP (2010). Throughout the 1980s and 1990s several countries took bold steps to phase out the use of lead in petrol. The first countries to do so were Japan (1980), Austria and Canada (1993), Slovakia (1994), Denmark and Sweden (1995), Germany and The United States (1996) (UNITEP, 2010). South Africa was counted among the highest users of tetraethyl lead in petrol as late as the late 1990s. Christchurch, New Zealand and Cape Town, South Africa are reported to have had the highest concentration (0.84 g/L) of tetraethyl lead in petrol. In Cape Town these levels were maintained through 1984 (Thomas et al., 1999). In January 2006 use of tetraethyl lead in petrol was phased out in South Africa (Mathee, 2014).

By the same token, the use of lead in paint has had a profound impact on the lives of young children. Despite the well known saying "crazy as a painter" (Gilfillan, 1965) lead was used for pigment in paint and to speed up drying for a very long time. Adverse health effects of leaded paint in children were first reported in 1904 by an Australian, Dr J. Lockhart Gibson. He specialized in children's diseases, particularly eye diseases in Brisbane close to a lead mining town around Queensland. In his seminal article titled "A Plea for Painted Railings and Painted Walls of Rooms as the Source of Lead Poisoning Amongst Queensland Children", Dr Gibson outlined four cases of children diagnosed with "ocular neuritis" (Gibson, 2005). Like many other physicians who had diagnosed paediatric lead poisoning for years, he could not identify the source of the problem. By a process of elimination, Gibson was able to link seasonality of most of the cases that came to the hospital (mainly the hottest months in Brisbane), chipped leaded paint, mouth-to-mouth tendency in children, ingestion and observed lead related symptoms. At first, paediatric lead poisoning was disputed with some arguing that the problem was not with lead paint, but rather with the children. At the time, children who consumed chipped paint were diagnosed with pica, which some physicians regarded as a mental disorder described as "mental retardation". They concluded that there was no need to ban use of lead in paint because of a few cases of children with psychological problems (Rosner and Markowitz, 2005). Nonetheless, due to Dr Gibson and other Australian researchers' work, the use of lead paint for households was banned in Brisbane in 1920 (Needleman, 2004, Rosner and Markowitz, 2005). Similarly, the United States banned residential use of leaded paint in 1978 (Gaitens et al., 2009) and in South Africa, the use of white lead in paint was abolished and promulgation of regulations to curb the use of other forms of lead (other than white lead) took place in 1940 and 2009, respectively. However, the paint manufacturing industry was given a year to fully comply; as such, the regulations came into full effect only in 2010 (Mathee Angela, Chapter 4).

In many parts of the world where use of lead in petrol, paint and other household items was lowered or phased out there has been drastic reductions in the blood lead levels of children. However, advances in research employing improved analytical and epidemiologic research methods (Needleman, 2004) have revealed harmful effects of lead exposure at much lower concentration levels than previously expected. As a result, the Centers for Disease Control and Prevention (CDC) Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP) recommended that the use of the term "blood lead levels of concern" for blood lead levels $\geq$ 10 μg/dL be discontinued. The recommended reference values based on the 97.5^{th} percentile of the distribution of blood lead levels of children from 1 to 5 years old from the National Health and Nutritional Examination Survey (NHANES) study are blood lead concentration levels < 5μg/dL (Centers for Disease Control and Prevention, 2012c). The WHO purports that environmental lead is one of the top ten chemicals that requires public health attention and Member States are urged to take action in order to protect the health of lead exposed workers, children and young women (World Health Organization, 2015).

1.2.3 SOURCES AND ROUTES OF LEAD EXPOSURE

In its natural state lead exists as immobile and non-toxic to humans (World Health Organization, 2010b). However, because of its versatility it has been introduced into the environment through various activities including mining, use in the form of TEL in petrol, in paint (as has been noted) and for "smelting, manufacturing and recycling activities" (World Health Organization, 2010b, World Health Organization, 2015). Even though the use of lead for certain products has been banned in most countries for decades, it remains a public health problem because of its stubborn persistence in media such as air, dust, soil and water (World

Health Organization, 2009). Once introduced into the environment, it remains and it is almost impossible to totally eliminate it from the environment again.

Humans are exposed to lead through various routes. These include ingestion, respiration or inhalation, endogenous exposure and dermal contact which is more prevalent in occupational settings (Centers for Disease Control and Prevention, 2012c, White et al., 1998).

1.2.3.1 Lead exposure through ingestion

Ingestion is the major route of exposure, while leaded paint is the main source of lead exposure in children. This is partly due to children's natural curiosity to taste everything and "hand to mouth" behaviour. The risk of exposure is heightened in children who exhibit pica (World Health Organization, 2010b). In houses where leaded paint was used, specifically older houses, when they deteriorate or undergo renovation, lead contaminated dust is generated. In such instances, children are exposed directly or indirectly to lead through exposure to the paint if lead-based, lead contaminated dust both inside and outside the house, as well as lead contaminated soil outside the house. Children are also be exposed to lead through the use of lead contaminated toys and playgrounds (World Health Organization, 2010b, Mathee et al., 2009).

Ingestion may also be a pathway for lead exposure through drinking lead contaminated water from taps with lead solder and pipes; eating vegetables grown from lead contaminated soil or canned food with lead solder, use of lead-based traditional medicines, cosmetics and

jewellery (World Health Organization, 2010b, Centers for Disease Control and Prevention, 2012c).

1.2.3.2 Lead exposure through inhalation/ respiration

Airborne lead exposure is more common in adults than in children, even though it also occurs in children to some extent. This is due to the large size of the particulate matter in airborne lead. Where particulate matter is less than 10 μm in diameter (PM_{10}), for example from smoke from open fires or car exhausts where leaded gasoline is used, inhalation can occur in children (World Health Organization, 2010b). Additionally, lead exposure through inhalation is a concern for children living close to high risk areas (White et al., 1998) such as mining or smelting regions.

The risk of occupational lead exposure is greater in employees who work in lead battery manufacturing factories, construction workers, lead smelting and soldering, pottery and other lead contaminated consumer products (Centers for Disease Control and Prevention, 2012c). Their families may also be at risk of secondary exposure through contact with their contaminated clothes and hands and informal sector work such as cottage industries (Teare et al., 2015).

1.2.3.3 Endogenous lead exposure

Since lead is able to cross the placenta barrier, pregnant women with 'elevated' blood lead concentrations pose a serious risk to their unborn children as lead can be transferred from

mother to baby in utero (World Health Organization, 2000). In addition, cumulative lead stored in bone mobilizes to the bloodstream during pregnancy thus exposing the foetus to lead (Centers for Disease Control and Prevention, 2012c). Pregnant mothers who practise geophagia can expose their unborn children. For example, in a study conducted at the Rahima Moosa Mother and Child Hospital in Westbury, Johannesburg, South Africa involving pregnant women 18 years and above with a gestational age of at least 20 weeks, almost 20% of the study participants reported geophagia habits (Mathee et al., 2014).

1.2.3.4 Dermal lead exposure

Dermal exposure is not considered a common route of lead exposure for the general public. Occupational lead exposure poses a much greater risk in this regard. It can contribute to exposure by ingestion as lead can be transferred from the fingers to the mouth (Sleeuwenhoek and van Tongeren, 2006).

1.2.4 PHARMACOKINETICS OF LEAD EXPOSURE

1.2.4.1 Lead absorption

Lead enters the body through the gastrointestinal tract (GIT) or is inhaled from the air into the lungs to the GIT. However, only minute amounts of lead entering the body are actually absorbed into the bloodstream (White et al., 1998). The amount of lead absorbed into the body is influenced by various factors such as: chronological age, health status, and nutritional status of the individual. For instance, lead absorption is affected by levels of calcium, iron and zinc in the body - when they are low more lead is absorbed into the bloodstream (Hu et

al., 2007, World Health Organization, 2000). The timing of the last meal including how well the ingested lead was broken down by the digestive enzymes can also affect its absorption levels (Abadin et al., 2007b).

Lead absorbed through inhalation must be deposited in order for pulmonary absorption to take place. Lead contaminated particles that are inhaled but not deposited are either exhaled or remain in the muco-ciliary lift mechanism and ingested, and those greater than 2.5 μm in diameter are deposited in the nasopharyngeal and tracheobronchial airways ciliated areas and through the muco-ciliary lift mechanism are passed to the GIT. If the inhaled particles are small enough to go through the alveolar region, they get completely absorbed which may be the reason why lead does not accumulate in the lungs. As such, the size and density of particulate lead inhaled is inversely proportional to the amount of lead that is absorbed into the bloodstream and directly proportional to the deposition amount, individual's respiratory volume and physical activity (White et al., 1998, Hu et al., 2007). In occupational settings lead is inhaled in the form of fumes or vapour particulate with a respiration size of less than 1 μm (Hu et al., 2007). Dermal absorption is also possible when lead contaminated dust and soil get on one's skin but usually on a very small scale unless the skin is broken. Human skin is much more permeable to organic lead like TEL (Järup, 2003, Abadin et al., 2007b).

Children are more vulnerable to the negative health effects of lead exposure compared to adults because their physiological rates of lead uptake are higher. They absorb far more ingested lead into the bloodstream than adults. They can absorb as much as 50% of the water soluble ingested lead versus 3 to 10% for adults (World Health Organization, 2000, Abadin et

al., 2007b). These variations in lead uptake between children and adults can be explained as follows:

- Children have less developed organs compared to adults, for example, the blood brain barrier (BBB) in children is still developing and therefore more permeable to lead (World Health Organization, 2000);
- Their breathing zone is closer to the ground compared to adults (Bearer, 1995);
- Children have a larger surface-to-volume ratio and thus higher breathing rate and oxygen intake than adults (Bearer, 1995);
- Their lead intake for body weight is much higher than that in adults (World Health Organization, 2000).

It is important to note that children from the same neighbourhood may be exposed at different levels to environmental lead due to variations such as: paving at home where children play, thus less exposure to lead compared to children who play in soil areas; exposure to indoor dust at home depending on the levels of cleanliness within the household; different playing habits, some children are more prone to hand to mouth habits; eating food outside and increased exposure to soil and dust; and biological factors (White et al., 1998).

1.2.4.2 Lead distribution

Lead that is absorbed into the body is mainly distributed into the bloodstream and transported to the soft tissues and organs such as "liver, kidneys, lungs, brain, spleen, muscles and heart" (Abadin et al., 2007b) and mineralized tissues like teeth and bones (World Health Organization, 2000). It has a half-life of about 30 days in the blood (Mason et al., 2014), years to decades in the trabecular bone and decades in the cortical bone. Blood lead levels are

used as a proxy for current lead exposure whilst bone lead levels are used as a proxy for cumulative lead exposure (Hu et al., 2007).

Less than 1% of lead is found in plasma (Hu et al., 2006). Red blood cells (RBC) are known to have a very high affinity for lead (Gurer and Ercal, 2000), approximately 99% of the lead in blood is bound in the RBCs where it displaces zinc from the active sites of some hematopoietic enzymes (Bressler et al., 1999, Reddy and Zawia, 2000). Once in the bloodstream, lead is able to cross the BBB (Hu et al., 2007), altering the normal brain mechanisms and pathways. This may lead to neurotoxicity whereby lead alters neurotransmitters and hormonal systems in the brain (Järup, 2003, Hu et al., 2006).

1.2.4.3 Lead excretion

Lead is excreted from the body via urine and faeces, with a small amount excreted via breast milk, sweat, saliva, skin, hair and nails (White et al., 1998, Hu et al., 2007, Abadin et al., 2007b). Lead that has not been absorbed is excreted through urine and faeces, and this includes dietary lead or airborne lead (World Health Organization, 1995). Additionally, in animals lead that is not retained in the body is eliminated through biliary excretion. About 90% of the excreted lead is excreted in faeces. There is a direct relationship between lead excreted and age and the character of the source of lead exposure of the individual, however, more research is still necessary in this regard (World Health Organization, 1995).

1.2.5 HEALTH EFFECTS OF LEAD EXPOSURE ON THE CENTRAL NERVOUS SYSTEM

In 1931 Major wrote of an observation by Dioscorides in the 2nd Century BC that "lead makes the mind give away" (Needleman, 1990) and as cited in (White et al., 2007). Lead is a bivalent cation and has a high affinity for sulfhydryl groups (Needleman, 2004). Its toxicity affects cell membranes and their functions (Donaldson and Knowles, 1993). This increases membrane susceptibility by changing their integrity through degradation of their components (Gurer and Ercal, 2000), subsequently disrupting "calcium regulatory actions" in the cell functions resulting in the breakdown of "homeostatic cellular mechanisms" (Finkelstein et al., 1998).

There is strong evidence that lead disrupts the haem synthesis pathway by inhibiting delta aminolevulinic acid dehydratase. This results in increased levels of precursor δ aminolevulinic acid (ALA), which in turn decreases the release of *gamma*-Aminobutyric acid (GABA) thus suppressing GABA-mediated neurotransmission in the central nervous system (Needleman, 2004, Lidsky and Schneider, 2003). ALA is a neurotoxin and is associated with psychosis at increased levels in adults (Opler et al., 2004); whilst inhibition of the GABA-mediated neurotransmission is associated with schizophrenia (Benes, 1997). Because of its versatility, lead has many other targets (Needleman, 2004) in the body.

Using findings from four laboratory studies White and colleagues outlined four potential biological mechanisms through which childhood lead exposure attacks the development and function of the central nervous system leading to the neuro-degeneration (White et al., 2007).

The first theory relates to the impact of the interaction between lead exposure and stress. This theory starts from the premise that in the United States it has been shown that children from lower socio-economic communities are more likely to have elevated blood lead levels compared to their counterparts living in comparatively affluent communities. The former are more likely to live and go to school in areas with residual contamination of lead from soil, dust and air (White et al., 2007). Similar observations have been reported in South Africa (von Schirnding et al., 1991, Mathee et al., 2002, Mathee et al., 2006). In relation to stress, first it is important to note that all populations, regardless of their socio-economic backgrounds, are susceptible to stress. However, individuals from lower socio-economic status (SES) independent of race, age and gender are at an increased risk because of where they work, their status at work and where they live (Adler and Newman, 2002). Higher SES is associated with reduced levels of stress hormones such as cortisol and epinephrine (Cohen et al., 2006). Exposure to physiological or psychosocial stressors leads to production of adrenal cortical glucocorticoids by the hypothalamic-pituitary adrenal (HPA) axis, whereby corticotrophin-releasing hormone (CRH) and arginine vasopressin are released from the periventricular nucleus (PVN) in the hypothalamus (White et al., 2007). This triggers the secretion of adrenal pituitary of the adrenocorticotropin (ACTH) which activates the adrenal cortex receptors to increase cortisol and other plasma glucocorticoids. There are two types of receptors through which glucocorticoids act: Type 1 or mineralocorticoid receptors in the septo-hippocampal and Type II or glucocorticoid which are spread all over the brain. Release of glucocorticoid is mediated by the feedback mechanism between the pituitary, hypothalamus and hippocampus. Exposure to lead changes the HPA axis function, and the dysfunction of the HPA axis affects several organs and systems in the body upon which glucocorticoids act; these include the adrenals, lungs, bone, erythropoeisis, skin, macrophages, liver, thymus and particularly the brain. The HPA and glucocorticoids are able

to influence complicated cognitive functions through the hippocampus and amygdala in the prefrontal cortex (White et al., 2007).

The authors suggest that there is a possibility of "multiple hit hypothesis" as both lead exposure and glucocorticoid act on the same area of the brain, the mesocorticolimbic systems responsible for complex cognitive function, albeit using different mechanisms. In this case, simultaneous attacks from these different mechanisms may make it more difficult for the mesocorticolimbic system to evoke compensatory and homeostastic mechanisms thus causing extensive damage to the system. If the "multiple hit hypothesis" is true - the question that arises is if studies examining health effects of environmental toxicants such as lead without modelling interaction with psychosocial environmental factors like stress give a true picture of the health risks caused by the toxicants (White et al., 2007). These laboratory findings showed gender differences in the effects of lead exposure in different parts of the brain ten months following maternal exposure to lead, stress, and the combination of the two. In the nucleus accumbens, lead exposure alone resulted in reduced dopamine levels and increased dopamine turnover in lead only group and the combination of lead with environmental stress group in female offspring. In contrast, there was a slight increase in dopamine turnover in lead only and lead combined with stress groups in males compared to the group exposed to stress only. In the frontal cortex, when lead exposure was combined with exposure to environmental stress there was an increase in levels of dopamine levels in females but not in males. In the striatum, exposure to stress alone and combined lead and environmental stress showed increased levels of dopamine and reduced level of dopamine turnover in males. On the other hand, in females, exposure to lead only resulted in increased dopamine levels with increased dopamine turnover in lead only and stress only groups (White et al., 2007).

The second theory relates to a possible mechanism of action which links exposure to lead and reduced cognition due to structural changes in the hippocampus. One of the known mechanisms through which lead exposure negatively affects the CNS is its ability to mimic and substitute calcium actions and therefore cross the BBB (Lidsky and Schneider, 2003, White et al., 2007). This leads to "apoptosis, excitotoxicity, influences on neurotransmitter storage and release processes, mitochondria, second messengers, cerebrovascular endothelial cells, and both astroglia and oligodendroglia" (Lidsky and Schneider, 2003). Study findings using physiological and neurochemical approaches/explanations suggest that in rodents the actions of developmental lead exposure on the presynaptic transmitter release measured using *in vivo* microdialysis are associated with levels of cognition as measured by "cellular model of learning called long term potential (LTP)". In lead exposed rodents there was a direct relationship between reduction in LTP magnitude and impairment of the cellular mechanisms supporting cognitive function in the hippocampus. Lead exposure was associated with permanent alteration of the presynaptic release of glutamate in the hippocampus even after exposure to lead was terminated. The authors suggest that this shows that lead exposure at critical stages of brain development is extremely harmful as the damage may be irreversible. Additionally, chronic lead exposure was associated with altered neurogenesis in the hypothalamus (White et al., 2007).

The third theory suggests that lead influences protein "expression, conformation, and accumulation" in the brain. Lead has a high affinity for the binding immunoglobin protein also called chaperone GRP78. The binding of chaperone GRP78 to lead reduces its efficiency in functioning as a chaperone and this is linked to intracellular build-up of proteins in "neurons and glia" of the brain as a results of unfolded protein response failure (White et al.,

2007). The impact of these lead-based chaperone malfunctions is hypothesised to be one of the underlying causes of age-related degenerative diseases such as Alzeimer's disease and Parkinson's disease, and neuro-behavioural and developmental disorders (White et al., 2007).

The fourth theory related postnatal exposure of rodents to lead is associated with increase in cellular uptake of amyloid precursor protein (APP) messenger ribonucleic acid (mRNA) expression and its levels of amyloidogenic cleavage product beta ameloid (Aβ). The authors hypothesize that the increase in cellular uptake of APP mRNA in the brains of the older rodents indicates that the latent expression was a pre-programmed event following developmental exposure to lead (White et al., 2007).

More recently, neuroepigenetics studies are beginning to unravel the association between lead exposure and CNS development due to epigenetic dysregulation; which again links to development of neuro-degenerative diseases such as Alzheimer's diseases. Eid et al (2016) showed that there is a statistically significant decrease in levels of Deoxyribonucleic acid methylation regulators DNMT1 and MeCP2 protein in male mice exposed to lead during early postnatal development compared to untreated mice. DNMT1 is a DNA methyltransferase responsible for the regulation of DNA methylation and MECP2 protein is also pivotal for DNA methylation. Additionally, there were reduced levels of H3K9Ac and H3K4me2 proteins but, elevated levels of H3K27me in lead exposed mice; symbolizing histone modification (Eid et al., 2016, Senut et al., 2012). Histones are crucial for gene activation and expression (Eid et al., 2016, Senut et al., 2012) and histone modification is known for regulating "developing brain processes" (Senut et al., 2012). In addition, Sen et al (2015) reported that maternal DNA methylation changes associated with lead exposure in

pregnant women can be multigenerational. Their study showed that there is an association between elevated neonatal blood lead levels of the mother and altered DNA methylation of her child's neonatal blood, thus suggesting maternal lead exposure "has epigenetic effects on the DNA methylation in the grandchildren". However, these changes in DNA methylation normalize postnatally within the first 3-5 years of a child's life (Sen et al., 2015b). Nonetheless, permanent damage to the brain may have been done as major fiber pathways including the "thalamocortical pathway" are complete by the end of prenatal period (Stiles and Jernigan, 2010) thus setting in motion patterns and trajectories of development.

The alteration of the CNS associated with lead exposure is linked to reduced cognitive function, aggressive behaviour and violent behaviour both in children and adults as discussed in the next sections.

1.2.5.1 Lead exposure and reduced cognitive function

In a landmark study Byers and Lord (1943) showed that the neurological effects of childhood lead exposure remain long after the children cease to be exposed to lead. Their study was one of the first studies to refute the notion that the manifestation of the effects of childhood lead exposure subsides after exposure is terminated. They reported the delayed effects of lead poisoning on mental development in young children who had been hospitalized due to lead poisoning. The main source of lead exposure in their study was leaded paint and almost all the children exhibited pica habits. Between four and ten years later, 20 of the children participated in a follow-up study examining their mental development. In part, the findings showed limited levels of cognitive deficiency exhibited by lower intelligence quotient (IQ), poor writing and reading skills and challenges in arithmetic (Byers and Lord, 1943).

However, it is important to mention that one of the affected study participants did not show intellectual challenges at school (Byers and Lord, 1943). Since then there has been a growing body of evidence confirming these findings.

Schwartz (1994) suggests that lead exposure in the first three years of life in children is the most critical time for brain injury. In school age children, there is an association between an increase of blood lead levels from 10 μg/dL to 20 μg/dL and a decrease in IQ by 2.57 points ($P<0.001$) (Schwartz, 1994). Similarly, a longitudinal Port Pire cohort study of 494 Australian 7 to 8 year olds associated an increase in blood lead levels from 10 to 30 μg/dL with reduction in verbal IQ by 5.5 to 6.4 points, dependent on the age of the child. The elevated blood lead levels were associated with a reduction in full scale IQ by 4.4 (95% CI, 2.2 to 6.6) to 5.3 points (95% CI, 2.8 to 7.8) (Baghurst et al., 1992). A 1 μg/dL unit increase in blood lead levels below 10 μg/dL is associated with 1.37 points reduction in IQ (95% CI, -2.56 to -0.17) (Canfield et al., 2003). More studies have confirmed that there is an inverse relationship between blood lead levels and IQ in children (Needleman and Gatsonis, 1990, Schwartz, 1994, Lanphear et al., 2000, Lanphear et al., 2005).

In adults, Mazumdar et al (2011) examined a relationship between childhood lead exposure and health outcomes later in life. The study included adults enrolled in the cohort as infants born at the Brigham and Women's hospital in Boston, United States. Full scale IQ was associated with blood lead concentrations at 6 months, 4 years, 10 years and mean blood lead levels ($P = 0.03$, $P = 0.04$, $P = 0.02$ and $P= 0.03$), respectively. The mean late childhood blood lead concentration levels (defined by ages 4 and 10 years) showed the strongest associations ($P = 0.01$) (Mazumdar et al., 2011). In the elderly women 47 to 74 years old,

there is relationship between one SD increase in tibia bone lead levels and 0.051 units reduction in cognition scores (95% CI, –0.099 to –0.003; P = 0.04) (Weuve et al., 2009). Likewise, in a study of male former workers exposed to TEL and methaethyl lead compared to controls (non-exposed study participants), former lead employees had greater age-related annual declines for 17 out of 19 cognitive tests, and they performed worst in neuro-behavioural test scores. Additionally, there was an inverse relationship between peak tibia lead and test scores over time (Schwartz et al., 2000), thus showing reduced cognition due to past lead exposure long after lead exposure had been terminated. In a follow up study involving the same former chemical plant workers Stewart and colleagues (2006) reported a direct relationship between peak tibia lead and white matter lesions ($P< 0.01$). These are important findings since brain volume is associated with cognitive function (Schwartz et al., 2007) and neuro-behavioural disorders.

1.2.5.2 Lead exposure and neuro-behavioural disorders characterized by - (Aggressive and Violent behaviour)

Childhood lead exposure is associated with "unreliable impulsive behaviour", "cruel impulsive behaviour"; and "short attention span" (Byers and Lord, 1943). A longitudinal study of African Americans from Pennsylvania followed from birth to about 22 years old showed that in male offspring childhood lead exposure is strongly associated with a number of juvenile and delinquent offences, disciplinary problems at school, being in a disciplinary program, a number of adult offenses and aggressive behaviour. These associations were limited to males only (Denno, 1990, Denno, 1997). Following up on these findings by Denno, Needleman et al. (1996) conducted a study involving 12 year old boys enrolled in the Pittsburgh Youth Study to examine a relationship between lead exposure at ubiquitous levels to children in schools and the risk of anti-social behaviour. Bone lead levels were measured

using K X-ray fluorescence spectroscopy of tibia and antisocial and delinquent behaviour were measured using the Child Behaviour Checklist (CBCL), behaviour reports from parents and teachers, and self-report of delinquent and anti-social behaviour at ages 7 and 11 years old completed by the study participants. In part, the study findings showed a significant association between elevated bone lead levels and increased somatic complaints (P=0.08), increased delinquent behaviour (P=0.04) and aggressive behaviour (P=0.09) at 11 years of age (Needleman et al., 1996) –corroborating earlier findings by Denno.

Furthermore, there is a strong link between lifetime blood lead exposure and emotional and behavioural problems. For example, in children aged 11 to 13 years an increase in lifetime blood lead levels from 10 μg/dL to 30 μg/dL increased the likelihood of having aggressive behavior by 1.6 (95% CI, 1.0 – 1.6) (Burns et al., 1999). Additionally blood lead levels >15 μg/dL were associated with aggressive behaviour, delinquent behaviour, attention problems, thought problems, social problems, anxiety and depression, and withdrawal, in both males and females. However, in girls there was also a correlation between elevated blood lead levels and somatic complaints. The scale of the correlation differed slightly between the two sexes, behavioural problems relating to aggression, delinquent behaviour and attention problems were somewhat more pronounced in boys (Burns et al., 1999). Many more studies have confirmed that there is a relationship between lead exposure and behavioural problems. Nevin (2000) conducted a study where he showed a correlation between periods of high lead exposure through use of leaded petrol in cities such as New York City and elevated crime rates in the cities 20 plus years later; meaning that young adults who were infants (a critical time for brain development) at times when levels of use of leaded petrol were high were shown to be at a higher risk of becoming offenders in young adulthood (Nevin, 2000) due to brain injury and gray matter reduction (P<0.001) (Cecil et al., 2008) associated with

childhood lead exposure, a possible explanation for the elevated crime rates in the periods when they were young adults (Nevin, 2000). Nevin (2000) also linked racial differences in juvenile arrest in the United States of America. For example, he looked at time periods when preschool blood lead levels of Whites were higher than those of Blacks which correlated with higher juvenile arrest in Whites than Blacks and vice versa. Likewise, a study by Stretesky and Lynch (2001) showed that homicide rates in a county with the maximum air lead concentration levels (0.17 μg/ m^3) were almost four times higher than in a county with air lead concentration levels equivalent to 0 μg/ m^3. Thus showing a correlation between air lead concentration levels and homicide rates. Wright et al. (2008) posit that developmental lead exposure "is a purported risk factor for antisocial behaviour". In a Cincinnati Lead Study (CLS) 250 study participants aged 19 to 24 years old participated in a follow up study that examined association between lead exposure and criminal arrests since the age of 18. The results showed a significant association between average childhood blood lead levels and blood lead levels at six years old and violent criminal arrests in adulthood (95 % CI, 1.15 – 1.89) (Wright et al., 2008). These study findings are indeed a reason for great concern because they show how lead exposure can negatively influence and indirectly define who young children become based on environmental factors outside of their control and that of their parents or caregivers.

Interestingly, in 1992 Denno (1993) published a journal article titled "Considering Lead Poisoning as a Criminal Defence", where she explored whether insanity defence due to lead poisoning and diminished capacity could be viable in the court of law. She argued for example that "internal causes" such as brain cysts (where brain scans can be used to show the functioning of the defendant's brain or lack thereof at the time a crime was committed) or postnatal depression which are more aligned with middle class tend to be more acceptable

defences than those she calls "external causes" such as environmental lead poisoning more prevalent in poorer sectors of our societies. However, she suggests that these "internal" factors may appeal much better because they seem more "tangible" and therefore "causal". Even though lead poisoning is "external" in its origin it can also be "internal" in that it has been associated with alteration of the brain structure and dysfunction. However, to what extent is lead poisoning responsible for neurological damage related anti-social behaviour? That may still need to be further examined as there may be other unexplained factors. Additionally, epidemiological data associating environmental lead exposure and negative neuro-behavioural effects have not proved causality yet. In short, the opinion put forward is that when considering an insanity plea both "internal" and "external" factors should be given consideration (Denno, 1993).

That noted; could science be closer to proving causality with regard to the association between lead exposure and anti-social behaviour including aggressive behaviour and violent behaviour? In addition to epigenetics studies previously mentioned, contemporary data using neuro-imaging is shedding more insight into the effects of environmental lead exposure on the brain. At about 23 years old, 91 study participants from the CLS birth cohort participated in a study examining the long-term impact of lead exposure on white matter architecture in young adulthood (Brubaker et al., 2009). Their findings suggest that childhood lead exposure is associated with "significant and persistent" multiple insults in the brain ($P<0.05$) as demonstrated by abnormalities in white matter diffusion in adult brain later in life. These abnormal changes in white matter diffusion imply alterations in myelination and integrity of the axon (Brubaker et al., 2009). Additionally, using the same CLS birth cohort Brubaker and colleagues (2010) conducted another follow up study examining the association between childhood lead exposure and reduction in the volume of gray matter volume later in life

($p<0.001$). Instead of using mean childhood blood lead levels they used yearly mean blood lead levels from the age of one to six years old in both males and females using votex-wise analysis on high resolution volumetric MRI. In line with previously reported findings from cross sectional studies, neurological effects of childhood lead exposure affect males more than they do females. At all ages the association between childhood lead exposure and gray matter loss in adulthood is stronger in males than in females and these are more prominent in the frontal lobes ($p < 0.001$). In addition, these associations are more significant in later ages (years 5 and 6) than they are in earlier ages. This suggests that earlier childhood blood lead concentrations may not give a full picture of the negative neurological effects of lead exposure in childhood later in life (Brubaker et al., 2010).

Furthermore, to try and understand the neuro-anatomical factors underlying the association between lead exposure and behaviour deficits due to dysfunction of the CNS, Cecil et al. (2008) examined the relationship between childhood lead exposure and brain volume reduction in adulthood using MRI. One hundred and fifty seven study participants from the CLS birth cohort participated in this follow up study (Cecil et al., 2008). The results showed an inverse relationship between elevated mean childhood blood lead concentrations and reduction in gray matter volume in the "ventro-lateral prefrontal cortex" ($P<0.001$) and "anterior cingulated cortex" ($P<0.0001$). Decrements in brain volume were also observed in the "postcentral gyri" ($P<0.0001$), the "inferior parietal lobe" ($P<0.001$) and the "cerebellar hemisphere" ($P<0.05$). With regard to the white matter and the cerebrospinal fluid, there were no significant changes in volume observed (Cecil et al., 2008). The authors posit that the associated reduction in all the aforementioned brain areas is consistent with the neuro-behavioural problems associated with lead exposure (Needleman, 1999, Needleman et al., 1996, Stretesky and Lynch, 2001, Dietrich et al., 2001, Bellinger, 2008).

To assess these associations even further, Cecil and colleagues (2011) examined the association between childhood lead exposure and changes in human brain metabolism which may be the underlying factor linked to neuro-behavioural changes associated with childhood lead exposure. Their study examined the association between average childhood blood lead concentration and *in vivo* brain metabolite in 159 adults using proton magnetic resonance spectroscopy (MRS). The results demonstrated an association between increasing mean blood lead levels and reduction in brain metabolic concentrations in three gray matter areas i.e. left basal ganglia related to a decrease of N-acetyl aspartate (β = –0.05; 95% CI, –0.1 to –0.01 and creatine and phosphocreatine (β = –0.05; 95% CI, –0.09 to –0.01) concentration levels, left cerebellar hemisphere related to a reduction of N-acetyl aspartate (β = –0.05; 95% CI, –0.11 to –0.01) concentration levels, and the cerebellar vermis in relation to decrease in composite of glutamate and glutamine (β = –0.07; 95% CI, –0.12 to –0.02) and two white matter areas i.e. left frontal and left parietal related to decrease of N-acetyl aspartate (β = –0.01; 95% CI, –0.02 to -0.001) and glycerolphosphocholine and phosphocholines (β = –0.01; 95% CI, –0.01 to –0.001), respectively. The authors argue that the observed reduction in the gray matter regions is consistent with the notion that chronic childhood lead exposure results in irreversible damage of neuronal function. Furthermore, the observed changes in the white matter are indicative of permanent changes to myelin architecture (Cecil et al., 2011).

1.2.5.2.1 Definition of aggressive behaviour and violent behaviour

Aggressive behaviour and violent behaviour are interconnected (Liu, 2004). While aggressive behaviour is described as physical violence, verbal and/ or psychological abuse, and various other ways that are harmful towards others (Liu, 2004, Penders et al., 2013). Violence is defined as an "overt physical aggression that has the potential consequence of physical harm to another person or object" (Penders et al., 2013). This definition is consistent with that of Liu (2004) who defines violence as "excessive negative aggression". As such, aggression can be defined as another form of violence and vice versa.

It is important to consider the state of mind of the perpetrator at time the offense took place, as is usually observed in forensic investigations. Aggressive and violent behaviour can also be classified into two different states of mind; that is, impulsive versus premeditated aggressive and violent acts. Findings by Barratt et al. (1999) confirm that impulsive and premeditated aggressive acts are two different constructs. Impulsive aggressive/ violent behaviour are classified as acts committed without thinking, "associated with a high degree of affective arousal" (Barratt et al., 1999). On the other hand, premeditated aggressive/ violent behaviour is defined as acts that are planned, "predatory and not associated with high levels of affect" (Barratt and Felthous, 2003). Empirical evidence shows that individuals with lead exposure-associated aggressive or violent behaviour tend to display impulsive acts (Byers and Lord, 1943, Ris et al., 2004); a concept similar to that of murderers exhibiting injury in the prefrontal cortex area of the brain (Raine, 2001, Ramirez, 2006).

1.2.5.2.2 Risk factors for aggressive behaviour and violent behaviour

Risk factors associated with aggressive and violent behaviour can be categorized into three categories, environmental, biological and psychosocial risk factors.

Box 1.1: Risk factors for aggressive behaviour and violent behaviour

Risk factors for aggressive/violent behaviour	Examples
1. Environmental risk factors	Exposure to heavy metals such as lead.
2. Biological risk factors:	i. Medical illness or brain injury: Psychotic disorders; bipolar disorder; personality disorders; impulse control disorder; substance use disorders; developmentally disabled; delirium; dementia; brain disorders; traumatic brain injury and epilepsy (Penders et al., 2013) ii. Stress (White et al., 2007) iii. Genetic factors: Levels of testosterones ((Batrinos, 2012) Serotonin (Duke et al., 2013) iv. Maternal pathophysiological processes (Liu Jianghong, 2011)
3. Psychosocial risk factors	i. Physical or emotional trauma ii. Birth to teenage parents iii. Poor parenting iv. Social information processing v. Community level risk factors: low levels of employment and poverty; community crime and violence; easy access to drugs and guns; domestic violence; poor parenting; birth to teenage mothers (Liu Jianghong, 2011, Burton and Leoschut, 2012)

1.2.5.2.3 MITIGATING FACTORS AGAINST AGGRESSIVE AND VIOLENT BEHAVIOUR

It is recommended that pregnant mothers should have access to efficient prenatal care, avoid of exposure to environmental hazards, observe proper nutrition and eliminate all substance abuse; among others (Liu et al., 2013). A South African study showed that children and adolescents with positive educator role models - have motivation to do well at school, proper parental guidance, positive friends, access and participation in positive social activities are less likely to commit violent acts (Burton and Leoschut, 2012).

The next section discusses brain injury in violent crime offenders to examine similarities between findings from the lead associated brain imaging studies and brain imaging studies of convicted violent offenders in the United States.

1.2.6 LINK BETWEEN BRAIN INJURY AND NEURO-BEHAVIOURAL DISORDERS

Findings from brain imaging studies of men incarcerated for violent crimes and negative social behaviour seem to mirror those of individuals who had been exposed to lead. Raine and colleagues (2000) conducted a study to assess differences in volumetric measures of prefrontal gray and white matter using structural magnetic resonance in 21 men diagnosed with antisocial personality disorder (APD) ("APD group"), 34 men with no APD, alcohol or drug abuse dependency ("control group") and 27 men with a lifetime diagnosis of alcohol and drug abuse dependency but no APD ("substance-dependent group") (Raine et al., 2000). APD was associated with significant reduction in prefrontal gray matter. This group had 11% reduction in prefrontal gray matter volume compared to the control group and 13.9%

reduction compared to the substance-dependent group. The findings from this study add to the growing body of evidence linking reduction in prefrontal gray matter and behavioural problems. However, Raine and colleagues caution that not all people with lesions in the pre-frontal cortex exhibit "social and psychopathic behaviour" (Raine et al., 2000).

On several occasions people committed with serious crimes such as murder have pleaded not guilty by reason of insanity (NGRI). To try and understand the link between brain dysfunction and violent behaviour Raine et al (1997) undertook another study to test the direct measures of "cortical and subcortical function" in relation to homicide (Raine et al., 1997). In the cortical regions murderers had lower glucose metabolism in the lateral and prefrontal cortex when compared to the control group. Furthermore, murderers had significantly lower glucose metabolism in the "left and right medial superior frontal cortex", "left anterior medial cortex", "right orbitofrontal cortex" and "lateral middle frontal gyri of both left and right hemispheres". In the parietal lobe murderers had lower glucose metabolism than controls in the "left angular gyrus and bilateral superior parietal regions" and "left and right superior parietal gyri". On the contrary, in the occipital lobe (primarily involved in visual processing as opposed to executive functions), mostly there was higher glucose metabolism in the murderers than controls. There was no significant difference between the two groups in glucose metabolism in the temporal lobe (Raine et al., 1997). In the subcortical regions, murderers had lower glucose metabolism in the corpus callosum than controls. In the amygdala there was "relatively" reduced activity in the "left and greater right" areas compared to the control group. Additionally, murderers had reduced activity in the left than right amygdala than controls (Raine et al., 1997).

reduction compared to the substance-dependent group. The findings from this study add to the growing body of evidence linking reduction in prefrontal gray matter and behavioural problems. However, Raine and colleagues caution that not all people with lesions in the pre-frontal cortex exhibit "social and psychopathic behaviour" (Raine et al., 2000).

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Of great interest is that again these findings confirm the association between deficits in brain function specifically in the prefrontal cortex and antisocial and violent behaviour as exhibited in lead exposed individuals. The authors point out that these results give prudence to the "pre-existing biological theories of violence" (Raine et al., 1997). However, they caution that their study findings do not prove that murderers pleading NGRI are innocent of the crimes they have committed or that positron emission tomography (PET) is the correct diagnostic tool as it has its limitations (Raine et al., 1997). I used these examples to highlight some similarities in areas of brain damage in lead associated brain injury and those of convicted criminal offenders; and not to suggest that all violent offenders or murders with a history of lead exposure are innocent of their crimes. The next section looks at why brain injury in the aforementioned regions of the brain matter in relation to neuro-behavioural disorders and social behaviour.

1.2.7 BIOLOGICAL SIGNIFICANCE OF PRE-FRONTAL CORTEX AND OTHER RELEVANT REGIONS OF THE BRAIN IN NEURO-BEHAVIOURAL FUNCTIONS

In order to put these data into context, Table 1.1 below shows the biological functional significance of prefrontal cortex and other relevant regions of the brain (Yang et al., 2008).

Table 1.1: Regional brain functions linked to neuro-behavioural disorders (Young et al., 2008)

Region of the brain	Functional significance
Prefrontal Cortex: Orbitofrontal regions i.e. orbitofrontal cortex; ventromedial prefrontal cortex	• Execution of executive functions i.e. planning, organization, regulation, strategizing, and control response impulse. • "Receive and process information concerning emotion and reward values", and help transmit that information to the "dorsolateral prefrontal cortex" for completion. • Mediates reasoning. • Responsible for making ethical and moral decisions.
Superior Temporal gyrus:	• Moral reasoning. • Mediates "theory of mind". • Communication and interaction with others.
Amygdala-hippocampal complex:	• Processing of "affective stimuli" i.e. "threat or fear related stimuli".
Anterior cingulated cortex:	• Processing and regulation of emotional information and responses. • Regulates aggression. • Responsible for empathy, "shallow effect", monitoring error and "response inhibition".

It is important to note that as much as brain imaging studies are assisting in addressing the biological gap from epidemiological studies linking brain injury to negative social behaviour; Button et al. (2013) warn that sample size and thus low statistical power is a problem in neuroimaging studies. However, from the public health perspective these data are of great value as they can better inform policies by governments and other relevant institutions about the possible negative impact of environmental toxins on human social behaviour. If lead

exposure predisposes children to aggressive behaviour, violent behaviour and other negative social behaviours as has been shown – this indeed raises some "uncomfortable, philosophical, legal and moral questions" as environmental lead exposure can be controlled if made a health priority by the governments. The World Health Organization defines violence as a "global public health problem". Globally, violence is one of the leading causes of death in people between the ages of 15 and 44 years (Dahlberg and Krug, 2002).

The next section reviews how the signalling from the CNS to the hypothalamus results in a chain of actions in the endocrine system that influence onset of puberty and tempo of progression; and how lead exposure may alter the regulation of puberty development in both males and females.

1.2.8 LEAD EXPOSURE AND PUBERTAL DEVELOPMENT

1.2.8.1 Puberty

Puberty is a time of sexual and physical maturation from childhood to adolescence due to hormonal changes in young girls and boys. A variety of endocrine changes i.e. adrenarche and gonadarche contribute to the physical signs of puberty (Blondell et al., 1999) represented by the development of secondary sexual characteristics (Louis et al., 2008). Tanner stages are a standard clinical system used to describe physical measurements of development as shown in Tables 1.2 and 1.3 for girls and boys, respectively (Blondell et al., 1999). Puberty is marked by breast development, pubic hair development and menarche in girls; and by the size and volume of the testicles, penile and pubic hair growth in boys.

Table 1.2 Pubertal Development and Tanner Stages in girls (Blondell et al., 1999)

	PUBERTAL DEVELOPMENT IN GIRLS	
	Breasts	**Pubic Hair**
TANNER STAGES		
Stage 1	Prepubertal	Prepubertal; villus hair visible.
Stage 2	Elevated breast bud; enlarged areola; approximately 11 years old.	"Sparse growth of slightly pigmented hair along the labia"; approximately 12 years old.
Stage 3	No contour separation but breast tissue enlarged beyond areola; approximately 12 years old.	Coarser, curled and pigmented hair; spread over the pubes; approximately 13 years old.
Stage 4	Areola and papilla projection forms a secondary mould; approximately 13 years old.	Hair similar to adult type; "no spread to medial thigh"; approximately 13years old.
Stage 5	Mature breast contour; only projection of papilla; approximately 14 years old.	Hair adult in type; "spread to medial thigh but not up linea alba"; approximately 14 and a half years old.

Table 1.3 Pubertal development and tanner stages in boys (Blondell et al., 1999)

	PUBERTAL DEVELOPMENT IN BOYS	
	Genitalia	**Pubic Hair**
TANNER STAGES		
Stage 1	Prepubertal.	Prepubertal; villus hair visible.
Stage 2	Scrotum thinning and reddening; "testes: 2.5 to 3.2 cm (1 to 1.28 in)"; approximately 12 years old.	"Sparse growth of slightly pigmented hair ay base of penis"; approximately 12 years old.
Stage 3	Penis enlargement, especially in length; "testes: 3.3 to 4.0 cm (1.32 to 1.6 in); approximately 13 years old.	"Thicker, curlier hair spread to the mons pubis"; approximately 14 years old.
Stage 4	Increased penis size; growth of glands; scrotum darkening; "testes: 4.1 to 4.5 cm (1.64 to 1.8 in)"; approximately 14 years old.	Hair adult type; no spread to medial thigh; approximately 15 years old.
Stage 5	"Adult genitalia"; testes: > 4.5 cm (1.8 in)"; approximately 15 years old.	Hair adult type; "spread to medial thighs but not up to linea alba"; approximately 15 years old.

1.2.8.2 Normal onset of puberty

The activation of the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) result in the onset and progression of puberty in young girls and boys (Louis et al., 2008, Brämswig and Dübbers, 2009). HPG axis is operational during the "midfetal, neonatal and early infancy" and relatively inactive in childhood. It is activated by the signals from the CNS to the hypothalamus resulting in the release of gonadotropin release hormone (GnRH) by the hypothalamus. The GnRH stimulates the secretion of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary. It

is these gonadotropins that produce sex hormones resulting in pubertal changes such as the onset of menarche in girls. Before ovulation, the ovarian follicle secretes ovarian androgens from theca cells and oestradiol from granulosa cells; and after ovulation the corpus luteum secretes progesterone. Oestrogen initiates developmental changes of breasts, ovaries and uterus. In boys, LH release results in secretion of testosterone and androstenedione by the testicular Leydig cells. Subsequently, androgen action initiates developmental changes of the penis, pubic hair and testes (Louis et al., 2008).

To activate the HPA axis, the CNS signals the hypothalamus resulting in it releasing the adrenocorticotropin releasing hormone (CRH), which in turn stimulates the release of the adrenocortical tropic hormone (ACTH) by the pituitary. ACTH initiates secretion of androstenedione and dehydroepiandrosterone (DHEA) by the adrenal cortex; initiating developmental changes of pubic hair, armpit hair and acne in both girls and boys (Louis et al., 2008). This is the initiation of gonadal endocrine function called gonadarche. It is suspected that these gonadal changes of puberty are influenced by both genetic and environmental factors (Louis et al., 2008).

Almstrup et al (2016) posit that methylation patterns could be better predictors of pubertal development compared to chronological age. In their study of healthy study participants blood samples were collected for DNA isolation during pre- mid- and post puberty and testicular volume of ≥4 mL and Tanner breast stage ≥2 (B2) for boys and girls, respectively were used as measures of pubertal timing. During pre-puberty the children had lower DNA methylation levels in "open sea and in shores and shelf of CpG islands" than in post-puberty ($p=0.001$) with higher levels of CpG islands observed in girls compared to boys (Almstrup et

al., 2016). To identify differentially methylated CpGs specifically linked to pubertal progression, individual time points for DNA collection were assigned specific "pubertal age" based on puberty timing. Four hundred and fifty seven CpGs with a false discovery rate (FDR) <0.05 were found in a combined analyses of both sexes. In addition, analyses to identify methylated CpGs associated with reproductive hormone levels compared alterations in DNA methylation to those of reproductive hormones during pubertal transition using an FDR of <0.05, and these were only significantly associated in boys. These results show sex differences with regard to gene expression during pubertal transition.

When data were examined for genomic regions where DNA methylation is linked to pubertal age, the most significant genomic region that was differentially methylated for both sexes individually and collectively was on chromosome 7 located between *SLC12A9* and Thyroid Hormone Receptor Interactor 6 (*TRIP6).* When *TRIP6* expression was analysed for pubertal transition in males *TRIP6* expression was "prominent and highly specific" in post-pubertal testosterone producing Leydig cells and was also found in pre-pubertal Sertoli cells; conversely, *TRIP6* staining was absent in pre-pubertal Leydig cells and post-pubertal Sertoli cells. Using ovarian tissue in females, weak *TRIP6* staining was found in oocytes and granulose cells but was absent in theca cells. When circulation levels of *TRIP6* were compared in pre-, mid- and post-pubertal children, results showed significant increases as children progressed through pubertal stages. *TRIP6* is mainly expressed in reproductive tissues and thyroid hormones are vital for the regulation of genital development (Almstrup et al., 2016).

1.2.8.3 Lead exposure and altered pubertal development

Lead is considered one of the endocrine disrupting compounds (EDCs). EDCs are harmful because of their activities and are described as any compound that disturbs endocrine action (Zawatski and Lee, 2013, Zoeller et al., 2012). Environmental factors associated with pubertal development have "oestrogenic or androgenic properties" (Zawatski and Lee, 2013) known to have hormone disrupting effects. As mentioned earlier, during onset of puberty GnRH is released from the hypothalamus in the brain, which stimulates secretion of the LH and FSH secretion by the pituitary. LH activates testicular Leydig cells to produce testosterone in males. FSH helps seminiferous tubules and spermatogonia maturation in the Sertoli cells in males and regulates development of follicles in the granulosa cells in females. The sertoli cells produce inhibin. Subsequently testosterone and inhibin suppress the secretion of LH and FSH through a feedback mechanism to the hypothalamus-pituitary.

In altered puberty environmental lead disrupts the pituitary function potentially inhibiting suppression of LH and FSH secretion by inhibin and testosterone (Doumouchtsis et al., 2009, Zawatski and Lee, 2013). In girls, lead levels build up in the granulosa cells of the ovary resulting in structural alterations which in turn cause developmental delays in growth and pubertal development and affects fertility in females (Doumouchtsis et al., 2009). In addition, the authors speculate that lead exposure may delay growth in exposed individuals by suppressing growth hormone (GH) and subsequently insulin-like growth factor1 (IGF-I) secretion (Doumouchtsis et al., 2009) crucial for child development.

Epidemiological studies show that there is a relationship between lead exposure and altered puberty in girls and boys as discussed in the next section.

1.2.8.4 Lead exposure and altered puberty in girls

In the United States using data from the NHAHES III were used to assess the relationship between blood lead levels and onset of puberty in girls (Selevan et al., 2003). A total of 2186 girls aged 8 through to 18 years made up of 600 non-Hispanic whites, 805 non-Hispanic African Americans and 781 Mexican American fulfilled the inclusion criteria and were enrolled in the study. Onset of puberty was marked by stages of pubic hair development, breast development and menarche. In African Americans and Mexican Americans the results showed an association between elevated blood lead levels (3 μg/dL compared to 1 μg/dL) and delayed onset of puberty. These findings were not statistically significant in the non-Hispanic white study group. In non-Hispanic African American girls there was a significant association between elevated blood lead levels and delay in onset of puberty in all three pubertal measures examined. In Mexican American girls elevated blood lead levels were associated with delayed onset in breast and pubic hair development ($P<0.05$). There was an inverse relationship between blood lead levels and the likelihood of reaching each Tanner stage of breast development ($P<0.05$). Also, in non-Hispanic African American girls there was a significant association between elevated blood lead levels and delay in age of menarche ($P<0.05$) (Selevan et al., 2003).

These study findings by Selevan et al. (2003) confirmed earlier findings within the same cohort by Wu et al. (2003). The only slight difference in their results was that Wu et al. (2003) found a statistically significant association between elevated blood lead levels and delayed onset of menarche and public hair development (95% CI, 0.28 to 0.97) and (95% CI, 0.32 to 0.91), respectively – but not breast development. On the contrary, in an Egyptian pilot study involving 10 to 13 year old girls, the results showed a significant association between

elevated blood lead levels and delayed onset in breast development only and not in pubic hair development (Tomoum et al., 2010).

Similarly, in South Africa, a study to examine the association between elevated blood lead levels and delayed onset of puberty in adolescent girls (Naicker et al., 2010a) showed that there is a statistically significant association between elevated blood lead levels and delayed onset of menarche ($P<0.001$), breast development ($P<0.01$) and public hair development ($P<0.05$) in Black South African adolescents (Naicker et al., 2010a).

Overall, all these studies show that there is an association between lead exposure and delayed onset of puberty in girls albeit slight differences in measures of puberty associated with lead exposure and altered puberty.

1.2.8.5 Lead exposure and altered puberty in boys

In a Russian study to examine the association between lead exposure and onset of puberty in boys aged 8 to 9 years, the odds of being in stage 2 were reduced by 43% in boys with elevated blood lead levels (95% CI, 0.34 to 0.95; $P = 0.03$) (Hauser et al., 2008). Using the same cohort of boys, Williams et al. (2010) conducted a longitudinal analysis to evaluate the association between blood lead levels and onset of puberty using a different statistical method - Cox proportional hazards model. The results confirmed a statistically significant association between elevated blood lead levels and delayed onset of puberty in all three measures i.e. pubarche (95% CI, 0.39 to 0.94; $P= 0.03$) adjusted for birth weight, gestational age, caloric intake, percent fat, percent protein, low income, low parental education, maternal alcohol during pregnancy, BMI and height at study entry; genitalia (95% CI,0.59–0.98; $P=0.04$) and testicular volume (95% CI, 0.55 to 0.97; $P=0.03$) adjusted for birth weight, gestational age,

caloric intake, percent fat, percent protein, low income, low parental education, and maternal alcohol during pregnancy (Williams et al., 2010). However, there is a huge gap in available empirical data regarding environmental lead exposure and altered puberty in boys. More research studies need to be conducted in this regard.

1.2.8.6 Consequences of altered pubertal development in girls and boys

Earlier onset in growth spurt development in boys is associated with taller height (P=0.02) and in girls later onset of breast development and mernache is linked to taller adult height at age 18 years (P=0.01 and P=0.004), respectively (Yousefi et al., 2013). Additionally, early onset of puberty in girls is associated with an increased risk of obesity, type-2 diabetes, breast cancer and other chronic diseases (Golub et al., 2008). In boys, it is less clear if earlier or later onset of puberty increases the risk of developing testicular cancer as both are associated with developing the disease (MOSS et al., 1986, Wilkinson et al., 1992, Wilkinson and Colls, 1994). Animal studies have linked delayed onset of puberty to reduced development of bone strength (Yingling and Taylor, 2008), which increases the risk of osteoporosis.

Furthermore, there is a relationship between delay in puberty and an increased risk of being victimized, increased risk of developing an eating disorder and being depressed (Zawatski and Lee, 2013). In boys the psychological consequences of delayed pubertal development are more severe than in girls (Rosen and Foster, 2001). Boys who look younger than their peers of the same age have a problem interacting socially, dating and are usually unpopular (Rosen and Foster, 2001).

1.3 SUGGESTED MEASURES FOR PREVENTION OF LEAD EXPOSURE IN CHILDREN

Environmental lead exposure is an environmental health problem that can be prevented (Centers For Disease Control and Prevention, 1997). What can be done to prevent lead exposure in children in middle and low income countries?

Box 1.2 outlines some of the preventive measures that can be implemented to drastically reduce the levels of lead exposure in children from lower socio-economic communities, in particular.

Box 1.2: Prevention and/or control of environmental lead exposure in children

Identify communities and children at increased risk of lead exposure.
Implement a national plan for blood lead screening.
Develop screening and follow up testing guidelines.
Monitor blood lead levels in at risk communities.
Develop standardized treatment guidelines for those already exposed.
Educate the public on how to prevent lead exposure.
Educate families about risks of elevated blood lead levels.
Educate communities about adequate nutrition, impact of pica habits, and other risk factors.
Enforce and monitor regulations on phase-out use of leaded paint in household items.

Adapted from (Centers For Disease Control and Prevention, 1997, Tong et al., 2000, World Health Organization, 2010b)

Additionally, educating communities about the dangers of lead exposure should not be an annual one-time event. More awareness programs throughout the year should be implemented. The CDC emphasizes that prevention; control and elimination of lead exposure are the best ways to tackle lead poisoning in young people. Education of elected officials at local and national levels about the importance of primary prevention is paramount (Centers For Disease Control and Prevention, 2012b).

1.4 SUMMARY OF LITERATURE REVIEW

In our literature review, the following data were highlighted:

- Despite massive decline in the commercial use of lead worldwide, lead remains a stubborn health problem and a threat to the lives, and wellbeing of young people in particular.

- Exposure to lead alters biological systems and controls involved in the functioning of the CNS resulting in neurological damage which may be irreversible.

- Neuroimaging studies are beginning to address the gap in knowledge on how lead affects the structure and function of the brain as evidenced by epidemiological studies associating it to deficit in cognition and social behaviour.

- The similarities in neuroimaging study findings of the brain abnormalities of individuals convicted of violent crimes as shown in the American studies by Raine et al (2000) and those with reduced brain volume associated with childhood lead exposure as shown by Cecil et al (2008), should be further examined.

They are consistent with the opinion that exposure to environmental lead could be "precursors of physiological and neurological instability" in young children and later in adulthood.

- Lead is an EDC. EDCs are associated with altered puberty in both girls and boys. Altered puberty has major adverse physiological and psychological ramifications for adolescents.
-
- New developments in the study of epigenetics show epigenetic mechanisms that regulate brain development and puberty development.

1.5 GAPS IN LITERATURE REVIEW

Most of the empirical data evaluating the health effects of lead exposure come from the developed countries, yet the WHO (World Health Organization, 2009) estimates that almost 100% of children and adults exposed to lead and with elevated blood lead levels live in developing countries. More empirical data from developing countries such as South Africa are crucial to help highlight the detrimental health effects of lead exposure in children.

It is still poorly understood what the nature of neuro-behavioural disorders associated with early lead exposure is, especially in low and middle countries.

Much research has been conducted with regard to environmental exposures and onset of puberty, however, there is still paucity of longitudinal studies examining the association

between lead exposure and pubertal transition/ progression. As far as we know no such research has been conducted in South Africa.

1.6 RELEVANCE OF THE STUDY

To effect change regarding monitoring of blood lead levels in children from low and middle income countries, research from these communities is required to address gaps in evidence required by policy makers.

Findings from this study will assist in addressing the gap in information with regard to the nature of neuro-behavioural effects of lead exposure and pubertal longitudinal analyses of lead exposure associated altered puberty development in girls and boys low and middle income countries.

1.7AIMS AND OBJECTIVES OF THE STUDY

1.7.1 Aim of the study

The main aim of this study is to examine the association between lead exposure and specific types of aggressive and violent behaviour during adolescence; and the association between lead exposure and pubertal transition using latent classes of puberty development in girls and boys.

- **1.7.2 Objectives of the study:**
- To assess the dimensionality of aggressive behaviour by sex.
- To assess the relationship between lead exposure and dimensions of aggression in mid-adolescence.
- To examine the association between lead exposure and violent behaviour during adolescence and determine specific type(s) of violent behaviour associated with lead exposure.
- To assess the impact of lead exposure during early adolescence on pubertal progression in South African boys and girls. Data are further analyzed for an association between cord blood lead levels and pubertal progression in girls and boys.

PART 2

JOURNAL ARTICLES

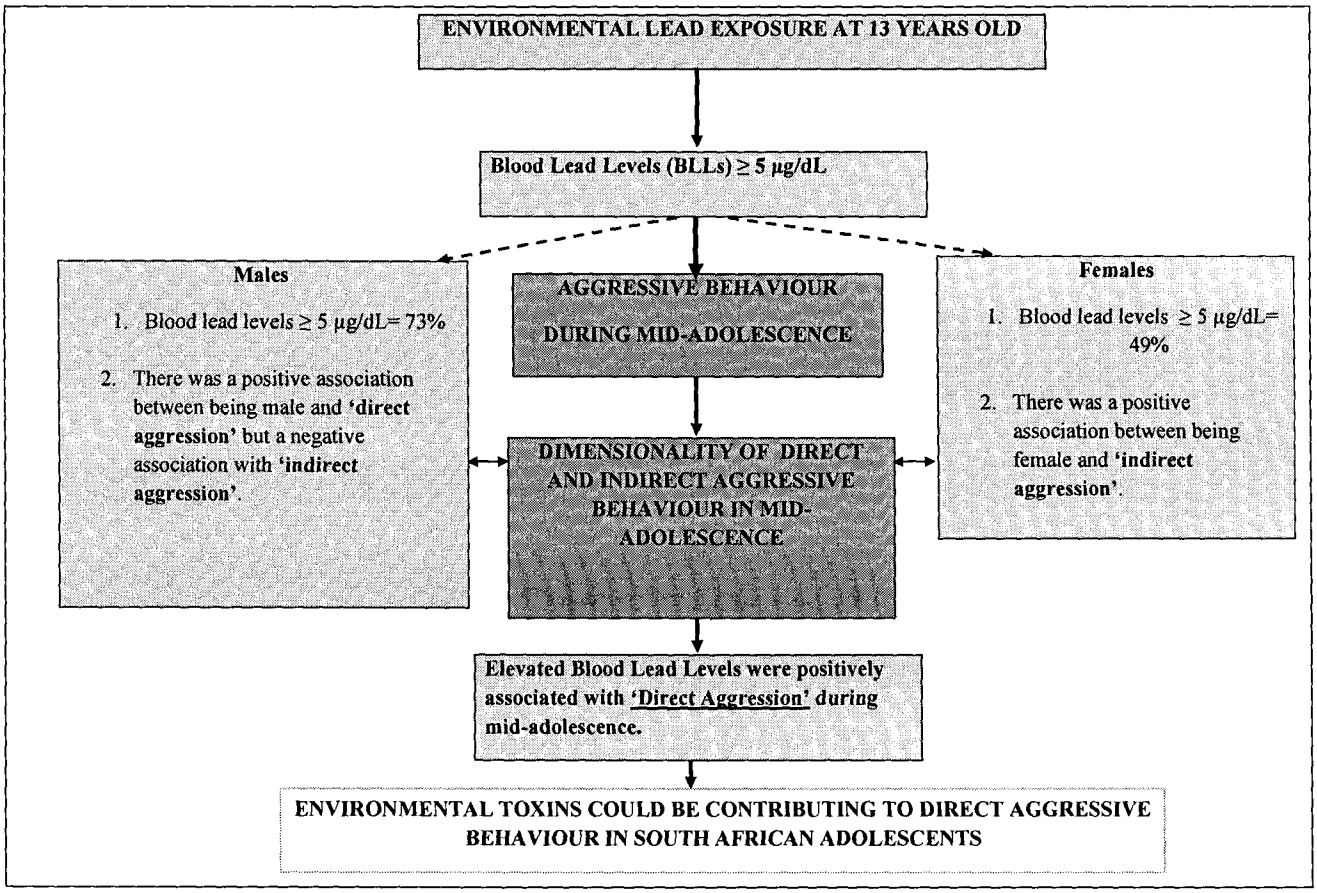
CHAPTER 2

The association between environmental lead exposure with aggressive behaviour, and dimensionality of direct and indirect aggression during mid-adolescence: Birth to Twenty Plus Cohort.

Publication journal: ***Science of the Total Environment.*** **2018 Jan 15; 612:472-9.**

This journal article is published in the Science of the Total Environment, aims to answer objectives 1 and 2 of the thesis. The association between lead exposure in early adolescence and aggressive behaviour during mid-adolescence was examined using PCA derived components. Additional analyses conducted after the journal article was published have been included in this chapter. A copy of the published journal article is attached in the Appendices section (Appendix A).

Figure 2.1 GRAPHIC ABSTRACT: The association between environmental lead exposure with aggressive behaviour, and dimensionality of direct and indirect aggression during mid-adolescence: Birth to Twenty Plus Cohort.


ENVIRONMENTAL LEAD EXPOSURE AT 13 YEARS OLD
Blood Lead Levels (BLLs) ≥ 5 µg/dL
Males
1. Blood lead levels ≥ 5 µg/dL= 73%
2. There was a positive association between being male and 'direct aggression' but a negative association with 'indirect aggression'.
AGGRESSIVE BEHAVIOUR DURING MID-ADOLESCENCE
DIMENSIONALITY OF DIRECT AND INDIRECT AGGRESSIVE BEHAVIOUR IN MID-ADOLESCENCE
Females
1. Blood lead levels ≥ 5 µg/dL= 49%
2. There was a positive association between being female and 'indirect aggression'.
Elevated Blood Lead Levels were positively associated with 'Direct Aggression' during mid-adolescence.
ENVIRONMENTAL TOXINS COULD BE CONTRIBUTING TO DIRECT AGGRESSIVE BEHAVIOUR IN SOUTH AFRICAN ADOLESCENTS

2.1 Introduction

The World Health Organization (WHO) estimates that lead exposure accounts for 0.6% of the global burden of disease (World Health Organization, 2009). Lead is also considered an important environmental health hazard contributing to the environmental disease burden in Africa (Nweke and Sanders III, 2009, Centers for Disease Control and Prevention, 2012c). A known cumulative toxicant, lead affects the "neurological, haematological, gastrointestinal, cardiovascular and renal systems" (World Health Organization, 2010b). Epidemiologic studies have shown evidence of lead-related adverse health outcomes, most notably acting on the central nervous system (CNS) (Needleman and Gatsonis, 1990, Hernberg, 2000, Lanphear et al., 2005, Schwartz et al., 2007, Shih et al., 2007, Yuan et al., 2006, Cecil et al., 2008, Cecil et al., 2011). The effects of lead exposure on the CNS in children are associated with reduced cognitive function, aggressive, violent, delinquent and criminal behaviour, amongst others (Needleman et al., 1979, Needleman and Gatsonis, 1990, Needleman et al., 2002, Bellinger et al., 1992, Canfield et al., 2003, Lanphear et al., 2005, Mazumdar et al., 2011, Wright et al., 2008). The indirect costs of childhood lead exposure speak to the economic burden it places on the general public (World Health Organization, 2010a). Its adverse effects on the intellectual function of young people (Lanphear et al., 2000, Lanphear et al., 2005) may in turn affect the level of education they attain and employment opportunities available to them in the future. Additionally, there is a link between reduced educational attainment and the probability of being arrested (Lochner and Moretti, 2004). As such, environmental lead exposure contributes to great *"lost opportunity costs"* (World Health Organization, 2010a) and robs young people of their full potential in life.

The Port Pirie Cohort was the first study to prospectively evaluate the association between lifetime blood lead exposure in children and "emotional and behavioural problems" (Burns et al., 1999). Children 11 to 13 years from a lead smelting neighbourhood in Port Pirie, Australia were included in the study. Using a cut-off score of 15 μg/dL, in both sexes a correlation between higher cumulative blood lead levels and total behaviour problems was found. In boys, the highest correlation was between elevated lifetime blood lead levels and "aggressive behaviour", "delinquent behaviour", and "attention problems"; and in girls with "aggressive behaviour", "delinquent behaviour", "attention problems", "social problems", "anxious/ depressed", and "withdrawn" (Burns et al., 1999).

South African children particularly from poor communities are exposed to environmental lead through various mediums such as use of lead based paint in children's toys (Mathee et al., 2007) and playground equipment for children in Johannesburg, Ekurhuleni, and Tshwane (Mathee et al., 2009); use of lead in subsistence fishing where waste lead for example from "wheel balancing and alignment centres" is collected, melted and recycled to make new craft sinkers (Mathee, 2014); and pregnant women who practice geophaphia thus increasing the risk of prenatal lead exposure (Mathee et al., 2014) amongst others.

Naicker et al. (2012) examined the relationship between lead exposure and socio-behavioural adjustment including aggressive behaviour during early adolescence in the Birth to Twenty Plus (BT20+) cohort in South Africa. With regard to aggressive behaviour, blood lead levels at age 13 years were negatively associated with aggressive behaviour item 'I argue a lot' 95% CI [-0.23 to – 0.02] but positively associated with 'I attack other people' 95% CI [0.09 – 0.98] (Naicker et al., 2012). To further assess these findings at a later stage in adolescence and determine the specific type(s) of aggressive behaviour related to environmental lead

exposure, we set out to examine the association between lead exposure in early adolescence and aggression items in mid-adolescence (at ages 14 to 15 years old). The use of Principal Components Analysis (PCA) in this study allowed for a more comprehensive integration of aggressive behaviour. We examined lead exposure at age 13 years old and dimensions of aggression during mid-adolescence. These data are essential for public health and environmental health policymakers to address this important public health problem in the country.

2.2 Methods and materials

2.2.1 Study population

BT20+ is the largest and longest running longitudinal birth cohort in Africa. The cohort includes all singleton births at public health facilities during a seven-week period from April 23 to June 8, 1990 in Soweto/Johannesburg, South Africa. The birth cohort is representative of long-term urban residents. Over the years, the cohort has reported a very low attrition rate of less than 3% annually – with the highest rate reported in the first two years of the study; mostly due to movement away from the study area (Norris et al., 2007). The cohort is described in detail elsewhere (Richter et al., 2004, Richter et al., 2007).

For this study, Black African and Coloured (mixed race heritage) study participants with blood lead samples at age 13 years and who had completed the Youth Self Report(YSR) during mid-adolescence at ages 14 to 15 were included (n=1086). White and Indian study participants were excluded due to very low numbers. In addition, to test if study participants exhibiting aggressive behaviour during mid-adolescence have an early predisposition to

aggressive behaviour, study participants with YSR data for year 11 were included in the study.

2.2.2 Blood lead measurements

Venous samples of whole blood were collected at age 13 years into EDTA-containing tubes previously determined to be free of trace metals. Blood sampling was undertaken by professional health officials, using sterile equipment and aseptic techniques. Blood samples were vortexed and rolled on the coulter mixer for at least 10 minutes until properly mixed. They were diluted 10 times with 1,1 % (v/v) Triton X-100 using automatic Hamilton Microlab 500 diluter into disposable 10 ml Sterilin plastic tubes covered with screw caps and mixed well using a vibration mixer. Blood lead levels were measured using Perkin Elmer 600 AAnalyst atomic absorption spectrometer with a THGA graphite furnace, Zeeman background correction and AS-800 Autosampler. Both blood samples and samples for quality control were prepared and measured in-house.

2.2.3 Measurement of aggressive behaviour in mid-adolescence and potential confounders

Data were collected from the cohort at age 14 to 15 years using the YSR questionnaire. This questionnaire is a self-report measure used to assess social and behavioural problems in children and adolescents aged 11 to 17 years. These include aggressive behaviour, substance abuse, breaking rules, social problems, thinking disorders, anxiety disorders, depression, mood disorders, impulsive behaviour and others (Achenbach, 1991). Seventeen of the 112 questionnaire items examine aggressive behaviour. Each item has four response options: not true, sometimes true, true and very true. The aggressive behaviour response options were collapsed into two categories referred to as 'negative response' for not true and sometimes true and 'positive response' for true and very true.

To measure possible confounding, socio-demographic factors such as sex, maternal education at birth (which was categorized into four levels, i.e. grade 7 or less, grade 8-10, grade 9-12 and post school education) – the South African education system is divided into primary school (grade 1 -7), secondary school (grade 8 – 10), high school (grade 11 – 12), and tertiary (college/ university); maternal marital status at birth was dichotomized into married/ living with a partner, or single/ widowed/ divorced/ separated. It has been shown that children raised from two-parent households have less aggressive behaviour tendencies compared to their counterparts from single parent households (Usakli, 2013) most of which are headed by single mothers (Fields, 2003); maternal age at birth was categorized as < 20 (representing teenage mothers), 20 - 29 (representing younger mothers in their 20's), 30 – 39 (representing older mothers in their 30's) and ≥ 40 years old (representing mother aged 40 and above) – children born to teenage mothers are more likely to exhibit aggressive and antisocial behaviour later in life (Fergusson and Woodward, 1999, Nagin and Tremblay, 2001); residential area of birth was collapsed into two categories, Soweto/Diepmeadow or former Coloured/ Asian and Inner City/ Suburb – in former *apartheid*[1] South Africa inner cities and suburbs were places of residence mainly for white South Africans; and hospital of birth was categorized as public or private – health services in *apartheid* South Africa were segregated according to each racial group (Kautzky and Tollman, 2008). In addition, socio-economic status (SES) at birth was calculated using binary household items, such as type of home, access to water inside the dwelling, sole use of water, access to flush toilet, sole use of toilet, and ownership of household appliances including television, motor car, fridge, washing machine and telephone.

[1]*Apartheid* was a system of "institutionalized segregation and discrimination" in South Africa.

PCA with varimax rotation was used as the dimensionality reduction technique to create an SES variable. The sample size was adequate with a Kaizer-Meyer-Olkin score of 0.68 and the Bartlett's test was statistically significant ($p<0.001$). One PCA component was extracted based on Scree Plot analysis and eigenvalue of > 1 (Supplementary Table 1). The extracted principle components using SPSS software are continuous variable that can be used in regression analysis. The SES variable ranges from -1.59836 to 4.28605.

2.3 Statistical analysis

2.3.1 Descriptive Statistics

For the purposes of this study, blood lead levels at age 13 years were stratified into three categories: <5 μg/dL as the reference level, 5 - 9.99 μg/dL and ≥10 μg/dL. In part, these categories were selected in line with the new recommendations from the Centers for Disease Control and Prevention (CDC)' Advisory Committee for Childhood Lead Poisoning Prevention (ACCLPP) (Centers For Disease Control and Prevention, 2012b). In light of the overwhelming scientific evidence showing detrimental health effects of blood lead levels at < 10 μg/dL in children, CDC's ACCLPP recommended the use of reference value of 5 μg/dL based on the 97.5th percentile of the current blood lead level distribution among children aged 1 to 5 years in the United States of America (Centers For Disease Control and Prevention, 2012b). Blood lead levels were also classified by sex of the study participant using measures of spread and central tendency. Socio-demographic factors were stratified by sex of the study participant, as was aggressive behaviour profile i.e. (positive response for aggressive behaviour questions). For categorical variables, Chi-square tests were performed to establish the significance of association, and for continuous variables, t-tests were used to compare the means.

2.3.2 Analytical Statistics

Data were first assessed for possible predisposition to aggressive behaviour at an earlier age. Because blood lead levels at age 13 years were skewed they were log transformed to ensure normal distribution. Using logistic regression analysis the association between blood lead levels at age 13 years and aggressive behaviour during mid-adolescence (characterized by a positive and a negative response for each aggressive behaviour item) was examined, controlling for the effect of aggressive behaviour at age 11 years old.

To determine dimensions of aggression; using binary variables of aggressive behaviour items PCA was used as the dimensionality reduction technique to create suitable principal components. PCA with varimax rotation was used to reduce the original 17 items of aggressive behaviour items. PCA was conducted separately for males and females to assess the comparability of the patterns. A model including both sexes was selected as the best model; and PCA was conducted using both sexes combined. The sample size was adequate with a Kaiser-Meyer-Olkin (KMO) score of 0.71 and Bartlett's test was statistically significant ($p<0.001$). Three principal components with an eigenvalue of > 1 were extracted. All three principal components were retained based on the Scree plot analysis, sound interpretability of the patterns, and percentage of total variance explained (32%) (Field, 2009). The retained principal components were named according to the aggressive behaviour patterns observed as demonstrated in Supplementary Table 2. Principal component 1 increased with increasing stubbornness, suspiciousness, having a hot temper, loudness, screaming, moodiness, argumentativeness, teasing others, and attention seeking; and the aggressive behaviour pattern was interpreted as a measure of 'indirect aggression' perpetrated

by the study participant. Principal component 2 increased with increased destruction of things, attacking others, meanness, threatening to hurt others, and getting into fights and the aggressive behaviour pattern was interpreted as a measure of 'direct aggression' perpetrated by the study participant. Principal component 3 increased with increasing disobedience and was interpreted as a measure of 'disobedience'. Component scores represent "a composite score for each individual on a particular factor". They inform on an individual's score on a subset of measurable variables (Field, 2009). The SPSS statistical software converts the extracted principal component scores into continuous variable which can be used in a regression analysis. The ranges for indirect aggression, direct aggression and disobey were -3.16553 to 5.82261, -3.50782 to 6.37942 and -2.39748 8.15515, respectively.

Linear regression analysis was used to assess the relationship between lead exposure and dimensions of aggressive behaviour in mid-adolescence. In Model 1 data were examined for the association between blood lead levels and aggressive behaviour controlling for sex of the study participant. In Model 2, data were re-examined for the association between blood lead levels and aggressive behaviour controlling for sex, race and possible confounding. Confounding was defined by statistical significance of ($p<0.05$) or a $\geq$ 10% difference in crude and adjusted coefficients. In addition, data were evaluated for an association between socio-demographic factors at birth and aggressive behaviour during mid-adolescence. Data analyses were conducted using STATA 14 statistical package and SPSS version 22.

2.4 Ethical issues

Ethical approval was obtained from the University of the Witwatersrand Committee for Research on Human Subjects (Medical). Only consented individuals were enrolled in the study and participants were informed of their right to withdraw at any time without penalty.

The original BT20+ cohort study received clearance from the University of the Witwatersrand Ethics Committee on Human Subjects (M010556); and the Federal Wise Assurance registration number of the University of the Witwatersrand Ethics Committee on Human Subjects is FWA00000715.

2.5 Results

2.5.1 Distribution of blood lead levels (BLLs) at age 13 years

The distribution of blood lead levels at age 13 years differed significantly between males and females ($p < 0.001$) (Table not shown). Overall, blood lead concentrations ranged from 1.0 to 28.1 µg/dL with a mean and standard deviation of 5.6 ± 2.3 µg/dL, a geometric mean of 5.1 µg/dL and a median of 5.4 µg/dL. The mean blood lead levels were 6.4 ± 2.5 µg/dL and 4.9 ±1.9 for males and females, respectively. Forty one percent of the study participants and 27.8% of males and 52.3% of females had blood lead levels < 5 µg/dL. The proportion of males, females and both with blood lead levels ranging from 5 to 9.99 µg/dL was 66.9%, 47.1% and 56.4%, respectively. Also, 5.3% of males, 0.7% of females and total sample of 2.9% had blood lead levels ≥ 10 µg/dL.

2.5.2 Characteristics of members of the analytical sample by sex

There was no difference between males and females with regard to race, maternal education at birth, maternal age at birth, residential area of birth, hospital of birth and maternal marital status at birth of the study participant. However, on average, males had higher socio-economic status than females at birth as shown in Table 2.1.

Table 2.1 Socio-demographic characteristics of the analytical sample by sex

	Males (n=508) Total (%)	**Females** (n=578) Total (%)	**Total** (n=1086) Total (%)
Race			
Black African	439 (86.4)	499 (86.3)	938 (86.3)
Mixed Ancestral	69 (13.6)	79 (13.7)	148 (13.6)
$\chi_{(1)}^2 = 0.0017$, p = 0.967, n = 1086			
Hospital of Birth			
Public	467 (91.9)	524 (90.8)	991 (91.3)
Private	41 (8.1)	53 (9.2)	94 (8.66)
$\chi_{(1)}^2 = 0.4241$, p = 0.515, n = 1085			
Place of Birth			
Soweto/Diepkloof	485 (95.5)	545 (94.3)	1030 (94.8)
Former Coloured/Asian/ Inner City /Suburban	23 (4.5)	33 (5.7)	56 (5.2)
$\chi_{(1)}^2 = 0.7721$, p = 0.380, n = 1086			
Maternal Education At Birth			
Grade7 and less	58(12.5)	64 (11.8)	122 (12.2)
Grade 8-10	232(50.1)	245 (45.3)	477 (47.5)
Grade 11-12	138 (29.8)	188 (34.8)	326 (32.5)
Post school training	35(7.6)	44 (8.1)	79 (7.9)
$\chi_{(3)}^2 = 3.3036$, p = 0.347, n = 1004			
Maternal Age at Birth			
< 20	76 (15.0)	104 (18.0)	180 (16.6)
20 – 29	282 (55.5)	305 (52.8)	587 (54.0)
30 – 39	136 (26.7)	155 (26.8)	291 (26.8)
>=40	14 (2.8)	14 (2.4)	28 (2.6)
$\chi_{(3)}^2 = 1.9936$, p = 0.574, n = 1086			

Maternal Marital Status at Birth			
Married/ Living Partner	195 (38.5)	209 (36.6)	404 (37.5)
Single/ Widowed/ Divorced/ Separated	312 (61.5)	362 (63.4)	674 (62.5)
$\chi_{(1)}^2 = 0.3961$, p = 0.529, n = 1078			
Socio-Economic Status at Birth			
Minimum	-1.598	-1.598	-1.598
Maximum	4.286	3.715	4.286
Mean	0.058	-0.051	0.000000286
$t_{(1084)} = 1.8623$, p= 0.03, n = 1086			

2.6 Aggressive behaviour by sex during mid-adolescence

Table 2.2 shows the aggressive behaviour profile in mid-adolescence stratified by sex. More females than males reported a positive response for 'I argue a lot', 'I scream a lot', 'I am stubborn', 'my moods and feelings change suddenly', 'I am louder than other kids'. On the other hand, more males than females gave a positive response to aggressive behaviour items such as 'I try to get a lot of attention', 'I destroy things belonging to others', 'I get into many fights', 'I physically attack people', 'I am suspicious', 'I tease others a lot' and 'I threaten to hurt people'.

Table 2.2 Aggressive behaviour profile in mid-adolescence by sex

	Male (%) n = 508	Female (%) n = 578	Total (%) n = 1086	P-Value
I argue a lot	144 (28.6)	195 (33.7)	339 (31.2)	0.06
I am mean to others	46 (9.1)	40 (6.9)	86 (7.9)	0.19
I try to get a lot of attention	112 (22.1)	92 (15.9)	204 (18.8)	**0.01***
I destroy my own things	16 (3.2)	11 (1.9)	27 (2.5)	0.19
I destroy things belonging to others	11 (2.2)	4 (0.7)	15 (1.4)	**0.04***
I disobey my parents	7 (1.4)	6 (1.0)	13 (1.2)	0.61
I disobey at school	12 (2.4)	8 (1.4)	20 (1.8)	0.23
I get into many fights	38 (7.5)	19 (3.3)	57 (5.3)	**0.002***
I physically attack people	15 (2.9)	6 (1.0)	21 (1.9)	**0.02***
I scream a lot	19 (3.7)	79 (13.7)	98 (9.0)	**<0.001***
I am stubborn	51 (10.0)	136 (23.5)	187 (17.2)	**<0.001***
My moods and feeling change suddenly	94 (18.5)	151 (26.1)	245 (22.6)	**0.003***
I am suspicious	141 (27.8)	110 (19.0)	251 (23.1)	**0.001***
I tease others a lot	74 (14.6)	35 (6.1)	109 (10.0)	**<0.001***
I have a hot temper	78 (15.4)	95 (16.4)	173 (15.9)	0.63
I threaten to hurt people	23 (4.5)	6 (1.0)	29 (2.7)	**<0.001***
I am louder than other kids	50 (9.8)	88 (15.2)	138 (12.7)	**0.01***

Chi-square test used to determine statistical difference between males and females
* signifies statistically significant differences

2.7 Is the aggressive behaviour exhibited during mid-adolescence influenced by a predisposition to aggressive behaviour during early adolescence?

To examine if aggressive behaviour shown in this study during mid-adolescence could be influenced by a predisposition to aggressive behaviour at an earlier age (age 11 years), the association between blood lead levels at age 13 years and aggressive behaviour in mid-adolescence was examined adjusting for the effect of aggressive behaviour during early adolescence as shown in Table 2.3.

After adjusting for the effect of aggressive behaviour at age 11 years, sex, race and SES; study participants who responded positively for aggressive behaviour item 'I argue a lot' during mid-adolescence were 34% less likely to have increased blood lead concentration levels at age 13 years old compared to those who gave a negative response. On the contrary, the odds of having increased blood lead levels were 3.7 times higher for study participants who responded positively for aggressive behaviour item 'I threaten to hurt other' compared to those who responded negatively to the same question. Therefore, the results show that aggressive behaviour in mid-adolescence is not influenced by a predisposition to aggressive behaviour during early adolescence.

Table 2.3 Odds Ratios for blood lead levels at age 13 years and aggressive behaviour during mid-adolescence controlling for aggressive behaviour during early adolescence

OUTCOME	EXPOSURE			
Aggressive Behaviour Items	**Blood Lead Levels at 13 years old**			
	Unadjusted OR (95% CI)	p-value	Adjusted OR (95%CI)	p-value
I argue a lot	0.68 (0.47 - 0.97)	0.036	0.66 (0.46 - 0.96)	0.03
I threaten to hurt people	3.74 (1.46 - 9.56)	0.006	3.75 (1.46 - 9.59)	0.006

2.8 Association between gender and blood lead levels at age 13 years

Data were further analyzed for an association between gender and blood lead levels. Again, data confirmed that being male was associated with blood lead levels at age 13 years compared to being female as shown in Supplementary Table 2.4.

Table 2.4 Association between gender and blood lead levels

Exposure variable	Outcome variable		
Gender		**BLLs**	
	β	Std error	P-value
Female			
Male	1.52	0.133	<0.000

2.9 Association between blood lead levels at age 13 years and Direct Aggressive Behaviour, Indirect Aggressive Behaviour and Disobedience during mid-adolescence

Data were analyzed for the association between blood lead levels at age 13 years and aggressive behaviour during mid-adolescence using PCA derived components to assess dimensionality of direct and indirect aggressive behaviour, and disobedience. As shown in

Table 2.5, by comparison blood lead levels < 5 μg/dL versus blood lead levels > 10 μg/dL were positively associated with direct aggression after adjusting for the effects of sex, race, maternal age and education at birth of the study participant. Indirect aggressive behaviour was not significantly associated with lead exposure.

Furthermore, after adjusting for the effect of race and SES at birth compared to being female, being male on average was negatively associated with 'indirect aggression'; but positively associated with 'direct aggression'. Additionally, being born to a mother who is single, widowed, divorced or separated (adjusting for sex and race), 'disobedience' decreased by 0.15 on average during mid-adolescence. Birth to a mother with a higher level of education was protective of direct aggressive behaviour in mid-adolescence, adjusting for the effect of sex, race and maternal age at birth. After controlling for the effect of sex, race and maternal marital status at birth, birth to mothers in their 20's and 30's was protective of direct aggressive behaviour during mid-adolescence compared to children born to teenage mothers. Socio-economic status was negatively associated with indirect aggressive behaviour, controlling for the effect of sex and race.

Table 2.5 Association between blood lead levels & socio-demographic factors and aggressive behaviour: Linear Regression Analysis

EXPOSURE VARIABLES	OUTCOME VARIABLES																	
	Model 1 (Unadjusted Coefficients)									Model 2 (Adjusted Coefficients)								
	Indirect Aggression			Direct Aggression			Disobedience			Indirect Aggression			Direct Aggression			Disobedience		
	β	Std Error	p-value	β	Std Error	p-value	β	Std Error	p-value	β	Std Error	p-value	β	Std Error	p-value	β	Std Error	p-value
BLL																		
<5 μg/dL= (low – reference category)																		
5 – 9.99μg/dL	0.004	0.06	0.95	-0.10	0.06	0.11	0.08	0.07	0.22	0.01	0.06	0.94	-0.07	0.06	0.28	0.10	0.07	0.14
≥10μg/dL	0.26	0.19	0.17	0.37	0.18	**0.04**	-0.26	0.19	0.19	0.26	0.18	0.16	0.43	0.18	**0.02**	-0.28	0.20	0.17
R^2	0.0149			0.0390			0.0042			0.0189			**0.0516**			0.0121		
Sex																		
Female																		
Male										-0.21	0.06	**<0.001**	0.34	0.06	**<0.001**	0.01	0.06	0.92
Maternal education at birth																		
Grade7 and less																		

Grade 8-10				-0.11	0.10	0.27	-0.11	0.10	0.29	0.11	0.11	0.32
Grade 11-12				-0.11	0.11	0.33	-0.23	0.10	**0.04**	0.08	0.11	0.45
Post school training				-0.22	0.15	0.14	-0.20	0.14	0.15	0.28	0.15	0.07
Maternal age at birth												
<20 (teen moms)												
20 - 29				-0.01	0.09	0.93	-0.18	0.09	**0.04**	0.02	0.09	0.80
30 - 39				-0.003	0.10	0.98	-0.24	0.10	**0.02**	0.03	0.11	0.80
≥ 40				-0.05	0.20	0.80	-0.31	0.20	0.12	-0.28	0.21	0.18
Marital Status												
Married/Living with partner												
Single/Widowed/ Divorced/Separated				0.10	0.06	0.10	-0.04	0.06	0.56	-0.15	0.07	**0.02**
Hospital of Birth												
Public												
Private				0.09	0.11	0.40	-0.17	0.11	0.10	0.05	0.11	0.67
SES				-0.07	0.03	**0.04**	0.03	0.03	0.37	-0.05	0.03	0.16

Bold signals statistical significance

2.10 Discussion

This study investigated the association between lead exposure in early adolescence and aggressive behaviour during mid-adolescence in the BT20+ cohort in Johannesburg, South Africa. Elevated blood lead levels were positively associated with direct aggression in mid-adolescence among Black African and Mixed ancestral youth. These study findings are highly significant and vital in that they show that lead exposure is associated with the most severe form of aggressive behaviour in this study which is 'direct aggression' and not associated with indirect aggression. In addition, our results also showed that the associated link between blood lead levels and aggressive behaviour during mid-adolescence is not confounded by a predisposition to aggressive behaviour at an earlier age. After adjusting for the effect of aggressive behaviour during early adolescence and covariates, lead exposure in early adolescence was shown to almost quadruple the risk to 'threaten to hurt others', during mid-adolescence - lending support to the type of aggressive behaviour 'direct aggression' associated with environmental lead exposure in South African youth. Stretesky and Lynch linked lead exposure to homicide "the most extreme outcome associated with aggression" (Stretesky and Lynch, 2001). As mentioned in the introduction section, lead exposure is associated with aggressive behaviour in early adolescence in South Africa (Naicker et al., 2012). As such, in addition to identifying the nature of aggressive behaviour associated with lead exposure in mid-adolescence; our results signal to what should possibly be a worrying trend of aggressive antisocial behaviour among the country's adolescents. Likewise, other international epidemiological studies have shown an association between lead exposure and aggressive and/or anti-social behaviour (Byers and Lord, 1943, Denno, 1990, Needleman et al., 1996, Needleman et al., 2002, Dietrich et al., 2001, Stretesky and Lynch, 2001, Wright et

al., 2008, Mazumdar et al., 2011). However, there is dearth of reliable data from developing countries regarding this major public health problem.

The identification of specific type(s) of aggressive behaviour associated with environmental lead exposure in South African youth is important because it highlights: i) the violent nature of aggressive behaviour associated with lead exposure ii) possible environmental toxicological contribution to 'contact crime' in the country iii) possible environmental lead contribution to the national burden of disease in the country iv) its negative impact on the country's economy and v) the need for appropriate modulating mechanisms when implementing measures to combat aggressive behaviour in adolescence.

Sex differences in blood lead levels found in this study were consistent with those previously reported in international studies, in the United States (Lanphear et al., 2000, Muntner et al., 2005), Korea (Kim et al., 2017) and Sweden (Bárány et al., 2002); where males had higher blood lead concentrations than females. Given that factors associated with increased levels of absorption, distribution and excretion of pharmacological agents are greater in men than women (Soldin and Mattison, 2009); this may explain the observed increased levels of blood lead in males. On average males have higher body mass, length and surface area, intracellular and extracellular water, total body water, greater pulmonary function, renal clearance and cardiac output compared to females (Soldin and Mattison, 2009). Lead acts as a pharmacological agent in the central nervous system resulting in pharmacological effects (Mason et al., 2014) and anatomic and physiological differences between males and females are known to influence the pharmacokinetics and pharmacodynamics of pharmacological agents in the body (Soldin and Mattison, 2009).

In addition, the positive association between being male and direct aggression, and being female and indirect aggression found in this study speaks to sex differences in the manifestation of aggressive behaviour among South African adolescents. Our results are consistent with those reported in a Finish study of 8, 11 and 15 year olds, where indirect aggression was prevalent in girls aged 11 and 15 years and physical aggression more common in boys of all three age groups (Bjrkqvist et al., 1992). Similarly, meta-analytic reviews of aggression and sex differences show evidence of physical and direct aggression in the male direction (Archer, 2004, Campbell, 2006). Bjrkqvist et al. (1992) posit that the indirect aggression patterns associated with girls could be due to the fact that teenage girls are known to mature verbally faster than their male counterparts, which in-turn may facilitate verbal aggression as opposed to physical aggression (Bjrkqvist et al., 1992). However, a British study found no difference in indirect aggression between males and females (Forrest et al., 2005). Nonetheless, our results suggest that to effect change it is important that public health policymakers and other relevant stakeholders take cognisance of the need for sex-tailored programs when implementing measures to combat aggressive behaviour among adolescents.

Furthermore, socio-demographic factors were associated with aggressive behaviour among adolescents in this study. Parental educational level, a proxy for socio-economic status predicts children's attainment later life (Haveman and Wolfe, 1995, Davis-Kean, 2005). Low parental educational levels are associated with behaviour problems such as aggressive behaviour in children (Dubow et al., 2009). In line with these predictions, in this study birth to a mother with a higher educational level was negatively associated with direct aggressive behaviour and better socio-economic status at birth was protective of indirect aggressive behaviour in mid-adolescence. Also, there was a negative association between maternal age

(older) at birth and direct aggressive behaviour in mid-adolescence; consistent with other reports where a higher proportion of children born to a younger mother or younger mothers at first birth displayed aggressive and conduct disorder problems (Wakschlag et al., 2000, Nagin and Tremblay, 2001, Tremblay et al., 2004). Surprisingly, birth to a single mother headed home was negatively associated with disobedience in mid-adolescence in this study, contrary to findings by Ryan et. al. (2013) showed that children from low or middle income homes who experienced changes in family structure from two-parent homes to single parent homes exhibit higher behaviour problems than those who experienced no changes in their family structures (Ryan et al., 2013). Anecdotal evidence suggests that children born to single parents in some South African communities are usually raised in extended family settings where in fact they may be exposed to much stronger social support and stability. Therefore, from a South African point of view we predict that this may explain the seemingly lower acts of disobedience in adolescents from single parent homes found in this study. In support of this, South African census data from 2001 showed that in Black African and Coloured households, there were more single parents living with extended family than those living on their own (Amoateng et al., 2007).

As mentioned earlier, there is a paucity of empirical data from low or middle-income countries regarding health and behavioural effects of lead exposure. Findings from this study will help address this gap in knowledge. The implications of these findings are far reaching in that they speak to the biological disruption of normal developmental processes, particularly in children from low income communities as they are the most affected. As such, childhood lead exposure and the associated 'direct' aggressive behaviour in South African young people is not only a major public health problem but a socio-political problem as well.

There were some limitations to this study. Firstly, a much more inclusive study sample is required to evaluate further the adverse health effects of lead exposure in children among all different South African ethnic groups, especially in this post-apartheid era. In the past the white population group had relatively low blood lead levels (von Schirnding et al., 1991); and there is dearth of information regarding lead exposure among Indian/ Asian population groups in South Africa.

Secondly, this was a cross sectional study. As such, we were not able to control for variables such as nutrition or progesterone which may contribute to blood lead levels or aggressive behaviour, respectively (Olweus et al., 1988, Archer, 2004, WHO, 2006). These data were not available for this study's specified timelines.

Thirdly, the YSR was the only aggressive behaviour scale used to measure aggressive behaviour. Use of more than one measuring tool would have helped in this study to assess the consistency or lack thereof of aggressive behaviour patterns within the study group.

Fourthly, blood lead levels were only measured at one time point; measuring recent lead exposure. For future studies we recommend using blood lead levels and bone lead levels so that associated aggressive behaviour outcome can be examined for both recent and cumulative lead exposure.

Finally, data for testosterone levels were not available for the timelines examined in this study, as such, testosterone levels were not adjusted for in the models. The question that may arise is whether higher testosterone levels in males may predispose them to 'direct aggression'. Studies are inconclusive on this matter; some have shown that there is no evidence that higher testosterone levels facilitate aggressive behaviour in males (Archer,

2004), but nonetheless suggest that testosterone levels do influence the intensity and/ or frequency of aggressive responses when provoked or threatened (Olweus et al., 1988).

2.11 Conclusion

This study examined the role of environmental lead exposure in aggressive behaviour during mid-adolescence among young male and female South Africans. The results showed that there is a positive association between elevated blood lead levels and direct aggression in South African adolescents. There was a positive relationship between adolescent males and direct aggressive behaviour, but a negative association with indirect aggressive behaviour. In contrast, indirect aggressive behaviour was positively associated with the female sex. As such, our findings show how environmental lead exposure potentially contributes to anti-social behaviour patterns among South African youth. Our results also showed a link between socio-demographic factors at birth and aggressive behaviour during mid-adolescence, which is imperative in that it shows an indirect role of socio-demographic factors in the aggressive behaviour of adolescents. Further investigation to ascertain whether the identified aggressive behaviour in early and mid-adolescence associated with lead exposure can escalate to violent behaviour in late adolescence and indeed adulthood is essential, since a correlation does not imply causation.

Supplementary Table 2a **Factor-loading matrix for the Socio-Economic Status (SES) derived from varimax rotation - Principal Component Analysis**

	Principal Component (SES)
	1
Home ownership	**.106**
Access to water inside the dwelling	**.178**
Sole use of water dwelling	**.221**
Access to flush toilet	**.060**
Sole use of toilet	**.205**
Access to electricity	**.149**
Ownership of television	**.199**
Ownership of a motor car	**.155**
Ownership of a refrigerator	**.230**
Ownership of a washing machine	**.153**
Ownership of a telephone	**.218**

Supplementary Table 2b **Factor-loading matrix for the aggression patterns derived from varimax rotation - Principal Component Analysis**

	Principal Components		
	Indirect Aggression	**Direct Aggression**	**Disobedience**
I am stubborn	**.575**		
I am suspicious	**.545**		
I have a hot temper	**.539**		
I am louder than other kids	**.515**		
I scream a lot	**.515**		
My moods or feelings change suddenly	**.473**		
I argue a lot	**.445**		
I tease others a lot	**.414**		
I try to get a lot of attention	**.324**		
I destroy things belonging to others		**.601**	
I physically attack others		**.547**	
I am mean to others		**.469**	
I destroy my own things		**.416**	
I threaten to hurt others		**.396**	
I get into a lot of fights		**.337**	
I disobey at school			**.723**
I disobey my parents			**.695**

CHAPTER 3

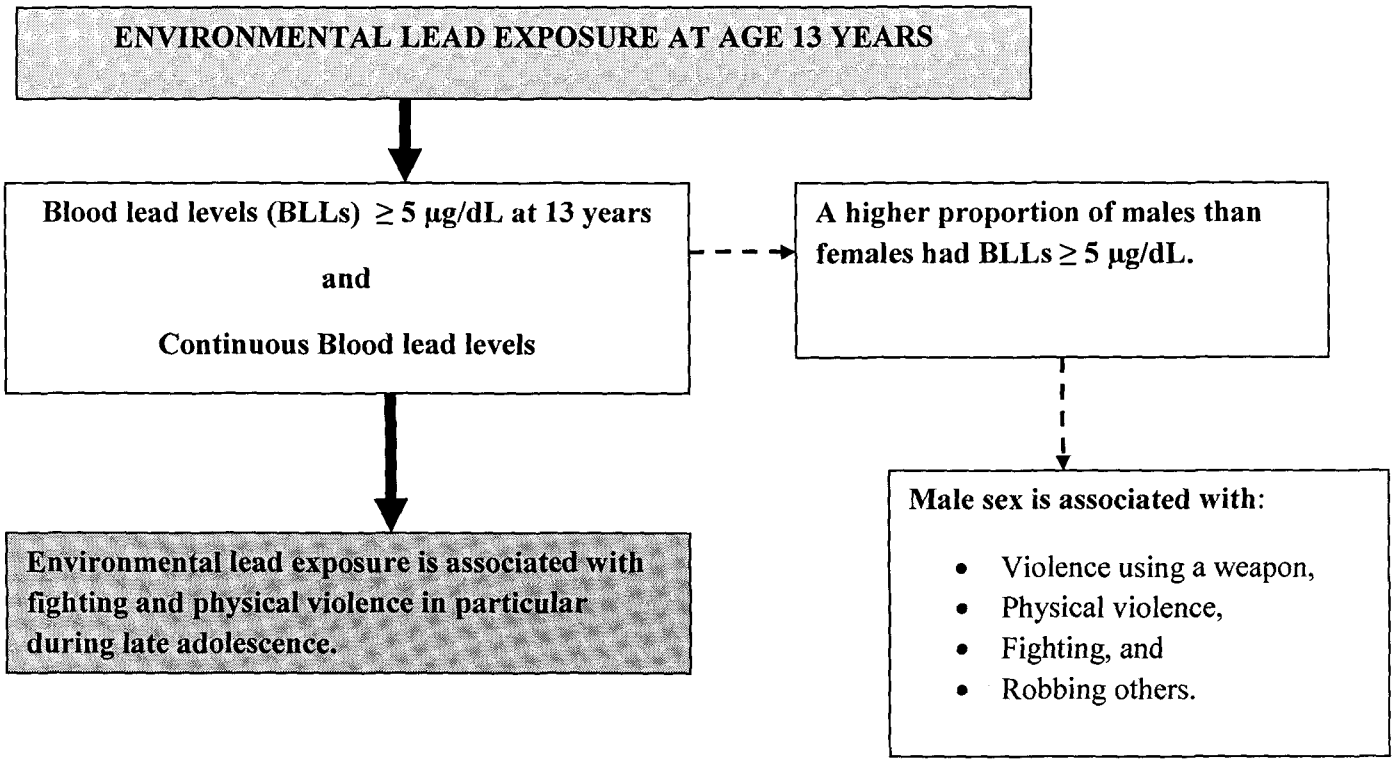
The association between elevated blood lead levels and violent behaviour during late adolescence: the South African Birth to Twenty Plus Cohort

Publication journal: ***Environment international.*** **2017 Dec 1; 109:136-45.**

This journal article was published in the Environment International, it aims to answer objective 3 of the thesis. The association between blood lead levels at 13 years and violent behaviour in late-adolescence was assessed using components derived using Principal Component Analysis. This chapter includes additional analyses conducted after the journal article was published.

A copy of the published journal article is attached in the Appendices (Appendix B).

Figure 3.1 GRAPHIC ABSTRACT: the association between elevated blood lead levels and violent behaviour during late adolescence: the South African Birth to Twenty plus Cohort



3.1 Introduction

Lead is one of ten chemicals identified by the World Health Organization (WHO) as being of "major public health concern" and in need of action by Member States (World Health Organization, 2010b). Approximately 600 000 new cases of children with intellectual disability are attributed to childhood lead exposure annually (Prüss-Ustün et al., 2011). In recent decades there has been a steady increase in epidemiological studies showing a possible link between childhood lead exposure and lower socio-economic status (Morrens et al., 2012); altered pubertal development in girls and boys (Naicker et al., 2010a, Williams et al., 2010, Den Hond et al., 2011); and intellectual impairment and antisocial behaviour (Needleman et al., 1979, Needleman and Gatsonis, 1990, Needleman et al., 2002, Bellinger et al., 1992, Dietrich et al., 2001, Canfield et al., 2003, Lanphear et al., 2005, Wright et al., 2008) among others.

Contemporary research studies involving brain-imaging show possible neuro-anatomical bases underlying the neuro-behavioural changes associated with lead exposure. In a Cincinnati Lead study, analyses of childhood lead exposure and adult brain volume using magnetic resonance imaging (MRI) showed that elevated mean childhood blood lead levels were significantly associated with 1.2% reduction of the grey matter ($p<0.001$) (Cecil et al., 2008). The affected areas of the brain included prefrontal cortical areas such as the "medial and superior frontal gyri" with the "ventrolateral prefrontal cortex and anterior cingulated cortex", and in the "postcentral gyri, the inferior parietal lobule", and the cerebellar hemispheres. It is important to note that this grey matter loss was only significant among males (Cecil et al., 2008). Childhood lead exposure has also been reported to alter the integrity of white matter in adulthood (Brubaker et al., 2009). Other brain imaging studies

have supported these findings (Stewart et al., 2006, Cecil et al., 2011, Caffo et al., 2008, Brubaker et al., 2010, Schwartz et al., 2010), suggesting that exposure to lead changes the structure and function of the brain, affecting executive functions and consequently resulting in neuro-behavioural changes such as violent behaviour. Prefrontal cortex dysfunction is associated with aggressive and violent behaviour (Brower and Price, 2001, Siever, 2008, Hawkins and Trobst, 2000, Grafman et al., 1996).

In South Africa lead has been used in, amongst other items, petrol, paint, batteries, solder, electrical appliances, fishing weights and road markings (Mathee et al., 2009). Lead continues to be used in traditional medicines (Mathee et al., 2015), and leaded ammunition (Mathee, 2014, Mathee et al., 2017) amongst others. Given that South Africa has a long history of blood lead concentrations above the Centers for Disease and Control and Prevention (CDC)'s recommended reference level of 5 µg/dL in children (von Schirnding et al., 1991, von Schirnding et al., 2003, Mathee et al., 2006, Naicker et al., 2010b, Mathee et al., 2013) and violent behaviour characterized by physical violence, violence using a weapon, bullying, emotional violence and sexual violence during adolescence (Burton and Leoschut, 2012, Mncube and Harber, 2013), there is good reason to examine the association between childhood lead exposure and violent behaviour among young people. In addition, in view of the fact that 98% of children exposed to lead live in low or middle income countries such as South Africa (World Health Organization, 2009), it is vital that more research is conducted in these communities to examine its deleterious health effects. Currently, most of the empirical data demonstrating the detrimental effects of lead exposure comes from the developed countries. More locally-generated empirical data are essential to inform decisions and policies related to prevention and control of lead exposure in South Africa and other low or middle income countries. To our knowledge, no study has been conducted in South Africa

showing the relationship between lead exposure and violent behaviour during adolescence. In this study we hypothesized that there is an association between lead exposure at 13 years old and violent behaviour during late adolescence in South Africa. Principal Component Analysis (PCA) derived components were used to determine the type or types of violent behaviour associated with lead exposure among South African adolescents.

3.2 Materials and methods

3.2.1 Study Population

Study participants were selected from the Birth to Twenty Plus (BT20+) cohort in Johannesburg, South Africa. BT20+ is the largest and longest running birth cohort in Africa. It was initiated at the cusp of democracy in South Africa with the intention to address the foreseeable health problems as a result of heightened demand for access to health care services in urban areas due to increased urbanization. A total of 3273 study participants were enrolled in the birth cohort from 23 April to 8 June 1990. The cohort is representative of the South African racial demographics as defined by the *"Apartheid"* system[1]; comprised of 78% Black Africans, 6% Whites, 12% Coloureds and 4% Indians. Initially the White population group was under-represented mainly because during the time of enrollment most White families used the private health practitioners and facilities. This imbalance was later rectified at age 10 years by enrolling a supplementary sample of 120 White children born during the cohort enrolment dates.

[1]The racial categories Black African, White, Coloured (mixed ancestral descent) and Indian/ Asian were enforced through legislation in Apartheid South Africa. Even though they are no longer enforced, to a great extent they remain part of South African vocabulary.

Even though the cohort has a very low attrition rates, White families have shown a higher attrition compared to others. The study is still in contact with more than 70% of the original study participants. For more details regarding the cohort, see (Richter et al., 2007, Richter et al., 2004).

Of the 3273 BT20+ study participants, 1457 had data for blood lead levels at age 13 years and 2004 had data for violent behaviour at ages 15/16 years. Availability of study participants during data collection cycles was one of the main contributing factors with regard to reduced numbers for data collected at these time points for blood samples and violent behaviour. To be included in the current study, study participants needed to have blood lead measurements at 13 years old and violent behaviour data collected at age 15 to 16 years (late adolescence). With these criteria, a total of 1332 study participants (684 females) comprised of 87.2% Black African and 10.4% Coloured adolescents were included in the study. White and Indian study participants were excluded due to very low numbers, 1.54% and 0.88% respectively.

3.2.2 Blood lead measurement

Venous samples of whole blood were collected at age 13 years into EDTA-containing tubes. Blood sampling was undertaken by professional health officials, using sterile equipment and aseptic techniques. Blood sample analysis was conducted as described in (Nkomo et al., 2018).

3.2.3 Measurement of violent behaviour in late adolescence and socio-demographic factors

Data on violent behaviour were collected in the 15th year data collection waves using the Youth Self Report (YSR) questionnaire. Information for the YSR questionnaire for violent behaviour was collected at two time points, 11/12 and 15/16 years old. Study participants were contacted by telephone at home; work, or through nominated contactable family members or friends to secure appointment dates for data collection. Study participants came to the BT20+ data collection site and were compensated a minimum of R50 for transport. The YSR questionnaires were administered by trained field workers - most of whom have been with the cohort since its inception and have a very long trusting relationship with the study participants (Richter et al., 2004).

The YSR is a self-report questionnaire comprising 112 items assessing behavioural competency and problems of children and adolescents aged 11 to 17. It assesses aggressive and oppositional behaviour attention seeking problems, as well as psychotic, impulsive, social interaction, and conduct problems among others (Achenbach, 1991). Regarding the sensitivity and specificity of the YSR questionnaire, the Achenbach System of Empirically Based Assessment (ASEBA) scales for internalizing and externalizing for YSR are 0.90 for alpha, 0.85 for test-retest reliability and 0.56 for long term stability for the United States. In general psychometric results from different cultural backgrounds have approximated those from the United States (Achenbach et al., 2008). Furthermore, YSR was used in adolescents from different cultural backgrounds to compare ratings for self-reported behavioural and emotional problems. The selected 7,137 adolescents from the general population samples were from the United States, Turkey, Australia, Netherlands, China, Jamaica and Israel. The

effect size of culture was very small (4%) for externalizing problems. Across all seven countries sex differences were similar for externalizing problems, but higher for girls compared to boys (Verhulst et al., 2003). In general, YSR has been validated and has been used in many populations including South Africa (Verhulst et al., 2003, Ivarsson et al., 2005, Cluver et al., 2007, Sabet et al., 2009, Naicker et al., 2012).

The violent behaviour variable comprised 21 items with four response options: never, once or twice, a few times, and many times. The first eleven questions assessed violent behaviour perpetrated by the study participant at school and an additional 10 similar questions pertained to violent behaviour perpetrated outside of school. The four response options were collapsed into two: 'negative response' denoted by never and 'positive response' denoted by once or twice, a few times, and many times. These 2 categories were selected to reflect whether the study participant has propensity to perpetrate violent behaviour.

Socio-demographic factors including sex; maternal education at birth of the cohort child, categorized into four levels, i.e. grade 7 or less, grade 8-10, grade 9-12 and post school education; maternal marital status at birth of the child divided into two categories, married/living with a partner, or single/widowed/divorced/separated; maternal age at birth was divided into four categories < 20 years old (representing teenage mothers), 20 – 29 years old (representing younger mothers in their 20's), 30 – 39 years old (representing older mothers in their 30's) and ≥ 40 years old (representing mothers 40 and above); residential area of birth was dichotomized into Soweto/Diepmeadow or former Coloured/Asian and Inner City/Suburb, hospital of birth was categorized as public or private. Socio-economic status (SES) was calculated using binary items of household commodities at birth of the

child, including home ownership, type of home, access to water inside the dwelling, sole use of water, access to flush toilet, sole use of toilet, ownership of television, motor car, fridge, washing machine and telephone. PCA with varimax rotation was used as the dimensionality reduction technique to create an SES variable. The sample size was adequate with a Kaizer-Meyer-Olkin score of 0.68 and the Bartlett's test was statistically significant ($p < 0.001$). One PCA component was extracted based on Scree Plot analysis and eigenvalue of > 1.

3.2.4 Statistical analysis

Blood lead concentration levels were categorized into three levels: <5 μg/dL (used as a reference category), 5 – 9.99 μg/dL and ≥10 μg/dL. The use of < 5 μg/dL as the reference level is in line with the recommendations by the Advisory Committee for Childhood Lead poisoning Prevention (ACCLPP) of the CDC to use the reference value of 5 μg/dL based on the 97.5th percentile of the current blood lead level distribution among children aged 1 to 5 years in the United States of America (Centers For Disease Control and Prevention, 2012b). Continuous blood lead concentration were skewed and were natural log-transformed for normality.

The distribution of violent behaviour was stratified by sex. For categorical variables, Chi-square tests were performed to establish the significance of association and for continuous variables, t-tests were used to compare the means.

Using SPSS composite variables for violent behaviour were derived using PCA. PCA with varimax rotation was used to reduce the data. Analyses were performed on the correlation matrix. The sample size was adequate with a Kaiser-Meyer-Olkin (KMO) of 0.84 and the Bartlett's test was statistically significant ($p < 0.001$). Six factors with an eigenvalue > 1 were

extracted. They were all retained based on the Scree plot analysis and sound interpretability of the patterns (Fields, 2009). The retained principal components accounted for 67.1% of the total variance explained. They were named according to the violent behaviour patterns observed, violence using a weapon, physical violence, fighting, sexual harassment, robbing and verbal & emotional abuse. All the retained principal components showed good internal consistency with Cronbach's alpha coefficients for violence using a weapon, physical violence, fighting, sexual harassment, robbing and verbal & emotional abusive of 0.88, 0.81, 0.53, 0.80, 0.83, and 0.78, respectively. Component scores represent "a composite score for each individual on a particular factor". They inform on an individual's score on a subset of measurable variables. Factor scores can be used in regression analysis (Fields, 2009). *Supplementary Table 3a* shows factor loadings for the violence patterns. The ranges for 'violence using a weapon', 'physical violence', 'fighting', 'sexual harassment', 'robbing' and 'verbal and emotional abuse' components were -2.5454 to 11.9719, -1.9151 to 4.6136, -4.14016 to 8.42092, -3.28421 to 11.55645, -4.83406 to 7.4105 and -2.71956 to 4.56067, respectively.

First, sex comparison of outcomes of individual violent behaviour items during late adolescence (i.e. positive versus negative) with respect to geometric mean blood lead levels in early adolescence using bivariate analysis was conducted. Second, data were assessed for possible influence of sex and socio-demographic factors at birth on violent behaviour during late adolescence controlling for blood lead levels at age 13 years and race. Statistically significant ($p < 0.05$) covariates were included in the linear regression models that sought to examine the association between lead exposure during early adolescence and the six violent behaviour components extracted using PCA, accordingly. Confounding was also defined as a difference of 10% or more in coefficients. Data were examined for an association between

elevated BLLs and violent behaviour in late adolescence controlling for sex and race first (Model 1). In Model 2 the association was further examined to see if the associations still held after controlling for sex, race and covariates as possible confounders. Furthermore, data were analyzed using continuous BLLs adjusting for possible confounding as above in Models 1 and 2. Data analyses were conducted using the STATA 14 statistical package and SPSS version 20.

3.3 Ethical oversight

Permission to conduct the study was sought from the BT20 Plus birth cohort and their parents. Only consented individuals were enrolled and participants were informed of their right to withdraw at any time without being penalized. Clearance was sought and granted from the University of the Witwatersrand University Ethics Committee on Human Subjects (M010556). The Federal Wise Assurance registration number of The Witwatersrand University Ethics Committee on Human Subjects is FWA00000715.

3.4 Results

3.4.1 Characteristics of members of the analytical sample and BT20+ members excluded from the analytical sample

First, data were checked if systematic bias was introduced during the selection of study participants. The study sample was compared to the BT20+ cohort members excluded from the study as shown in *Supplementary Table 3b*. The distribution of males to females was similar with no statistically significant difference between the two samples. However, study participants were more likely to be Black African than Mixed ancestry; born in a public hospital than private, born in Soweto/ Diepmeadow/ Former Indian/Mixed ancestry areas,

born to mother less than 20 years old, born to mothers with secondary level education, and born to mothers who are either single, widowed, divorced or separated from the partner.

3.4.2 Characteristics of the analytical sample by sex

In the analytical sample there was no statistically significant difference between males and females with regard to maternal education at birth, maternal age at birth, residential area of birth, hospital of birth, maternal marital status at birth and SES of the study participant as shown in Table 3.1.

Table 3.1 Socio-demographic characteristics of the analytical sample by sex

Socio-Demographic Factor	Male (n= 648) Total (%)	Female (n=684) Total (%)	Total (n= 1332) Total (%)	P-value
Race				0.49
Black African	575 (88.7)	615 (89.9)	1190 (89.3)	
Mixed Ancestral	73 (11.3)	69 (10.1)	142 (10.7)	
Hospital of Birth				0.58
Private	46 (7.1)	54 (7.9)	100 (7.5)	
Public	602 (92.9)	629 (92.1)	1231 (92.5)	
Place of Birth				0.32
Soweto/Diepkloof	623 (96.1)	650 (95.0)	1273 (95.6)	
Former Mixed Ancestral/Asian/Inner City/Suburb	25 (3.9)	34 (5.0)	59 (4.4)	
Maternal Education at Birth				0.42
Grade 7 or less	71 (11.8)	84 (13.1)	155 (12.5)	
Grade 8 – 10	300 (50.1)	291 (45.3)	591 (47.6)	
Grade 11 – 12	185 (30.9)	218 (34.0)	403 (32.5)	
Post school education	43 (7.2)	49 (7.6)	92 (7.4)	
Maternal Age at Birth				0.63
< 20	104 (16.0)	125 (18.3)	229 (17.2)	
20 – 29	362 (55.9)	360 (52.6)	722 (54.2)	
30 – 39	168 (25.9)	183 (26.8)	351 (26.4)	
≥40	14 (2.2)	16 (2.3)	30 (2.2)	
Maternal Marital Status at Birth				0.37
Married/ Living with partner	227 (35.1)	222 (32.8)	449 (33.9)	
Single/ Widowed/ Separated/ Divorced	419 (64.9)	455 (67.2)	874 (66.1)	
Socio-Economic Status (SES) at Birth				0.76
Minimum	-3.391	-3.186	-3.391	
Maximum	1.979	1.979	1.979	
Mean(SD)	-0.025(1.02)	0.023(0.98)	0.0000003(1.0)	

Chi-square test used to determine statistical significance between males and females ($p<0.05$)

3.4.3 Distribution of blood lead levels at age 13 years by sex

The mean and median whole blood lead levels were much higher in males than females (Table 3.2). The proportion of study participants with BLLs ≥5 μg/dL was relatively high at 62%, of these; more than 75% were males.

Table 3.2 Blood lead levels at 13 years old

	Males (%)	Females (%)	Total (%)	P-value
Blood lead categories (μg/dL)*				<0.0001
<5 (reference level)	161 (24.8)	342 (50.0)	503 (37.8)	
5–9.99	449 (69.3)	334 (48.8)	783 (58.8)	
≥10	38 (5.9)	8 (1.2)	46 (3.4)	
Blood lead levels (μg/dL)				
Mean (SD)**	6.55 (2.60)	5.02 (1.96)	5.76 (2.42)	<0.0001
Range	1.3 – 28.1	1.0 – 16.3	1.0 -28	
Q1	5.0	3.54	4.16	
Median***	6.35	4.90	5.62	<0.0001
Q3	7.91	6.25	7.08	

*Chi-square test used to determine statistical significance between males and females.
**t-test used to determine statistical significance between males and females.
***Nonparametric equality-of-medians test used to determine statistical significance between males and females.

3.4.4 Violent behaviour items stratified by sex during late adolescence

The differences between the proportion of males and female who gave a positive response for perpetration of violence in school and outside of school are outlined in Table 3.3. For all the violent behaviour items, the proportion of males perpetrating violence was higher than females except for one question where they were asked if they have "verbally or emotionally abused someone" i.e. calling someone names or having things said to them that make them feel bad about themselves or afraid where a slightly higher proportion of females (16.1%)

responded positively versus 15.6% of males, the results were however not statistically significant. Males showed highest levels of violent behaviour for violent behaviour items 'hit or kicked someone', 'pushed or shoved someone when angry', 'badly beaten up someone' - both at school and outside of school - and suspension from school. Although at a lower scale, the same pattern was observed in females except for the behaviour item 'badly beaten up someone'. Sixteen males and one female admitted to having "threatened someone with a gun" at school and outside of school ($p<0.001$). Eleven males and two females reported that they had "attacked someone with a knife or a sharp weapon" at school and fourteen males and three females admitted to have done the same outside of school ($p<0.05$).

Table 3.3 Violent behaviour profile in late-adolescence by sex

Positive Response for Violence Behaviour	Male (%) n = 648	Female (%) n = 684	Total (%) n = 1332	P-Value
Violence at school				
Hit or kicked someone	304 (46.91)	171 (25.00)	475 (35.66)	**<0.001***
Pushed or shoved someone when angry	345 (53.24)	272 (39.77)	617 (46.32)	**<0.001***
Badly beaten someone up	75 (11.57)	39 (5.70)	114 (8.56)	**<0.001***
Threatened someone with a knife or sharp weapon	23 (3.55)	9 (1.32)	32 (2.40)	**0.01***
Attacked someone with a knife or sharp weapon	11 (1.70)	2 (0.29)	13 (0.98)	**0.01***
Threatened someone with a gun	16 (2.47)	1 (0.15)	17 (1.28)	**<0.001***
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	98 (15.12)	110 (16.08)	208 (15.62)	0.63
Sexually harassed someone	20 (3.09)	4 (0.58)	24 (1.80)	**0.001***
Robbed someone	47 (7.25)	14 (2.05)	61 (4.58)	**<0.001***
Been suspended from school	96 (14.81)	57 (8.33)	153 (11.49)	**<0.001***
Gotten into a fight after drinking or getting high	33 (5.09)	9 (1.32)	42 (3.15)	**<0.001***
Violence outside of school				
Hit or kicked someone	256 (39.51)	128 (18.71)	384 (28.83)	**<0.001***
Pushed or shoved someone when angry	282 (43.52)	201 (29.39)	483 (36.26)	**<0.001***
Badly beaten someone up	88 (13.58)	31 (4.53)	119 (8.93)	**<0.001***
Threatened someone with a	30 (4.63)	6 (0.88)	36 (2.70)	**<0.001***

knife or sharp weapon				
Attacked someone with a knife or sharp weapon	14 (2.16)	3 (0.44)	17 (1.28)	**0.01***
Threatened someone with a gun	16 (2.47)	1 (0.15)	17 (1.28)	**<0.001***
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	89 (13.73)	79 (11.55)	168 (12.61)	0.23
Sexually harassed someone	19 (2.93)	4 (0.58)	23 (1.73)	**0.001***
Robbed someone	35 (5.40)	10 (1.46)	45 (3.38)	**<0.001***
Gotten into a fight after drinking or getting high	32 (4.94)	9 (1.32)	41 (3.08)	**0.001***

Chi-square test used to determine statistical difference between males and females.
* Symbolizes statistically significant difference between males and females.

3.4.5 Comparison of outcomes of individual violent behaviour (positive/ negative) with respect to the geometric mean BLLs (μg/dl) by sex

Bivariate analysis was conducted to examine the link between geometric mean BLLs at age 13 years and violent behaviour during late adolescence as shown in Table 3.4. Elevated geometric mean BLLs were significantly associated with violent behaviour items 'hit or kicked someone' in the total sample and in females; 'pushed or shoved someone' in the total sample; 'badly beaten someone up' in the total sample and males; 'sexually harassed someone' in the total sample; and 'been suspended from school' in the total sample and males ($p<0.05$).

Table 3.4 Comparison of outcomes of individual ***violent behaviour*** (positive/ negative) with respect to the geometric mean **BLLs (µg/dl)** by sex

Violent Behaviour Type	Males n = 648				Females n = 684				Total n = 1332			
	Geometric mean BLL				Geometric mean BLL				Geometric mean BLL			
	Pos	Neg	95%(CI)	p-value	Pos	Neg	95%(CI)	p-value	Pos	Neg	95%(CI)	p-value
Violent Behaviour at School												
Hit or kicked someone	6.17	6.04	(0.92-1.04)	0.50	5.05	4.49	(0.82-0.95)	**0.001**	5.74	5.05	(0.84-0.92)	**<0.001**
Pushed or shoved someone when angry	6.18	6.02	(0.92-1.03)	0.38	4.74	4.54	(0.90-1.02)	0.19	5.50	5.12	(0.89-0.97)	**0.002**
Badly beaten someone up	6.72	6.03	(0.81-0.98)	**0.02**	5.24	4.59	(0.76-1.00)	0.06	6.17	5.21	(0.78-0.92)	**<0.001**
Threatened someone with a knife or sharp weapon	6.17	6.10	(0.84-1.16)	0.88	5.08	4.62	(0.69-1.20)	0.50	5.84	5.28	(0.78-1.05)	0.18
Attacked someone with a knife or sharp weapon	7.10	6.09	(0.68-1.07)	0.18	4.70	4.62	(0.54-1.77)	0.95	6.66	5.28	(0.63-1.00)	0.05
Threatened someone with a gun	5.65	6.11	(0.90-1.31)	0.41	4.16	4.62	—	—	5.55	5.29	(0.78-1.17)	0.64
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	6.21	6.08	(0.90-1.06)	0.60	4.66	4.62	(0.91-1.08)	0.87	5.33	5.28	(0.93-1.05)	0.77
Sexually harassed someone	6.44	6.09	(0.80-1.12)	0.51	6.99	4.61	(0.43-1.00)	0.05	6.53	5.27	(0.68-0.98)	**0.01**
Robbed someone	5.89	6.12	(0.93-1.16)	0.51	4.53	4.62	(0.81-1.28)	0.85	5.55	5.29	(0.85-1.06)	0.37
Been suspended from school	6.59	6.02	(0.84-0.99)	**0.03**	4.98	4.59	(0.82-1.03)	0.17	5.93	5.21	(0.82-0.94)	**<0.001**
Gotten into a fight after drinking or getting high	6.13	6.10	(0.87-1.13)	0.93	5.30	4.61	(0.66-1.15)	0.33	5.95	5.27	(0.77-1.01)	0.07

Violence outside of school												
Hit or kicked someone	6.36	5.94	(0.88-0.99)	**0.02**	4.96	4.55	(0.84-0.99)	**0.03**	5.85	5.08	(0.82-0.91)	**<0.001**
Pushed or shoved someone when angry	6.29	5.96	(0.89-1.00)	0.08	4.84	4.53	(0.87-1.00)	0.06	5.64	5.10	(0.86-0.95)	**<0.001**
Badly beaten someone up	6.75	6.01	(0.82-0.97)	**0.01**	5.21	4.59	(0.75-1.03)	0.11	6.31	5.20	(0.76-0.89)	**<0.001**
Threatened someone with a knife or sharp weapon	5.91	6.11	(0.90-1.19)	0.64	4.07	4.63	(0.81-1.60)	0.46	5.55	5.28	(0.82-1.09)	0.49
Attacked someone with a knife or sharp weapon	6.03	6.10	(0.83-1.24)	0.91	3.94	4.62	(0.72-1.90)	0.51	5.60	5.29	(0.77-1.16)	0.58
Threatened someone with a gun	5.51	6.12	(0.92-1.34)	0.27	4.16	4.62	___	___ ǂ	5.42	5.29	(0.80-1.20)	0.81
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	5.89	6.14	(0.96-1.13)	0.35	4.88	4.59	(0.85-1.04)	0.23	5.39	5.28	(0.91-1.05)	0.53
Sexually harassed someone	5.92	6.12	(0.87-1.23)	0.71	6.73	4.61	(0.45-1.04)	0.07	6.05	5.28	(0.73-1.04)	0.12
Robbed someone	6.01	6.11	(0.89-1.16)	0.81	5.36	4.61	(0.66-1.12)	0.26	5.86	5.27	(0.78-1.01)	0.10
Gotten into a fight after drinking or getting high	5.81	6.12	(0.92-1.20)	0.45	5.43	4.61	(0.64-1.12)	0.25	5.72	5.28	(0.81-1.05)	0.23

Pos = positive response to violent behaviour item

Neg = negative response to violent behaviour item; ǂ very low response =1

3.4.6 Association between sex and socio-demographic factors at birth with violent behaviour during late adolescence

Results of the analyses of covariates for potential influence on violent behaviour are summarized in Table 3.5. After adjusting for the effect of race (Model 1) sex was positively associated with violence using a weapon, fighting, physical violence and robbing (95% CI [0.024, 0.240], 95% CI [0.328, 0.538], 95% CI [0.005, 0.214], 95% CI [0. 002, 0.217]; respectively. In Model 2 controlling for blood lead levels at age 13 years and race, sex was only positively associated with violence using a weapon, physical violence and robbing; 95% CI [0.039, 0.267], 95% CI [0.248, 0.469], 95% CI [0.003, 0.231]; respectively – but not fighting.

For maternal education at birth adjusting for sex and race (Model 1): compared to birth to a mother with a grade 7 and below level of education, birth to a mother with a grade 8 to 10 level of education was positively associated with fighting others during late adolescence 95% CI [0.015, 0.358] and birth to a mother with a grade 9 to 12 and post school level of education were positively associated with verbally and emotionally abusing others during late adolescence 95% CI [0.069, 0.441], 95% CI [0.199, 0.717], respectively. After adjusting for the effect of sex, blood lead levels at age 13 years and race in Model 2, birth to mothers with a grade 8 to 10 level of education was positively associated with fighting others in late adolescence 95% CI [0.017, 0.360]; and maternal education level of grade 11 to 12 and post school education at birth were positively associated with verbal and emotional abusiveness towards others during late adolescence 95% CI [0.067, 0.440], 95% CI [0.199, 0.716], respectively.

Adjusting for the effect of sex and race (Model 1) birth at a private hospital was negatively associated with physical violence but positively associated with associated with verbal and emotional abusiveness to others during late adolescence 95% CI [-0.43, -0.03], 95% CI [0.104, 0.516]; respectively. In Model 2 after adjusting for the effects of sex, race and blood lead levels at age 13 years, birth at a private hospital remained negatively associated with physical violence 95% CI [-0.408, -0.006] and positively associated with verbally and emotionally abusing others during late adolescence 95% CI [0.101, 0.514]. Furthermore, there was a positive association between birth to a single mother and physical violence, adjusting for the effect of sex and race (Model 1) 95% CI [0.025, 0.249] and the association remained significant after controlling for sex, race and blood lead levels at age 13 years 95% CI [0.009, 0.233] (Model 2). Socio-economic status at birth was positively associated with being verbally and emotionally abusive to others in late adolescence, controlling for sex (Model1) 95% CI [0.037, 0.174], and it remained so after adjusting for the effect of sex, race and blood lead levels at age 13 years (Model 2) 95% CI [0.036, 0.173].

Table 3.5 Association between sex and socio-demographic factors at birth with violent behaviour during late adolescence

	MODEL 1						MODEL 2					
	Violence using a weapon	Physical violence	Fighting	Sexual Harass-ment	Robbing	Verbal & emotional Abusive	Violence using a weapon	Physical violence	Fighting	Sexual Harass-ment	Robbing	Verbal & emotional Abusive
	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Sex												
Female												
Male	0.13 (0.05) *	0.43(0.05)***	0.11 (0.05) *	0 .07 (0.05)	0.11 (0.05)*	-0.02 (0.05)	0.15(0.06)**	0.36(0.06)***	0 .09 (0.05)	0 .07 (0.06)	0.12 (0.06) *	-0.01 (0.06)
Maternal educati-on												
Grades: 7 & <												
8-10	0.02 (0.08)	-0.04 (0.09)	0.19 (0.09)*	0.13 (0.09)	0.12 (0.09)	0.14 (0.09)	0.02 (0.08)	-0.03 (0.09)	0.18 (0.08)*	0.13 (0.09)	0.12 (0.09)	0.15 (0.09)
9-12	0.06 (0.09)	-0.15 (0.09)	0.03 (0.09)	0.02 (0.09)	0.05 (0.09)	0.25(0.09)**	0.06 (0.09)	-0.14 (0.09)	0.03 (0.09)	0.03 (0.09)	0.05 (0.09)	0.25(0.09)**
Post school training	0.08 (0.12)	-0.11 (0.13)	0.04 (0.13)	-0.04 (0.13)	-0.02 (0.13)	0.46(0.13)**	0.08 (0.12)	-0.11 (0.13)	0.03 (0.12)	-0.04 (0.13)	-0.02 (0.13)	0.46(0.13)**
Maternal age												
<20												
20- 29	-0.08 (0.08)	0.02 (0.07)	-0.07 (0.07)	0.01 (0.08)	-0.14 (0.08)	-0.10 (0.08)	-0.08 (0.08)	0.01 (0.07)	-0.07 (0.07)	0.01 (0.07)	-0.14 (0.08)	-0.10 (0.07)
30-39	-0.02 (0.08)	-0.06 (0.08)	-0.08 (0.08)	0.07 (0.08)	-0.13 (0.08)	-0.14 (0.08)	-0.02 (0.08)	-0.06 (0.08)	-0.08 (0.08)	0.07 (0.08)	-0.13 (0.08)	-0.14 (0.08)
>=40	-0.09 (0.20)	-0.02 (0.19)	-0.19 (0.19)	-0.16 (0.20)	-0.15 (0.20)	-0.35(0.20)	-0.09 (0.20)	0.003 (0.19)	-0.19 (0.19)	-0.16 (0.20)	-0.15 (0.20)	-0.36 (0.20)
Hospital of birth												

Public Private	0.03 (0.10)	-0.23 (0.10)*	0.05 (0.10)	-0.07 (0.10)	-0.06 (0.10)	0.31(0.10)**	0.02 (0.10)	-0.21(0.10)*	0.06 (0.10)	-0.07 (0.10)	-0.06 (0.10)	0.31(0.10)**
Maternal marital status												
Married												
Single/ divorced/ widowed/ separated	-0.01 (0.06)	0.14 (0.06) *	-0.01 (0.06)	-0.05 (0.06)	0.09 (0.06)	-0.004(0.06)	-0.01 (0.06)	0.12 (0.06) *	-0.01 (0.06)	-0.05 (0.06)	0.09 (0.06)	-0.001(0.06)
SES	0.04 (0.03)	-0.03 (0.03)	-0.01 (0.03)	0.02 (0.03)	-0.02 (0.03)	0.10 (0.03)**	0.04 (0.03)	-0.02 (0.03)	0 .01 (0.03)	0.02 (0.03)	-0.02 (0.03)	0 .10(0.03)**

***p < 0.0001

**p < 0.02

*p < 0.05

3.4.7 Association between gender and blood lead levels at age 13 years

Males are positively associated with blood lead levels compared to females as demonstrated in Table 3.6 below.

Table 3.6 Association between gender and blood lead levels

Exposure variable	Outcome variable		
Gender		BLLs	
	β	Std error	P-value
Female			
Male	1.53	0.126	<0.000

3.4.8 Association between blood lead levels at age 13 years and violent behaviour in late adolescence

As shown in Table 3.5 sex, maternal education at birth, maternal age at birth, maternal marital status at birth, hospital of birth and SES were found to be influential covariates. As such, they were adjusted for accordingly in the linear regression analyses to examine the association between BLLs at age 13 years and violent behaviour in late adolescence. Model 1 adjusted for sex and race in all models regardless of significance; and Model 2 adjusted for sex and race in all models regardless of significance, and significant covariates (Table 3.7).

In Model 1 blood lead levels 5–9.99 μg/dL at age 13 years and ≥10 μg/dL were positively associated with perpetration of physical violence 95% CI [0.020, 0.246] and 95% CI [0.070, 0.699], respectively. The positive association remained significant when the effect was examined using continuous blood lead levels at age 13 years 95% CI [0.138, 0.398]. Additionally, blood lead levels ≥10 μg/dL were positively associated with fighting in late adolescence 95% CI [0.093, 0.689]; however, when data were further examined using

continuous BLLs the association between blood lead levels at age 13 years and fighting was not significant.

Table 3.7 Association between BLLs at age 13 years and violent behaviour during late adolescence

Exposure variable	MODEL 1						MODEL 2					
	Violence using a weapon	Physical violence	Fighting	Sexual Harassment	Robbing	Verbal & emotional Abusive	Violence using a weapon	Physical violence	Fighting	Sexual Harassment	Robbing	Verbal & emotional Abusive
	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
BLL (µg/dL)												
<5 = (reference category)												
5-9.99	-0.07 (0.06)	0.13 (0.06) *	0.03 (0.06)	-0.01 (0.06)	-0.03 (0.06)	0.03 (0.06)	-0.07 (0.06)	0.12 (0.06) *	0.03 (0.06)	0.01 (0.06)	-0.03 (0.06)	0.06 (0.07)
≥10	0.01 (0.16)	0.37 (0.15) *	0.39(0.15)**	-0.19 (0.17)	-0.22 (0.16)	-0.22 (0.16)	0.01 (0.16)	0.36 (0.15) *	0.41(0.15)**	-0.19 (0.16)	-0.22 (0.16)	-0.31 (0.19)
R^2	0.0055	0.0559	0.0087	0.0059	0.0045	0.0023	0.0055	0.0594	0.0153	0.0046	0.0031	0.0022
Continuous BLLs	-0.07(0.07)	0.27(0.07)***	0.07(0.07)	0 .01(0.07)	-0.02(0.07)	-0.04(0.07)	-0.07(0.01)	0.26(0.01)***	0 08(0.07)	0.01(0.07)	-0.03(0.07)	-0.05(0 08)

***p < 0.0001

**p<0.02

*p < 0.0

3.5 Discussion

The main purpose of this study was to examine the association between blood lead levels during early adolescence and violent behaviour in late adolescence. Using a dimension-reduction technique this study sought to identify the specific types of violent behaviour associated with lead exposure. We found that blood lead levels at age 13 years are positively associated with physical violence in particular, later in adolescence in Black African and Mixed Ancestral South Africans. Both categorical and continuous blood lead levels were positively associated with physical violence. The change in significance for the association between blood lead levels at age 13 years and fighting in late adolescence when data were analyzed using continuous blood lead levels could be a consequence of "increased risk of positive results being a false positive" associated with the use of categorical variables and reduced sample size (Altman and Royston, 2006, van Walraven and Hart, 2008). However, it is important to note that categories are useful when examining the effects of lead exposure because they show if there is a dose-response effect and provide evidence of its detrimental health effects at various levels including those ubiquitous to the general population.

In addition, elevated geometric mean blood lead levels at age 13 years were positively associated with violent behaviour items 'hit or kicked someone', 'badly beaten up someone', 'pushed or shoved someone' – all of which describe violence pattern for "physical violence", in line with study findings using PCA derived components. Our results add a very important component to the international literature linking lead exposure to perpetration of violent acts (Denno, 1990, Stretesky and Lynch, 2001, Nevin, 2000, Wright et al., 2008, Reyes, 2007); in that they show that lead exposure is associated with one of the most extreme forms of violent behaviour in South African youth. Given that lead exposure is associated with aggressive

behaviour in early adolescence (Naicker et al., 2012) and 'direct' aggressive behaviour in mid-adolescence (Nkomo et al., 2018) - our results point to an increased risk of violent lifestyle and poor quality of life for young people with a history of chronic lead exposure in South Africa.

The proportion of study participants with blood lead levels ≥5 µg/dL was much higher in our study (62.2%) compared to those reported in developed countries such the United States of America (3.1%) (Raymond et al., 2014). This should be a cause for concern because as shown by Nevin early lead exposure is a good predictor of future violent behaviour (Nevin, 2007). Similarly, Wright et al. associated average childhood blood lead and six years blood lead concentrations with violent offenses later in life (Wright et al., 2008). Furthermore, Needleman and colleagues reported that arrested and adjudicated African American and White youth from Philadelphia were four times more likely to have elevated bone lead levels 95% CL [1.4 – 11.1] (Needleman et al., 2002).

Consistent with international literature, in this study being a male was a predictor of having elevated blood lead levels (Raymond et al., 2009). It has been shown that sex differences in blood lead concentrations are influenced by bioavailability, capacity to absorb and toxicokinetics (Vahter et al., 2007). After lead is absorbed into the bloodstream, approximately 99% of it is bound to the erythrocytes because of their high affinity for lead, the remaining 1% resides in blood plasma and is available for circulation to the different tissues and organs of the body (Holstege et al., 2013, Rabinowitz, 1991). In addition to higher pulmonary function, intestinal motility, and greater body surface area than women which are associated with increased absorption capacity, on average men have greater red blood cell

and plasma volume than women (Soldin and Mattison, 2009). These may be some of the contributing factors in sex differences regarding blood lead concentration levels.

Furthermore, males were more likely to engage in violent behaviour acts compared to females. Additionally, this study showed that the patterns of violent behaviour observed at school, although heightened, do mirror those perpetrated outside of school. Elliot and colleagues posit that violence in school is a known indication of violence outside of school in the communities (Elliott et al., 1998). However, findings from a multi-national country study utilizing data from the Third International Math and Science Study (TIMMS) survey including 32 nations (Akiba et al., 2002) showed that there is no direct link between community crime rates and school violence. The authors suggest that use of adult crime rates as opposed to juvenile crime rates may or may not be a contributing factor in their negative study findings in this regard (Akiba et al., 2002). In general, it is reported that violence in South Africa is "overwhelmingly perpetrated by men" (Jewkes et al., 2009); which may imply that if no interventions are put in place violent adolescents will grow to become violent adult men. Our results support the growing body of evidence showing that violence is a public health problem both in developing and developed countries.

Interestingly elevated geometric mean BLLs were significantly associated with sexual harassment in the total sample at school but not outside of school. Which begs the question – what is it about the school environment that fosters this type of violent behaviour among adolescents? Similarly, an American study of adolescents attending middle and high school reported increased prevalence of peer-on-peer sexual harassment at school compared to the adolescent's house, someone else's house, at a party or other location (Young et al., 2009).

The perpetrator at school was two times more likely to be a friend than outside of school ($p<0.05$).

Perpetration of violent behaviour by adolescents is a huge concern in South Africa. One in five secondary learners in South African schools had succumbed to one form or another of violence while at school (Burton and Leoschut, 2012). From a social science point of view, in the 2012 report titled 'The Dynamics of violence in schools in South Africa', Mncube et al. wrote "....it is also the understanding of this report that the basis of violence is social rather than genetic or biological, and therefore there are ways and means of reducing human violence" (Mncube and Harber, 2013). It is without a doubt that social issues play very important roles in violent behaviour. According to the WHO, violent behaviour can be controlled. There are modulating social and environmental factors that either deter or enhance propensity to criminal behaviour (Mercy et al., 2002) .

On the other hand, new developments in neuroscience using brain imaging are now beginning to unravel the possible underlying links between altered brain structure and antisocial behaviour, including violent behaviour in humans. Empirical data show an association between lead exposure and subsequent structural changes in different areas of the brain (Stewart et al., 2006, Brubaker et al., 2009, Brubaker et al., 2010, Schwartz et al., 2010, Cecil et al., 2011). The affected brain areas include those responsible for "executive functions, mood regulation and decision-making" (Cecil et al., 2008). Even more concerning is that these structural changes appear to be permanent (Cecil et al., 2008). These findings suggest that exposure to lead may result in changes in the structure and function of the brain which explains the underlying neuro-anatomical basis of the link between lead exposure and

cognitive dysfunction, aggressive and violent behaviour among others. These results complement epidemiological study findings, including our current study findings, suggesting a possible biological link between environmental lead exposure and violent behaviour in adolescents. Scientific evidence is pivotal for public health policy making (Krug et al., 2002). We hope that our study findings will add to reliable empirical evidence from mainly developed countries showing a link between biology and violent behaviour. This will provide the necessary information required by policy makers in low and middle income countries such as South Africa to mobilize for the essential public health priorities such as lead screening in children and other preventive measures.

The Director General of the WHO states that "while public health does not offer all the answers to this complex problem, we are determined to play our role in the prevention of violence worldwide" (Krug et al., 2002). To find a solution regarding the root causes of aggressive behaviour requires an integrated approach including evidence from biology and social sciences (Ramirez, 2006) – so is the case with violent behaviour. To help avert this public health hazard the CDC's ACCLPP recommends primary prevention where all homes are lead-free as the main practical way to prevent high BLLs in children (Centers for Disease Control and Prevention, 2012c).

There were some limitations to this study. Blood lead levels were measured at only one time point (age 13 years). It is recommended that estimates from both blood lead levels for recent exposure and bone lead levels for cumulative lead exposure be used (Hu et al., 2007). Our study findings are valuable in that they fill a gap in evidence and our findings can be further examined using bone lead levels. The exposure variable was categorized into 3 levels which

may risk loss of power due to reduced sample size. To address and prevent or reduce possible bias in results, data were also analyzed using continuous blood lead levels. Furthermore, use of more than one scale to evaluate violent behaviour is recommended in research studies, only YSR was the used to assess violent behaviour in this study. Only Black African and Mixed Ancestral study participants were included in our study. For future studies inclusion of all South African population groups will improve the applicability of the study findings. Also, study participants were not evaluated for mental health, a known risk factor for violent behaviour. Even though, none of the study participants included in the study had known mental health problems, this may have been a limitation in our study. Extremely violent behaviour such as 'violence using a weapon' could not be properly assessed in relation to lead exposure as the study sample had a very small proportion of participants who reported to have perpetrated 'violence using a weapon'. It would be of great interest to further examine our study findings in environments where the study sample is comprised of young people convicted of violent crime in South Africa and control for other possible confounding variables such as alcohol intake and substance abuse which were not able to in this study.

3.6 Conclusions

Because lead is persistent in the environment, it is likely to remain a significant public health problem for a very long time, especially in developing countries (Tong et al., 2000). Findings from this study show that lead exposure in early adolescence is positively associated with 'physical violence' during late adolescence in South African young people. Males were associated with perpetration of the most severe forms of violent behaviour in this study, such as violence using a weapon, physical violence and robbing others.

Supplementary Table 3a **Factor-loading matrix for the violence patterns derived from varimax rotation - Principal Component Analysis**

	Components					
	Violence using a weapon	Physical violence	Fighting	Sexual Harassment	Robbing	Abusive
Threatened someone with a gun at outside of school	.832					
Threatened someone with a gun at school	.810					
Attacked someone with a knife or sharp weapon outside of school	.790					
Attacked someone with a knife or sharp weapon at school	.753					
Threatened someone with a knife or sharp weapon outside of school	.691					
Threatened someone with a knife or sharp weapon at school	.660					
Pushed or shoved someone outside of school		.757				
Hit or kicked someone outside of school		.746				
Pushed or shoved someone at school		.738				
Hit or kicked someone at school		.721				
Badly beaten up someone outside of school		.607				
Badly beaten up someone at school		.566				
Gotten into a fight after drinking or getting high outside of school			.856			
Gotten into a fight after drinking or getting high at school			.826			
Sexually harassed someone outside of school				.857		
Sexually harassed someone at school				.814		
Robbed someone outside of school					.789	
Robbed someone at of school					.782	
Verbally or emotionally abused someone at school						.887
Verbally or emotionally abused someone outside of school						.852

Supplementary Table 3b: **Characteristics of members of the analytical sample and BT20+ members excluded from the analytical sample**

	BT20+ members excluded from analytical sample	Analytical Sample	P-Value
Race			<0.0001
White	207 (10.7)	0 (0)	
Black	1378 (71.0)	1190 (89.3)	
Coloured	241 (12.4)	142 (10.7)	
Indian	115 (5.9)	0 (0)	
Sex			0.97
Male	943 (48.6)	648 (48.6)	
Female	998 (51.4)	684 (51.4)	
Hospital of birth			<0.0001
Private	341 (17.6)	100 (7.5)	
Public	1600 (82.4)	1231 (92.5)	
Place of birth			<0.0001
Soweto/ Diepkloof/Former Coloured/Asian	122 (6.3)	1328 (99.7)	
Suburb	1819 (93.7)	4 (0.3)	
Maternal education at birth			<0.0001
Grade 7 or less	300 (17.7)	155 (12.5)	
Grade 8 – 10	665 (39.3)	591 (47.6)	
Grade 11 – 12	490 (29.0)	403 (32.5)	
Post education schooling	236 (14.0)	92 (7.4)	
Maternal age at birth			0.004
<20	255 (13.1)	229 (17.2)	
20 – 29	1148 (59.1)	722 (54.2)	
30 – 36	505 (26.0)	351 (26.3)	
≥37	33 (1.7)	30 (2.3)	
Maternal marital status at birth			<0.0001
Married/ Living with partner	966 (50.1)	449 (33.9)	
Single/Widowed/ Separated/ Divorced	962 (49.9)	874 (66.1)	

CHAPTER 4

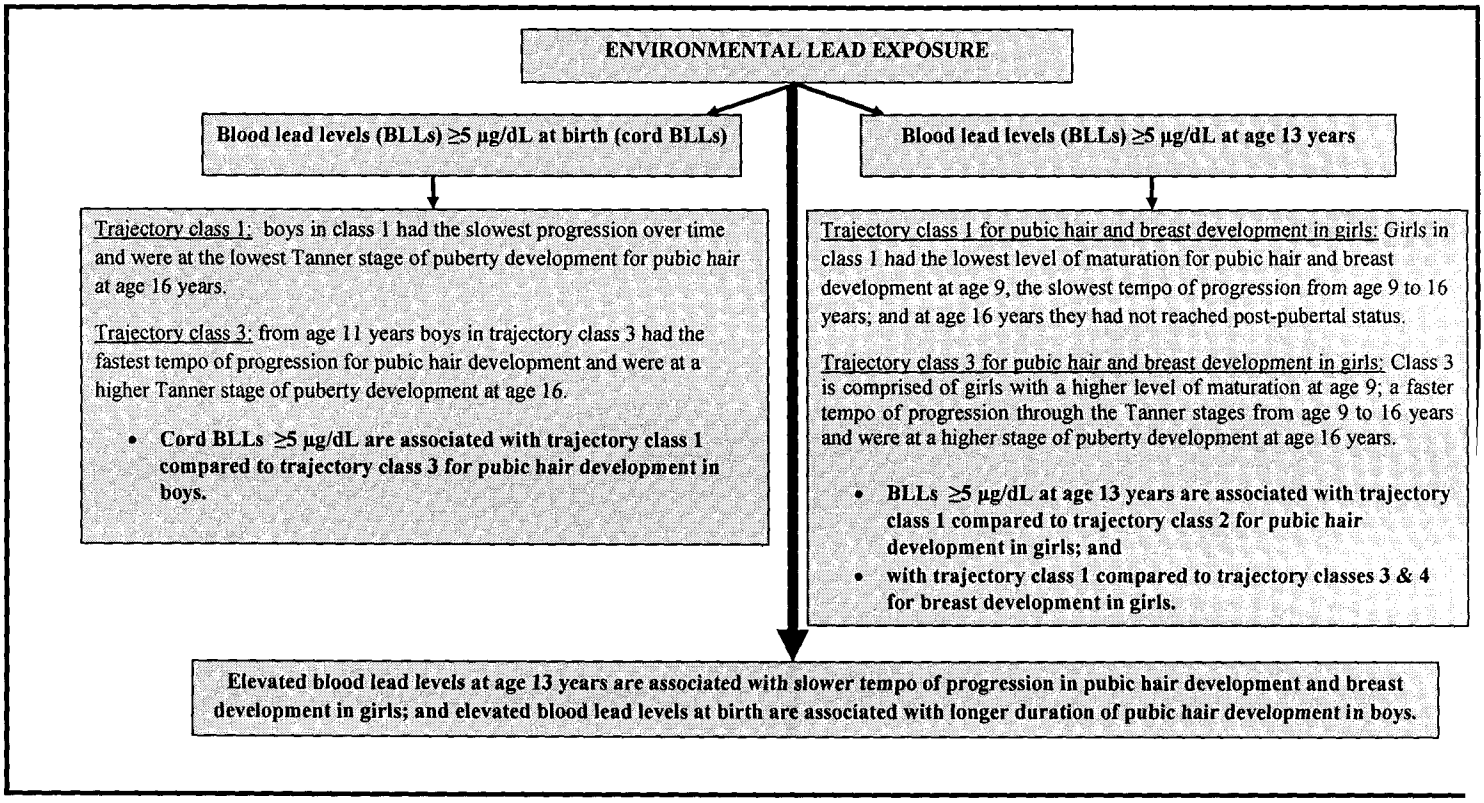
Environmental lead exposure and pubertal trajectory classes in South African adolescent males and females

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This journal article was published in the Science of the Total Environment. It aims to answer objective 4 of the thesis. It examined the association between blood lead levels at age 13 years old and latent classes of puberty development in South African boys and girls. In addition, data were evaluated for an association between lead exposure at birth and pubertal progression. The journal article in this chapter has been modified slightly to include additional analysis conducted after the paper was published.

A copy of the published journal article is attached in the Appendices (Appendix C).

Figure 4.1 GRAPHIC ABSTRACT: Environmental lead exposure and pubertal trajectory classes in South African adolescent males and females


ENVIRONMENTAL LEAD EXPOSURE
Blood lead levels (BLLs) ≥5 µg/dL at birth (cord BLLs)
Blood lead levels (BLLs) ≥5 µg/dL at age 13 years
Trajectory class 1: boys in class 1 had the slowest progression over time and were at the lowest Tanner stage of puberty development for pubic hair at age 16 years.
Trajectory class 3: from age 11 years boys in trajectory class 3 had the fastest tempo of progression for pubic hair development and were at a higher Tanner stage of puberty development at age 16.
• Cord BLLs ≥5 µg/dL are associated with trajectory class 1 compared to trajectory class 3 for pubic hair development in boys.
Trajectory class 1 for pubic hair and breast development in girls: Girls in class 1 had the lowest level of maturation for pubic hair and breast development at age 9, the slowest tempo of progression from age 9 to 16 years; and at age 16 years they had not reached post-pubertal status.
Trajectory class 3 for pubic hair and breast development in girls: Class 3 is comprised of girls with a higher level of maturation at age 9; a faster tempo of progression through the Tanner stages from age 9 to 16 years and were at a higher stage of puberty development at age 16 years.
• BLLs ≥5 µg/dL at age 13 years are associated with trajectory class 1 compared to trajectory class 2 for pubic hair development in girls; and
• with trajectory class 1 compared to trajectory classes 3 & 4 for breast development in girls.
Elevated blood lead levels at age 13 years are associated with slower tempo of progression in pubic hair development and breast development in girls; and elevated blood lead levels at birth are associated with longer duration of pubic hair development in boys.

4.1 Introduction

Epidemiological studies show secular trends in puberty timing and tempo (Euling et al., 2008, Jones et al., 2009, Sørensen et al., 2010, Sørensen et al., 2012, Parent et al., 2015). Altered pubertal timing in both girls and boys is linked to nutritional factors, various chronic illnesses and more recently endocrine disrupting chemicals (EDCs) (Rosen and Foster, 2001, Pozo and Argente, 2002, Selevan et al., 2003, Williams et al., 2010, Naicker et al., 2010a, Zawatski and Lee, 2013), among others. However, little research has been conducted to evaluate the association between environmental lead exposure and tempo of progression through the Tanner stages of pubertal development in low or middle-income countries.

Puberty is a time of transition from childhood to adolescence and is marked by sexual and physical maturation due to hormonal changes in young girls and boys. Onset of puberty is mainly characterized by breast budding, pubic hair development and menarche in girls, and testicular volume and size, penile and pubic hair development and voice breaking in boys (Blondell et al., 1999, Golub et al., 2008, Jones et al., 2009, Day et al., 2015). Pubertal onset and progression is regulated via hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes (Louis et al., 2008). To activate the HPG and HPA axes requires a signal from the central nervous system (CNS) to the hypothalamus (Louis et al., 2008). Stimulation of the HPG axis initiates the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, activating the release of gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary (Louis et al., 2008, Roy et al., 2009, Zawatski and Lee, 2013). Release of gonadotropin activates the gonad. In girls, this leads to the production of ova and subsequently onset of menarche. In addition, FSH initiates secretion of androgens, which facilitates the development of breasts, ovaries and uterus. In

boys, LH activates the secretion of androgen, which in turn initiates penile, pubic hair and testicular size and volume development. Stimulation of the HPA axis initiates the release of adrenocorticotropin releasing hormone (CRH) by the hypothalamus, which activates secretion of adrenocortical tropic hormone (ACTH) by the pituitary. ACTH activates adrenal cortex resulting in androstenedione and DHEA secretion which initiates development of pubic hair, armpit hair and acne (Louis et al., 2008).

Exposure to EDCs such as lead, soy phytoestrogens and cadmium, among others (Zawatski and Lee, 2013) during developmental stages of the CNS and sexual differentiation can alter puberty development. EDCs are defined as any chemical or mixture of chemicals that disrupts any part of hormone action (Zoeller et al., 2012). Exposure to lead interferes with various aspects of the HPG axis including disruption of hormonal pathways (Doumouchtsis et al., 2009, Zawatski and Lee, 2013), reduction in GnRH levels, testosterone levels in males, estradiol levels in females, LH and FSH levels (Doumouchtsis et al., 2009). Furthermore, it alters the neurotransmitter systems and consequently the HPA function (Doumouchtsis et al., 2009). Both animal (Sokol et al., 1985, Sokol et al., 2002) and human studies (Selevan et al., 2003, Wu et al., 2003, Hauser et al., 2008, Naicker et al., 2010a, Williams et al., 2010) have shown a relationship between lead exposure and altered pubertal timing in males and females.

Altered puberty development poses potential health risks to the young people. A study in New Zealand linked longer duration of pubertal development (Wilkinson and Colls, 1994) to higher incidents of testicular cancer in Moaris compared to non-Moaris (Wilkinson et al., 1992). However, the authors posit that there could be other contributing factors such as lower birth weight which is common in Maoris infants and high rate of obese mothers which

exposes the foetus to oestrogen (Wilkinson and Colls, 1994). Furthermore, a UK Biobank study involving 250,037 women and 197,714 men showed that in females early pubertal timing increases the risk of breast cancer, angina, hypertension, obesity, early menopause and allergy to food, among others by 13%, 23%, 13%, 82%, 36%, and 39%, respectively in adulthood. Late puberty timing increases risk of early menopause, coeliac disease, low intelligence, asthma and poor overall health, among others by 16%, 62%, 32%, 11% and 19%, respectively (Day et al 2015). In males early onset of puberty increases the odds of having angina, heart attack, obesity, Type 2 diabetes, depression, irritable bowel syndrome and short stature in adulthood, among others by 39%, 26%, 58%, 24%, 28%, 49% and 39%, respectively. While, delayed pubertal timing increases the risk of having anxiety/panic attacks, depression, asthma and poor overall health, among others by 43%, 36%, 22% and 25%, respectively (Day et al., 2015). These data highlight the significance of altered pubertal development as a public health problem.

In 1984 the limit on use of lead in petrol in South Africa was 0.84 g/L. This was reduced to 0.40 g/L in 1986 and phasing in of unleaded petrol was introduced in 1989 (Nriagu, 1990). In addition, lead mining and previous use of lead in paint are some of the well known sources of lead exposure in South African children (Mathee et al., 2009, Mathee, 2014). More recently, studies have shown that children continue to be exposed to lead because of limited knowledge about dangers of lead exposure in subsistence fishing communities (Mathee et al., 2013) and cottage industries (Teare et al., 2015) among others. Consequently, South African children had been shown to have elevated blood lead levels (von Schirnding et al., 1991, von Schirnding et al., 2003, Mathee, 2014). Even though blood lead concentrations in children have dropped significantly globally, including South Africa, since the ban of use of lead in

paint and tetraethyl lead in petrol (Mathee et al., 2006, Centers for Disease Control and Prevention, 2012c); there are "no safe blood lead levels in children" (Centers for Disease Control and Prevention, 2012c).

Previous study findings using a sub-sample from the Birth to Twenty Plus (BT20+) cohort, showed that female study participants with blood lead levels ≥5 µg/dL at age 13 years were 2.34, 1.81 and 2.01 times more likely to have delayed onset of breast development, pubic hair development and age of menarche attainment, respectively, compared to those with blood lead levels <5 µg/dL ($p<0.001$) (Naicker et al., 2010a). To our knowledge, no study has been conducted in South Africa to examine the association between lead exposure and puberty development in males. For this study we seek to conduct longitudinal examination of the effects of lead exposure on pubertal development. Using Latent Class Growth Analysis (LCGA), pubertal progression information from the BT20+ study collected from age 9 to 16 years old was used to generate distinct classes according to a "common developmental trajectory" for the Tanner Sexual Maturation Scale (SMS) indicators of pubertal stage (Lundeen et al., 2016). We examined the association between both cord and adolescent lead concentrations and latent classes of puberty development in girls and boys.

4.2 Methods

4.2.1 Study population

Study participants were selected from the BT20+ cohort in Johannesburg, South Africa. BT20+ is made up of all singleton births from 23 April to 8 June 1990 in Soweto and Johannesburg. Inclusion criteria included that the infant must reside in the Johannesburg area

for at least six months after birth. The reason for this was that at the time pilot studies had shown that some pregnant women from the rural areas came to the Johannesburg metropolis to deliver their babies in order to access better health facilities and for other family reasons. A total of 3273 (1682 females) study participants fulfilled the inclusion criteria and were enrolled in the study (Richter et al., 2004, Richter et al., 2007).

Study participants were included in the current analysis if they had data for blood lead levels at age 13 years and pubertal growth trajectory classes for pubic hair development and breast development in girls and pubic hair development and genital development in boys. Of the 1682 girls enrolled in the original BT20+ study, 1135 had data for pubertal growth trajectories and 749 had data for blood lead levels at age 13 years. A total of 728 Black African and Mixed ancestry girls fulfilled the inclusion criteria. Of the 1591 boys enrolled in the original study sample, 1060 had data for pubertal growth trajectory for pubic hair development and genital development and 708 had data for blood lead levels at age 13 years. A total of 683 Black African and Mixed ancestry boys fulfilled the inclusion criteria. White and Indian/ Asian study participants were excluded because of low numbers.

Additionally, a subsample of study participants with data for blood lead levels at birth (cord blood lead levels) and pubertal development trajectory classes for pubic hair development and breast development in girls and pubic hair development and genital development in boys was included to examine the association between lead exposure at birth and pubertal progression. This was to determine if there are any differences between the relationship prenatal and postnatal lead exposure and pubertal progression. Two hundred and thirty five females and 234 males of Black African and Mixed ancestry fulfilled the inclusion criteria.

4.2.2 Blood lead measures

Whole blood samples were collected at birth (umbilical cord blood) and at age 13 years into EDTA-containing tubes free of metal traces. Trained healthcare professionals performed blood sampling. "Blood samples were vortexed and rolled on the coulter mixer for at least 10 minutes until properly mixed. They were diluted 10 times with 1,1 % (v/v) Triton X-100 using automatic Hamilton Microlab 500 diluter into disposable 10 ml Sterilin plastic tubes covered with screw caps and mixed well using a vibration mixer. Blood lead levels were measured using Perkin Elmer 600 Aanalyst atomic absorption spectrometer with a THGA graphite furnace, Zeeman background correction and AS- 800 Autosampler" (Nkomo et al., 2017). All blood samples and samples for quality control were prepared and measured in-house at the National Institute for Occupational Health, Johannesburg, South Africa.

4.2.3 Anthropometric measures and socio-demographic factors

Birth weight, height and Body Mass Index (BMI) at age 8 years were measured using standard methods (Cameron, 1984). BMI was calculated as weight in kilograms divided by height in square meters (Lundeen et al., 2016). Height was converted into height-for-age and BMI into BMI-for-age using the World Health Organization (WHO) standards (World Health Organization, 2006, Onis, 2006, Onis et al., 2007). Data for household income at birth were divided into quintiles ranging from 1 (poorest) to 5 (highest) (Lundeen et al., 2016) and for ethnic group of the child were collected at birth classified as Black African and Mixed ancestry (Richter et al., 2007).

4.2.4 Growth trajectory classes for pubertal development at age 9 to 16 years

From age 9 to 16 years data for pubertal development were collected annually using a validated self-reported Tanner-stage pubertal development questionnaire from boys and girls. At ages 9 and 10 data were collected by trained medical practitioners and from age 11 years onwards were through self-assessment (Norris and Richter, 2005). Tanner stages of pubertal development refer to a standard clinical method used to describe physical measurements of secondary sexual characteristics using drawings to signal stage of pubertal development where stage 1 signifies lowest level of pubertal maturation and stage 5 denotes highest level of pubertal maturation in girls and boys (Blondell et al., 1999).

Pubertal growth trajectory classes were grouped using Mplus to perform LCGA. Study participants had to have at least one Tanner Sexual Maturation Scale measurement to be included in the analyses for trajectory classes. Full Information Maximum Likelihood technique (Jung and Wickrama, 2008) was used to account for the missing data (Lundeen et al., 2016). LCGA is a latent growth modeling method that is helpful in identifying meaningful classes of individuals and modeling their longitudinal development trajectories (Jung and Wickrama, 2008). Using LCGA to describe both the level of pubertal development at age 9 years tempo of progression through the Tanner stages in the BT20+ study, analyses for breast development and pubic hair development in girls and genital development and pubic hair development in boys were conducted separately (Lundeen et al., 2016). Three trajectory classes for pubic hair development for both males and females, and four trajectory classes for breast development in females and four trajectory classes for genital development in males were identified.

4.2.5 Classification of pubertal growth trajectory groups

(Adapted from Lundeen et al, 2016)

The trajectory classes in this study comprise of three stages of pubertal development: i) level of maturation at age 9 years, ii) tempo of progression from through the Tanner stages from age 9 to 16 years and iii) postpubertal status at age 16 years. In all cases, trajectory class 1 is a reference category representing the least level of pubertal growth in all three stages as described above. Tables 4.1 and 4.2 summarize classification of pubertal growth trajectory classes in girls and boys, respectively.

Table 4.1 Pubertal growth trajectory classes for girls

Trajectory Classes	Pubic Hair Development	Breast Development
Class 1	Reference category	Reference category
Class 2	Girls in class 2 had a higher level of pubertal maturation for pubic hair development at age 9 years than those in class 1 but a lower level than those in class 3. Their tempo of progression from age 9 to 16 years was faster than those in class 1 but slower than those in class 3. At age 16 years they had not reached post puberty status and were at a higher Tanner stage than those in class 1, but at a lower Tanner stage than those in class 3.	At age 9 years pubertal maturation level for girls in class 2 was similar to those in class 1, but were less developed than those in classes 3 and 4 for breast development. Their tempo of progression from age 9 to 16 years was faster than those in class 1 but slower than those in classes 3 and 4. At age 16 years they had not reached postpubertal status; they were at a higher Tanner stage than those in class 1; at a similar Tanner stage as those in class 3, but at a lower Tanner stage than those in Class 4.
Class 3	Class 3 is comprised of girls with the highest level of pubertal maturation at age 9 years; fastest tempo of progression through the Tanner stages from age 9 to 16 years; and at age 16 they were at the highest stage of puberty development and had reached postpubertal status for pubic hair development compared to girls in classes 1 and 2.	At age 9 years girls in class 3 were at a higher level of pubertal maturation compared to those in classes 1 and 2; but at a similar level as those in class 4 for breast development. They had the fastest tempo of progression from age 9 to 11years, followed by girls in class 4, then class 2 and lastly girls in class 1. From age 11 to 16 years their pubertal transition was faster than that of girls in classes 1 and 2, but slower than those in class 4. At age 16 they were at a higher Tanner stage than those in class 1; similar Tanner stage as those in class 2, at a lower Tanner stage than girls in class 4; and had not reached post puberty status.
Class 4		At the age 9 years old girls in class 4 were at a higher level of puberty maturation for breast development than those in classes 1 and 2; but at a similar level as those in classes 3. They had the fastest tempo of progression from age 9 to 16 years followed by girls in classes 2 & 3. At age 16 years they were at the highest Tanner stage, followed by those in classes 2 & 3, with those in class 1 at

		the lowest Tanner stage. They were the only ones who had reached postpubertal status for breast development at age 16.

Table 4.2 Pubertal growth trajectory classes for boys

Trajectory Classes	Pubic Hair Development	Genital Development
Class 1	Reference category	Reference category
Class 2	At age 9 years boys in class 2 had pubertal maturation levels for pubic hair development similar to those of boys in classes 1 and 3. Their tempo of progression from age 9 to 16 years was faster than those in class 1 but slower than those in class 3. At age 16 years they fared between than those in class 1 but, were at a lower Tanner stage compared to those in class 3. They also had not reached post puberty status.	At age 9 years boys in class 2 were at the same level of puberty maturation for genital development as those in classes 1 and 4, but at a lower level than those in class 3. They had a faster pubertal progression from age 9 to 16 years than those in class 1 but slower than those in classes 3 and 4. At age 16 years they were at a lower Tanner stage compared to girls in class 4, but at a higher Tanner stage than those in classes 1 and 2; and had not reached postpubertal status.
Class 3	Boys in class 3 had a similar level of pubertal maturation for pubic hair development as those in classes 1 and 2 at 9 years old. From age 9 to 16 years their tempo of progression was faster than both those in classes 1 and 2. At age 16 years they were at the highest Tanner stage, followed by those in class 2; and they had reached post puberty status.	At age 9 years boys in class 3 were at a slightly higher level of puberty maturation for genital development than those in classes 1, 2 and 4. From age 10 to 16 years their tempo of progression was faster than those in classes 1 and 2, but slower than those in class 4. At age 16 years they had not reached post puberty status, and were at a higher Tanner stage than those in classes1 and 2, but at a lower Tanner stage than those in class 4.
Class 4		At age 9 years boys in class 4 were at the same level of puberty maturation for genital development as those in classes 1 and 2. They had the fastest tempo of progression from age 9 to 16 years, followed by boys in class 3, then class 2, with those in class 1 the slowest. At age 16 years they had reached postpubertal status.

4.2.6 Statistical analysis

First data were analyzed for statistically significant differences between the analytical sample and BT20+ study population excluded in the current study. In the analytical sample cord blood lead levels and blood lead levels at age 13 years were divided into <5 μg/dL, 5-9.99 μg/dL and ≥10 μg/dL for descriptive analysis. For cross tabulation between pubertal growth trajectory classes and blood lead levels; and inferential statistics blood lead levels were dichotomized into <5 and ≥5 μg/dL. The cut point of <5 μg/dL was chosen in line with the recommendations by the Centers for Disease Control and Prevention (CDC) Advisory Committee for Childhood Lead poisoning Prevention to use the reference value of 5 μg/dL which is based on the 97.5th percentile of the current blood lead level distribution among children aged 1 to 5 years in the United States of America (Centers For Disease Control and Prevention, 2012b). In this study cord blood lead levels and blood lead levels at age 13 years are indicators of prenatal and postnatal lead exposure, respectively.

Lundeen et al (2016) showed that there is an association between anthropometric measures such as height and BMI and pubertal development. BMI was calculated as weight in kilograms divided by height in meters squared. Measurements for height and weight were converted to height-for-age (HAZ) and BMI-for-age (BMIZ) z-scores, respectively (Lundeen et al., 2016). Household income at birth was divided into quintiles ranging from 1 (lowest) to 5 (highest) (Lundeen et al., 2016). Bivariate analyses were conducted for anthropometric measures and socio-demographic covariates by sex. To compare the significance of the associations; t-tests were used in the case of continuous variables and chi-square tests for the categorical variables. Multinomial logistic regression was used to predict pubertal growth trajectory class based on blood lead levels at age 13 years and cord blood lead levels.

Covariates for the adjusted models were defined by a statistical significance of $p<0.05$ or a crude and adjusted models difference of 10%. Bolding is used in the tables to indicate statistical significant results. Data analyses were conducted using STATA version 14.

4.2.7 Ethics

Consent for all study procedures was sought from the BT20+ birth cohort. Ethical approval was obtained from the University of the Witwatersrand Ethics Committee on Human Subjects (M010556). The Federal Wise Assurance registration number of the Witwatersrand University Ethics Committee on Human Subjects is FWA00000715. Only consented individuals were enrolled in the study.

4.3 Results

4.3.1 Differences between the characteristics of analytical sample and excluded members of the BT20+ cohort

For study participants with data for blood lead levels at age 13 years and pubertal growth trajectory classes there were no statistically significant differences between the analytical sample and the cohort study participants excluded from the current study with regard to key variables such as distribution of blood lead levels at age 13 years; trajectory classes for pubic hair and breast development in females; trajectory classes for pubic hair and genital development in males; race; height at age 8 years and birth weight in both males and females ($p > 0.05$). Data not shown.

For study participants with data for cord blood lead levels and pubertal growth trajectory classes there were no statistically significant differences between the analytical sample and the excluded cohort members regarding distribution of blood lead levels at birth; trajectory classes for pubic hair and breast development in females; trajectory classes for pubic hair and genital development in males; and birth weight in both males and females ($p > 0.05$). However, there were more Black Africans than Mixed ancestry study participants in the analytical sample; and on average males in the analytical sample were 1.22cm taller at age 8 years compared to the excluded cohort members ($p < 0.05$). Data not shown.

4.3.2 Analytical sample characteristics

Table 4.3 shows comparison analyses between males and females in the analytical sample. There was no statistically significant difference between males and females in Black African and Mixed ancestry study participants. There were slight differences in birth weight and household income between males and females at birth. The average difference in height at age 8 years was about 1 cm in favour of males. There was no difference between males and females regarding BMI at birth.

Table 4.3 Distribution of selected characteristics of the study population by sex

	Females (732) Total (%)	Males (683) Total (%)	P-value
Race			0.9
Black African	645(88.1)	601(88.0)	
Mixed ancestry	87(11.9)	82(12.0)	
Birth weight (kg)			
Mean ± SD	3.02 ± 0.5	3.13± 0.5	**<0.0001**
Range	1.07 – 4.9	1.12 - 4.8	
<2.5	91 (12.5)	57 (8.4)	**<0.0001**
2.5 – 3	268 (36.7)	203 (29.8)	
>3	372 (50.9)	421 (61.8)	
Height (8y), mean ± SD (cm)	123.6± 5.9	124.6± 5.9	**0.01**
Height-for-age z-score (8y) mean ± SD	-0.7 ± 0.9	-0.7 ± 1.0	1.0
Body mass index (kg/m^2) mean ± SD	15.9 ± 2.1	15.8± 1.4	0.4
Body mass index z-score mean ± SD	-0.1± 0.9	-0.1 ± 0.9	1.0
Household income in quintiles (range 1 to 5)			**<0.0001**
1	100 (14.8)	110 (17.4)	
2	117 (17.3)	110 (17.4)	
3	237 (35.1)	217 (34.4)	
4	138 (20.4)	13 (20.8)	
5	83 (12.3)	63(10.0)	

4.3.3 Distribution of cord blood lead levels and blood lead levels at age 13 years

At birth there were no statistically significant differences in blood lead levels between males and females as demonstrated in Table 4.4a. Blood lead levels at age 13 years ranged from 1.3 to 28.1 µg/dL in males and 1.0 to 16.3 µg/dL in females. Compared to females, a higher proportion of males had blood lead levels ≥5 µg/dL and vise versa with regard to blood lead levels <5 µg/dL as shown in Table 4.4b.

Table 4.4a Distribution of cord blood lead levels: n=234 (males) and n=235 (females)

	Cord blood lead levels		
	Male n (%)	**Female** n (%)	**P-value**
Blood lead categories (µg/dL)			0.87
<5	59 (25.2)	64 (27.1)	
5 – 9.99	164 (70.1)	160 (67.8)	
≥10	11 (4.7)	12 (5.1)	
Blood lead levels(µg/dL)			
Mean (SD)	5.9 (2.0)	5.8 (2.1)	0.60
Range	2.0-13	2.0-16.0	
Q1	4.0	4.0	
Median	6.0	6.0	
Q3	7.0	7.0	

Table 4.4b Distribution of blood lead levels at age 13 years by sex: n=684 (males) and n=732 (females)

	Blood lead levels at age 13 years		
	Male n (%)	**Female** n (%)	**P-value**
Blood lead categories (μg/dL)			**< 0.0001**
< 5	171 (25.0)	370 (50.5)	
5 – 9.99	470 (68.7)	354 (48.4)	
≥ 10	43 (6.3)	8 (1.1)	
Blood lead levels (μg/dL)			**<0.0001**
Mean (SD)	6.6(2.6)	5.0 (1.9)	
Range	1.3-28.1	1.0-16.3	
Q1	5.0	3.5	
Median	6.5	4.8	
Q3	6.0	7.9	

4.3.4 Pubertal growth trajectory classes by blood lead levels at birth and blood lead levels at age 13 years by sex

Data were analyzed for differences in the proportion of study participants in each trajectory class by blood lead levels. Almost 31% of boys with blood lead levels ≥5 μg/dL at birth were in trajectory class 1 compared to 18.6% of boys with blood lead levels <5 μg/dL in the same trajectory class (Table 4.5a). There were no statistically significant differences in trajectory classes for pubic hair development and breast development between girls with cord blood lead levels <5 μg/dL and those with cord blood lead levels ≥5 μg/dL; and trajectory classes for genital development between boys with cord blood lead levels <5 μg/dL compared to those with cord blood lead levels ≥5 μg/dL.

More than 40% of females with blood lead levels ≥5 μg/dL during adolescence had a slower tempo of progression through the Tanner stages for pubic hair development from age 9 to 16 years and had not reached postpubertal status for pubic hair development at age 16 compared to 27% of those with blood lead levels <5 μg/dL (Table 4.5b). Twenty seven percent of girls with blood lead levels ≥5 μg/dL at age 13 years had a slower rate of pubertal transition for breast development from age 9 to 16 years and were at the lowest Tanner stage for breast development at age 16 years compared to their counterparts versus 18.5% of those with blood lead levels <5 μg/dL. There were no statistically significant differences in trajectory classes for pubic hair development and genitalia development between boys with blood lead levels <5 μg/dL and those with blood lead levels ≥5 μg/dL at age 13 years.

Table 4.5a **Cross tabulation between pubertal trajectory class and cord blood levels for girls and boys**

	Cord blood lead categories (μg/dL)			
	< 5	≥ 5	Total	p-value
GIRLS n(%)				
Pubic Hair				0.136
Class 1	5 (7.8)	30 (17.4)	35 (14.8)	
Class 2	35 (54.7)	92 (53.5)	127 (53.8)	
Class 3	24 (37.5)	50 (29.1)	74 (31.4)	
Breast				0.239
Class 1	6 (9.4)	31 (18.0)	37 (15.7)	
Class 2	29 (45.3)	69 (40.1)	98 (41.5)	
Class 3	18 (28.1)	35 (20.3)	53 (22.5)	
Class 4	11 (17.2)	37 (21.5)	48 (20.3)	
BOYS n(%)				
Pubic Hair				**0.031**
Class 1	11 (18.6)	54 (30.9)	65 (27.8)	
Class 2	35 (59.3)	103 (58.9)	138 (59.0)	
Class 3	13 (22.0)	18 (10.3)	31 (13.2)	
Genital				0.251
Class 1	1 (1.7)	12 (6.9)	13 (5.6)	
Class 2	18 (30.5)	59 (33.7)	77 (32.9)	
Class 3	33 (55.9)	93 (53.1)	126 (53.8)	
Class 4	7 (11.9)	11 (6.3)	18 (7.7)	

Table 4.5b Cross tabulation between pubertal trajectory class and blood lead levels at 13 years for girls and boys

	Blood lead categories at age 13 years (µg/dL)			
	< 5	≥ 5	Total	p-value
GIRLS n(%)				
Pubic Hair				**<0.0001**
Class 1	99 (27.0)	149(41.3)	248(34.1)	
Class 2	221(60.2)	170(47.1)	391(53.7)	
Class 3	47(12.8)	42(11.6)	89(12.2)	
Breast				**0.012**
Class 1	68(18.5)	98(27.1)	166(22.8)	
Class 2	91(24.8)	94(26.0)	185(25.4)	
Class 3	146(39.8)	128(35.5)	274(37.6)	
Class 4	62(16.9)	41(11.4)	103(14.2)	
BOYS n(%)				
Pubic Hair				0.45
Class 1	51(29.8)	151(29.5)	202(29.6)	
Class 2	102(59.7)	288(56.2)	390(57.1)	
Class 3	18(10.5)	73(14.3)	91(13.3)	
Genital				0.76
Class 1	8(4.7)	28(5.5)	36(5.3)	
Class 2	70(40.9)	189(36.9)	259(37.9)	
Class 3	81(47.4)	251(49.0)	332(48.6)	
Class 4	12(7.0)	44(8.6)	56(8.2)	

4.3.5 The association between cord blood lead levels and blood lead levels at age 13 years

As shown in Table 4.6 below there is a positive association between cord blood lead levels and blood lead levels at age 13 years in males ($P<0.05$).

Table 4.6 The association between blood lead levels at birth and blood lead levels at age 13 years

Blood lead levels at age 13 years

	Males			Females		
	β	Std error	p-value	β	Std error	p-value
Cord Blood lead levels	0.147	0.066	**0.027**	0.65	0.084	0.441

4.3.6 The association between pubertal growth trajectory classes and blood lead levels at 13 years old and blood lead levels at birth

Data were analyzed for an association between blood lead levels at age 13 years and pubertal growth trajectory classes; and umbilical cord blood lead levels and pubertal growth trajectory classes in girls and boys. In females after adjusting for confounders elevated blood lead levels (≥5 µg/dL) at 13 years old were associated with significantly decreased RRR for class 2 compared to class 1 for pubic hair development ($p<0.001$) as demonstrated in Table 4.7a. Elevated blood lead levels at age 13 years relative to blood lead levels <5 µg/dL were associated with a 37% reduction in the risk of being in class 3 compared to class 1 ($p<0.05$), and a 54% reduction in the risk of being in class 4 compared to class 1 for breast development ($p<0.01$). In males, elevated blood lead levels at age 13 years were not significantly associated with puberty development as shown in Table 4.7b.

However, when data were analyzed for association between blood lead levels at birth and pubertal development; there was a 72% reduction in risk of being in class 3 compared to class 1 for pubic hair development in males ($p<0.05$) as shown in Table 4.7c. In females, there was no statistically significant association between elevated cord blood lead levels and tempo of progression (results not shown)

Table 4.7a **Multinomial logistic regression analysis to predict the risk of being in trajectory class 2 versus 1 or trajectory class 3 versus 1 or trajectory class 4 versus 1 in females with elevated blood lead levels (≥ 5 µg/dL) at age 13 years**

	Unadjusted										Adjusted [a]									
Exposure Variable	**Pubic hair development Trajectory classes**				**Breast development Trajectory classes**						**Pubic hair development Trajectory classes**				**Breast development Trajectory classes**					
	3 vs. 1		**2 vs.1**		**4 vs. 1**		**3 vs. 1**		**2 vs. 1**		**3 vs. 1**		**2 vs.1**		**4 vs. 1**		**3 vs. 1**		**2 vs.**	
	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)
Blood lead levels at age 13 years																				
<5 µg/dL																				
≥5 µg/dL	0.59	0.36-0.97†	0.51	0.37-0.70‡	0.45	0.28-0.76*	0.61	0.41-0.90†	0.72	0.47-1.09	0.55	0.26-1.17	0.45	0.29-0.68‡	0.46	0.27-0.77*	0.63	0.42-0.94†	0.72	0.47-1.11

[a] Adjusted for race and height at 8 years.

*p<0.01

†p<0.05

‡p<0.001

Table 4.7b **Multinomial logistic regression analysis to predict the risk of being in trajectory class 2 versus 1 or trajectory class 3 versus 1 or trajectory class 4 versus 1 in males with elevated blood lead levels (≥ 5 µg/dL) at age 13 years**

	Unadjusted										Adjusted [a]									
Exposure Variable	Pubic hair development Trajectory classes				Genital development Trajectory classes						Pubic hair development Trajectory classes				Genital development Trajectory classes					
	3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs. 1		3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs.	
	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)
Blood lead levels at age 13 years																				
<5 µg/dL																				
≥5 µg/dL	1.12	0.64-1.96	00.93	0.63-.137	0.98	0.36-2.62	0.83	0.37-1.90	0.76	0.33-1.75	1.35	0.73-2.47	0.94	0.63-1.39	1.02	0.37-2.83	0.88	0.38-2.01	0.77	0.33-1.77

[a] Adjusted for race and height at 8 years.

There were no statistically significant results in males.

Table 4.7c Multinomial logistic regression analysis to predict the risk of being in trajectory class 2 versus 1 or trajectory class 3 versus 1 or trajectory class 4 versus 1 in males with elevated blood lead levels (≥5 µg/dL) at birth

	Unadjusted										Adjusted [a]									
Exposure Variable	Pubic hair development Trajectory classes				Genital development Trajectory classes						Pubic hair development Trajectory classes				Genital development Trajectory classes					
	3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs. 1		3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs.	
	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)
Blood lead levels at age 13 years																				
<5 µg/dL																				
≥5 µg/dL	0.28	0.11-0.74*	0.60	0.28-1.27	0.13	0.01-1.24	0.23	0.03-1.88	0.27	0.03-2.25	0.28	0.11-0.74*	0.61	0.25-1.43	0.13	0.01-1.24	0.24	0.03-1.89	0.27	0.03-2.26

[a] Adjusted for race.

Height at age 8 years was not included in the final model because it was not statistically significant.

*p=0.01

4.4 Discussion

This study used longitudinal data to investigate the association between exposure to lead and latent classes of puberty development among urban African Black and Mixed ancestry girls and boys in South Africa. Blood lead measures ≥5 μg/dL at age 13 years were associated with lower levels of pubertal maturation at age 9 years, slower tempo of progression from age 9 through to 16 years and lower Tanner stage attainment of pubic hair development and breast development at age 16 years in females. On the other hand, cord blood lead levels ≥5 μg/dL were associated with low pubertal maturation levels at age 9 years, longer duration of puberty transition from age 9 to 16 years and lower Tanner stage attainment of pubic hair development at age 16 years in males only. As such, our findings show a link between prenatal lead exposure and slower tempo of progression in males; and postnatal lead exposure and slower tempo of progression in females; suggesting sex differences in tempo of pubertal progression based on the timing of lead exposure.

Previous studies have linked lead exposure to altered pubertal timing. NHANES studies have reported an inverse relationship between blood lead levels and onset of breast development in girls between the ages of 8 and 18 years, and an inverse association between blood lead concentrations and onset of pubic hair development and menarche in girls between the ages of 8 and 16 years (Selevan et al., 2003, Wu et al., 2003). Likewise, in Russia a cross-sectional study involving 489 boys aged 8 to 9 years showed an association between blood lead levels ≥ 5 μg/dL and delayed onset of genital development (Hauser et al., 2008). These findings were later confirmed using Cox proportional hazards model (Williams et al., 2010). Even though empirical data show that there is a link between postnatal lead exposure and delay in onset of genital development in boys; on the contrary, in this study there was no association

between adolescent lead exposure in boys and altered puberty development. These results were also interesting in that males were shown to have higher blood lead levels than females during adolescence; which could be interpreted as a sign of higher susceptibility to lead exposure in males. Therefore, it was expected that lead exposure during adolescence will be associated with tempo of progression in pubertal development in males. Instead, prenatal lead exposure in males was associated with slower transition through the Tanner stages; a significant finding since this is a period when epigenetic changes to environmental changes are most likely to occur thus setting in motion patterns and trajectories of development (Kundakovic and Jaric, 2017). As explained in the introduction section of this paper, EDCs disrupt hormonal actions during sexual differentiation which may explain the sex differences in the effects of lead exposure during pubertal progression found in this study. Almstrup et al (2016) reported lower methylation levels of CpG islands during pre-puberty compared to post-puberty in a study of healthy Danish girls and boys, with girls showing higher levels of DNA methylation than boys. During pubertal transition CpGs associated with changes in circulating reproductive hormone levels were significant in boys only, however, the authors suggest that these findings should be further examined because the number of girls (n=20) enrolled in the study was lower than that of boys (n=31) (Almstrup et al., 2016).

In a study of infants aged 3 months to 5 years blood lead levels ≥5 μg/dL were associated with a higher number of differentially methylated clusters related to lead exposure in females compared to males (Sen et al., 2015a). This was contrary to their hypothesis where males were expected to have more lead exposure related DNA methylation changes; as males have been shown to be more sensitive to lead exposure compared to females (Cecil et al., 2008, Brubaker et al., 2010). The authors propose that the unexpected findings could be suggestive of the "adaptive and protective nature" of DNA methylation changes. Our results need to be

further examined to investigate the possible underlying biological programs influencing the established sex differences in the effects of EDCs during onset of puberty, pubertal transition and post puberty.

One of the limitations of this study was that it did not include all ethnic South African population groups which may limit the generalizability of the study findings in the country. Evidence of elevated blood lead levels in Black African and Mixed ancestry children is well reported in South Africa (von Schirnding et al., 1991, Mathee et al., 2002). However, there is dearth of information regarding blood lead levels of children of Asian and Indian descent. Previous study findings showed that blood lead levels in White children were much lower than those of Mixed ancestry children in the Western Cape (von Schirnding et al., 1991). Nonetheless, there have been great developments in the new democratic South Africa and its peoples; as such it is prudent that lead exposure studies include children from all ethnic backgrounds. We also suggest that our findings be further investigated using bone lead levels as a proxy for cumulative lead exposure.

Second, pubertal development data from 11 to 16 years old were self reported. Rasmussen and colleagues recommend that self reported data be augmented by a physical assessment (Rasmussen et al., 2015). However, other studies have shown substancial agreement between the two instruments (Chan et al., 2008). In South Africa self-rating pubertal development data has been validated among Black African adolescents (Norris and Richter, 2005).

4.5 Conclusion

The results in this study suggest that environmental lead exposure is associated with slower tempo of progression in South African young girls and boys. More longitudinal research is needed to better understand the consequence of this association for later health in low and middle income countries.

PART 3

INTEGRATION OF RESULTS

CHAPTER 5: DISCUSSION

The objective of this chapter is to summarize the results of the studies which form part of this thesis, focusing attention on the key points and research theme of the study. A comprehensive framework of the theoretical and contextual relevance of the study results will be conducted. A summary of study limitations and possible future research opportunities will be discussed.

5.1 Summary of study findings

The overall aim of this study was to examine the negative health effects of environmental lead exposure in adolescents living in Soweto-Johannesburg, South Africa. To address these aims there were 3 main objectives of this study. The papers published in international peer reviewed journals answered the questions posed in these objectives. The main objectives of this study were achieved as outlined below in Table 5.1.

Table 5.1 Consolidated research findings of the three journal articles

Study objectives	Journal article	Research Findings
To examine the association between lead exposure during early adolescence and dimensionality of aggressive behaviour during mid-adolescence.	1	i. Elevated blood lead levels (blood lead levels ≥ 5 µg/dL) were positively associated with direct aggression during mid-adolescence. ii. Males were positively associated with blood lead level at age 13 years. iii. There was a higher proportion of males with blood lead levels ≥ 5 µg/dL compared to females. iv. Being male was positively associated with direct aggression, and negatively associated with indirect aggression v. The association between blood lead levels and direct aggression was driven by the male sex. vi. Being female was positively associated with indirect aggression, but not with direct aggression. **<u>In addition:</u>** vii. Maternal education at birth (mothers with a grade 11-12 education level compared to mothers with lower education levels) and being born to a young mother were negatively associated with direct aggression during mid-adolescence. viii. Birth to a single mother was negatively associated with disobedience during

		mid-adolescence. ix. Socio-economic status of the family at birth was negatively associated with indirect aggression.
To investigate a possible link between lead exposure during early adolescence and violent behaviour during late-adolescence.	2	i. Blood lead levels and blood lead categories (≥5 μg/dL) at age 13 years were associated with fighting and physical violence. ii. As in mid-adolescence, during late adolescence there was a higher proportion of males than females with blood lead levels ≥ 5 μg/dL. iii. Males were positively associated with blood lead levels at age 13 years. iv. Males were shown to be more likely to perpetrate violent behaviour acts such as 'violence using a weapon', 'physical violence', 'fighting' and 'robbing others' against other adolescents during late adolescence compared to females. **In addition:** v. Birth to a mother with a grade 9 education level and higher, socio-economic status and birth in a private hospital were positively associated with being verbally and emotionally abusive to other adolescents during late adolescence. vi. Birth to a single mother was positively associated with perpetration of physical violence during late adolescence. vii. Adolescents born in a private hospital

		were negatively associated with perpetration of violent behaviour in late adolescence.
To assess the effects of lead exposure during early adolescence on pubertal transition in females and males. And also examine the link between cord blood lead levels and pubertal progression in females and males.	3	i. Cord blood lead levels were positively associated with blood lead levels at age 13 years in males. ii. There was a difference in the association between prenatal and adolescent blood lead levels, and pubertal transition: • Cord blood lead levels ≥ 5 μg/dL were associated with pubertal transition in boys; and • Blood lead levels at age 13 years were associated with pubertal transition in girls.

Race was controlled for in all the journal articles. Previous studies conducted in South Africa showed variations in blood lead levels of school children across racial lines. For example, in Cape Town children from Mixed ancestry background have higher blood lead levels compared to their White counterparts (von Schirnding et al., 1991). Because of South Africa's history of segregation and discrimination, race influenced where people lived, their access to healthcare and different other variables. The study participants in this study were born in 1990, at the dawn of democracy in South Africa. As such, it was important to control for race even though there are no studies that we know of where blood lead levels of Black African children were compared to those of children from Mixed ancestry in South Africa. In this study there was no statistically significant difference between blood lead levels of Black African and Mixed ancestry children ($p > 0.05$).

5.2 Key findings from the journal articles

- **The four major points highlighted in this study were:**

i. Sex differences in relation to dimensionality of aggressive behaviour during mid-adolescence.

ii. The possible neuro-behavioural effects of early lead exposure later in adolescence.

iii. Possible sex differences in "neurotoxic effects" and neuroendocrine effects of postnatal lead exposure.

iv. Sex specific response in relation to lead exposure at birth and pubertal progression.

5.2.1 Dimensionality of aggressive behaviour between direct aggression and indirect aggression during mid-adolescence.

In this study descriptive statistics showed that a much higher proportion of females than males exhibited positive evidence for indirect aggression relative to direct aggressive behaviour which was much higher in males. Accordingly, when data were tested for an association between being female and indirect aggression, using male sex as a reference category, there was a statistically significant positive association between being female and indirect aggression (data not shown). With regard to use of indirect aggression, our results are consistent with those from another South African study which showed a slightly higher perpetration of online aggression by adolescent girls than boys (Burton, 2012). Online aggression is a form of aggressive behaviour which "transcends physical boundaries" (Burton and Leoschut, 2012); thus more in line with the theoretical constructs of indirect aggression. Similarly, international literature shows a higher likelihood of perpetration of indirect aggression by female adolescents (Bjrkqvist et al., 1992, Archer, 2004). However, it is important to note that empirical data on sex differences regarding use of indirect aggression is inconclusive (Forrest et al., 2005, Moroschan et al., 2009, Card et al., 2008). Card et al (2008) suggests that it is a misperception that girls are more indirectly aggressive than boys, but rather that the "general pattern is of similarities than differences" among girls and boys in relation to indirect aggression. However, in our study there were clear sex differences in this regard. On the hand, the data are almost conclusive in relation to the link between male sex and direct aggression.

These findings are significant in that the consequences of both direct and indirect aggression can be quite severe for adolescents. Direct aggression is strongly associated with "emotional

dysregulation, conduct problems, low peer acceptance, and peer rejection". Whilst indirect aggression is more strongly associated with "internalizing problems" (Card et al., 2008). Furthermore, Card et al (2008) report that there is a unique association between direct aggression and "low prosocial behaviour" and indirect aggression and "high prosocial behaviour". Indirect aggression in females for example is hurtful in that it seeks to damage the victim's social reputation and her close friendships among other things (Card et al., 2008); the main intention being to isolate the victim, which is where the perpetrator's "high prosocial behaviour" becomes an asset. In general, consistent acts of aggression could be a sign of maladjustment (Card et al., 2008). When trying to address issues of aggressive behaviour among adolescents, it seems that direct aggression may be easier to identify and address, but indirect aggression may pose a more serious problem in that it may be difficult to pin point the perpetrators and even worse for the victims to prove their ordeal as the actions of the perpetrator may not be as overt. The important question is what mediating factors can be put in place to reduce the risk of adolescents becoming either perpetrators or victims of aggressive behaviour?

5.2.2 Lead exposure and neuro-behavioural effects: From aggressive behaviour during mid-adolescence to violent behaviour during late adolescence

Compared to perpetration of aggressive behaviour during mid-adolescence (Chapter 3), the proportion of adolescents responding positively for perpetration of violence in late-adolescence (Chapter 4) was much higher within the BT20+ cohort. These findings imply a possible progression from aggressive behaviour in mid-adolescence to violent behaviour in late adolescence. It is important that these results are further examined to identify age trends in aggressive and violent behaviour among South African youth. Early aggressive behaviour

and antisocial behaviour have been shown to be risk factors for violent and criminal behaviour in late adolescence and adulthood (Huesmann et al., 2002, Huesmann et al., 2009). Masango also points out that in unhealthy societies anger leads to aggression which manifests into violence (Masango, 2004).

Even though males were the main perpetrators of violent behaviour, it is important to note that the prevalence of violent behaviour in females was much high than expected. For example, 39.8% of females admitted to having pushed or shoved someone when angry; and 25% to having hit or kicked someone. With regard to females and violent behaviour; estimates from another South African study were much lower than in this study. A study on violence in schools reported that males were the main perpetrators (90%) of gender-based violence (Burton and Leoschut, 2012). There is a slight difference of course in that our study did not directly examine gender-based violence within the cohort. Nonetheless, our results do highlight that violent behaviour is a serious health problem among South African youth.

5.2.3 Are males more susceptible to "neurotoxic effects" and females to neuroendocrine effects of postnatal lead exposure?

Vahter et al. (2007) posit that there are gender differences in how health outcomes related to exposure to lead and other toxic metals such as cadmium, arsenic and mercury manifest due to variations in "kinetics, mode of action or susceptibility". Males are more susceptible to "neurotoxic effects" and females more susceptible to "immunotoxic effects" of lead (Vahter et al., 2007).

In our study blood lead levels at age 13 years were higher in males than females; and male sex was associated with blood lead levels. When data were examined for an association between blood lead levels at age 13 years and neuro-behavioural effects such as aggressive behaviour and violent behaviour; with regard to aggressive behaviour it was evident that the positive association between lead exposure and direct aggression was driven by the male sex. There was a statistically significant association between being male and direct aggression, and as mentioned in the previous sub-section being female was positively associated with indirect aggression. Similarly, lead exposure at age 13 years was positively associated with physical violence and fighting during late adolescence, and so was being male. These results are consistent with the above stated assertion by Vahter et al (2007) that males are more vulnerable to "neurotoxic effects" of lead than females. Sen et al (2015) showed that differentially-methylated regions associated with lead exposure in males, among others, "show an enrichment of genes associated with calcium ion transport". Lead is known for its ability to substitute for various divalent cations including calcium thus able to substitute for calcium affecting CNS regulatory systems (Garza et al., 2006). Furthermore, as previously alluded to, the reduction in adult brain volume associated with childhood lead exposure is statistically significant in males only (Cecil et al., 2008). On the contrary, in this study when the effects of lead exposure at age 13 years were assessed in relation to the tempo of pubertal progression; the negative health effects of lead exposure were only statistically significant in females. These research findings were not expected because as mentioned earlier, males in this study had higher blood lead levels compared to females; and epidemiologic studies have shown that there is a link between lead exposure and delayed onset of puberty in males (Hauser et al., 2008, Williams et al., 2010). These findings suggest that the effects of lead exposure may be influenced by the stage of pubertal development. Could these differences be influenced by different hormonal changes and regulatory systems specific to the different

stages of pubertal development? Almstrup et al (2016) showed that there are differentially methylated CpGs associated with changes in specific circulating hormones during pubertal transition; and these were only significant in males. Blood lead levels ≥5 μg/dL are mostly associated with hypermethylation (Sen et al., 2015a). These findings raise the question of whether the identified DNA methylation in males during pubertal progression may be part of the explanation for the lack of association between lead exposure and altered puberty during pubertal transition.

Our findings generate a hypothesis that postnatal lead exposure is associated with "neurotoxic effects" driven by male sex and characterized by direct aggression and physical violence and fighting; and that lead exposure is associated with neuroendocrine effects characterized by longer duration of pubertal progression in females. It is well established that lead exposure related neuro-behavioural disorders are more pronounced in males compared to females. However, our research findings raise a novel and very important question about possible sex specific biological mechanisms related to postnatal lead exposure and neuroendocrine mechanisms at different stages of pubertal development. These results should be further examined using bone lead levels and more than one test to measure aggressive and violent behaviour.

5.2.4 Lead exposure at birth and tempo of pubertal progression

It is important to mention that there were no statistically significant differences in cord blood lead levels between males and females in this study (data not shown). However, there was a positive association between cord blood lead levels and blood lead levels at age 13 years. When cord blood lead levels <5 μg/dL and ≥5 μg/dL were measured against trajectory classes for pubic hair development the results were statistically significant (p=0.03) in males

only as shown in Table 4.4a. Cord blood lead levels were significantly associated with altered pubertal progression in males only. There is a gap in evidence regarding cord blood lead levels and pubertal development. Cord blood lead levels represent the measurement of lead exposure during foetal development. This is a very sensitive period in child development when epigenetic dyregulation due to lead exposure is most likely to take place. If epigenetic foetal development through DNA methylation can explain the neuroendocrine effects of lead exposure (Pilsner et al., 2009); our findings need to be further examined using bone lead levels in addition to cord blood lead levels to further determine if the effects of maternal burden of lead on pubertal progression differ from those of current lead exposure. Pilsener et al (2009) showed that there is no statistically significant association between cord blood lead levels and DNA methylation in cord blood but a significant association between elevated maternal patella lead levels and lower cord genomic DNA methylation.

5.3 Emerging research theme

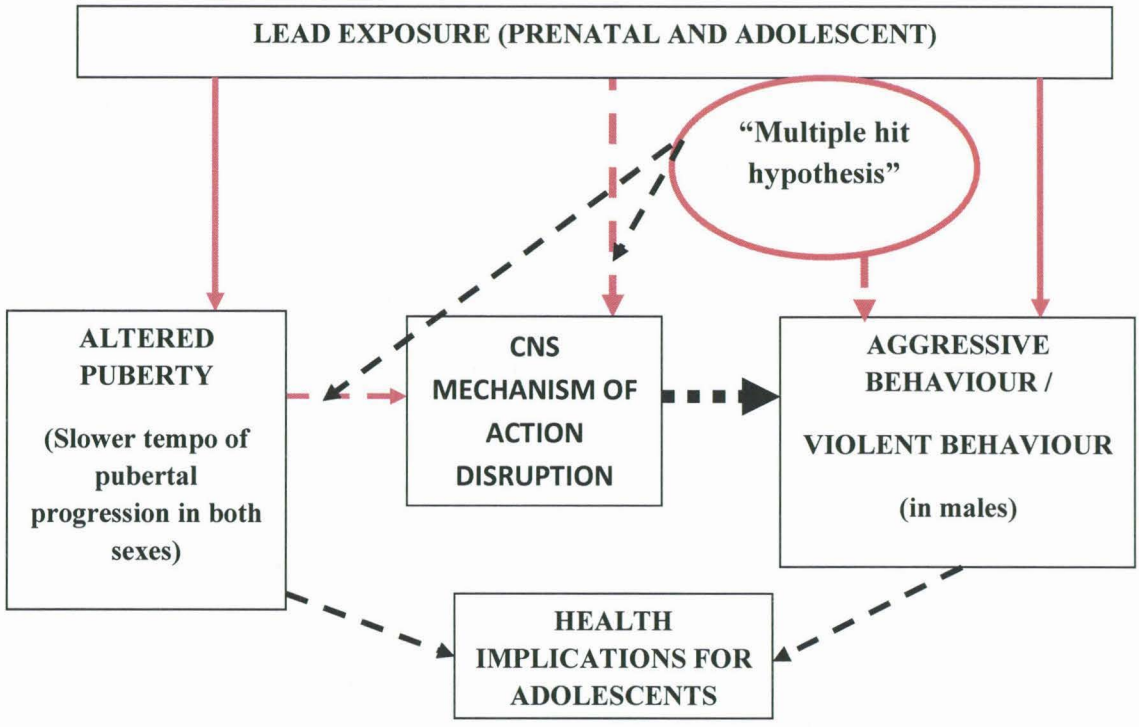
5.3.1 The relationship between lead exposure and slow pubertal transition and aggressive/ violent behaviour

This study examined the effects lead exposure during pubertal transition and mid- and late adolescence. Adolescence and puberty are intricately intertwined because they are influenced by brain development and hormonal events associated with it. While puberty is a period of individual growth to become sexually reproductive; adolescence is a period between childhood and adulthood encompassing pubertal transition, cognitive and emotional development and social maturation (Sisk and Zehr, 2005). Puberty starts from ages 8 and 9 in girls and boys respectively (Blondell et al., 1999) and its end is characterized by epiphyseal closure at age 16 years in girls and age 17 years in boys (Roenneberg et al., 2004). Adolescence starts at age 10 years and its conclusion is defined by a combination of factors

including "physical, psychological, mental and social measures" at approximately 19 years old (Bahadur and Hindmarsh, 2000) as cited by (Roenneberg et al., 2004) and (UNICEF., 2011). As shown, there is an overlap in the ages during the two transitional stages which means that they are not mutually exclusive.

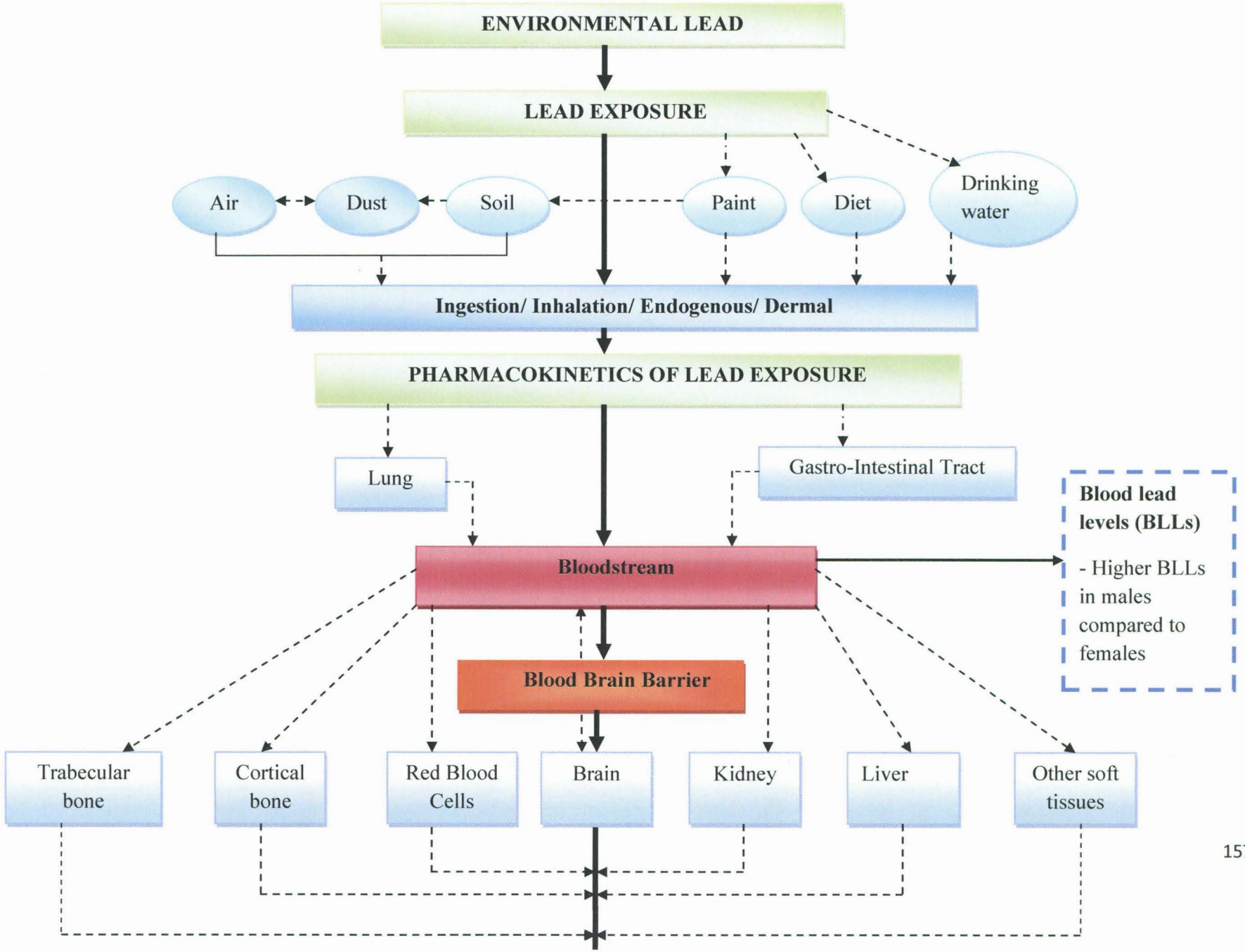
As discussed in the introduction section, exposure to lead may lead to disruptions in the CNS mechanisms of action leading to abnormal neurodevelopment particularly in the prefrontal cortex of the brain. In turn, this may have adverse neuro-behavioural effects such as aggressive behaviour and violent behaviour. Neuro-imaging studies such as the MRI show that there is a link between sex hormones responsible for pubertal progression and structural development of the brain in humans (Blakemore et al., 2010); predominantly the prefrontal cortex and the limbic brain regions (Spear, 2000); in addition to the physical transformation associated with puberty. Vital for this research is that altered pubertal transition could trigger dysfunction in the regions of brain similar to those related to lead exposure and aggressive and violent behaviour (Spear, 2000, Sisk and Zehr, 2005, Cecil et al., 2008, Blakemore et al., 2010). This suggests the possibility of "multiple hit hypothesis" in the CNS as demonstrated in Figure 5.1. More research needs to be conducted in this regard and about the link between slower tempo of pubertal progression and aggressive or violent behaviour during adolescence.

Figure 5.1 Possible "Multiple hit hypothesis" in the CNS due to environmental exposure



5.4 Conceptual relevance

Results from this study contribute in addressing the gaps in available reliable data on the possible role of environmental toxins on adolescent health in low and middle income countries. In addition, they raise questions about what is known or not known about the effects of lead exposure and how these are either augmented or impeded by the different stages of development in males and females. The main research findings as discussed in detail in previous sections are incorporated into the conceptual framework that was presented in Chapter 1 (Figure 1.1) as demonstrated in Figure 5.2.


ENVIRONMENTAL LEAD
LEAD EXPOSURE
Air
Dust
Soil
Paint
Diet
Drinking water
Ingestion/ Inhalation/ Endogenous/ Dermal
PHARMACOKINETICS OF LEAD EXPOSURE
Lung
Gastro-Intestinal Tract
Bloodstream
Blood lead levels (BLLs)
- Higher BLLs in males compared to females
Blood Brain Barrier
Trabecular bone
Cortical bone
Red Blood Cells
Brain
Kidney
Liver
Other soft tissues

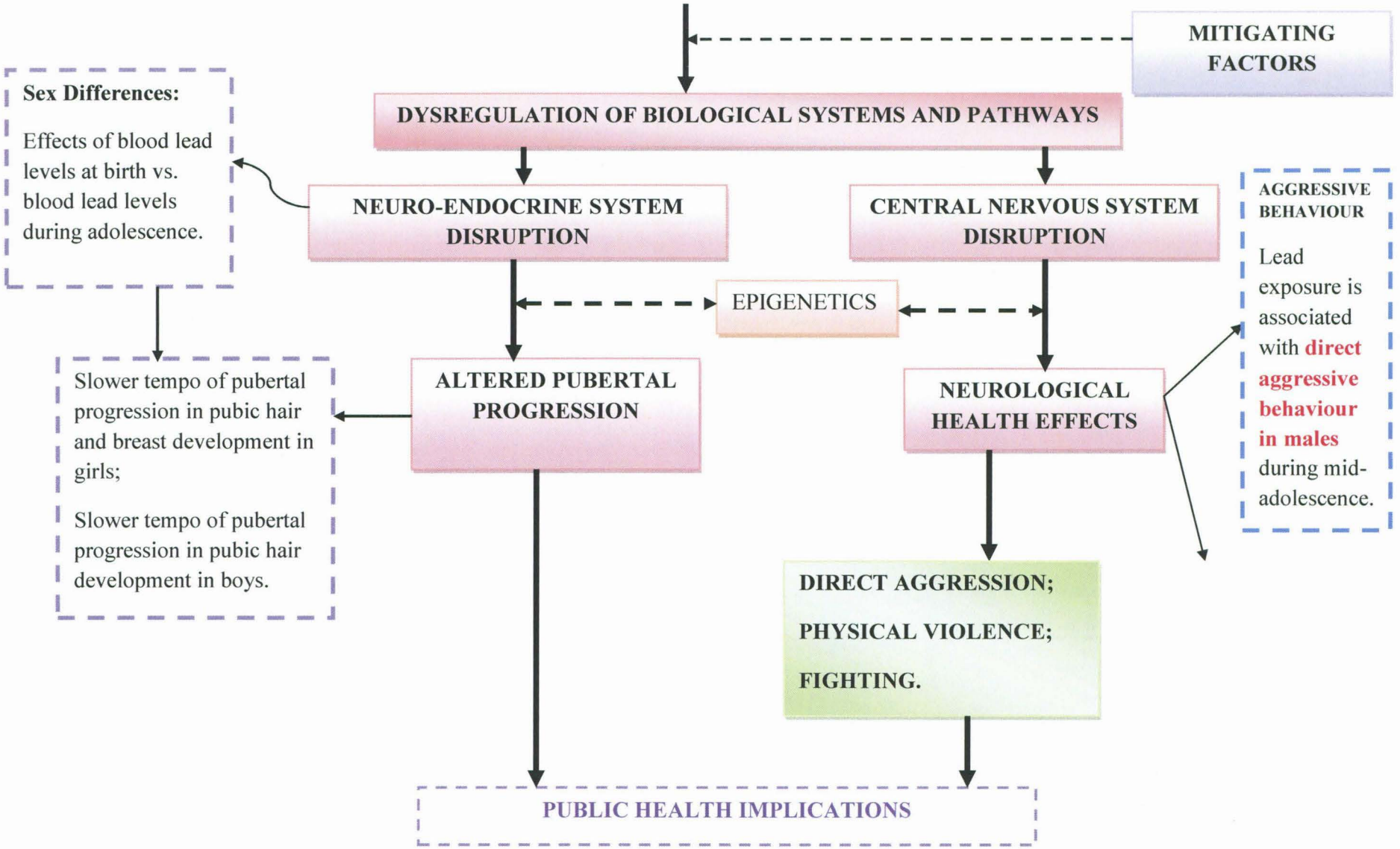


Figure 5.2 Conceptual relevance of the study

5.5 Contextual relevance

The sustainable development goal 3 of the Sustainable Development Goals 2030 aims to "ensure health and well-being for all, at every stage of life" with one of its major health priorities seeking to address "non-communicable diseases and environmental diseases" (United Nations, 2013). Environmental lead exposure increases the burden of human diseases. An NHANES II study examined the link between blood lead levels and mortality; blood lead levels > 10 μg/dL were associated with a 68% increase in cancer mortality, 39% increase in circulatory disease mortality and 46% increase in all other diseases mortality (Lustberg and Silbergeld, 2002). In 2013, the Institute for Health Metrics and Evaluation attributed 853 000 deaths and 16.8 million DALYs on health to lead exposure, most of which were in developing countries (World Health Organization, 2016b). According to Dr. Maria Neira, Director at the Department of Public Health, Environmental and Social Determinants of Health at the World Health Organization "the release of lead into the environment poses significant risks to human health and the environment. We've known for centuries that lead is poisonous, and we know lead exposure is a serious threat to our kids.....No level of lead exposure is considered safe for children" (World Health Organization, 2016a). It is therefore very clear that lead exposure continues to be a global health problem.

The Global Alliance to Eliminate Lead Paint called for the fifth International Lead Poisoning Prevention Week (ILPPW) in October 2017. Its main objective was to mobilize for the phasing-out of lead paint and its manufacturing with a clear focus on prevention. Additionally, it sought to educate communities about the dangers of lead exposure especially in children (World Health Organization, 2017) and minimize occupational lead exposure from lead paint (World Health Organization, 2016a). In the WHO and United Nations Environment survey in June 2016, South Africa and Algeria were the only 2 countries in

Africa whose governments confirmed that they have "legally binding control measures on lead paint" (World Health Organization, 2017). As mentioned earlier, lead paint is one of the major sources of lead exposure in children. In South Africa previous studies have found use of lead paint in homes with young children (Montgomery and Mathee, 2005). Even though use of lead in household paint has been banned in South Africa since 2010, the level of compliance among paint manufacturers in still uncertain (Mathee, 2014). Currently, there is no national blood lead surveillence programme in place in South Africa.

However, it is important to note that the world has come a long way since the first cases of lead poisoning from paint in children were documented as outlined in Box 5.2.

Box 5.2 – History of lead poisoning in children

1831	Paris, France – Dr Louis Jean-Charles-Marie-Tanquerel des Planches begins his work on lead poisoning.
1848	Use of toys painted with lead paint is linked to lead colic in children by Dr Tanquerel des Planches.
1892	Brisbane, Australia - first reported cases of childhood lead poisoning.
1897	Queensland, Australia – lead poisoning in children documented.
1904	Queensland, Australia – Dr Lockhart Gibson links leaded paint used in homes to lead poisoning epidemic in children.
1909	France, Belgium and Australia - use of white-lead interior paint banned.
1914	United States – first documented description of lead poisoning in children from eating crib-paint.
1920	Brisbane, Australia - use of lead in paint is banned.
1922	White-lead interior paint banned by the League of Nations. United States did not adopt the banning.
1922-50	Use of white-lead interior paint banned in more than 20 countries.
1922	Queensland, Australia – use of lead paint banned for certain household surfaces.
1924	United Sates – first cases of lead poisoning in children documented at Boston Infants' and Children's Hospital.
1926	United Sates – Dr Charles McKhann states "Lead poisoning is of relatively frequent occurrence in children."
Early 1930s	Toronto, Canada – 23 cases of lead poisoning in children are reported including 2 deaths in two years.
1931-40	Baltimore - United States, 135 cases of child plumbism with 41 deaths are documented.
1940	Use of white lead abolished in South Africa.

1943	United States, Randolph K. Byers and Elizabeth E. Lord – publish the first empirical evidence of childhood lead exposure and its late-effects such as long-term behavioural and cognitive deficits in children.
1960-70	CDC recommends 60 µg/dL as the 'blood lead level of concern' for lead exposure in children.
1970-85	CDC lowers the 'blood lead level of concern' in children for lead exposure to 30 µg/dL.
1985-91	CDC lowers the 'blood lead level of concern' in children for lead exposure to 25 µg/dL.
1991-2012	CDC lowers the 'blood lead level of concern' in children to 10 µg/dL.
2009	Regulations for use of lead in paint were promulgated in South Africa.
2010	Regulations against use of lead in paint came into full effect in South Africa.
2012	CDC recommended a "childhood BLL reference value" of 5 µg/dL.
2018	South Africa has not developed blood lead standards of its own, nor officially endorsed any developed by international institutions. Researchers have generally followed the CDC reference levels.

Compiled from: (Dana, 1848, Blackfan, 1917, Byers and Lord, 1943, Rabin, 1989, Needleman, 2004, Centers For Disease Control and Prevention, 2012a, Bochynska Katarzyna, 2013, World Health Organization, 2017)

Global annual lead awareness campaigns such as the ILPPW are evidence that environmental lead exposure is still a problem in many societies. It is therefore desirable that more research is conducted to highlight its detrimental health effects especially in children from low and middle income countries.

This study shows the possible link between environmental toxicity and biological disruptions in developing youth. Papers 1 and 2 showed the adverse neuro-behavioural effects of lead

exposure during early adolescence. Paper 3 highlighted the effects of foetal and adolescent lead exposure on the tempo of pubertal progression and how these may manifest in a sex specific manner. These results imply that lead exposure, be it prenatal or postnatal, may negatively impact the entire life and future of the affected young person. It is important to note that the available data have only shown associations and not causality.

As outlined in Box 5.2, it took reliable evidence to shift the awareness of lead exposure associated health effects in children from speculation to scientific facts. As discussed earlier, this lead to the phasing out of the use of lead in paint and petrol and later compelling CDC to shift the 'lead level of concern' from 10 to 5 μg/dL as the "upper reference range value for blood lead levels in children" (Centers For Disease Control and Prevention, 2012b).

It is hoped that these findings will add value to evidence-based data necessary for lobbyists to advocate for screening and monitoring of blood lead levels, especially in high risk communities.

5.6 Limitations of this study

i) As mentioned earlier, the main limitation of this study is that it only included Black African and Mixed ancestral study participants. This limits the generalizability of the study findings pertaining to other racial groups. It is therefore necessary that these data are further investigated using a sample that is inclusive of all South African ethnic groups.

ii) In this study recent exposure measures taken at one time point both for prenatal and postnatal blood samples were used. In addition to current blood lead levels,

cumulative blood lead levels are recommended when evaluating effects of lead exposure.

iii) Nutrition is an important factor in measuring lead exposure levels. As stated in the literature review section levels of zinc, iron and calcium levels influence the level of lead absorption. These were not controlled for in the research studies as possible confounding variables.

5.7 Identified research gaps and future research

i. The role of epigenetics in neuroendocrine effects of lead exposure during pre-puberty, onset of puberty, pubertal transition/ tempo of pubertal progression and post puberty needs to be examined? Including information on how do these differ and why?

ii. More research is required to establish if females are more susceptible to postnatal lead exposure and its effects on pubertal progression compared to males. Additionally, to examine if males are more susceptible to prenatal lead exposure and its effects on pubertal progression?

iii. The association between slower tempo of pubertal progression and aggressive/ violent behaviour in lead exposed adolescents.

iv. The literature review showed that delayed onset of puberty in boys is associated with low-self esteem, and aggression, amongst others. In this study lead exposure during early adolescence was linked to direct aggressive behaviour in mid-adolescence. It would be interesting to examine if there is a relationship between lead exposure and

altered puberty, including delayed onset and lower tempo of progression in pubertal development, and low-self esteem. That is, to determine if outcomes such low-self esteem are dependent on the exposure variable.

CHAPTER 6: CONCLUSION

The aim of this thesis was to examine the health effects of prenatal and postnatal lead exposure during pubertal transition, mid-adolescence and late adolescence. In low and middle income countries including South Africa there is huge deficit in information regarding dimensionality of aggression as a consequence of environmental lead exposure during childhood. Furthermore, in South Africa there were no empirical data showing the link between prenatal and adolescent blood lead levels and pubertal progression from early to late pubertal development in girls and boys. Findings from this thesis are vital to inform decisions on the need for national driven lead screening and monitoring systems in South Africa and other countries of similar economies.

The study findings showed a relationship between prenatal lead exposure and longer pubertal transition in boys. Postnatal lead exposure was associated with slower pubertal progression in girls; direct aggressive behaviour during mid-adolescence and physical violence during late adolescence.

The implications of lead exposure in young people are dire in that – as alluded to earlier in the literature review section, children from lower socio-economic environments have been shown to have higher blood lead levels compared to their counterparts from affluent communities because of variables such as where they live, among others. This means that they face high probability of being trapped in poverty cycles for generations. Lead exposure

can stunt the development of children i) physically: slower pubertal progression can negatively affect young people when they are not developing at the same rate as their counterparts; ii) academically: display of aggressive and violent behaviour towards others can lead to expulsion from school; iii) socially: due to unruly and unacceptable behaviour; and iv) economically: because with low levels of education young people have limited employment opportunities.

Developed countries such as the United States, which for the most part have much lower blood lead concentration in children, have national programs in place to address lead exposure. South Africa and many other low and middle income countries need to make blood lead screening and monitoring in children a matter of priority. However, in implementing measures to prevent and control lead exposure in children, it is important that these are put into practise in a manner that is fair and does not infringe on dignity and basic human rights of the children. They should be devoid of profiling, labelling and stigmatization of young people.

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APPENDICES

Appendix 1: Journal Article 1

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The association between environmental lead exposure with aggressive behavior, and dimensionality of direct and indirect aggression during mid-adolescence: Birth to Twenty Plus cohort

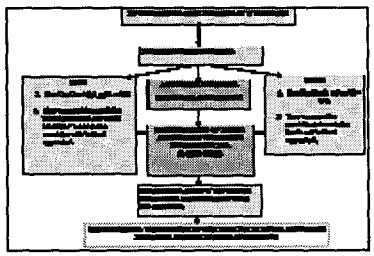
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HIGHLIGHTS

- Higher blood lead levels are associated with direct aggression in mid-adolescence.
- Males have higher blood lead levels than females during mid-adolescence.
- Adolescent males are positively associated with direct aggressive behavior.
- Adolescent females are positively associated with indirect aggressive behavior.
- Sociodemographic factors at birth influence dimensions of aggression later in life.

GRAPHICAL ABSTRACT



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ABSTRACT

Chronic lead exposure is associated with neurological ill-health including anti-social behavior such as aggressive behavior. The main aim of this study was to examine the association between lead exposure at 13 years old and dimensions of aggressive behavior during mid-adolescence.

The study sample included 508 males and 578 females in mid-adolescence (age 14 to 15 years) from the Birth to Twenty Plus cohort in Johannesburg, South Africa. Blood samples collected at age 13 years were used to measure blood lead levels. Seventeen items characterizing aggression from the Youth Self Report questionnaire were used to examine aggressive behavior. Principal Component Analysis was used to derive composite variables from the original data for aggressive behavior, and data were examined for an association between blood lead levels and dimensionality of direct and indirect aggression and disobedience during mid-adolescence. We also examined the dimensions of aggression during mid-adolescence in relation to gender and socio-demographic factors.

Blood lead levels ranged from 1 to 28.1 μg/dl. Seventy two percent of males and 47.7% of females in the study had blood lead levels ≥5 μg/dl. There was a positive association between elevated blood lead levels and direct aggression ($p < 0.05$). Being male was positively associated with direct aggression ($p < 0.001$) but, negatively associated with indirect aggression ($p < 0.001$). Maternal education and age at birth were negatively associated with direct aggression during mid-adolescence.

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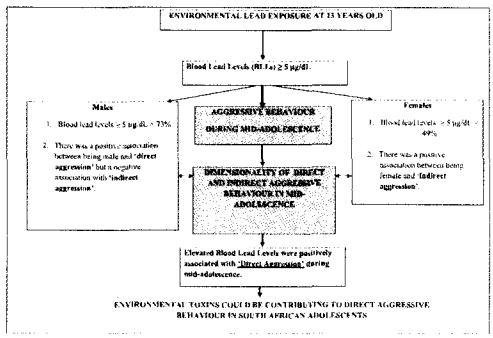
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The significant association between elevated blood lead levels and direct aggressive behavior observed in this study may shed light on a possible environmental toxicological contribution to aggressive behavior in South African youth; and most importantly the type of aggressive behavior associated to lead exposure.
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1. Introduction

The World Health Organization (WHO) estimates that lead exposure accounts for 0.6% of the global burden of disease (World Health Organization, 2009). Lead is also considered an important environmental health hazard contributing to the environmental disease burden in Africa (Nweke and Sanders, 2009; Centers for Disease Control and Prevention, 2012b). A known cumulative toxicant, lead affects the "neurological, haematological, gastrointestinal, cardiovascular and renal systems" (World Health Organization, 2010b). Epidemiologic studies have shown evidence of lead-related adverse health outcomes, most notably acting on the central nervous system (CNS) (Needleman and Gatsonis, 1990; Hernberg, 2000; Lanphear et al., 2005; Schwartz et al., 2007; Shih et al., 2007; Yuan et al., 2006; Cecil et al., 2008; Cecil et al., 2011). The effects of lead exposure on the CNS in children are associated with reduced cognitive function, aggressive, violent, delinquent and criminal behavior, among others (Needleman et al., 1979; Needleman and Gatsonis, 1990; Needleman et al., 2002; Bellinger et al., 1992; Canfield et al., 2003; Lanphear et al., 2005; Mazumdar et al., 2011; Wright et al., 2008). The indirect costs of childhood lead exposure speak to the economic burden it places on the general public (World Health Organization, 2010a). Its adverse effects on the intellectual function of young people (Lanphear et al., 2000; Lanphear et al., 2005) may in turn affect the level of education they attain and employment opportunities available to them in the future. Additionally, there is a link between reduced educational attainment and the probability of being arrested (Lochner and Moretti, 2004). As such, environmental lead exposure contributes to great "*lost opportunity costs*" (World Health Organization, 2010a) and robs young people of their full potential in life.

The Port Pirie Cohort was the first study to prospectively evaluate the association between lifetime blood lead exposure in children and "emotional and behavioral problems" (Burns et al., 1999). Children 11 to 13 years from a lead smelting neighbourhood in Port Pirie, Australia were included in the study. Using a cut-off score of 15 μg/dL, in both sexes a correlation between higher cumulative blood lead levels and total behavior problems was found. In boys, the highest correlation was between elevated lifetime blood lead levels and "aggressive behavior", "delinquent behavior", and "attention problems"; and in girls with "aggressive behavior", "delinquent behavior", "attention problems", "social problems", "anxious/depressed", and "withdrawn" (Burns et al., 1999).

South African children particularly from poor communities are exposed to environmental lead through various mediums such as use of lead based paint in children's toys (Mathee et al., 2007) and playground equipment for children in Johannesburg, Ekurhuleni, and Tshwane (Mathee et al., 2009); use of lead in subsistence fishing where waste lead for example from "wheel balancing and alignment centers" is collected, melted and recycled to make new craft sinkers (Mathee, 2014); and pregnant women who practice geophaphia thus increasing the risk of prenatal lead exposure (Mathee et al., 2014) among others.

Naicker et al. (2012) examined the relationship between lead exposure and socio-behavioral adjustment including aggressive behavior during early adolescence in the Birth to Twenty Plus (BT20+) cohort in South Africa. With regards to aggressive behavior, blood lead levels at age 13 years were negatively associated with aggressive behavior item 'I argue a lot' 95% CI [−0.23 to −0.02] but positively associated with 'I attack other people' 95% CI [0.09–0.98] (Naicker et al., 2012). To further examine these findings at a later stage in adolescence and determine the specific type(s) of aggressive behavior related to environmental lead exposure, we set out to determine the association between lead exposure in early adolescence and aggression items in mid-adolescence (at ages 14 to 15 years old). The use of Principal Components Analysis (PCA) in this study allowed for a more comprehensive integration of aggressive behavior. We examined lead exposure at age 13 years old and dimensions of aggression during mid-adolescence. These data are essential for public health and environmental health policymakers to address this important public health problem in the country.

2. Methods and materials

2.1. Study population

BT20+ is the largest and longest running longitudinal birth cohort in Africa. The cohort includes all singleton births at public health facilities during a seven-week period from April 23 to June 8, 1990 in Soweto/Johannesburg, South Africa. The birth cohort is representative of long-term urban residents. Over the years, the cohort has reported a very low attrition rate of <3% annually – with the highest rate reported in the first two years of the study; mostly due to movement away from the study area (Norris et al., 2007). The cohort is described in detail elsewhere (Richter et al., 2004; Richter et al., 2007).

For this study, Black African and Coloured (mixed race heritage) study participants with blood lead samples at age 13 years and who had completed the Youth Self Report(YSR) during mid-adolescence at ages 14 to 15 were included (n = 1086). White and Indian study participants were excluded due to very low numbers. In addition, to test if study participants exhibiting aggressive behavior during mid-adolescence have early predisposition to aggressive behavior, study participants with YSR data for year 11 were included in the study.

2.2. Blood lead measurements

Venous samples of whole blood were collected at age 13 years into EDTA-containing tubes previously determined to be free of trace metals. Blood sampling was undertaken by professional health officials, using sterile equipment and aseptic techniques. Blood samples were vortexed and rolled on the coulter mixer for at least 10 min until properly mixed. They were diluted 10 times with 1,1% (v/v) Triton X-100 using automatic Hamilton Microlab 500 diluter into disposable 10 mL Sterilin plastic tubes covered with screw caps and mixed well using a vibration mixer. Blood lead levels were measured using Perkin Elmer 600 AAnalyst atomic absorption spectrometer with a THGA graphite furnace, Zeeman background correction and AS-800 Autosampler. Both blood samples and samples for quality control were prepared and measured in-house.

2.3. Measurement of aggressive behavior in mid-adolescence and potential confounders

Data were collected from the cohort at age 14 to 15 years using the YSR questionnaire. This questionnaire is a self-report measure used to examine social and behavioral problems in children and adolescents aged 11 to 17 years. These include aggressive behavior, substance abuse, breaking rules, social problems, thinking disorders, anxiety disorders, depression, mood disorders, impulsive behavior and others (Achenbach, 1991). Seventeen of the 112 questionnaire items examine aggressive behavior. Each item has four response options: not true, sometimes true, true and very true. The aggressive behavior response

options were collapsed into two categories referred to as 'negative response' for not true and sometimes true and 'positive response' for true and very true.

To measure possible confounding, socio-demographic factors such as gender, maternal education at birth (which was categorized into four levels, i.e. grade 7 or less, grade 8–10, grade 9–12 and post school education) – the South African education system is divided into primary school (grade 1–7), secondary school (grade 8–10), high school (grade 11–12), and tertiary (college/university); maternal marital status at birth was dichotomized into married/living with a partner, or single/widowed/divorced/separated. It has been shown that children raised from two-parent households have less aggressive behavior tendencies compared to their counterparts from single parent households (Usakli, 2013) most of which are headed by single mothers (Fields, 2003); maternal age at birth was categorized as ˜20 (representing teenage mothers), 20–29 (representing younger mothers in their 20's), 30–39 (representing older mothers in their 30's) and ≥40 years old (representing mother aged 40 and above) – children born to teenage mothers are more likely to exhibit aggressive and antisocial behavior later in life (Fergusson and Woodward, 1999; Nagin and Tremblay, 2001); residential area of birth was collapsed into two categories, Soweto/Diepmeadow or former Coloured/Asian and Inner City/Suburb – in former *apartheid*[1] South Africa inner cities and suburbs were places of residence mainly for white South Africans; and hospital of birth was categorized as public or private – health services in *apartheid* South Africa were segregated according to each racial group (Kautzky and Tollman, 2008). In addition, socio-economic status (SES) at birth was calculated using binary household items, such as type of home, access to water inside the dwelling, sole use of water, access to flush toilet, sole use of toilet, and ownership of household appliances including television, motor car, fridge, washing machine and telephone. PCA with varimax rotation was used as the dimensionality reduction technique to create an SES variable. The sample size was adequate with a Kaizer-Meyer-Olkin score of 0.68 and the Bartlett's test was statistically significant ($p < 0.001$). One PCA component was extracted based on Scree Plot analysis and eigenvalue of >1 (Supplementary Table 1).

2.4. Statistical analysis

2.4.1. Descriptive statistics

For the purposes of this study, blood lead levels at age 13 years were stratified into three categories: <5 μg/dL as the reference level, 5–9.99 μg/dL and ≥10 μg/dL. In part, these categories were selected in line with the new recommendations from the 'Centers for Disease Control and Prevention (CDC)' Advisory Committee for Childhood Lead Poisoning Prevention (ACCLPP) (Centers For Disease Control and Prevention, 2012a). Where, in light of the overwhelming scientific evidence showing detrimental health effects of blood lead levels at <10 μg/dL in children, CDC's ACCLPP recommended the use of reference value of 5 μg/dL based on the 97.5th percentile of the current blood lead level distribution among children aged 1 to 5 years in the United States of America (Centers For Disease Control and Prevention, 2012a). Blood lead levels were also classified by gender of the study participant using measures of spread and central tendency. Socio-demographic factors were stratified by gender of the study participant, as was aggressive behavior profile i.e. (positive response for aggressive behavior questions). For categorical variables, chi-squared tests were performed to establish the significance of association, and for continuous variables, *t*-tests were used to compare the means.

2.4.2. Analytical statistics

Data were first examined for possible predisposition to aggressive behavior at an earlier age. Because blood lead levels at age 13 years were skewed they were log transformed to ensure normal distribution. Using logistic regression analysis the association between blood lead levels at age 13 years and aggressive behavior during mid-adolescence (characterized by a positive and a negative response for each aggressive behavior item) was examined, controlling for the effect of aggressive behavior at age 11 years old.

To determine dimensions of aggression; using binary variables of aggressive behavior items PCA was used as the dimensionality reduction technique to create suitable principal components. PCA with varimax rotation was used to reduce the original 17 items of aggressive behavior items. PCA was conducted separately for males and females to examine the comparability of the patterns. A model including both sexes was selected as the best model; and PCA was conducted using both sexes combined. The sample size was adequate with a Kaiser-Meyer-Olkin (KMO) score of 0.71 and Bartlett's test was statistically significant ($p < 0.001$). Three principal components with an eigenvalue of >1 were extracted. All three principal components were retained based on the Scree plot analysis, sound interpretability of the patterns, and percentage of total variance explained (32%) (Field, 2009). The retained principal components were named according to the aggressive behavior patterns observed as demonstrated in Supplementary Table 2. Principal component 1 increased with increasing stubbornness, suspiciousness, having a hot temper, loudness, screaming, moodiness, argumentativeness, teasing others, and attention seeking; and the aggressive behavior pattern was interpreted as a measure of 'indirect aggression' perpetrated by the study participant. Principal component 2 increased with increased destruction of things, attacking others, meanness, threatening to hurt others, and getting into fights and the aggressive behavior pattern was interpreted as a measure of 'direct aggression' perpetrated by the study participant. Principal component 3 increased with increasing disobedience and was interpreted as a measure of 'disobedience'. Component scores represent "a composite score for each individual on a particular factor". They inform on an individual's score on a subset of measurable variables (Field, 2009). The factor scores of the extracted principal components can then be used in a regression analysis.

Linear regression analysis was used to examine the relationship between lead exposure and dimensions of aggressive behavior in mid-adolescence. In Model 1 data were examined for the association between blood lead levels and aggressive behavior controlling for gender of the study participant. In Model 2, data were re-examined for the association between blood lead levels and aggressive behavior controlling for gender, ethnicity and possible confounding. Confounding was defined by statistical significance of ($p < 0.05$) or a ≥10% difference in crude and adjusted coefficients. In addition, data were evaluated for an association between socio-demographic factors at birth and aggressive behavior during mid-adolescence. Data analyses were conducted using STATA 14 statistical package and SPSS version 22.

2.5. Ethical issues

Ethical approval was obtained from the University of the Witwatersrand Committee for Research on Human Subjects (Medical). Only consented individuals were enrolled in the study and participants were informed of their right to withdraw at any time without penalty. The original BT20 + cohort study received clearance from the University of the Witwatersrand Ethics Committee on Human Subjects (M010556); and the Federal Wise Assurance registration number of the University of the Witwatersrand Ethics Committee on Human Subjects is FWA00000715.

3. Results

3.1. Distribution of blood lead levels at age 13 years

The distribution of blood lead levels at age 13 years differed significantly between males and females ($p < 0.001$) (table not shown).

[1] *Apartheid* was a system of "institutionalized segregation and discrimination" in South Africa.

Overall, blood lead concentrations ranged from 1.0 to 28.1 μg/dL with a mean and standard deviation of 5.6 ± 2.3 μg/dL, a geometric mean of 5.1 μg/dL and a median of 5.4 μg/dL. The mean blood lead levels were 6.4 ± 2.5 μg/dL and 4.9 ± 1.9 for males and females, respectively. Forty one percent of the study participants and 27.8% of males and 52.3% of females had blood lead levels <5 μg/dL. The proportion of males, females and both with blood lead levels ranging from 5 to 9.99 μg/dL was 66.9%, 47.1% and 56.4%, respectively. And, 5.3% of males, 0.7% of females and total sample of 2.9% had blood lead levels ≥10 μg/dL.

3.2. Characteristics of members of the analytical sample by gender

There was no difference between males and females with regard to ethnicity, maternal education at birth, maternal age at birth, residential area of birth, hospital of birth and maternal marital status at birth of the study participant. However, on average, males had higher socio-economic status than females at birth as shown in Table 1.

3.3. Aggressive behavior by gender during mid-adolescence

Table 2 shows the aggressive behavior profile in mid-adolescence stratified by gender. More females than males reported a positive response for 'I argue a lot', 'I scream a lot', 'I am stubborn', 'my moods and feelings change suddenly', 'I am louder than other kids'. On the other hand, more males than females gave a positive response to aggressive behavior items such as 'I try to get a lot of attention', 'I destroy things belonging to others', 'I get into many fights', 'I physically attack people', 'I am suspicious', 'I tease others a lot' and 'I threaten to hurt people'.

3.4. Is the aggressive behavior exhibited during mid-adolescence influenced by a predisposition to aggressive behavior during early adolescence?

To examine if aggressive behavior shown in this study during mid-adolescence could be influenced by a predisposition to aggressive behavior at an earlier age (age 11 years), the association between blood lead levels at age 13 years and aggressive behavior in mid-adolescence was examined adjusting for the effect of aggressive behavior during early adolescence as shown in Table 3.

After adjusting for the effect of aggressive behavior at age 11 years, gender, ethnicity and SES; study participants who responded positively for aggressive behavior item 'I argue a lot' during mid-adolescence were 34% less likely to have increased blood lead concentration levels at age 13 years old compared to those who gave a negative response. On the contrary, the odds of having increased blood lead levels were 3.7 times higher for study participants who responded positively for aggressive behavior item 'I threaten to hurt other' compared to those who responded negatively to the same question. Therefore, the results show that aggressive behavior in mid-adolescence is not influenced by a predisposition to aggressive behavior during early adolescence.

3.5. Association between blood lead levels at age 13 years and direct aggressive behavior, indirect aggressive behavior and disobedience during mid-adolescence

Data were analyzed for the association between blood lead levels at age 13 years and aggressive behavior during mid-adolescence using PCA derived components to examine dimensionality of direct and indirect aggressive behavior, and disobedience. As shown in Table 3, an increase in blood lead levels from <5 μg/dL to >10 μg/dL was positively associated with direct aggression after adjusting for the effects of gender, ethnicity, maternal age and education at birth of the study participant. Indirect aggressive behavior was not significantly associated with lead exposure.

Furthermore, after adjusting for the effect of ethnicity and SES at birth compared to being female, being male on average was negatively associated with 'indirect aggression'; but positively associated with 'direct aggression'. And, being born to a mother who is single, widowed, divorced or separated (adjusting for gender and ethnicity), 'disobedience' decreased by 0.15 on average during mid-adolescence. Birth to a mother with a higher level of education was protective of direct aggressive behavior in mid-adolescence, adjusting for the effect of gender, ethnicity and maternal age at birth. After controlling for the effect of gender, ethnicity and maternal marital status at birth, birth to mothers in their 20's and 30's was protective of direct aggressive behavior during mid-adolescence compared to children born to teenage mothers. Socio-economic status was negatively associated with indirect aggressive behavior, controlling for the effect of gender and ethnicity (Table 4).

Table 1
Socio-demographic characteristics of the analytical sample.

	Males (n = 508)	Females (n = 578)	Total (n = 1086)
	Total (%)	Total (%)	Total (%)
Ethnicity			
Black African	439 (86.4)	499 (86.3)	938 (86.3)
Coloured	69 (13.6)	79 (13.7)	148 (13.6)
$\chi^2_{(1)} = 0.0017$, p = 0.967, n = 1086			
Hospital of birth			
Public	467 (91.9)	524 (90.8)	991 (91.3)
Private	41 (8.1)	53 (9.2)	94 (8.66)
$\chi^2_{(1)} = 0.4241$, p = 0.515, n = 1085			
Place of birth			
Soweto/Diepkloof	485 (95.5)	545 (94.3)	1030 (94.8)
Former Coloured/Asian/Inner City/Suburban	23 (4.5)	33 (5.7)	56 (5.2)
$\chi^2_{(1)} = 0.7721$, p = 0.380, n = 1086			
Maternal education at birth			
Grade7 and less	58(12.5)	64 (11.8)	122 (12.2)
Grade 8–10	232(50.1)	245 (45.3)	477 (47.5)
Grade 11–12	138 (29.8)	188 (34.8)	326 (32.5)
Post school training	35(7.6)	44 (8.1)	79 (7.9)
$\chi^2_{(3)} = 3.3036$, p = 0.347, n = 1004			
Maternal age at birth			
<20	76 (15.0)	104 (18.0)	180 (16.6)
20–29	282 (55.5)	305 (52.8)	587 (54.0)
30–39	136 (26.7)	155 (26.8)	291 (26.8)
≥40	14 (2.8)	14 (2.4)	28 (2.6)
$\chi^2_{(3)} = 1.9936$, p = 0.574, n = 1086			
Maternal marital status at birth			
Married/living partner	195 (38.5)	209 (36.6)	404 (37.5)
Single/widowed/divorced/separated	312 (61.5)	362 (63.4)	674 (62.5)
$\chi^2_{(1)} = 0.3961$, p = 0.529, n = 1078			
Socio-economic status at birth			
Minimum	−1.598	−1.598	−1.598
Maximum	4.286	3.715	4.286
Mean	0.058	−0.051	0.000000286
$t_{(1084)} = 1.8623$, p = 0.03, n = 1086			

4. Discussion

This study investigated the association between lead exposure in early adolescence and aggressive behavior during mid-adolescence in the BT20 + cohort in Johannesburg, South Africa. Elevated blood lead levels were positively associated with direct aggression in mid-

Table 2
Aggressive behavior profile in mid-adolescence by gender.

	Male (%) n = 508	Female (%) n = 578	Total (%) n = 1086	p-Value
I argue a lot				0.06
Pos	144 (28.6)	195 (33.7)	339 (31.2)	
Neg	364 (71.7)	383 (66.3)	747 (68.8)	
I am mean to others				0.19
Pos	46 (9.1)	40 (6.9)	86 (7.9)	
Neg	462 (90.9)	538 (93.1)	1000 (92.1)	
I try to get a lot of attention				0.01*
Pos	112 (22.1)	92 (15.9)	204 (18.8)	
Neg	396 (78.9)	486 (84.1)	882 (81.2)	
I destroy my own things				0.19
Pos	16 (3.2)	11 (1.9)	27 (2.5)	
Neg	492 (96.8)	567 (98.1)	1059 (97.5)	
I destroy things belonging to others				0.04*
Pos	11 (2.2)	4 (0.7)	15 (1.4)	
Neg	497 (97.8)	574 (99.3)	1071 (98.6)	
I disobey my parents				0.61
Pos	7 (1.4)	6 (1.0)	13 (1.2)	
Neg	501 (98.6)	572 (90.0)	1073 (98.8)	
I disobey at school				0.23
Pos	12 (2.4)	8 (1.4)	20 (1.8)	
Neg	496 (97.6)	570 (98.6)	1066 (98.2)	
I get into many fights				0.002*
Pos	38 (7.5)	19 (3.3)	57 (5.3)	
Neg	470 (92.5)	559 (96.7)	1029 (94.7)	
I physically attack people				0.02*
Pos	15 (2.9)	6 (1.0)	21 (1.9)	
Neg	493 (97.1)	572 (99.0)	1065 (98.1)	
I scream a lot				<0.001*
Pos	19 (3.7)	79 (13.7)	98 (9.0)	
Neg	489 (96.3)	499 (86.3)	988 (91.0)	
I am stubborn				<0.001*
Pos	51 (10.0)	136 (23.5)	187 (17.2)	
Neg	457 (90.0)	442 (76.5)	899 (82.8)	
My moods and feeling change suddenly				0.003*
Pos	94 (18.5)	151 (26.1)	245 (22.6)	
Neg	414 (81.5)	427 (73.9)	841 (77.4)	
I am suspicious				0.001*
Pos	141 (27.8)	110 (19.0)	251 (23.1)	
Neg	367 (72.2)	468 (80.9)	835 (76.9)	
I tease others a lot				<0.001*
Pos	74 (14.6)	35 (6.1)	109 (10.0)	
Neg	434 (85.4)	543 (93.9)	977 (90.0)	
I have a hot temper				0.63
Pos	78 (15.4)	95 (16.4)	173 (15.9)	
Neg	430 (84.6)	483 (83.6)	913 (84.1)	
I threaten to hurt people				<0.001*
Pos	23 (4.5)	6 (1.0)	29 (2.7)	
Neg	485 (95.5)	572 (99.0)	1057 (97.3)	
I am louder than other kids				0.01*
Pos	50 (9.8)	88 (15.2)	138 (12.7)	
Neg	458 (90.2)	490 (84.8)	948 (87.3)	

Chi-square test used to determine statistical difference between males and females.
Pos – positive response; Neg – negative response.
* Statistically significant results.

adolescence among Black African and Coloured youth. These study findings are highly significant and vital in that they show that lead exposure is associated with the most severe form of aggressive behavior in this study which is 'direct aggression' and not associated with indirect aggression. In addition, our results also showed that the associated link between blood lead levels and aggressive behavior during mid-adolescence is not confounded by a predisposition to aggressive behavior at an earlier age. After adjusting for the effect of aggressive behavior during early adolescence and covariates, lead exposure in early adolescence was shown to almost quadruple the risk to 'threaten to hurt others', during mid-adolescence - lending support to the type of aggressive behavior 'direct aggression' associated with environmental lead exposure in South African youth. Stretesky and Lynch linked lead exposure to homicide "the most extreme outcome associated with aggression" (Stretesky and Lynch, 2001). As mentioned in our introduction section, lead exposure is associated with aggressive behavior in early adolescence in South Africa (Naicker et al., 2012). As such, in addition to identifying the nature of aggressive behavior associated with lead exposure in mid-adolescence; our results signal to what should possibly be a worrying trend of aggressive antisocial behavior among the country's adolescents. Likewise, other international epidemiological studies have shown an association between lead exposure and aggressive and/or anti-social behavior (Byers and Lord, 1943; Denno, 1990; Needleman et al., 1996; Needleman et al., 2002; Dietrich et al., 2001; Stretesky and Lynch, 2001; Wright et al., 2008; Mazumdar et al., 2011). However, there is dearth of reliable data from developing countries regarding this major public health problem.

The identification of specific type(s) of aggressive behavior associated with environmental lead exposure in South African youth is important because it highlights: i) the violent nature of aggressive behavior associated with lead exposure ii) possible environmental toxicological contribution to 'contact crime' in the country iii) possible environmental lead contribution to the national burden of disease in the country iv) its negative impact on the country's economy and v) the need for appropriate modulating mechanisms when implementing measures to combat aggressive behavior in adolescence.

Gender differences in blood lead levels found in this study were consistent with those previously reported in international studies, in the United States (Lanphear et al., 2000; Muntner et al., 2005), Korea (Kim et al., 2017) and Sweden (Bárány et al., 2002); where males had higher blood lead concentrations than females. Given that factors associated with increased levels of absorption, distribution and excretion of pharmacological agents are greater in men than women (Soldin and Mattison, 2009); may explain the observed increased levels of blood lead in males. On average males have higher body weight, length and surface area, intracellular and extracellular water, total body water, greater pulmonary function, renal clearance and cardiac output compared to females (Soldin and Mattison, 2009). Lead acts as a pharmacological agent in the central nervous system resulting in pharmacological effects (Mason et al., 2014) and anatomic and physiological differences between males and females are known to influence the pharmacokinetics and pharmacodynamics of pharmacological agents in the body (Soldin and Mattison, 2009).

In addition, the positive association between being male and direct aggression, and being female and indirect aggression found in this study speaks to gender differences in the manifestation of aggressive behavior among South African adolescents. Our results are consistent with those reported in a Finish study of 8, 11 and 15 year olds, where indirect aggression was prevalent in girls aged 11 and 15 years and physical aggression more common in boys of all three age groups (Bjrkqvist et al., 1992). Similarly, meta-analytic reviews of aggression and gender differences show evidence of physical and direct aggression in the male direction (Archer, 2004; Campbell, 2006). Bjrkqvist and colleagues posit that the indirect aggression patterns associated with girls could be due to the fact that teenage girls are known to mature verbally faster than their male counterparts, which in-turn may facilitate verbal aggression as opposed to physical aggression (Bjrkqvist et al., 1992). However, a British study found no difference in indirect aggression between males and females (Forrest et al., 2005). Nonetheless, our results

Table 3
Odds Ratios for blood lead levels at age 13 years and aggressive behavior during mid-adolescence controlling for aggressive behavior during early adolescence.

Outcome	Exposure			
Aggressive behavior items	Blood lead levels at 13 years old			
	Unadjusted OR (95% CI)	p-Value	Adjusted OR (95%CI)	p-Value
I argue a lot	0.68 (0.47–0.97)	0.036	0.66 (0.46–0.96)	0.03
I threaten to hurt people	3.74 (1.46–9.56)	0.006	3.75 (1.46–9.59)	0.006

Table 4
Association between blood lead levels & socio-demographic factors and aggressive behavior: linear regression analysis.

Exposure variables	Outcome variables																	
	Model 1 (unadjusted coefficients)									Model 2 (adjusted coefficients)								
	Indirect aggression			Direct aggression			Disobedience			Indirect aggression			Direct aggression			Disobedience		
	β	Std error	p-Value	β	Std error	p-Value	β	Std error	p-Value	β	Std error	p-Value	β	Std error	p-Value	β	Std error	p-Value
BLL																		
<5 μg/dL = (low – reference category)																		
5–9.99 μg/dL	0.004	0.06	0.95	−0.10	0.06	0.11	0.08	0.07	0.22	0.01	0.06	0.94	−0.07	0.06	0.28	0.10	0.07	0.14
≥10 μg/dL	0.26	0.19	0.17	0.37	0.18	**0.04**	−0.26	0.19	0.19	0.26	0.18	0.16	0.43	0.18	**0.02**	−0.28	0.20	0.17
R^2	0.0149	0.0390	0.0042	0.0189	**0.0516**	0.0121												
Gender																		
Female																		
Male										−0.21	0.06	<**0.001**	0.34	0.06	<**0.001**	0.01	0.06	0.92
Maternal education at birth																		
Grade 7 and less																		
Grade 8–10										−0.11	0.10	0.27	−0.11	0.10	0.29	0.11	0.11	0.32
Grade 11–12										−0.11	0.11	0.33	−0.23	0.10	**0.04**	0.08	0.11	0.45
Post school training										−0.22	0.15	0.14	−0.20	0.14	0.15	0.28	0.15	0.07
Maternal age at birth																		
<20 (teen moms)																		
20–29										−0.01	0.09	0.93	−0.18	0.09	**0.04**	0.02	0.09	0.80
30–39										−0.003	0.10	0.98	−0.24	0.10	**0.02**	0.03	0.11	0.80
≥40										−0.05	0.20	0.80	−0.31	0.20	0.12	−0.28	0.21	0.18
Marital status																		
Married/living with partner																		
Single/widowed/divorced/separated										0.10	0.06	0.10	−0.04	0.06	0.56	−0.15	0.07	**0.02**
Hospital of birth																		
Public																		
Private										0.09	0.11	0.40	−0.17	0.11	0.10	0.05	0.11	0.67
SES										−0.07	0.03	**0.04**	0.03	0.03	0.37	−0.05	0.03	0.16

suggest that to effect change it is important that public health policymakers and other relevant stakeholders take cognisance of the need for gender-tailored programs when implementing measures to combat aggressive behavior among adolescents.

Furthermore, socio-demographic factors were associated with aggressive behavior among adolescents in this study. Parental educational level, a proxy for socio-economic status predicts children's attainment later life (Haveman and Wolfe, 1995; Davis-Kean, 2005). Low parental educational levels are associated with behavior problems such as aggressive behavior in children (Dubow et al., 2009). In line with these predictions, in this study birth to a mother with a higher educational level was negatively associated with direct aggressive behavior and better socio-economic status at birth was protective of indirect aggressive behavior in mid-adolescence. Also, there was a negative association between maternal age (older) at birth and direct aggressive behavior in mid-adolescence; consistent with other reports where a higher proportion of children born to a younger mother or younger mothers at first birth displayed aggressive and conduct disorder problems (Wakschlag et al., 2000; Nagin and Tremblay, 2001; Tremblay et al., 2004). Surprisingly, birth to a single mother headed home was negatively associated with disobedience in mid-adolescence in this study, contrary to findings by Ryan and colleagues which showed that children from low or middle income homes who experienced changes in family structure from two-parent homes to single parent homes exhibit higher behavior problems than those who experienced no changes in their family structures (Ryan et al., 2013). Anecdotal evidence suggests that children born to single parents in some South African communities are usually raised in extended family settings where in fact they may be exposed to much stronger social support and stability. Therefore, from a South African point of view we predict that this may explain the seemingly lower acts of disobedience in adolescents from single parent homes found in this study. In support of this, South African census data from 2001 showed that in Black African and Coloured households, there were more single parents living with extended family than those living on their own (Amoateng et al., 2007).

As mentioned earlier, there is a paucity of empirical data from low or middle-income countries regarding health and behavioral effects of lead exposure. Findings from this study will help address this gap in evidence. The implications of these findings are far reaching in that they speak to the biological disruption of normal developmental processes, particularly in children from low income communities as they are the most affected. As such, childhood lead exposure and the associated 'direct' aggressive behavior in South African young people is not only a major public health problem but a socio-political problem as well.

There were some limitations to this study. First, a much more inclusive study sample is needed to evaluate further the adverse health effects of lead exposure in children among all different South African ethnic groups, especially in this post-Apartheid era. In the past the White population group had relatively low blood lead distribution (von Schirnding et al., 1991); and there is dearth of information regarding lead exposure among Indian/Asian population groups in South Africa.

Second, the YSR was the only aggressive behavior scale used to measure aggressive behavior. Use of more than one measuring tool would have helped in this study to examine the consistency or lack thereof of aggressive behavior patterns within the study group.

Third, blood lead levels were only measured at one time point; measuring recent lead exposure. For future studies we recommend using blood lead levels and bone lead levels so that associated aggressive behavior outcome can be examined for both recent and cumulative lead exposure.

Fourth, data for testosterone levels were not available for the timelines examined in this study, as such, testosterone levels were not adjusted for in the models. The question that may arise is whether higher testosterone levels in males may predispose them to 'direct aggression'. Studies are inconclusive on this matter; some have shown that there is no evidence that higher testosterone levels facilitate aggressive behavior in males (Archer, 2004), but nonetheless suggest that testosterone levels do influence the intensity and/or frequency of aggressive responses when provoked or threatened (Olweus et al., 1988).

5. Conclusion

This study examined the role of environmental lead exposure in aggressive behavior during mid-adolescence among young male and female South Africans. The results showed that there is a positive association between elevated blood lead levels and direct aggression in South African adolescents. And, there is a positive relationship between adolescent males and direct aggressive behavior but a negative association with indirect aggressive behavior. In contrast, indirect aggressive behavior was positively associated with the female gender. As such, our findings show how environmental lead exposure potentially contributes to anti-social behavior patterns among South African youth.

Our results also showed a link between socio-demographic factors at birth and aggressive behavior during mid-adolescence, which is imperative in that it shows an indirect role of socio-demographic factors in the aggressive behavior of adolescents. Further investigation to determine whether the identified aggressive behavior in early and mid-adolescence associated with lead exposure can escalate to violent behavior in late adolescence and indeed adulthood is essential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2017.08.138.

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Appendix 2: Journal Article 2

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The association between elevated blood lead levels and violent behavior during late adolescence: The South African Birth to Twenty Plus cohort

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ABSTRACT

Epidemiological studies have shown the adverse neuro-behavioral health effects of lead exposure among children, in particular. However, there is lack evidence in this regard from developing countries. The main aim of this study was to assess the association between blood lead levels (BLLs) during early adolescence and violent behavior in late adolescence.

Our study sample from the Birth to Twenty Plus cohort in Soweto-Johannesburg, South Africa included 1332 study participants (684 females). BLLs were measured using blood samples collected at age 13 years. Violent behavior was evaluated using data collected at ages 15 to 16 years using the Youth Self Report questionnaire. First, bivariate analysis was used to examine data for an association between lead exposure in early adolescence and violent behavior items during late adolescence. Principal Component Analysis (PCA) was used for dimensionality reduction and six violent behavior components were derived. Data was further analysed for an association between BLLs at age 13 years and violent behavior using PCA derived components; to determine the specific type(s) of violent behavior associated with lead exposure.

Median whole BLLs were 5.6 μg/dL ($p < 0.001$). Seventy five percent of males and 50% of females had BLLs ≥ 5 μg/dL. BLLs ranging from 5 to 9.99 μg/dL were associated with physical violence ($p = 0.03$) and BLLs ≥ 10 μg/dL were associated physical violence and fighting ($p = 0.02$ and $p = 0.01$, respectively). When data were analysed using continuous BLLs physical violence was associated with lead exposure ($p < 0.0001$). Furthermore, males were more likely to be involved in violence using a weapon ($p = 0.01$), physical violence ($p < 0.0001$), and mobbing others ($p < 0.05$) compared to females.

The results from this study show the severe nature of violent behavior in late adolescence associated with childhood lead exposure. They highlight the urgent need for preventive measures against lead exposure among children in low or middle income countries such as South Africa.

1. Introduction

Lead is one of ten chemicals identified by the World Health Organization (WHO) as being of "major public health concern" and in need of action by Member States (World Health Organization, 2010). Approximately 600,000 new cases of children with intellectual disability are attributed to childhood lead exposure annually (Prüss-Ustün et al., 2011). In recent decades there has been a steady increase in epidemiological studies showing a possible link between childhood lead exposure and lower socio-economic status (Morrens et al., 2012); altered pubertal development in girls and boys (Naicker et al., 2010a; Williams et al., 2010; Den Hond et al., 2011); and intellectual impairment and antisocial behavior (Needleman et al., 1979; Needleman and Gatsonis, 1990; Needleman et al., 2002; Bellinger et al., 1992; Dietrich et al., 2001; Canfield et al., 2003; Lanphear et al., 2005; Wright et al., 2008) among others.

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1. Introduction

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Contemporary research studies involving brain-imaging show possible neuro-anatomical bases underlying the neuro-behavioral changes associated with lead exposure. In a Cincinnati Lead study, analyses of childhood lead exposure and adult brain volume using magnetic resonance imaging (MRI) showed that elevated mean childhood blood lead levels were significantly associated with 1.2% reduction of the grey matter ($p < 0.001$) (Cecil et al., 2008). The affected areas of the brain included prefrontal cortical areas such as the "medial and superior frontal gyri" with the "ventrolateral prefrontal cortex and anterior cingulated cortex", and in the "postcentral gyri, the inferior parietal lobule", and the cerebellar hemispheres. It is important to note that this grey matter loss was only significant among males (Cecil et al., 2008). Childhood lead exposure has also been reported to alter the integrity of white matter in adulthood (Brubaker et al., 2009). Other brain imaging studies have supported these findings (Stewart et al., 2006; Cecil et al., 2011; Caffo et al., 2008; Brubaker et al., 2010; Schwartz et al., 2010), suggesting that exposure to lead changes the structure and function of the brain, affecting executive functions and consequently resulting in neuro-behavioral changes such as violent behavior. Prefrontal cortex dysfunction is associated with aggressive and violent behavior (Brower and Price, 2001; Siever, 2008; Hawkins and Trobst, 2000; Grafman et al., 1996).

In South Africa lead has been used in, among other items, petrol, paint, batteries, solder, electrical appliances, fishing weights and road markings (Mathee et al., 2009). Lead continues to be used in traditional medicines (Mathee et al., 2015), and leaded ammunition (Mathee, 2014; Mathee et al., 2017) among others. Given that South Africa has a long history of blood lead concentrations above the Centers for Disease and Control and Prevention (CDC)'s recommended reference level of 5 μg/dL in children (von Schirnding et al., 1991; von Schirnding et al., 2003; Mathee et al., 2006; Naicker et al., 2010b; Mathee et al., 2013) and violent behavior characterized by physical violence, violence using a weapon, bullying, emotional violence and sexual violence during adolescence (Burton and Leoschut, 2012; Mncube and Harber, 2013), there is good reason to examine the association between childhood lead exposure and violent behavior among young people. In addition, in view of the fact that 98% of children exposed to lead live in low or middle income countries such as South Africa (World Health Organization, 2009), it is vital that more research is conducted in these communities to examine its deleterious health effects. Currently, most of the empirical data demonstrating the detrimental effects of lead exposure comes from the developed countries. More locally-generated empirical data are essential to inform decisions and policies related to prevention and control of lead exposure in South Africa and other low or middle income countries. To our knowledge, no study has been conducted in South Africa showing the relationship between lead exposure and violent behavior during adolescence. In this study we hypothesized that there is an association between lead exposure at 13 years old and violent behavior during late adolescence in South Africa. Principal Component Analysis (PCA) derived components were used to determine the type or types of violent behavior associated with lead exposure among South African adolescents.

2. Materials and methods

2.1. Study population

Study participants were selected from the Birth to Twenty Plus (BT20+) cohort in Johannesburg, South Africa. BT20+ is the largest and longest running birth cohort in Africa. It was initiated at the cusp of democracy in South Africa with the intention to address the foreseeable health problems as a result of heightened demand for access to health care services in urban areas due to increased urbanization. A total of 3273 study participants were enrolled in the birth cohort from 23 April to 8 June 1990. The cohort is representative of the South African racial demographics as defined by the "*Apartheid*" system[1]; comprised of 78% Black Africans, 6% Whites, 12% Mixed Ancestral and 4% Indians. Initially the White population group was under-represented mainly because during the time of enrollment most White families used the private health practitioners and facilities. This imbalance was later rectified at age 10 years by enrolling a supplementary sample of 120 White children born during the cohort enrolment dates.

Even though the cohort has a very low attrition rates, White families have shown a higher attrition compared to others. The study is still in contact with > 70% of the original study participants. For more details regarding the cohort, see Richter et al. (2007) and Richter et al. (2004).

To be included in the current study, study participants needed to have blood lead measurements at 13 years old and violent behavior data collected at ages 15 to 16 years (late adolescence). With these criteria, a total of 1332 study participants (684 females) comprised of 87.2% Black African and 10.4% Mixed Ancestral adolescents were included in the study. White and Indian study participants were excluded due to low numbers, 1.54% and 0.88% respectively.

2.2. Blood lead measurement

Venous samples of whole blood were collected at age 13 years into EDTA-containing tubes previously determined to be free of trace metals. Blood sampling was undertaken by professional health officials, using sterile equipment and aseptic techniques. Blood samples were vortexed and rolled on the coulter mixer for at least 10 min until properly mixed. They were diluted 10 times with 1,1% (v/v) Triton X-100 using automatic Hamilton Microlab 500 diluter into disposable 10 mL Sterilin plastic tubes covered with screw caps and mixed well using a vibration mixer. Blood lead levels were measured using Perkin Elmer 600 Aanalyst atomic absorption spectrometer with a THGA graphite furnace, Zeeman background correction and AS-800 Autosampler. Both blood samples and samples for quality control were prepared and measured in-house.

2.3. Measurement of violent behavior in late adolescence and socio-demographic factors

Data on violent behavior were collected in the 15th year data collection wave using the Youth Self Report (YSR) questionnaire. Information for the YSR questionnaire for violent behavior was collected at two time points, 11/12 and 15/16 years old. Study participants were contacted by telephone at home, work, or through nominated contactable family members or friends to secure appointment dates for data collection. Study participants came to the BT20+ data collection site and were compensated a minimum of R50 for transport. The YSR questionnaires were administered by trained field workers - most of whom have been with the cohort since its inception and have a very long trusting relationship with the study participants (Richter et al., 2004).

The YSR is a self-report questionnaire comprising 112 items assessing behavioral competency and problems of children and adolescents aged 11 to 17. It assesses aggressive and oppositional behavior attention seeking problems, as well as psychotic, impulsive, social interaction, and conduct problems among others (Achenbach, 1991). Regarding the sensitivity and specificity of the YSR questionnaire, the Achenbach System of Empirically Based Assessment (ASEBA) scales for internalizing and externalizing for YSR are 0.90 for alpha, 0.85 for test-retest reliability and 0.56 for long term stability for the United States. In general psychometric results from different cultural backgrounds have approximated those from the United States (Achenbach et al., 2008). Furthermore, YSR was used in adolescents from different cultural

[1] The racial categories Black African, White, Mixed Ancestral and Indian/Asian were enforced through legislation in Apartheid South Africa. Even though they are no longer enforced, to a great extent they remain part of South African vocabulary.

backgrounds to compare ratings for self-reported behavioral and emotional problems. The selected 7137 adolescents from the general population samples were from the United States, Turkey, Australia, Netherlands, China, Jamaica and Israel. The effect size of culture was very small (4%) for externalizing problems. Across all seven countries gender differences were similar for externalizing problems, but higher for girls compared to boys (Verhulst et al., 2003). In general, YSR has been validated and has been used in many populations including South Africa (Verhulst et al., 2003; Ivarsson et al., 2005; Cluver et al., 2007; Sabet et al., 2009; Naicker et al., 2012).

The violent behavior variable comprised 21 items with four response options: never, once or twice, a few times, and many times. The first eleven questions assessed violent behavior perpetrated by the study participant at school and an additional 10 similar questions pertained to violent behavior perpetrated outside of school. The four response options were collapsed into two: 'negative response' denoted by never and 'positive response' denoted by once or twice, a few times, and many times. These 2 categories were selected to reflect whether the study participant has propensity to perpetrate violent behavior.

Socio-demographic factors including gender; maternal education at birth of the cohort child, categorized into four levels, i.e. grade 7 or less, grade 8–10, grade 11–12 and post-school education; maternal marital status at birth of the child divided into two categories, married/living with a partner, or single/widowed/divorced/separated; maternal age at birth was divided into four categories < 20 years old (representing teenage mothers), 20–29 years old (representing younger mothers in their 20's), 30–39 years old (representing older mothers in their 30's) and > = 40 years old (representing mothers 40 and above); residential area of birth was dichotomized into Soweto/Diepmeadow or former Mixed Ancestral/Asian and Inner City/Suburb, hospital of birth was categorized as public or private. Socio-economic status (SES) was calculated using binary items of household commodities at birth of the child, including home ownership, type of home, access to water inside the dwelling, sole use of water, access to flush toilet, sole use of toilet, ownership of television, motor car, fridge, washing machine and telephone. PCA with varimax rotation was used as the dimensionality reduction technique to create an SES variable. The sample size was adequate with a Kaizer-Meyer-Olkin score of 0.68 and the Bartlett's test was statistically significant ($p < 0.001$). One PCA component was extracted based on Scree Plot analysis and eigenvalue of > 1.

2.4. Statistical analysis

Blood lead concentration levels were categorized into three levels: < 5 μg/dL (used as a reference category), 5–9.99 μg/dL and ≥ 10 μg/dL. The use of blood lead levels < 5 μg/dL as the reference level is in line with the recommendations by the Advisory Committee for Childhood Lead poisoning Prevention (ACCLPP) of the CDC to use the reference value of 5 μg/dL based on the 97.5th percentile of the current blood lead level distribution among children aged 1 to 5 years in the United States of America (Centers For Disease Control and Prevention, 2012a).

Continuous blood lead concentration were skewed and were natural log-transformed for normality. The distribution of violent behavior was stratified by gender. For categorical variables, chi-squared tests were performed to establish the significance of association and for continuous variables; *t*-tests were used to compare the means.

Using SPSS composite variables for violent behavior were derived using PCA. PCA with varimax rotation was used to reduce the data. Analyses were performed on the correlation matrix. The sample size was adequate with a Kaiser-Meyer-Olkin (KMO) of 0.84 and the Bartlett's test was statistically significant ($p < 0.001$). Six factors with an eigenvalue > 1 were extracted. They were all retained based on the Scree plot analysis and sound interpretability of the patterns (Fields, 2009). The retained principal components accounted for 67.1% of the total variance explained. They were named according to the violent behavior patterns observed, violence using a weapon, physical violence, fighting, sexual harassment, robbing and verbal & emotional abuse. All the retained principal components showed good internal consistency with Cronbach's alpha coefficients for violence using a weapon, physical violence, fighting, sexual harassment, robbing and verbal & emotional abusive of 0.88, 0.81, 0.53, 0.80, 0.83, and 0.78, respectively. Component scores represent "a composite score for each individual on a particular factor". They inform on an individual's score on a subset of measurable variables. Factor scores can be used in regression analysis (Fields, 2009). Supplementary Table 1 shows factor loadings for the violence patterns.

First, gender comparison of outcomes of individual violent behavior items during late adolescence (i.e. positive versus negative) with respect to geometric mean blood lead levels in early adolescence using bivariate analysis was conducted. Second, data were assessed for possible influence of gender and socio-demographic factors at birth on violent behavior during late adolescence controlling for blood lead levels at age 13 years and ethnicity. Statistically significant ($p < 0.05$) covariates were included in the linear regression models examining the association between lead exposure during early adolescence and the six violent behavior components extracted using PCA, accordingly. Confounding was also defined as a difference of 10% or more in coefficients. In Model 1 data were examined for an association between higher BLLs and violent behavior in late adolescence controlling for gender and ethnicity. In Model 2 the association was further examined to see if the associations still held after controlling for gender, ethnicity and covariates as possible confounders. Furthermore, data were analyzed using continuous BLLs adjusting for possible confounding as above in Models 1 and 2. Data analyses were conducted using the STATA 14 statistical package and SPSS version 20.

2.5. Ethical oversight

Permission to conduct the study was sought from the BT20 Plus birth cohort and their parents. Only consented individuals were enrolled and participants were informed of their right to withdraw at any time without being penalized. Clearance was sought and granted from the University of the Witwatersrand University Ethics Committee on Human Subjects (M010556). The Federal Wise Assurance registration number of The Witwatersrand University Ethics Committee on Human Subjects is FWA00000715.

3. Results

3.1. Characteristics of the analytical sample by gender

There was no statistically significant difference between males and females with regard to ethnicity, maternal education at birth, maternal age at birth, residential area of birth, hospital of birth, maternal marital status at birth and SES of the study participant as shown in Table 1.

3.2. Distribution of blood lead levels at age 13 years

The mean and median whole blood lead levels were much higher in males than females (Table 2). The proportion of study participants with BLLs ≥ 5 μg/dL was relatively high at 62%, of these; > 75% were males.

3.3. Violent behavior items stratified by gender during late adolescence

The differences between the proportion of males and female who gave a positive response for perpetration of violence in school and outside of school are outlined in Table 3. For all the violent behavior items, the proportion of males perpetrating violence was higher than females except for one question where they were asked if they have "verbally or emotionally abused someone" i.e. calling someone names

Table 1
Socio-demographic characteristics of the analytical sample.

Socio-demographic factor	Male (n = 648) Total (%)	Female (n = 684) Total (%)	Total (n = 1332) Total (%)	p-Value
Ethnicity				0.49
Black African	575 (88.7)	615 (89.9)	1190 (89.3)	
Mixed ancestral	73 (11.3)	69 (10.1)	142 (10.7)	
Hospital of birth				0.58
Private	46 (7.1)	54 (7.9)	100 (7.5)	
Public	602 (92.9)	629 (92.1)	1231 (92.5)	
Place of birth				0.32
Soweto/Diepkloof	623 (96.1)	650 (95.0)	1273 (95.6)	
Former mixed ancestral/Asian/ inner city/suburb	25 (3.9)	34 (5.0)	59 (4.4)	
Maternal education at birth				0.42
Grade 7 or less	71 (11.8)	84 (13.1)	155 (12.5)	
Grade 8–10	300 (50.1)	291 (45.3)	591 (47.6)	
Grade 11–12	185 (30.9)	218 (4.0)	403 (32.5)	
Post-school education	43 (7.2)	49 (7.6)	92 (7.4)	
Maternal age at birth				0.63
< 20	104 (16.0)	125 (18.3)	229 (17.2)	
20–29	362 (55.9)	360 (52.6)	722 (54.2)	
30–39	168 (25.9)	183 (26.8)	351 (26.4)	
≥ 40	14 (2.2)	16 (2.3)	30 (2.2)	
Maternal marital status at birth				0.37
Married/living with partner	227 (35.1)	222 (32.8)	449 (33.9)	
Single/widowed/ separated/ divorced	419 (64.9)	455 (67.2)	874 (66.1)	
Socio-economic status (SES) at birth				0.76
Minimum	− 3.391	− 3.186	− 3.391	
Maximum	1.979	1.979	1.979	
Mean (SD)	− 0.025 (1.02)	0.023 (0.98)	0.0000003 (1.0)	

Chi-squared test used to determine statistical significance between males and females ($p < 0.05$).

Table 2
Distribution of blood lead levels at 13 years old.

	Males (%)	Females (%)	Total (%)	p-Value
Blood lead levels (μg/dL)[a]				< 0.0001
< 5 (reference level)	161 (24.8)	342 (50.0)	503 (37.8)	
5–9.99	449 (69.3)	334 (48.8)	783 (58.8)	
≥ 10	38 (5.9)	8 (1.2)	46 (3.4)	
Continuous blood lead levels (μg/dL)				
Mean (SD)[b]	6.55 (2.60)	5.02 (1.96)	5.76 (2.42)	< 0.0001
Range	1.3–28.1	1.0–16.3	1.0–28	
Q1	5.0	3.54	4.16	
Median[c]	6.35	4.90	5.62	< 0.0001
Q3	7.91	6.25	7.08	

[a] Chi-squared test used to determine statistical significance between males and females.
[b] *t*-Test used to determine statistical significance between males and females.
[c] Nonparametric equality-of-medians test used to determine statistical significance between males and females.

or having things said to them that make them feel bad about themselves or afraid where a slightly higher proportion of females (16.1%) responded positively versus 15.6% of males, the results were however not statistically significant. Males showed highest levels of violent behavior for violent behavior items 'hit or kicked someone', 'pushed or shoved someone when angry', 'badly beaten up someone' - both at school and outside of school - and suspension from school. Although at a lower scale, the same pertain was observed in females except for the behavior item 'badly beaten up someone'. Sixteen males and one female admitted to having "threatened someone with a gun" at school and outside of school ($p < 0.001$). Eleven males and two females reported that they had "attacked someone with a knife or a sharp weapon" at school and fourteen males and three females admitted to have done the same outside of school ($p < 0.05$).

3.4. Comparison of outcomes of individual violent behavior items (positive/negative) with respect to the geometric mean BLLs (μg/dl) by gender

Bivariate analysis was conducted to examine the link between geometric mean BLLs at age 13 years and violent behavior during late adolescence as shown in Table 4. Elevated geometric mean BLLs were significantly associated with violent behavior items 'hit or kicked someone' in the total sample and in females; 'pushed or shoved someone' in the total sample; 'badly beaten someone up' in the total sample and males; 'sexually harassed someone' in the total sample; and 'been suspended from school' in the total sample and males ($p < 0.05$).

3.5. Association between gender and socio-demographic factors at birth with violent behavior during late adolescence

Results of the analyses of covariates for potential influence on violent behavior are summarized in Table 5. After adjusting for the effect of ethnicity (Model 1) gender was positively associated with violence using a weapon, fighting, physical violence and robbing (95% CI [0.024, 0.240], 95% CI [0.328, 0.538], 95% CI [0.005, 0.214], 95% CI [0. 002, 0.217]; respectively. In Model 2 controlling for blood lead levels at age 13 years and ethnicity, gender was only positively associated with violence using a weapon, physical violence and robbing; 95% CI [0.039, 0.267], 95% CI [0.248, 0.469], 95% CI [0.003, 0.231]; respectively – but not fighting.

For maternal education at birth adjusting for gender and ethnicity (Model 1): compared to birth to a mother with a grade 7 and below level of education, birth to a mother with a grade 8 to 10 level of education was positively associated with fighting others during late adolescence 95% CI [0.015, 0.358] and birth to a mother with a grade 11 to 12 and post-school level of education were positively associated with verbally and emotionally abusing others during late adolescence 95% CI [0.069, 0.441], 95% CI [0.199, 0.717], respectively. After adjusting for the effect of gender, blood lead levels at age 13 years and ethnicity in Model 2, birth to mothers with a grade 8 to 10 level of education was positively associated with fighting others in late adolescence 95% CI [0.017, 0.360]; and maternal education level of grade 11 to 12 and post-school education at birth were positively associated with verbal and emotional abusiveness towards others during late adolescence 95% CI [0.067, 0.440], 95% CI [0.199, 0.716], respectively.

Adjusting for the effect of gender and ethnicity (Model 1) birth at a private hospital was negatively associated with physical violence but positively associated with associated with verbal and emotional abusiveness to others during late adolescence 95% CI [− 0.43, − 0.03], 95% CI [0.104, 0.516]; respectively. In Model 2 after adjusting for the effects of gender, ethnicity and blood lead levels at age 13 years, birth at a private hospital remained negatively associated with physical violence 95% CI [− 0.408, − 0.006] and positively associated with verbally and emotionally abusing others during late adolescence 95% CI [0.101, 0.514]. Furthermore, there was a positive association between birth to a single mother and physical violence, adjusting for the effect of gender and ethnicity (Model 1) 95% CI [0.025, 0.249] and the association remained significant after controlling for gender, ethnicity and blood lead levels at age 13 years 95% CI [0.009, 0.233] (Model 2). Socio-economic status at birth was positively associated with being verbally and emotionally abusive to others in late adolescence,

Table 3
Violent behavior profile in late-adolescence by gender.

Positive response for violence behavior	Male (%) n = 648	Female (%) n = 684	Total (%) n = 1332	p-Value
Violence at school				
Hit or kicked someone	304 (46.91)	171 (25.00)	475 (35.66)	< 0.001
Pushed or shoved someone when angry	345 (53.24)	272 (39.77)	617 (46.32)	< 0.001
Badly beaten someone up	75 (11.57)	39 (5.70)	114 (8.56)	< 0.001
Threatened someone with a knife or sharp weapon	23 (3.55)	9 (1.32)	32 (2.40)	0.01*
Attacked someone with a knife or sharp weapon	11 (1.70)	2 (0.29)	13 (0.98)	0.01*
Threatened someone with a gun	16 (2.47)	1 (0.15)	17 (1.28)	< 0.001*
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	98 (15.12)	110 (16.08)	208 (15.62)	0.63
Sexually harassed someone	20 (3.09)	4 (0.58)	24 (1.80)	0.001*
Robbed someone	47 (7.25)	14 (2.05)	61 (4.58)	< 0.001*
Been suspended from school	96 (14.81)	57 (8.33)	153 (11.49)	< 0.001
Gotten into a fight after drinking or getting high	33 (5.09)	9 (1.32)	42 (3.15)	< 0.001*
Violence outside of school				
Hit or kicked someone	256 (39.51)	128 (18.71)	384 (28.83)	< 0.001
Pushed or shoved someone when angry	282 (43.52)	201 (29.39)	483 (36.26)	< 0.001*
Badly beaten someone up	88 (13.58)	31 (4.53)	119 (8.93)	< 0.001
Threatened someone with a knife or sharp weapon	30 (4.63)	6 (0.88)	36 (2.70)	< 0.001*
Attacked someone with a knife or sharp weapon	14 (2.16)	3 (0.44)	17 (1.28)	0.01*
Threatened someone with a gun	16 (2.47)	1 (0.15)	17 (1.28)	< 0.001*
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	89 (13.73)	79 (11.55)	168 (12.61)	0.23
Sexually harassed someone	19 (2.93)	4 (0.58)	23 (1.73)	0.001*
Robbed someone	35 (5.40)	10 (1.46)	45 (3.38)	< 0.001
Gotten into a fight after drinking or getting high	32 (4.94)	9 (1.32)	41 (3.08)	0.001

Chi-square test used to determine statistical difference between males and females.
* Signifies statistically significant difference between males and females.

Table 4
Comparison of outcomes of individual violent behavior (positive/negative) with respect to the geometric mean BLLs (μg/dl) by gender.

Violent behavior type	Males n = 648				Females n = 684				Total n = 1332			
	Geometric mean BLL				Geometric mean BLL				Geometric mean BLL			
	Pos	Neg	95% (CI)	p-Value	Pos	Neg	95% (CI)	p-Value	Pos	Neg	95% (CI)	p-Value
Violent behavior at school												
Hit or kicked someone	6.17	6.04	(0.92–1.04)	0.50	5.05	4.49	(0.82–0.95)	**0.001**	5.74	5.05	(0.84–0.92)	< **0.001**
Pushed or shoved someone when angry	6.18	6.02	(0.92–1.03)	0.38	4.74	4.54	(0.90–1.02)	0.19	5.50	5.12	(0.89–0.97)	**0.002**
Badly beaten someone up	6.72	6.03	(0.81–0.98)	**0.02**	5.24	4.59	(0.76–1.00)	0.06	6.17	5.21	(0.78–0.92)	< **0.001**
Threatened someone with a knife or sharp weapon	6.17	6.10	(0.84–1.16)	0.88	5.08	4.62	(0.69–1.20)	0.50	5.84	5.28	(0.78–1.05)	0.18
Attacked someone with a knife or sharp weapon	7.10	6.09	(0.68–1.07)	0.18	4.70	4.62	(0.54–1.77)	0.95	6.66	5.28	(0.63–1.00)	0.05
Threatened someone with a gun	5.65	6.11	(0.90–1.31)	0.41	4.16	4.62	–	–[a]	5.55	5.29	(0.78–1.17)	0.64
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	6.21	6.08	(0.90–1.06)	0.60	4.66	4.62	(0.91–1.08)	0.87	5.33	5.28	(0.93–1.05)	0.77
Sexually harassed someone	6.44	6.09	(0.80–1.12)	0.51	6.99	4.61	(0.43–1.00)	0.05	6.53	5.27	(0.68–0.98)	**0.01**
Robbed someone	5.89	6.12	(0.93–1.16)	0.51	4.53	4.62	(0.81–1.28)	0.85	5.55	5.29	(0.85–1.06)	0.37
Been suspended from school	6.59	6.02	(0.84–0.99)	**0.03**	4.98	4.59	(0.82–1.03)	0.17	5.93	5.21	(0.82–0.94)	< **0.001**
Gotten into a fight after drinking or getting high	6.13	6.10	(0.87–1.13)	0.93	5.30	4.61	(0.66–1.15)	0.33	5.95	5.27	(0.77–1.01)	0.07
Violence outside of school												
Hit or kicked someone	6.36	5.94	(0.88–0.99)	**0.02**	4.96	4.55	(0.84–0.99)	**0.03**	5.85	5.08	(0.82–0.91)	< **0.001**
Pushed or shoved someone when angry	6.29	5.96	(0.89–1.00)	0.08	4.84	4.53	(0.87–1.00)	0.06	5.64	5.10	(0.86–0.95)	< **0.001**
Badly beaten someone up	6.75	6.01	(0.82–0.97)	**0.01**	5.21	4.59	(0.75–1.03)	0.11	6.31	5.20	(0.76–0.89)	< **0.001**
Threatened someone with a knife or sharp weapon	5.91	6.11	(0.90–1.19)	0.64	4.07	4.63	(0.81–1.60)	0.46	5.55	5.28	(0.82–1.09)	0.49
Attacked someone with a knife or sharp weapon	6.03	6.10	(0.83–1.24)	0.91	3.94	4.62	(0.72–1.90)	0.51	5.60	5.29	(0.77–1.16)	0.58
Threatened someone with a gun	5.51	6.12	(0.92–1.34)	0.27	4.16	4.62	–	–[a]	5.42	5.29	(0.80–1.20)	0.81
Verbally or emotionally abused someone, i.e. being called names or having things said at you that make you feel bad about yourself or afraid	5.89	6.14	(0.96–1.13)	0.35	4.88	4.59	(0.85–1.04)	0.23	5.39	5.28	(0.91–1.05)	0.53
Sexually harassed someone	5.92	6.12	(0.87–1.23)	0.71	6.73	4.61	(0.45–1.04)	0.07	6.05	5.28	(0.73–1.04)	0.12
Robbed someone	6.01	6.11	(0.89–1.16)	0.81	5.36	4.61	(0.66–1.12)	0.26	5.86	5.27	(0.78–1.01)	0.10
Gotten into a fight after drinking or getting high	5.81	6.12	(0.92–1.20)	0.45	5.43	4.61	(0.64–1.12)	0.25	5.72	5.28	(0.81–1.05)	0.23

Pos = positive response to violent behavior item; Neg = negative response to violent behavior item.
[a] Very low positive response rate = 1.

Table 5
Association between gender and socio-demographic factors at birth with BLLs at age 13 years.

	Model 1						Model 2					
	Violence using a weapon	Physical violence	Fighting	Sexual harassment	Robbing	Verbal & emotional abusive	Violence using a weapon	Physical violence	Fighting	Sexual harassment	Robbing	Verbal & emotional abusive
	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
Gender												
Female												
Male	0.13 (0.05)*	0.43 (0.05)***	0.11 (0.05)	0.07 (0.05)	0.11 (0.05)*	−0.02 (0.05)	0.15 (0.06)**	0.36 (0.06)***	0.09 (0.05)	0.07 (0.06)	0.12 (0.06)*	−0.01 (0.06)
Maternal education Grades												
7 & <												
8–10	0.02 (0.08)	−0.04 (0.09)	0.19 (0.09)*	0.13 (0.09)	0.12 (0.09)	0.14 (0.09)	0.02 (0.08)	−0.03 (0.09)	0.18 (0.08)	0.13 (0.09)	0.12 (0.09)	0.15 (0.09)
11–12	0.06 (0.09)	−0.15 (0.09)	0.03 (0.09)	0.02 (0.09)	0.05 (0.09)	0.25 (0.09)**	0.06 (0.09)	−0.14 (0.09)	0.03 (0.09)	0.03 (0.09)	0.05 (0.09)	0.25 (0.09)**
Post-school training	0.08 (0.12)	−0.11 (0.13)	0.04 (0.13)	−0.04 (0.13)	−0.02 (0.13)	0.46 (0.13)***	0.08 (0.12)	−0.11 (0.13)	0.03 (0.12)	−0.04 (0.13)	−0.02 (0.13)	0.46 (0.13)***
Maternal age												
< 20												
20–29	−0.08 (0.08)	0.02 (0.07)	−0.07 (0.07)	0.01 (0.08)	−0.14 (0.08)	−0.10 (0.08)	−0.08 (0.08)	0.01 (0.07)	−0.07 (0.07)	0.01 (0.07)	−0.14 (0.08)	−0.10 (0.07)
30–39	−0.02 (0.08)	−0.06 (0.08)	−0.08 (0.08)	0.07 (0.08)	−0.13 (0.08)	−0.14 (0.08)	−0.02 (0.08)	−0.06 (0.08)	−0.08 (0.08)	0.07 (0.08)	−0.13 (0.08)	−0.14 (0.08)
≥ 40	−0.09 (0.20)	−0.02 (0.19)	−0.19 (0.19)	−0.16 (0.20)	−0.15 (0.20)	−0.35 (0.20)	−0.09 (0.20)	0.003 (0.19)	−0.19 (0.19)	−0.16 (0.20)	−0.15 (0.20)	−0.36 (0.20)
Hospital of birth												
Public	0.03 (0.10)	−0.23 (0.10)	0.05 (0.10)	−0.07 (0.10)	−0.06 (0.10)	0.31 (0.10)**	0.02 (0.10)	−0.21 (0.10)*	0.06 (0.10)	−0.07 (0.10)	−0.06 (0.10)	0.31 (0.10)**
Private												
Maternal marital status												
Married	−0.01 (0.06)	0.14 (0.06)*	−0.01 (0.06)	−0.05 (0.06)	0.09 (0.06)	−0.004 (0.06)	−0.01 (0.06)	0.12 (0.06)*	−0.01 (0.06)	−0.05 (0.06)	0.09 (0.06)	−0.001 (0.06)
Single/divorced/ widowed/ separated												
SES												
	0.04 (0.03)	−0.03 (0.03)	−0.01 (0.03)	0.02 (0.03)	−0.02 (0.03)	0.10 (0.03)***	0.04 (0.03)	−0.02 (0.03)	0.01 (0.03)	0.02 (0.03)	−0.02 (0.03)	0.10 (0.03)***

*** $p < 0.0001$.
** $p < 0.02$.
* $p < 0.05$.

Table 6
Association between BLLs at age 13 years and violent behavior during late adolescence.

	Model 1						Model 2					
	Violence using a weapon	Physical violence	Fighting	Sexual harassment	Robbing	Verbal & emotional abusive	Violence using a weapon	Physical violence	Fighting	Sexual harassment	Robbing	Verbal & emotional abusive
	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)	β (SE)
BLL (μg/dL)												
< 5 (reference category)												
5–9.99	− 0.07 (0.06)	0.13 (0.06)	0.03 (0.06)	− 0.01 (0.06)	− 0.03 (0.06)	0.03 (0.06)	− 0.07 (0.06)	0.12 (0.06)	0.03 (0.06)	0.01 (0.06)	− 0.03 (0.06)	0.06 (0.07)
≥ 10	0.01 (0.16)	0.37 (0.15)	0.39 (0.15)*	− 0.19 (0.17)	− 0.22 (0.16)	− 0.22 (0.16)	0.01 (0.16)	0.36 (0.15)*	0.41 (0.15)**	− 0.19 (0.16)	− 0.22 (0.16)	− 0.31 (0.19)
R^2	0.0055	0.0559	0.0087	0.0059	0.0045	0.0023	0.0055	0.0594	0.0153	0.0046	0.0031	0.0022
Continuous BLLs	− 0.07 (0.07)	0.27 (0.07)***	0.07 (0.07)	0.01 (0.07)	− 0.02 (0.07)	− 0.04 (0.07)	− 0.07 (0.01)	0.26 (0.01)***	0.08 (0.07)	0.01 (0.07)	− 0.03 (0.07)	− 0.05 (0.08)

*** $p < 0.0001$.
** $p < 0.02$.
* $p < 0.05$.

controlling for gender (Model1) 95% CI [0.037, 0.174], and it remained so after adjusting for the effect of gender, ethnicity and blood lead levels at age 13 years (Model 2) 95% CI [0.036, 0.173].

3.6. Association between blood lead levels at age 13 years and violent behavior in late adolescence

As shown in Table 5 gender, maternal education at birth, maternal age at birth, maternal marital status at birth, hospital of birth and SES were found to be influential covariates. As such, they were adjusted for accordingly in the linear regression analyses to examine the association between BLLs at age 13 years and violent behavior in late adolescence. Model 1 adjusted for gender and ethnicity in all models regardless of significance; and Model 2 adjusted for gender and ethnicity in all models regardless of significance, and significant covariates (Table 6).

In Model 1 blood lead levels 5–9.99 μg/dL at age 13 years and ≥ 10 μg/dL were positively associated with perpetration of physical violence 95% CI [0.020, 0.246] and 95% CI [0.070, 0.699], respectively. The positive association remained significant when the effect was examined using continuous blood lead levels at age 13 years 95% CI [0.138, 0.398]. Additionally, blood lead levels ≥ 10 μg/dL were positively associated with fighting in late adolescence 95% CI [0.093, 0.689]; however, when data were further examined using continuous BLLs the association between blood lead levels at age 13 years and fighting was not significant.

4. Discussion

The main purpose of this study was to examine the association between lead exposure during early adolescence and violent behavior in late adolescence. Using a dimension-reduction technique this study sought to identify the specific types of violent behavior associated with lead exposure. We found that blood lead levels at age 13 years are positively associated with physical violence in particular, later in adolescence in Black African and Mixed Ancestral South Africans. Both categorical and continuous blood lead levels were positively associated with physical violence. The change in significance for the association between blood lead levels at age 13 years and fighting in late adolescence when data were analyzed using continuous blood lead levels could be a consequence of "increased risk of positive results being a false positive" associated with the use of categorical variables and reduced sample size (Altman and Royston, 2006; van Walraven and Hart, 2008). However, it is important to note that categories are useful when examining the effects of lead exposure because they show if there is a dose-response effect and provide evidence of its detrimental health effects at various levels including those ubiquitous to the general population.

In addition, elevated geometric mean blood lead levels at age 13 years were positively associated with violent behavior items 'hit or kicked someone', 'badly beaten up someone', 'pushed or shoved someone' – all of which describe violence pattern for "physical violence", in line with study findings using PCA derived components. Our results add a very important component to the international literature linking lead exposure to perpetration of violent acts (Denno, 1990; Stretesky and Lynch, 2001; Nevin, 2000; Wright et al., 2008; Reyes, 2007); in that they show that lead exposure is associated with one of the most extreme forms of violent behavior in South African youth. Given that lead exposure is associated with aggressive behavior in early adolescence (Naicker et al., 2012) and 'direct' aggressive behavior in mid-adolescence (Nkomo et al., 2018) - our results point to an increased risk of violent lifestyle and poor quality of life for young people with a history of chronic lead exposure in South Africa.

The proportion of study participants with blood lead levels ≥ 5 μg/dL was much higher in our study (62.2%) compared to those reported in developed countries such the United States of America (3.1%) (Raymond et al., 2014). This should be a cause for concern because as

shown by Nevin early lead exposure is a good predictor of future violent behavior (Nevin, 2007). Similarly, Wright et al. associated average childhood blood lead and six years blood lead concentrations with violent offenses later in life (Wright et al., 2008). Furthermore, Needleman and colleagues reported that arrested and adjudicated African American and White youth from Philadelphia were four times more likely to have elevated bone lead levels 95% CL [1.4–11.1] (Needleman et al., 2002).

Consistent with international literature, in this study being a male was a predictor of having elevated blood lead levels (Raymond et al., 2009). It has been shown that gender differences in blood lead concentrations are influenced by bioavailability, capacity to absorb and toxicokinetics (Vahter et al., 2007). After lead is absorbed into the bloodstream, approximately 99% of it is bound to the erythrocytes because of their high affinity for lead, the remaining 1% resides in blood plasma and is available for circulation to the different tissues and organs of the body (Holstege et al., 2013; Rabinowitz, 1991). In addition to higher pulmonary function, intestinal motility, and greater body surface area than women which are associated with increased absorption capacity, on average men have greater red blood cell and plasma volume than women (Soldin and Mattison, 2009). These may be some of the contributing factors in gender differences regarding blood lead concentration levels.

Furthermore, males were more likely to engage in violent behavior acts compared to females. Additionally, this study showed that the patterns of violent behavior observed at school, although heightened, do mirror those perpetrated outside of school. Elliot and colleagues posit that violence in school is a known indication of violence outside of school in the communities (Elliott et al., 1998). However, findings from a multi-national country study utilizing data from the Third International Math and Science Study (TIMMS) survey including 32 nations (Akiba et al., 2002) showed that there is no direct link between community crime rates and school violence. The authors suggest that use of adult crime rates as opposed to juvenile crime rates may or may not be a contributing factor in their negative study findings in this regard (Akiba et al., 2002). In general, it is reported that violence in South Africa is "overwhelmingly perpetrated by men" (Jewkes et al., 2009); which may imply that if no interventions are put in place violent adolescents will grow to become violent adult men. Our results support the growing body of evidence showing that violence is a public health problem both in developing and developed countries.

Interestingly elevated geometric mean BLLs were significantly associated with sexual harassment in the total sample at school but not outside of school. Which begs the question – what is it about the school environment that fosters this type of violent behavior among adolescents? Similarly, an American study of adolescents attending middle and high school reported increased prevalence of peer-on-peer sexual harassment at school compared to the adolescent's house, someone else's house, at a party or other location (Young). And, the perpetrator at school was two times more likely to be a friend than outside of school ($p < 0.05$).

Perpetration of violent behavior by adolescents is a huge concern in South Africa. One in five secondary learners in South African schools had succumbed to one form or another of violence while at school (Burton and Leoschut, 2012). From a social science point of view, in the 2012 report titled 'The Dynamics of violence in schools in South Africa', Mncube et al. wrote "...it is also the understanding of this report that the basis of violence is social rather than genetic or biological, and therefore there are ways and means of reducing human violence" (Mncube and Harber, 2013). It is without a doubt that social issues play very important roles in violent behavior. According to the WHO, violent behavior can be controlled. There are modulating social and environmental factors that either deter or enhance propensity to criminal behavior (Mercy et al., 2002).

On the other hand, new developments in neuroscience using brain imaging are now beginning to unravel the possible underlying links between altered brain structure and antisocial behavior, including violent behavior in humans. Empirical data show an association between lead exposure and subsequent structural changes in different areas of the brain (Stewart et al., 2006; Brubaker et al., 2009; Brubaker et al., 2010; Schwartz et al., 2010; Cecil et al., 2011). The affected brain areas include those responsible for "executive functions, mood regulation and decision-making" (Cecil et al., 2008). Even more concerning is that these structural changes appear to be permanent (Cecil et al., 2008). These findings suggest that exposure to lead may result in changes in the structure and function of the brain which explains the underlying neuro-anatomical basis of the link between lead exposure and cognitive dysfunction, aggressive and violent behavior among others. These results complement epidemiological study findings, including our current study findings, suggesting a possible biological link between environmental lead exposure and violent behavior in adolescents. Scientific evidence is pivotal for public health policy making (Krug et al., 2002). We hope that our study findings will add to reliable empirical evidence from mainly developed countries showing a link between biology and violent behavior. And, provide the necessary information required by policy makers in low and middle income countries such as South Africa to mobilize for the essential public health priorities such as lead screening in children and other preventive measures.

The Director General of the WHO states that "while public health does not offer all the answers to this complex problem, we are determined to play our role in the prevention of violence worldwide" (Krug et al., 2002). To find a solution regarding the root causes of aggressive behavior requires an integrated approach including evidence from biology and social sciences (Ramirez, 2006) – so is the case with violent behavior. To help avert this public health hazard the CDC's ACCLPP recommends primary prevention where all homes are lead-free as the main practical way to prevent high BLLs in children (Centers for Disease Control and Prevention, 2012b).

There were some limitations to this study. Blood lead levels were measured at only one time point (age 13 years). It is recommended that estimates from both blood lead levels for recent exposure and bone lead levels for cumulative lead exposure be used (Hu et al., 2007). Our study findings are valuable in that they fill a gap in evidence and our findings can be further examined using bone lead levels. The exposure variable was categorized into 3 levels which may risk loss of power due to reduced sample size. To address and prevent or reduce possible bias in results, data were also analyzed using continuous blood lead levels. Furthermore, use of more than one scale to evaluate violent behavior is recommended in research studies, only YSR was the used to assess violent behavior in this study. Only Black African and Mixed Ancestral study participants were included in our study. For future studies inclusion of all South African population groups will improve the applicability of the study findings. Also, study participants were not evaluated for mental health, a known risk factor for violent behavior. Even though, none of the study participants included in the study had known mental health problems, this may have been a limitation in our study. Extremely violent behavior such as 'violence using a weapon' could not be properly assessed in relation to lead exposure as the study sample had a very small proportion of participants who reported to have perpetrated 'violence using a weapon'. It would be of great interest to further examine our study findings in environments where the study sample is comprised of young people convicted of violent crime in South Africa.

5. Conclusions

Because lead is persistent in the environment, it is likely to remain a significant public health problem for a very long time, especially in developing countries (Tong et al., 2000). Findings from this study show that lead exposure in early adolescence is positively associated with 'physical violence' during late adolescence in South African young

people. And males were associated with perpetration of the most severe forms of violent behavior in this study, such as violence using a weapon, physical violence and robbing others.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2017.09.004.

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Appendix 3: Journal Article 3

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Environmental lead exposure and pubertal trajectory classes in South African adolescent males and females

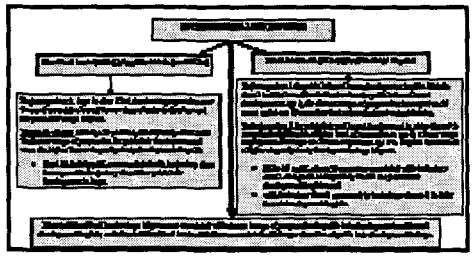
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HIGHLIGHTS

- BLLs at age 13 are linked to slower progression in pubic hair development in girls.
- BLLs at age 13 years are associated with longer duration of breast development.
- Cord BLLs are associated with slower progression in pubic hair development in boys.

GRAPHICAL ABSTRACT



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ABSTRACT

The effects of environmental lead exposure in the neuro-endocrine system have been shown to impact the maturation and tempo of puberty development in adolescents. In low and middle income countries very little is known regarding the detrimental health effects of childhood lead exposure with regard to the tempo of puberty development. To help address this gap in data, we examined the association between lead exposure and puberty progression in males and females.

Study participants from the urban Birth to Twenty Plus (BT20+) birth cohort in Soweto-Johannesburg, South Africa with data for blood lead levels at age 13 years, cord blood lead levels, pubic hair development and breast development in females, and pubic hair development and genital development in males, were included in this study. The sample comprised 1 416 study participants ($n = 684$ females). Pubertal development trajectory classes were defined using Latent Class Growth Analysis. Data were examined for (i) an association between cord blood lead levels and pubertal trajectory classes; and (ii) an association between blood lead levels at age 13 years and pubertal trajectory classes.

In females, there was an association between adolescent elevated blood lead levels (≥5 μg/dL) and lower level of maturation at age 9 years and slower progression of pubic hair and breast development (relative risk ratio (RRR) = 0.45, $p < 0.0001$; 95% CI (0.29–0.88)) and (RRR = 0.46, $p < 0.01$; 95% CI (0.27–0.77)), respectively. In males, elevated blood lead levels at birth were associated with slower tempo of pubic hair development (RRR = 0.20, $p < 0.05$).

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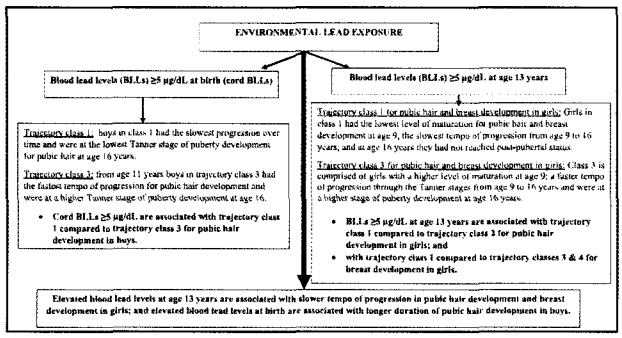
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Findings from this study suggest a possible role for environmental lead in altering pubertal development in South African adolescents as shown by slower tempo of progression through the Tanner stages pubertal development in females and males. There were also gender-differences between the effects of prenatal and postnatal lead exposure during pubertal development.

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1. Introduction

Epidemiological studies show secular trends in puberty timing and tempo (Euling et al., 2008; Jones et al., 2009; Sørensen et al., 2010; Sørensen et al., 2012; Parent et al., 2015). Altered pubertal timing in both girls and boys is linked to nutritional factors, various chronic illnesses and more recently endocrine disrupting chemicals (EDCs) (Rosen and Foster, 2001; Pozo and Argente, 2002; Selevan et al., 2003; Williams et al., 2010; Naicker et al., 2010; Zawatski and Lee, 2013), among others. However, little research has been conducted to evaluate the association between environmental lead exposure and tempo of progression through the Tanner stages of pubertal development in low or middle-income countries.

Puberty is a time of transition from childhood to adolescence and is marked by sexual and physical maturation due to hormonal changes in young girls and boys. Onset of puberty is mainly characterized by breast budding, pubic hair development and menarche in girls, and testicular volume and size, penile and pubic hair development and voice breaking in boys (Blondell et al., 1999; Golub et al., 2008; Jones et al., 2009; Day et al., 2015). Pubertal onset and progression is regulated via hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes (Louis et al., 2008). To activate the HPG and HPA axes requires a signal from the central nervous system (CNS) to the hypothalamus (Louis et al., 2008). Stimulation of the HPG axis initiates the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, activating the release of gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary (Louis et al., 2008; Roy et al., 2009; Zawatski and Lee, 2013). Release of gonadotropin activates the gonad. In girls, this leads to the production of ova and subsequently onset of menarche. In addition, FSH initiates secretion of androgens, which facilitates the development of breasts, ovaries and uterus. In boys, LH activates the secretion of androgen, which in turn initiates penile, pubic hair and testicular size and volume development. Stimulation of the HPA axis initiates the release of adrenocorticotropin releasing hormone (CRH) by the hypothalamus, which activates secretion of adrenocortical tropic hormone (ACTH) by the pituitary. ACTH activates adrenal cortex resulting in androstenedione and DHEA secretion which initiates development of pubic hair, armpit hair and acne (Louis et al., 2008).

Exposure to EDCs such as lead, soy phytoestrogens and cadmium, among others (Zawatski and Lee, 2013) during developmental stages of the CNS and sexual differentiation can alter puberty development. EDCs are defined as any chemical or mixture of chemicals that disrupts any part of hormone action (Zoeller et al., 2012). Exposure to lead interferes with various aspects of the HPG axis including disruption of hormonal pathways (Doumouchtsis et al., 2009; Zawatski and Lee, 2013), reduction in GnRH levels, testosterone levels in males, estradiol levels in females, LH and FSH levels (Doumouchtsis et al., 2009). Furthermore, it alters the neurotransmitter systems and consequently the HPA function (Doumouchtsis et al., 2009). Both animal (Sokol et al., 1985; Sokol et al., 2002) and human studies (Selevan et al., 2003; Wu et al., 2003; Hauser et al., 2008; Naicker et al., 2010; Williams et al., 2010) have shown a relationship between lead exposure and altered pubertal timing in males and females.

Altered puberty development poses potential health risks to the young people. A study in New Zealand linked longer duration of pubertal development (Wilkinson and Colls, 1994) to higher incidents of testicular cancer in Moaris compared to non-Moaris (Wilkinson et al., 1992). However, the authors posit that there could be other contributing factors such as lower birth weight which is common in Maoris infants and high rate of obese mothers which exposes the foetus to estrogen (Wilkinson and Colls, 1994). Furthermore, a UK Biobank study involving 250,037 women and 197,714 men showed that in females early pubertal timing increases the risk of breast cancer, angina, hypertension, obesity, early menopause and allergy to food, among others by 13%, 23%, 13%, 82%, 36%, and 39%, respectively in adulthood. And late puberty timing increases risk of early menopause, coeliac disease, low intelligence, asthma and poor overall health, among others by 16%, 62%, 32%, 11% and 19%, respectively (Day et al., 2015). In males early onset of puberty increases the odds of having angina, heart attack, obesity, Type 2 diabetes, depression, irritable bowel syndrome and short stature in adulthood, among others by 39%, 26%, 58%, 24%, 28%, 49% and 39%, respectively. While, delayed pubertal timing increases the risk of having anxiety/panic attacks, depression, asthma and poor overall health, among others by 43%, 36%, 22% and 25%, respectively (Day et al., 2015). These data highlight the significance of altered pubertal development as a public health problem.

In 1984 the limit on use of lead in petrol in South Africa was 0.84 g/L. This was reduced to 0.40 g/L in 1986 and phasing in of unleaded petrol was introduced in 1989 (Nriagu, 1990). In addition, lead mining and previous use of lead in paint are some of the well known sources of lead exposure in South African children (Mathee et al., 2009; Mathee, 2014). More recently, studies have shown that children continue to be exposed to lead because of limited knowledge about dangers of lead exposure in subsistence fishing communities (Mathee et al., 2013) and cottage industries (Teare et al., 2015) among others. Consequently, South African children had been shown to have elevated blood lead levels (von Schirnding et al., 1991; von Schirnding et al., 2003; Mathee, 2014). Even though blood lead concentrations in children have dropped significantly globally, including South Africa, since the ban of use of lead in paint and tetraethyl lead in petrol (Mathee et al., 2006; Centers for Disease Control and Prevention, 2012b); there are "no safe blood lead levels in children" (Centers for Disease Control and Prevention, 2012b).

Previous study findings using a sub-sample from the Birth to Twenty Plus (BT20+) cohort, showed that female study participants with blood lead levels ≥5 μg/dL at age 13 years were 2.34, 1.81 and 2.01 times more likely to have delayed onset of breast development, pubic hair development and age of menarche attainment, respectively, compared to those with blood lead levels <5 μg/dL ($p < 0.001$) (Naicker et al., 2010). To our knowledge, no study has been conducted in South Africa to examine the association between lead exposure and puberty development in males. For this study we seek to conduct longitudinal examination of the effects of lead exposure on pubertal development. Using Latent Class Growth Analysis (LCGA), pubertal progression information from the BT20+ study collected from age 9 to 16 years old was used to generate distinct classes according to a "common developmental trajectory" for the Tanner Sexual Maturation Scale (SMS) indicators of pubertal stage (Lundeen et al., 2016). We examined the association between both cord and adolescent lead concentrations and latent classes of puberty development in girls and boys.

2. Materials and methods

2.1. Study population

Study participants were selected from the BT20+ cohort in Johannesburg, South Africa. BT20+ is made up of all singleton births from 23 April to 8 June 1990 in Soweto and Johannesburg. Inclusion criteria included that the infant must reside in the Johannesburg area for at least six months after birth. The reason for this was that at the time pilot studies had shown that some pregnant women from the rural areas came to the Johannesburg metropolis to deliver their babies in order to access better health facilities and for other family reasons. A total of 3273 (1682 females) study participants fulfilled the inclusion criteria and were enrolled in the study (Richter et al., 2004; Richter et al., 2007).

Study participants were included in the current analysis if they had data for blood lead levels at age 13 years and pubertal growth trajectory classes for pubic hair development and breast development in girls and pubic hair development and genital development in boys. Of the 1682 girls enrolled in the original BT20+ study, 1135 had data for pubertal growth trajectories and 749 had data for blood lead levels at age 13 years. A total of 728 Black African and Mixed ancestry girls fulfilled the inclusion criteria. And of the 1591 boys enrolled in the original study sample, 1060 had data for pubertal growth trajectory for pubic hair development and genital development and 708 had data for blood lead levels at age 13 years. A total of 683 Black African and Mixed ancestry boys fulfilled the inclusion criteria. White and Indian/Asian study participants were excluded because of low numbers.

Additionally, a subsample of study participants with data for blood lead levels at birth (cord blood lead levels) and pubertal development trajectory classes for pubic hair development and breast development in girls and pubic hair development and genital development in boys was included to examine the association between lead exposure at birth and pubertal progression. This was to determine if there are any differences between the relationship prenatal and postnatal lead exposure and pubertal progression. Two hundred and thirty five females and 234 males of Black African and Mixed ancestry fulfilled the inclusion criteria.

2.2. Blood lead measures

Whole blood samples were collected at birth (umbilical cord blood) and at age 13 years into EDTA-containing tubes free of metal traces. Trained healthcare professionals performed blood sampling. "Blood samples were vortexed and rolled on the coulter mixer for at least 10 minutes until properly mixed. They were diluted 10 times with 1,1 % (v/v) Triton X-100 using automatic Hamilton Microlab 500 diluter into disposable 10 ml Sterilin plastic tubes covered with screw caps and mixed well using a vibration mixer. Blood lead levels were measured using Perkin Elmer 600 Aanalyst atomic absorption spectrometer with a THGA graphite furnace, Zeeman background correction and AS- 800 Autosampler" (Nkomo et al., 2017). All blood samples and samples for quality control were prepared and measured in-house at the National Institute for Occupational Health, Johannesburg, South Africa.

2.3. Anthropometric measures and socio-demographic factors

Birth weight, height and Body Mass Index (BMI) at age 8 years were measured using standard methods (Cameron, 1984). BMI was calculated as weight in kilograms divided by height in square meters (Lundeen et al., 2016). Height was converted into height-for-age and BMI into BMI-for-age using the World Health Organization (WHO) standards (World Health Organization, 2006; Onis, 2006; Onis et al., 2007). Data for household income at birth were divided into quintiles ranging from 1 (poorest) to 5 (highest) (Lundeen et al., 2016) and for ethnic group of the child were collected at birth classified as Black African and Mixed ancestry (Richter et al., 2007).

2.4. Pubertal growth trajectory classes for pubertal development from age 9 to 16 years old

From age 9 to 16 years data for pubertal development were collected annually using a validated self-reported Tanner-stage pubertal development questionnaire from boys and girls. At ages 9 and 10 data were collected by trained medical practitioners and from age 11 years onwards were through self-assessment (Norris and Richter, 2005). Tanner stages of pubertal development refer to a standard clinical method used to describe physical measurements of secondary sexual characteristics using drawings to signal stage of pubertal development where stage 1 signifies lowest level of pubertal maturation and stage 5 denotes highest level of pubertal maturation in girls and boys (Blondell et al., 1999).

Pubertal growth trajectory classes were grouped using Mplus to perform LCGA. Study participants had to have at least one Tanner Sexual Maturation Scale measurement to be included in the analyses for trajectory classes. Full Information Maximum Likelihood technique (Jung and Wickrama, 2008) was used to account for the missing data (Lundeen et al., 2016). LCGA is a latent growth modeling method that is helpful in identifying meaningful classes of individuals and modeling their longitudinal development trajectories (Jung and Wickrama, 2008). Using LCGA to describe both the level of pubertal development at age 9 years tempo of progression through the Tanner stages in the BT20+ study, analyses for breast development and pubic hair development in girls and genital development and pubic hair development in boys were conducted separately (Lundeen et al., 2016). Three trajectory classes for pubic hair development for both males and females, and four trajectory classes for breast development in females and four trajectory classes for genital development in males were identified.

2.5. Classification of pubertal growth trajectory groups

(Adapted from Lundeen et al., 2016)

The trajectory classes in this study comprise of three stages of pubertal development: i) level of maturation at age 9 years, ii) tempo of progression from through the Tanner stages from age 9 to 16 years and iii) postpubertal status at age 16 years. In all cases, trajectory class 1 is a reference category representing the least level of pubertal growth in all three stages as described above. Tables 1 and 2 summarize classification of pubertal growth trajectory classes in girls and boys, respectively.

2.6. Statistical analysis

First data were analyzed for statistically significant differences between the analytical sample and BT20+ study population excluded in the current study. In the analytical sample cord blood lead levels and blood lead levels at age 13 years were divided into <5 μg/dL, 5–9.99 μg/dL and ≥10 μg/dL for descriptive analysis. For cross tabulation between pubertal growth trajectory classes and blood lead levels; and inferential statistics blood lead levels were dichotomized into <5 and ≥5 μg/dL. The cut point of <5 μg/dL was chosen in line with the recommendations by the Centers for Disease Control and Prevention (CDC) Advisory Committee for Childhood Lead poisoning Prevention to use the reference value of 5 μg/dL which is based on the 97.5th percentile of the current blood lead level distribution among children aged 1 to 5 years in the United States of America (Centers For Disease Control and Prevention, 2012a). In this study cord blood lead levels and blood lead levels at age 13 years are indicators of prenatal and postnatal lead exposure, respectively.

Lundeen et al. (2016) showed that there is an association between anthropometric measures such as height and BMI and pubertal development. BMI was calculated as weight in kilograms divided by height

Table 1
Pubertal growth trajectory classes for girls.

Trajectory classes	Pubic hair development	Breast development
Class 1	Reference category	Reference category
Class 2	Girls in class 2 had a higher level of pubertal maturation for pubic hair development at age 9 years than those in class 1 but a lower level than those in class 3. Their tempo of progression from age 9 to 16 years was faster than those in class 1 but slower than those in class 3. At age 16 years they had not reached post puberty status and were at a higher Tanner stage than those in class 1, but at a lower Tanner stage than those in class 3.	At age 9 years pubertal maturation level for girls in class 2 was similar to those in class 1, but were less developed than those in classes 3 and 4 for breast development. Their tempo of progression from age 9 to 16 years was faster than those in class 1 but slower than those in classes 3 and 4. At age 16 years they had not reached postpubertal status; they were at a higher Tanner stage than those in class 1; at a similar Tanner stage as those in class 3, but at a lower Tanner stage than those in Class 4.
Class 3	Class 3 is comprised of girls with the highest level of pubertal maturation at age 9 years; fastest tempo of progression through the Tanner stages from age 9 to 16 years; and at age 16 they were at the highest stage of puberty development and had reached postpubertal status for pubic hair development compared to girls in classes 1 and 2.	At age 9 years girls in class 3 were at a higher level of pubertal maturation compared to those in classes 1 and 2; but at a similar level as those in class 4 for breast development. They had the fastest tempo of progression from age 9 to 11 years, followed by girls in class 4, then class 2 and lastly girls in class 1. From age 11 to 16 years their pubertal transition was faster than that of girls in classes 1 and 2, but slower than those in class 4. And at age 16 they were at a higher Tanner stage than those in class 1; similar Tanner stage as those in class 2, at a lower Tanner stage than girls in class 4; and had not reached post puberty status.
Class 4		At the age 9 years old girls in class 4 were at a higher level of puberty maturation for breast development than those in classes 1 and 2; but at a similar level as those in classes 3. They had the fastest tempo of progression from age 9 to 16 years followed by girls in classes 2 & 3. At age 16 years they were at the highest Tanner stage, followed by those in classes 2 & 3, with those in class 1 at the lowest Tanner stage. They were the only ones who had reached postpubertal status for breast development at age 16.

Table 2
Pubertal growth trajectory classes for boys.

Trajectory classes	Pubic hair development	Genital development
Class 1	Reference category	Reference category
Class 2	At age 9 years boys in class 2 had pubertal maturation levels for pubic hair development similar to those of boys in classes 1 and 3. Their tempo of progression from age 9 to 16 years was faster than those in class 1 but slower than those in class 3. At age 16 years they fared between than those in class 1 but, were at a lower Tanner stage compared to those in class 3. They also had not reached post puberty status.	At age 9 years boys in class 2 were at the same level of puberty maturation for genital development as those in classes 1 and 4, but at a lower level than those in class 3. They had a faster pubertal progression from age 9 to 16 years than those in class 1 but slower than those in classes 3 and 4. At age 16 years they were at a lower Tanner stage compared to girls in class 4, but at a higher Tanner stage than those in classes 1 and 2; and had not reached postpubertal status.
Class 3	Boys in class 3 had a similar level of pubertal maturation for pubic hair development as those in classes 1 and 2 at 9 years old. From age 9 to 16 years their tempo of progression was faster than both those in classes 1 and 2. At age 16 years they were at the highest Tanner stage, followed by those in class 2; and they had reached post puberty status.	At age 9 years boys in class 3 were at a slightly higher level of puberty maturation for genital development than those in classes 1, 2 and 4. From age 10 to 16 years their tempo of progression was faster than those in classes 1 and 2, but slower than those in class 4. At age 16 years they had not reached post puberty status. And they were at a higher Tanner stage than those in classes1 and 2, but at a lower Tanner stage than those in class 4.
Class 4		At age 9 years boys in class 4 were at the same level of puberty maturation for genital development as those in classes 1 and 2. They had the fastest tempo of progression from age 9 to 16 years, followed by boys in class 3, then class 2, with those in class 1 the slowest. At age 16 years they had reached postpubertal status.

in meters squared. Measurements for height and weight were converted to height-for-age (HAZ) and BMI-for-age (BMIZ) z-scores, respectively (Lundeen et al., 2016). Household income at birth was divided into quintiles ranging from 1 (lowest) to 5 (highest) (Lundeen et al., 2016). Bivariate analyses were conducted for anthropometric measures and socio-demographic covariates by gender. To compare the significance of the associations; *t*-tests were used in the case of continuous variables and chi-square tests for the categorical variables. Multinomial logistic regression was used to predict pubertal growth trajectory class based on blood lead levels at age 13 years and cord blood lead levels. Covariates for the adjusted models were defined by a statistical significance of $p < 0.05$ or a crude and adjusted models difference of 10%. Bolding is used in the tables to indicate statistical significant results. Data analyses were conducted using STATA version 14.

2.7. Ethics

Consent for all study procedures was sought from the BT20+ birth cohort. Ethical approval was obtained from the University of the Witwatersrand Ethics Committee on Human Subjects (M010556). The Federal Wise Assurance registration number of the Witwatersrand University Ethics Committee on Human Subjects is FWA00000715. Only consented individuals were enrolled in the study.

3. Results

3.1. Differences between the characteristics of analytical sample and excluded members of the BT20+ cohort

For study participants with data for blood lead levels at age 13 years and pubertal growth trajectory classes there were no statistically significant differences between the analytical sample and the cohort study participants excluded from the current study with regard to key variables such as distribution of blood lead levels at age 13 years; trajectory classes for pubic hair and breast development in females; trajectory classes for pubic hair and genital development in males; ethnicity; height at age 8 years and birth weight in both males and females ($p > 0.05$). Data not shown.

And for study participants with data for cord blood lead levels and pubertal growth trajectory classes there were no statistically significant differences between the analytical sample and the excluded cohort

members regarding distribution of blood lead levels at birth; trajectory classes for pubic hair and breast development in females; trajectory classes for pubic hair and genital development in males; and birth weight in both males and females ($p > 0.05$). However, there were more Black Africans than Mixed ancestry study participants in the analytical sample; and on average males in the analytical sample were 1.22 cm taller at age 8 years compared to the excluded cohort members ($p < 0.05$). Data not shown.

3.2. Analytical sample characteristics

Table 3 shows comparison analyses between males and females in the analytical sample. There was no statistically significant difference between males and females in Black African and Mixed ancestry study participants. There were slight differences in birth weight and household income between males and females at birth. The average difference in height at age 8 years was about 1 cm in favour of males. There was no difference between males and females regarding BMI at birth.

3.3. Distribution of cord blood lead levels and blood lead levels at age 13 years

At birth there were no statistically significant differences in blood lead levels between males and females as demonstrated in Table 4a. Blood lead levels at age 13 years ranged from 1.3 to 28.1 μg/dL in males and 1.0 to 16.3 μg/dL in females. Compared to females, a higher proportion of males had blood lead levels ≥5 μg/dL and vise versa with regard to blood lead levels <5 μg/dL as shown in Table 4b.

3.4. Pubertal growth trajectory classes by blood lead levels at birth and blood lead levels at age 13 years by gender

Data were analyzed for differences in the proportion of study participants in each trajectory class by blood lead levels. Almost 31% of boys with blood lead levels ≥5 μg/dL at birth were in trajectory class 1 compared to 18.6% of boys with blood lead levels <5 μg/dL in the same trajectory class (Table 5a). There were no statistically significant differences in trajectory classes for pubic hair development and breast development between girls with cord blood lead levels <5 μg/dL and those with cord blood lead levels ≥5 μg/dL; and trajectory classes for genital development between boys with cord blood lead levels <5 μg/dL compared to those with cord blood lead levels ≥5 μg/dL.

>40% of females with blood lead levels ≥5 μg/dL during adolescence had a slower tempo of progression through the Tanner stages for pubic hair development from age 9 to 16 years and had not reached postpubertal status for pubic hair development at age 16 compared to 27% of those with blood lead levels <5 μg/dL (Table 5b). Twenty seven percent of girls with blood lead levels ≥5 μg/dL at age 13 years had a slower rate of pubertal transition for breast development from age 9 to 16 years and were at the lowest Tanner stage for breast development at age 16 years compared to their counterparts versus 18.5% of those with blood lead levels <5 μg/dL. There were no statistically significant differences in trajectory classes for pubic hair development and genitalia development between boys with blood lead levels <5 μg/dL and those with blood lead levels ≥5 μg/dL at age 13 years.

3.5. The association between pubertal growth trajectory classes and blood lead levels at 13 years old and blood lead levels at birth

Data were analyzed for an association between blood lead levels at age 13 years and pubertal growth trajectory classes; and umbilical cord blood lead levels and pubertal growth trajectory classes in girls and boys. In females after adjusting for confounders elevated blood lead levels (≥5 μg/dL) at 13 years old were associated with significantly decreased RRR for class 2 compared to class 1 for pubic hair development ($p < 0.001$) as demonstrated in Table 6a. And elevated blood lead levels at age 13 years relative to blood lead levels <5 μg/dL were associated with a 37% reduction in the risk of being in class 3 compared to class 1 ($p < 0.05$), and a 54% reduction in the risk of being in class 4 compared to class 1 for breast development ($p < 0.01$). In males, elevated blood lead levels at age 13 years were not significantly associated with puberty development as shown in Table 6b.

Table 3
Distribution of selected characteristics of the study population by gender.

	Females (732)	Males (683)	*P*-value
	Total (%)	Total (%)	
Ethnicity			0.9
Black African	645(88.1)	601(88.0)	
Mixed ancestry	87(11.9)	82(12.0)	
Birth weight (kg)			
Mean ± SD	3.02 ± 0.5	3.13 ± 0.5	**<0.0001**
Range	1.07–4.9	1.12–4.8	
<2.5	91 (12.5)	57 (8.4)	**<0.0001**
2.5–3	268 (36.7)	203 (29.8)	
>3	372 (50.9)	421 (61.8)	
Height (8y), mean ± SD (cm)	123.6 ± 5.9	124.6 ± 5.9	**0.01**
Height-for-age z-score (8y) mean ± SD	−0.7 ± 0.9	−0.7 ± 1.0	1.0
Body mass index (kg/m^2) mean ± SD	15.9 ± 2.1	15.8 ± 1.4	0.4
Body mass index z-score mean ± SD	−0.1 ± 0.9	−0.1 ± 0.9	1.0
Household income in quintiles (range 1 to 5)			**<0.0001**
1	100 (14.8)	110 (17.4)	
2	117 (17.3)	110 (17.4)	
3	237 (35.1)	217 (34.4)	
4	138 (20.4)	13 (20.8)	
5	83 (12.3)	63(10.0)	

Table 4a
Distribution of cord blood lead levels: $n = 234$ (males) and $n = 235$ (females).

	Cord blood lead levels		
	Male n (%)	Female n (%)	P-value
Blood lead levels (μg/dL)			0.87
<5	59 (25.2)	64 (27.1)	
5–9.99	164 (70.1)	160 (67.8)	
≥10	11 (4.7)	12 (5.1)	
Continuous blood lead levels (μg/dL)			
Mean (SD)	5.9 (2.0)	5.8 (2.1)	0.60
Range	2.0–13	2.0–16.0	
Q1	4.0	4.0	
Median	6.0	6.0	
Q3	7.0	7.0	

Table 4b
Distribution of blood lead levels at age 13 years by gender: $n = 684$ (males) and $n = 732$ (females).

	Blood lead levels at age 13 years		
	Male n (%)	Female n (%)	P-value
Blood lead levels (μg/dL)			**< 0.0001**
<5	171 (25.0)	370 (50.5)	
5–9.99	470 (68.7)	354 (48.4)	
≥10	43 (6.3)	8 (1.1)	
Continuous blood lead levels (μg/dL)			**<0.0001**
Mean (SD)	6.6(2.6)	5.0 (1.9)	
Range	1.3–28.1	1.0–16.3	
Q1	5.0	3.5	
Median	6.5	4.8	
Q3	6.0	7.9	

Table 5a
Cross tabulation between pubertal trajectory class and cord blood levels for girls and boys.

	Cord blood lead levels (μg/dL)			*p*-value
	<5	≥5	Total	
GIRLS n(%)				
Pubic hair				0.136
Class 1	5 (7.8)	30 (17.4)	35 (14.8)	
Class 2	35 (54.7)	92 (53.5)	127 (53.8)	
Class 3	24 (37.5)	50 (29.1)	74 (31.4)	
Breast				0.239
Class 1	6 (9.4)	31 (18.0)	37 (15.7)	
Class 2	29 (45.3)	69 (40.1)	98 (41.5)	
Class 3	18 (28.1)	35 (20.3)	53 (22.5)	
Class 4	11 (17.2)	37 (21.5)	48 (20.3)	
BOYS n(%)				
Pubic hair				**0.031**
Class 1	11 (18.6)	54 (30.9)	65 (27.8)	
Class 2	35 (59.3)	103 (58.9)	138 (59.0)	
Class 3	13 (22.0)	18 (10.3)	31 (13.2)	
Genital				0.251
Class 1	1 (1.7)	12 (6.9)	13 (5.6)	
Class 2	18 (30.5)	59 (33.7)	77 (32.9)	
Class 3	33 (55.9)	93 (53.1)	126 (53.8)	
Class 4	7 (11.9)	11 (6.3)	18 (7.7)	

However, when data were analyzed for association between blood lead levels at birth and pubertal development; there was a 72% reduction in risk of being in class 3 compared to class 1 for pubic hair development in males ($p < 0.05$) as shown in Table 6c. In females, there was no statistically significant association between elevated cord blood lead levels and tempo of progression (results not shown).

4. Discussion

This study used longitudinal data to investigate the association between exposure to lead and latent classes of puberty development among urban African Black and Mixed ancestry girls and boys in South Africa. Blood lead measures ≥5 μg/dL at age 13 years were associated with lower levels of pubertal maturation at age 9 years, slower tempo of progression from age 9 through to 16 years and lower Tanner stage attainment of pubic hair development and breast development at age 16 years in females. On the other hand, cord blood lead levels ≥5 μg/dL were associated with low pubertal maturation levels at age 9 years,

Table 5b
Cross tabulation between pubertal trajectory class and blood lead levels at 13 years for girls and boys.

	Blood lead levels at age 13 years (μg/dL)			p-value
	<5	≥5	Total	
GIRLS n(%)				
Pubic hair				**<0.0001**
Class 1	99 (27.0)	149(41.3)	248(34.1)	
Class 2	221(60.2)	170(47.1)	391(53.7)	
Class 3	47(12.8)	42(11.6)	89(12.2)	
Breast				**0.012**
Class 1	68(18.5)	98(27.1)	166(22.8)	
Class 2	91(24.8)	94(26.0)	185(25.4)	
Class 3	146(39.8)	128(35.5)	274(37.6)	
Class 4	62(16.9)	41(11.4)	103(14.2)	
BOYS n(%)				
Pubic hair				0.45
Class 1	51(29.8)	151(29.5)	202(29.6)	
Class 2	102(59.7)	288(56.2)	390(57.1)	
Class 3	18(10.5)	73(14.3)	91(13.3)	
Genital				0.76
Class 1	8(4.7)	28(5.5)	36(5.3)	
Class 2	70(40.9)	189(36.9)	259(37.9)	
Class 3	81(47.4)	251(49.0)	332(48.6)	
Class 4	12(7.0)	44(8.6)	56(8.2)	

Table 6a
Multinomial logistic regression analysis to predict the risk of being in trajectory class 2 versus 1 or trajectory class 3 versus 1 or trajectory class 4 versus 1 in females with elevated blood lead levels (≥ 5 μg/dL) at age 13 years.

Exposure Variable	Unadjusted								Adjusted[a]							
	Pubic hair development			Breast development					Pubic hair development			Breast development				
	Trajectory classes			Trajectory classes					Trajectory classes			Trajectory classes				
	3 vs. 1		2 vs.1	4 vs. 1		3 vs. 1		2 vs. 1	3 vs. 1		2 vs.1	4 vs. 1		3 vs. 1		2 vs.
	RRR	95%(CI)	RRR	95%(CI)		RRR	95%(CI)		RRR	95%(CI)	RRR	95%(CI)		RRR	95%(CI)	RRR	95%(CI)
Blood lead levels at age 13 years																	
<5 μg/dL																	
≥5 μg/dL	0.59	0.36–0.97[†]	0.51	0.37–0.70[‡]	0.45	0.28–0.76[*]	0.61	0.41–0.90[†]	0.72	0.47–1.09	0.55	0.26–1.17	0.45	0.29–0.68[‡]	0.46	0.27–0.77[*]	0.63	0.42–0.94[†]	0.72	0.47–1.11

[a] Adjusted for ethnicity and height at 8 years.
[*] $p < 0.01$.
[†] $p < 0.05$.
[‡] $p < 0.001$.

Table 6b
Multinomial logistic regression analysis to predict the risk of being in trajectory class 2 versus 1 or trajectory class 3 versus 1 or trajectory class 4 versus 1 in males with elevated blood lead levels (≥ 5 μg/dL) at age 13 years.

Exposure Variable	Unadjusted										Adjusted[a]									
	Pubic hair development				Genital development						Pubic hair development				Genital development					
	Trajectory classes				Trajectory classes						Trajectory classes				Trajectory classes					
	3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs. 1		3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs.	
	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)
Blood lead levels at age 13 years																				
<5 μg/dL																				
≥5 μg/dL	1.12	0.64–1.96	00.93	0.63–0.137	0.98	0.36–2.62	0.83	0.37–1.90	0.76	0.33–1.75	1.35	0.73–2.47	0.94	0.63–1.39	1.02	0.37–2.83	0.88	0.38–2.01	0.77	0.33–1.77

[a] Adjusted for ethnicity and height at 8 years. There were no statistically significant results in males.

Table 6c
Multinomial logistic regression analysis to predict the risk of being in trajectory class 2 versus 1 or trajectory class 3 versus 1 or trajectory class 4 versus 1 in males with elevated blood lead levels (≥5 μg/dL) at birth.

Exposure variable	Unadjusted										Adjusted[a]									
	Pubic hair development				Genital development						Pubic hair development				Genital development					
	Trajectory classes				Trajectory classes						Trajectory classes				Trajectory classes					
	3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs. 1		3 vs. 1		2 vs.1		4 vs. 1		3 vs. 1		2 vs.	
	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)	RRR	95%(CI)
Blood lead levels at age 13 years																				
<5 μg/dL																				
≥5 μg/dL	0.28	0.11–0.74*	0.60	0.28–1.27	0.13	0.01–1.24	0.23	0.03–1.88	0.27	0.03–2.25	0.28	0.11–0.74.	0.61	0.25–1.43	0.13	0.01–1.24	0.24	0.03–1.89	0.27	0.03–2.26

[a] Adjusted for ethnicity. Height at age 8 years was not included in the final model because it was not statistically significant.
* $p = 0.01$.

longer duration of puberty transition from age 9 to 16 years and lower Tanner stage attainment of pubic hair development at age 16 years in males only. As such, our findings show a link between prenatal lead exposure and slower tempo of progression in males; and postnatal lead exposure and slower tempo of progression in females; suggesting gender differences in tempo of pubertal progression based on the timing of lead exposure.

Previous studies have linked lead exposure to altered pubertal timing. NHANES studies have reported an inverse relationship between blood lead levels and onset of breast development in girls between the ages of 8 and 18 years, and an inverse association between blood lead concentrations and onset of pubic hair development and menarche in girls between the ages of 8 and 16 years (Selevan et al., 2003; Wu et al., 2003). Likewise, in Russia a cross-sectional study involving 489 boys aged 8 to 9 years showed an association between blood lead levels ≥5 μg/dL and delayed onset of genital development (Hauser et al., 2008). These findings were later confirmed using Cox proportional hazards model (Williams et al., 2010). Even though empirical data show that there is a link between postnatal lead exposure and delay in onset of genital development in boys; on the contrary, in this study there was no association between adolescent lead exposure in boys and altered puberty development. These results were also interesting in that males were shown to have higher blood lead levels than females during adolescence; which could be interpreted as a sign of higher susceptibility to lead exposure in males. Therefore, it was expected that lead exposure during adolescence will be associated with tempo of progression in pubertal development in males. Instead, prenatal lead exposure in males was associated with slower transition through the Tanner stages; a significant finding since this is a period when epigenetic changes to environmental changes are most likely to occur thus setting in motion patterns and trajectories of development. As explained in the introduction section of this paper, EDCs disrupt hormonal actions during sexual differentiation which may explain the gender differences in the effects of lead exposure during pubertal progression found in this study. Almstrup et al. (2016) reported lower methylation levels of CpG islands during pre-puberty compared to post-puberty in a study of healthy Danish girls and boys, with girls showing higher levels of DNA methylation than boys. During pubertal transition CpGs associated with changes in circulating reproductive hormone levels were significant in boys only, however, the authors suggest that these findings should be further examined because the number of girls ($n = 20$) enrolled in the study was lower than that of boys ($n = 31$) (Almstrup et al., 2016).

In a study of infants aged 3 months to 5 years blood lead levels ≥5 μg/dL were associated with a higher number of differentially methylated clusters related to lead exposure in females compared to males (Sen et al., 2015). This was contrary to their hypothesis where males were expected to have more lead exposure related DNA methylation changes; as males have been shown to be more sensitive to lead exposure compared to females (Cecil et al., 2008; Brubaker et al., 2010). The authors propose that the unexpected findings could be suggestive of the "adaptive and protective nature" of DNA methylation changes. Our results need to be further examined to investigate the possible underlying biological programs influencing the established gender differences in the effects of EDCs during onset of puberty, pubertal transition and post puberty.

One of the limitations of this study was that it did not include all ethnic South African population groups which may limit the generalizability of the study findings in the country. Evidence of elevated blood lead levels in Black African and Mixed ancestry children is well reported in South Africa (von Schirnding et al., 1991; Mathee et al., 2002). However, there is dearth of information regarding blood lead levels of children of Asian and Indian descent. Previous study findings showed that blood lead levels in White children were much lower than those of Mixed ancestry children in the Western Cape (von Schirnding et al., 1991). Nonetheless, there have been great developments in the new democratic South Africa and its peoples; as such it is prudent that lead exposure studies include children from all ethnic backgrounds. We also suggest that our findings be further investigated using bone lead levels as a proxy for cumulative lead exposure.

Second, pubertal development data from 11 to 16 years old were self reported. Rasmussen and colleagues recommend that self reported data be augmented by a physical assessment (Rasmussen et al., 2015). However, other studies have shown substancial agreement between the two instruments (Chan et al., 2008). In South Africa self-rating pubertal development data has been validated among Black African adolescents (Norris and Richter, 2005).

5. Conclusion

The results in this study suggest that environmental lead exposure is associated with slower tempo of progression in South African young girls and boys. More longitudinal research is needed to better understand the consequence of this association for later health in low and middle income countries.

Acknowledgements

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Appendix 4: Ethics clearance certificate

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

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Palesa Nkomo

CLEARANCE CERTIFICATE	M110715
PROJECT	Association between Bone Lead Levels and Aggression in South Africa
INVESTIGATORS	Ms Palesa Nkomo.
DEPARTMENT	Department of Paediatrics
DATE CONSIDERED	29/07/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

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

DATE 05/09/2011 CHAIRPERSON .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor: Professor Shane Norris

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...

Appendix 5: Change of Title

Titled changed and approved to:

Association between lead levels and adverse health effects: Findings from the Birth to Twenty cohort.

Candidate name:

Palesa Manthabiseng Nkomo

Candidate signature:



Date:

30 March 2018

Appendix 6: Birth to Twenty Bara site: 14th year adolescent questionnaire



University of the Witwatersrand
Department of Paediatrics and Child Health

BIRTH TO TWENTY BARA SITE: 14TH YEAR ADOLESCENT QUESTIONNAIRE

DATE : Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER : ☐☐☐☐☐☐☐

BONE STUDY ID NUMBER : ☐☐☐

PHYSICAL ACTIVITY

Activities at school

1. Do you attend physical education/games lessons at school?
   (Exercise classes supervised by a teacher during school time)

Yes=1	No=0	

2. How often classes are held & how long are the classes?

Times / week	Hours / time

What are the three most frequent activities that you do during these classes?

Activities

3. Do your school teachers encourage you to participate in physical activity? **Y** **N**
4. Do your parents encourage you to participate in physical activity? **Y** **N**
5. Who (parent/caregiver or other) encourages you the most to participate in physical activities?



University of the Witwatersrand
Department of Paediatrics and Child Health

BIRTH TO TWENTY BARA SITE: 14TH YEAR ADOLESCENT QUESTIONNAIRE

DATE : Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER : ☐☐☐☐☐☐☐

BONE STUDY ID NUMBER : ☐☐☐

PHYSICAL ACTIVITY

Activities at school

1. Do you attend physical education/games lessons at school?
   (Exercise classes supervised by a teacher during school time)

Yes=1	No=0	

2. How often classes are held & how long are the classes?

Times / week	Hours / time

What are the three most frequent activities that you do during these classes?

Activities

3.	Do your school teachers encourage you to participate in **physical activity**?	**Y** / **N**
4.	Do your parents encourage you to participate in **physical activity**?	**Y** / **N**

5. Who (parent/caregiver or other) encourages you the most to participate in **physical activities?**

Informal activities

Do you engage in any physical activity during **school breaks** or **outside school**, for example riding a bike, playing in the street or yard? **NOT** activity as part of a sports team or club. Tick the three most frequent activities that you do, and time spent on each activity.

Activity		Mon	Tue	Wed	Thu	Fri	Sat	Sun
Riding a bike								
Playing with a ball								
Skipping								
Hop scotch								
Dibeke (tin game)								
Bhati (tennis ball game)								
Mgusha (panty hose game)								
Skateboarding								
Roller-skating								
Other (specify)								

Sedentary activities

Do you engage in any of the following activities before or after school, and if so, for how many hours?

Activity	Mon-Thur (hrs)	Fri-Sat (hrs)	Sun (hrs)
Watching TV & videos & movies			
Reading, drawing, homework			
Playing a musical instrument IF YES - please detail what musical instrument?			
Playing video/ TV/ computer games			
Internet surfing			
Listening to radio/ music			

What time do you go to bed on a <u>school night</u>?

What time do you go to bed on a <u>non-school</u> night (on a weekend or on holiday)?

What time do you wake up on a <u>school morning</u>?

What time do you wake up on a <u>non-school</u> morning (on a weekend or on holiday)?

Transport

How do you get to school and how long does it take to get there and back?

1. By car, bus, taxi, train etc.

Yes=1	No=2			
There:_____minutes				
Back:_____minutes				

2. Walking

Yes=1	No=2			
There:_____minutes				
Back:_____minutes				

When you walk, at what pace (how fast) do you usually walk?

At a pace, that makes me breathe much harder than normal	1	
At a pace that makes me breathe somewhat harder than normal	2	
At a pace where there is no change in my breathing	3	

3. Bicycle

Yes=1	No=2			
There:_____minutes				
Back:_____minutes				

When you cycle, at what pace (how fast) do you usually cycle?

At a pace, that makes me breathe much harder than normal	1	
At a pace that makes me breathe somewhat harder than normal	2	
At a pace where there is no change in my breathing	3	

NOTES:

EXTRA MURAL ACTIVITIES AT SCHOOL (LAST 12 MONTHS)

	How many months?	Prac/Wk	Hrs/Prac	Comp/Wk
Athletics (running)				
Athletics (other)				
Cricket				
Swimming				
Tennis				
Hockey				
Netball				
Rugby				
Soccer				
Badminton				
Basketball				
Ballet				
Cycling				
Dancing				
Gymnastics				
Judo / karate				
Squash				
Volleyball				
Other				
Musical instrument				

PRIVATE EXTRA MURAL ACTIVITIES (LAST 12 MONTHS)

	How many months?	Prac/Wk	Hrs/Prac	Comp/Wk
Athletics (running)				
Athletics (other)				
Cricket				
Swimming				
Tennis				
Hockey				
Netball				
Rugby				
Soccer				
Badminton				
Basketball				
Ballet				
Cycling				
Dancing				
Gymnastics				
Judo / karate				
Squash				
Volleyball				
Other				
Musical instrument				

SCHOOL REPORT	**Collected:** YES	NO	**School type:** PRIMARY	HIGH

Name of school:	
School address (NB - Suburb)	
Present Grade:	
Year of the report:	

FRIENDS

1. How many close friends do you have who are boys? ☐
2. How many close friends do you have who are girls? ☐
3. Are **most** of these close friends **(Select one only)**
   a. In your grade
   b. In a higher grade
   c. In a lower grade
   d. Not in school
   e. Don't have any close friends ☐
4. How often do you feel lonely and wish you had more friends? (**Select one only**)
   a. Often
   b. Sometimes
   c. Hardly ever ☐

SCHOOL RATING

1. How would you rate your school in general?

Excellent	Good	Ok	Not too good	Poor

2. How would you rate your own performance at school in general?

Excellent	Good	Ok	Not too good	Poor

PARENT WHEREABOUTS

1. Are you living with both your parents? No 0 Yes 1

If No,

Do you live with your mother No 0 Yes 1

If not living with mother,

Since what age in years have you not lived with your mother?

☐

Do you see your mother? No 0 Yes 1

If Yes, how often

Never	See her very seldom	More than once a year	More than once a month	More than once a week
0	1	2	3	4

Do you live with your father No 0 Yes 1

If not living with father,
Since what age in years have you not lived with your father?

Do you see your father? No 0 Yes 1

If Yes, how often

Never	See him very seldom	More than once a year	More than once a month	More than once a week
0	1	2	3	4

BULLYING

We say someone is being bullied when another pupil, or a group of pupils, says or does nasty and unpleasant things to him or her. It is also bullying when a pupil is teased repeatedly in a way he or she doesn't like. Common forms of bullying are name calling, taking things from a person, hurting a person. But it is not bullying when two pupils of about the same strength or status quarrel or fight.

1. How frequently have you been bullied at school in the **past three months**?

Many times a week	
About once a week	
Seldom	
Not at all	

2. How frequently have you bullied other pupils in the **past three months** either on your own, or with other peers ?

Many times a week	
About once a week	
Seldom	
Not at all	

ADOLESCENTS' SATISFACTION WITH THEIR RELATIONSHIP WITH THEIR MOTHER & FATHER OR CAREGIVER

Mother (if child has a relationship with biological mother) I am satisfied with...	**Strongly Disagree**	**Disagree**	**Neither Nor**	**Agree**	**Strongly Agree**
The way my mother and I Communicate with each other					
The love and affection my Mother shows me					
The emotional support my Mother gives me					
How many things my mother And I have in common					
The household responsibilities my Mother gives me					
The way my mother disciplines Me					
The amount of time my mother And I spend together					
The way my mother and I Resolve conflicts					
The respect my mother shows Me					
The fun my mother and I have Together					
My relationship with my mother					

Father (if child has a relationship with father. Not necessarily biological) I am satisfied with...	**Strongly Disagree**	**Disagree**	**Neither Nor**	**Agree**	**Strongly Agree**
The way my father and I Communicate with each other					
The love and affection my father shows me					
The emotional support my father gives me					
How many things my father And I have in common					
The household responsibilities my father gives me					
The way my father disciplines					

Me					
The amount of time my father And I spend together					
The way my father and I Resolve conflicts					
The respect my father shows Me					
The fun my father and I have Together					
My relationship with my father					

Caregiver (to be completed if Mother section not done) I am satisfied with...	**Strongly Disagree**	**Disagree**	**Neither Nor**	**Agree**	**Strongly Agree**
The way my caregiver and I Communicate with each other					
The love and affection my Caregiver shows me					
The emotional support my Caregiver gives me					
How many things my caregiver And I have in common					
The household responsibilities my caregiver gives me					
The way my caregiver disciplines Me					
The amount of time my caregiver And I spend together					
The way my caregiver and I Resolve conflicts					
The respect my caregiver shows Me					
The fun my caregiver and I have Together					
My relationship with my caregiver					

GENDER

How much would you agree or disagree with the following statements?

	Strongly Agree	Agree	Neither Nor	Disagree	Strongly Disagree	Do not know
Educating girls to a high level is of no use						
Girls should be educated so that they can operate on equal terms with boys in the modern world						
A job is alright, but what most woman really want is a home and children						
Being a housewife is just as fulfilling as working for pay						
Having a job is the best way for a woman to be an independent person						
Both the man and woman should contribute to the household income						
A woman's job is to look after the home and family rather than go out to work						
A man who is not bringing money into the household is a loser						

MORAL ISSUES

Answer the following questions	Not wrong at all	Only wrong sometimes	Almost always wrong	Always wrong	Do not know
Do you think it is wrong or not wrong if a man and a woman have sexual relations before marriage?					
Do you think it is wrong or not wrong for two adults of the same gender to have sexual relations?					
Do you think it is wrong or not wrong for a woman to have an abortion?					

NATIONAL IDENTITY

There are various ways in which you could describe yourself to another person: you could describe yourself in terms of your age, your gender (i.e. as a boy or a girl), in terms of being South African, being Zulu/English/Sotho/Afrikaans/Xhosa and being Black/White. But if you could choose only *one* of these five descriptions to describe yourself because it was *the most important to you*, which *one* would you choose? (Please rate the following from 1 to 5; 1 being the most important).

Age	
Gender	
South African	
Zulu/English/Sotho/Afrikaans/Xhosa/Shangaan etc	
Black/White etc	

Which one of these do you think best describes how you feel about yourself as a South African? (**Tick one only**)

Very South African	
Quite South African	
Little bit South African	
Not at all South African	
Don't know	

What in your view is the name of a song or a piece of music that is very, very South African?

What in your view is the name of a sport that is very, very South African?

What in your view is the name of a drink that is very, very South African?

What in your view is the name of a food that is very, very South African?

What in your view is the name of a place or a building that is very, very South African?

What in your view is the name of a person from history that was very, very South African?

What in your view is the name of something that happened in history that was very, very South African?

What in your view is the name of someone who is alive today who is very, very South African?

To what extent do you feel Attached to the following Types of people?	**Very attached**	**Slightly attached**	**Not very attached**	**Not at all**	**Do not know**
Those who speak the same language as you?					
Those who belong to the same race group as you?					
Those who belong to the same religious group as you?					
Those who go to the same school as you?					

Which, if any, group would you least want to come and live in South Africa? (Choose one group only)

Africans from other African countries	
Europeans	
Americans	
Indians from India	
Other Asians	
Australians	
Returning South Africans	
Other (specify)	
None (welcome all groups)	

We would like to know about how you see things in South Africa today. Please listen to each statement carefully and show how well it reflects your situation or feelings by marking the relevant column depending on whether you agree or disagree.

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
My family is having more money troubles now than in the past few years					
I worry that members of my family who are now employed may lose their jobs in the next year					
It is harder to find housing that my family can afford these days					
Pupils of different races get along well in my school					
We have more people of different "races" living in my neighbourhood now than two years ago					
My family and I are likely to leave South Africa because we do not like the way government runs the country					
Things in South Africa will improve under this government					
Other race groups have more advantages than my race group					
I think there is less violence in South Africa now than there was two years ago					
I think there is more crime now than there was two years ago					
South Africans are a free people and have many human rights					
The standard of education in schools is dropping in South Africa					
People are generally happy with life in South African today					

YOUTH SELF-REPORT FOR AGES 11-18 YEARS

	Not True	Sometimes True	True	Very True
1. I act too young for my age	0	1	2	3
2. I have an allergy	0	1	2	3
If YES, please describe:				
3. I argue a lot	0	1	2	3
4. I have asthma	0	1	2	3
5. I act like the opposite sex	0	1	2	3
6. I like animals	0	1	2	3
7. I brag (or show off)	0	1	2	3
8. I have trouble concentrating	0	1	2	3
9. I can't get my mind off certain thoughts	0	1	2	3
If YES, please describe:				
10. I have trouble sitting still	0	1	2	3
11. I'm too dependent on adults	0	1	2	3
12. I feel lonely	0	1	2	3
13. I feel confused or in a fog	0	1	2	3
14. I cry a lot	0	1	2	3
15. I am pretty honest	0	1	2	3

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

	Not True	Sometimes True	True	Very True
16. I am mean to others	0	1	2	3
17. I daydream a lot	0	1	2	3
18. I deliberately try to hurt or kill myself	0	1	2	3
19. I try to get a lot of attention	0	1	2	3
20. I destroy my own things	0	1	2	3
21. I destroy things belonging to others	0	1	2	3
22. I disobey my parents	0	1	2	3
23. I disobey at school	0	1	2	3
24. I don't eat as well as I should	0	1	2	3
25. I don't get along with other kids	0	1	2	3
26. I don't feel guilty after doing something I shouldn't	0	1	2	3
27. I am jealous of others	0	1	2	3
28. I am willing to help others when they need help	0	1	2	3
29. I am afraid of certain animals, situations or places other than school	0	1	2	3
If YES, please describe:				
30. I am afraid of going to school	0	1	2	3
31. I am afraid I might think or do something bad	0	1	2	3
32. I feel I have to be perfect	0	1	2	3
33. I feel that no one loves me	0	1	2	3

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

	Not True	Sometimes True	True	Very True
34. I feel that others are out to get me	0	1	2	3
35. I feel worthless or inferior	0	1	2	3
36. I accidentally get hurt a lot	0	1	2	3
37. I get in many fights	0	1	2	3
38. I get teased a lot	0	1	2	3
39. I hang around with kids who get into trouble	0	1	2	3
40. I hear sounds of voices that other people think aren't there	0	1	2	3
If YES, please describe:				
41. I act without stopping to think	0	1	2	3
42. I like to be alone	0	1	2	3
43. I lie or cheat	0	1	2	3
44. I bite my fingernails	0	1	2	3
45. I am nervous or tense	0	1	2	3
46. Parts of my body twitch or make nervous movements	0	1	2	3
If YES, please describe:				
47. I have nightmares	0	1	2	3

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

	Not True	Sometimes True	True	Very True
48. I am not liked by other kids	0	1	2	3
49. I can do certain things better than most kids	0	1	2	3
50. I am too fearful or anxious	0	1	2	3
52. I feel dizzy	0	1	2	3
53. I eat too much	0	1	2	3
54. I am overtired	0	1	2	3
55. I am overweight	0	1	2	3
56. I have physical problems without known medical cause:	0	1	2	3
Aches or pains	0	1	2	3
Headaches	0	1	2	3
Nausea, feel sick	0	1	2	3
Problems with eyes	0	1	2	3
If TRUE, please describe:				
Rashes or other skin problems	0	1	2	3
Stomach aches or cramps	0	1	2	3
Vomiting, throwing up	0	1	2	3
Other	0	1	2	3
If TRUE, please describe:				

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

	Not True	Sometimes True	True	Very True
57. I physically attack people	0	1	2	3
58. I pick my skin or other parts of my body	0	1	2	3
If TRUE, please describe:				
59. I can be pretty friendly	0	1	2	3
60. I like to try new things	0	1	2	3
61. My school work is poor	0	1	2	3
62. I am poorly coordinated or clumsy	0	1	2	3
63. I would rather be with older kids than kids my own age	0	1	2	3
64. I would rather be with younger kids than kids my own age	0	1	2	3
65. I refuse to talk	0	1	2	3
66. I repeat certain actions over and over	0	1	2	3
If TRUE, please describe:				
67. I run away from home	0	1	2	3
68. I scream a lot	0	1	2	3
69. I am secretive or keep things to myself	0	1	2	3
70. I see things that other people think aren't there	0	1	2	3
If TRUE, please describe:				

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

	Not True	Sometimes True	True	Very True
71. I am self-conscious or easily embarrassed	0	1	2	3
72. I set fires	0	1	2	3
73. I can work well with my hands	0	1	2	3
74. I show off or clown	0	1	2	3
75. I am shy	0	1	2	3
76. I sleep less than most kids	0	1	2	3
77. I sleep more than most kids during day and/or night	0	1	2	3
If TRUE, please describe:				
78. I have a good imagination	0	1	2	3
79. I have a speech problem	0	1	2	3
If TRUE, please describe:				
80. I stand up for my rights	0	1	2	3
81. I steal things at home	0	1	2	3
82. I steal things from places other than home	0	1	2	3
83. I store up things I don't need (describe)	0	1	2	3
If TRUE, please describe:				

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

	Not True	Sometimes True	True	Very True
84. I do things other people think are strange	0	1	2	3
If TRUE, please describe:				
85. I have thoughts that other people think are strange	0	1	2	3
If TRUE, please describe:				
86. I am stubborn	0	1	2	3
87. My moods or feelings change suddenly	0	1	2	3
88. I enjoy being with other people	0	1	2	3
89. I am suspicious	0	1	2	3
90. I swear or use dirty language	0	1	2	3
91. I think about killing myself	0	1	2	3
92. I like to make others laugh	0	1	2	3
93. I talk too much	0	1	2	3
94. I tease others a lot	0	1	2	3
95. I have a hot temper	0	1	2	3
96. I think about sex too much	0	1	2	3
97. I threaten to hurt people	0	1	2	3
98. I like to help others	0	1	2	3
99. I am too concerned about being neat or clean	0	1	2	3

YOUTH SELF-REPORT FOR AGES 11-18 YEARS
Continued

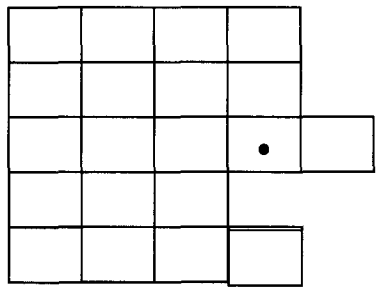
	Not True	Sometimes True	True	Very True
100. I have trouble sleeping	0	1	2	3
If TRUE, please describe:				
101. I cut / bunk classes or skip school	0	1	2	3
102. I don't have much energy	0	1	2	3
103. I am unhappy, sad or depressed	0	1	2	3
104. I am louder than other kids	0	1	2	3
105. I use alcohol or drugs for non-medical purposes	0	1	2	3
If TRUE, please describe:				
106. I try to be fair to others	0	1	2	3
107. I enjoy a good joke	0	1	2	3
108. I like to take life easy	0	1	2	3
109. I try to help other people when I can	0	1	2	3
110. I wish I were of the opposite sex	0	1	2	3
111. I keep from getting involved with others	0	1	2	3
112. I worry a lot	0	1	2	3

Interviewer:

ADOLESCENT MEASUREMENTS

SECTION A:

- STANDING HEIGHT: (mm)
- SITTING HEIGHT: (mm)
- WEIGHT: (kg)
- WAIST CIRCUMFERENCE: (mm)
- HIP CIRCUMFERENCE: (mm)





SECTION B: **DXA SCANS COMPLETED**

(Whole body, Hip, Spine, Radius)



SECTION C: COLLECTION OF SPECIMENS

- ULE URINE TEST

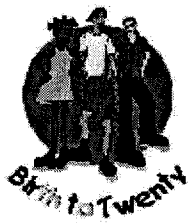
Y	*N*

SECTION D: PUBERTAL ASSESSMENT

- Pubertal assessment Questionnaire

Y	*N*

Appendix 7: Birth to Twenty Bara site: 15th year adolescent questionnaire



University of the Witwatersrand
Department of Paediatrics and Child Health

BIRTH TO TWENTY MEDICAL SCHOOL SITE: 15TH YEAR ADOLESCENT QUESTIONNAIRE

DATE : Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER : ☐☐☐☐☐☐☐

BONE STUDY ID NUMBER : ☐☐☐

There are 10 sections to this questionnaire that we are going to work through together, it will take about 35 minutes

The FIRST section of the questionnaire we are going to discuss is about your FAMILY

How many brothers and sisters do you have (children who have either one or both the biological mother and father as you) ☐

	Name	Age	Gender	Highest education level or current grade	Where do they live? (Suburb, City, Province, Country)
1					
2					
3					
4					
5					
6					



University of the Witwatersrand
Department of Paediatrics and Child Health

BIRTH TO TWENTY MEDICAL SCHOOL SITE: 15TH YEAR ADOLESCENT QUESTIONNAIRE

DATE : Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

BTT ID NUMBER : ☐☐☐☐☐☐☐

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How many brothers and sisters do you have (children who have either one or both the biological mother and father as you) ☐

	Name	Age	Gender	Highest education level or current grade	Where do they live? (Suburb, City, Province, Country)
1					
2					
3					
4					
5					
6					

Now we are going to talk about your PARENTS

Are you living with BOTH your parents?	NO	YES

If YES, skip the following question sand go to the section (Family bonging)

If NO, do you live with your MOTHER?				NO	YES
If NOT living with MOTHER, do you see your MOTHER?				NO	YES
If YES, how often?	See her very seldom	More than once a year	More than once a month		More than once a week

If NO, do you live with your FATHER?				NO	YES
If NOT living with FATHER, do you see your FATHER?				NO	YES
If YES, how often?	See him very seldom	More than once a year	More than once a month		More than once a week

Let us talk about your feelings towards your FAMILY

How strongly do you feel about each of the following sentences? A "YES!" is checked if the statement is very true, "yes" if it is somewhat true, "no" if it is somewhat false, and "NO!" if it is very false.

	YES!	**Yes**	**no**	**NO!**
I can tell my parents/caregivers the way I feel about things				
My family expects too much from me				
Sometimes I am ashamed of my parents/caregivers				
My family has let me down				
I like to do things with my family				
I enjoy talking with my family				

Who do you regard as your MAIN caregiver?

Answer the following questions with regard to your MAIN Caregiver.

	Not like him/her	**Somewhat like him/her**	**A lot like him or her**
Supports and encourages me			
Gives me attention and listens to me			
Shows me affection			
Praises me			
Comforts me			
Respects my sense of freedom			
Understands me			
Trusts me			
Gives me advice and guidance			
Provides for my necessities			
Gives me money			
Buys me things			
Has open communication with me			
Spends time with me			
Supports me in my school work			

The SECOND section of the questionnaire we are going to chat about your thoughts around your APPEARANCE

1. Have you tried to **lose weight** during the past year?

Yes	No

2. If YES, what was the **most important** reason (**mark only one**)

It is healthy	
I want to look better	
My clothes were too tight	
I am too fat compared to my friends	
I am unhappy with myself	
I dream of being a model or movie/TV star	
Any other reason, specify	

3. If you did try to **lose weight**, describe all the methods you have tried. Include any information on diet, exercise, pills or anything else that you have tried.

1. 2. 3. 4. 5.

4. Did you try to **build more muscles** or grow bigger during the past year?

Yes	No

5. If YES, what was the most important reason (mark only one)?

It is healthy	
I want to look better	
Compared to my friends I have too little muscle	
I am unhappy with myself	
I dream of being a model or movie/TV star	
Any other reason, specify	

6. If you did **try to build more muscles**, describe all the methods you have tried. Include any information on diet, exercise, pills or anything else that you have tried.

1. 2. 3. 4. 5.

Now I am going to ask you some questions about the way you feel about your body

	Never	Seldom	Sometimes	Often	Always
1. I like what I look like in pictures	0	1	2	3	4
2. Other people consider me good looking	0	1	2	3	4
3. I'm proud of my body	0	1	2	3	4
4. I'm preoccupied with trying to change my body weight	0	1	2	3	4
5. I like what I see when I look in the mirror	0	1	2	3	4
6. There are lots of things I'd like to change about my looks if I could	0	1	2	3	4
7. I am satisfied with my weight	0	1	2	3	4
8. I wish I looked better	0	1	2	3	4
9. I really like what I weigh	0	1	2	3	4
10. I wish I looked like someone else	0	1	2	3	4
11. People my own age like my looks	0	1	2	3	4
12. My looks upset me	0	1	2	3	4
13. I'm as nice looking as most people	0	1	2	3	4
14. I'm pretty happy about the way I look	0	1	2	3	4
15. I feel I weigh the right amount for my height	0	1	2	3	4
16. I feel ashamed of how I look	0	1	2	3	4
17. Weighing myself depresses me	0	1	2	3	4
18. My weight makes me unhappy	0	1	2	3	4
19. I worry about the way I look	0	1	2	3	4
20. I think I have a good body	0	1	2	3	4
21. I'm looking as nice as I'd like to	0	1	2	3	4

The THIRD section of the questionnaire is about your ACTIVITIES and CLUBS

How often during this year (last 12 months) did you participate in the following activities, or did you do the following things?

	Never	**A few times a year**	**Once or twice a month**	**Once a week**	**Daily or almost daily**
Supported a school sports team by attending their matches/games					
Participated in a school society or club					
Worked in a school garden or community garden					
Collected money or goods for your school, your church or a charitable organisation					
Participated/sang in a choir					
Been a member of a civic or community organisation					
Been a member of a dance or music group					
Read the newspaper or watched TV news					
Helped a friend with homework or some other project					
Gave money/food to someone who was poor or hungry					
Attended a church service					
Participated in a church activity other than a religious service					
Read the bible or another religious book					
Volunteered (offered) to help out around the house					

The FOURTH section of the questionnaire is about your SCHOOL HISTORY

Year	Grade	School	Notes
2006			
2005			
2004			
2003			
2002			
2001			
2000			
1999			
1998			
1997			
1996			
1995			
Preschool (formal; Grade 0)			
Preschool (informal)			
Day care (Informal)			

I am going to ask you questions around your move from PRIMARY (grade 7) to HIGH SCHOOL (grade 8 onwards)

Compared to your last year at primary school... (grade 7)	**Less**	**Same**	**More**
How much further (traveling distance) is your high school from home?			
How many pupils are in your high school?			
How many pupils are in your high school class?			
How difficult is the work at high school?			
To what extent are you coping with the work at High School?			
How much homework do you get at high school?			
How many people do you know at high school?			
Do you have more close friends at high school?			
Are you lonely at high school?			
Are there more rules at high school?			
Is it more difficult to get to know your teachers at high school?			
Are your teachers at high school more supportive			

The FIFTH section of the questionnaire is about your RELIGIOUS BELIEFS

Do you belong to any religious group?	NO	YES

If YES, which one?

ZCC ☐ Catholic ☐ Other Christian ☐ Hindu ☐

Muslim ☐ African traditional (Shembe) ☐

Other ☐

How often do you attend religious services?	**Never**	**Sometimes**	**Every week**

How important is religion in your life?	**Not important**	**Important**	**Very important**

How true are the following statements about your religious beliefs?	**Not true at all**	**Neutral (neither true nor false)**	**Very true**
My religious beliefs makes it important for me to help others			
My religious beliefs make me responsible for promoting fairness and justice			
My religious beliefs are similar to my parents			
I attend religious services/activities only because my parents expect this of me			
I feel that I am spiritual religious but I do not follow any organised religion			

The SIXTH section of the questionnaire we are going to talk about VIOLENCE in your community and school

How often do the following apply to you (not on TV or in movies)	**Never**	**Once or twice**	**A few times**	**Many times**
I have heard gun shots				
I have seen somebody arrested				
I have seen drug deals				
I have seen someone being beaten up				
My house has been broken into				
I have seen somebody get stabbed				
I have seen somebody get shot				
I have seen a gun in my home				
I have seen alcohol such as beer, wine, or hard liquor in my home				
I have seen gangs in my neighbourhood				
I have seen somebody pull a gun on another person				
I have seen someone in my home get shot or stabbed				

At school, how often have you been:	**Never**	**Once or twice**	**A few times**	**Many times**
Hit by a student				
Hit by school staff				
Kicked or pushed by a student				
Kicked or pushed by school staff				
Badly beaten up				
Threatened with a knife or sharp weapon				
Attacked with a knife or sharp weapon				
Threatened with a gun				
Verbally or emotionally abused by a student, that is, being called names or having things said to you that make you feel bad about yourself or afraid				
Verbally or emotionally abused by school staff				
Sexually harassed by a student (unwelcome advances which continue after saying no)				
Sexually harassed by school staff				
Sexually assaulted (attacked)				
Robbed				
In your neighbourhood, how often have you been:	**Never**	**Once or twice**	**A few times**	**Many times**
Hit				
Kicked				
Pushed or shoved				
Badly beaten up				
Threatened with a knife or sharp weapon				
Attacked with a knife or sharp weapon				
Threatened with a gun				
Verbally or emotionally abused, that is, being called names or having things said to you that make you feel bad about yourself or afraid				
Shot at				
Sexually harassed				
Sexually assaulted (attacked)				
Robbed				

At home, in the past, how often have you been:	**Never**	**Once or twice**	**A few times**	**Many times**
Hit				
Kicked				
Pushed or shoved				
Badly beaten up				
Threatened with a knife or sharp weapon				
Attacked with a knife or sharp weapon				
Threatened with a gun				
Verbally or emotionally abused, that is, being called names or having things said to you that make you feel bad about yourself or afraid				
Shot at				
Sexually harassed				
Sexually assaulted (attacked)				
Robbed				

At school, how often have **YOU** done these things:	**Never**	**Once or twice**	**A few times**	**Many times**
Hit or kicked someone				
Pushed or shoved someone when you were angry				
Badly beaten someone up				
Threatened someone with a knife or sharp weapon				
Attacked someone with a knife or sharp weapon				
Threatened someone with a gun				
Verbally or emotionally abused someone, that is, being called names or having things said to you that make you feel bad about yourself or afraid				
Sexually harassed someone				
Robbed someone				
Been suspended from school				
Gotten into a fight after drinking or getting high				

Outside of school, how often have **YOU** done these things:	**Never**	**Once or twice**	**A few times**	**Many times**
Hit or kicked someone				
Pushed or shoved someone when you were angry				
Badly beaten someone up				
Threatened someone with a knife or sharp weapon				
Attacked someone with a knife or sharp weapon				
Threatened someone with a gun				
Verbally or emotionally abused someone, that is, being called names or having things said to you that make you feel bad about yourself or afraid				
Sexually harassed someone				
Robbed someone				
Gotten into a fight after drinking or getting high				

The SEVENTH section of the questionnaire is about RELATIONSHIPS

Are you dating someone now (involved, steady boyfriend/girlfriend)?

YES	**NO**
Answer the following questions	Skip this section

Is this a serious relationship? YES or NO	
How long have the two of you been going together?	
Are you dating someone of the SAME or OPPOSITE sex?	
Do you feel this is the real love of your life, or do you think you are still to meet the one?	
Have your parents/caregiver met this person? YES or NO	
If YES, do they like the person? YES or NO	

The EIGHTH section of the questionnaire is about what you think of people that have HIV/AIDS

How strongly do you agree with the following statements?

Read each statement	**Strongly agree**	**Agree**	**Neutral**	**Disagree**	**Strongly disagree**	**Don't know**
I will sleep alongside someone who has HIV/AIDS						
I will share a meal with someone who is HIV positive						
I will talk to someone who has HIV/AIDS						
I will treat a family member with AIDS, well						
I will not get infected by being in the same room as an infected person						
Health workers should not write on a death certificate that a person died of HIV/AIDS related illness						

The LAST section of the questionnaire we are going to play the "ASPIRATIONS" game

Now suppose you were elected to be President of South Africa and could develop policies to solve social problems, but only had funds to tackle five problems. Which **FIVE** government issues would **YOU** support? (Please rate those five issues from 1 – 5).

Reduce the spread of AIDS	
Decrease homelessness in Johannesburg-Soweto	
Increase the availability of jobs	
Reduce the destruction of the environment and increasing pollution	
Decrease the extent of illegal alcohol use by young people	
Reduce the number of teen pregnancies	
Combat international terrorism	
Reduce the amount of racism and prejudice still in this country	
Improve the poor quality of schools	
Decrease crime	
Ban pornography	
Affirmative actions programmes	
Improve the availability of good quality health care	
Increase taxes on wealthy people	
Increase availability of condoms	
Increase availability of abortions	
Bring back the death penalty	
Sex education in schools	
Increase the minimum wage for workers	

EATING HABITS AND PRACTICES OF ADOLESCENTS

SECTION A: Breakfast habits

Think about a **usual school week and weekend** and try to answer the following questions about your eating habits as truthfully as possible. There are no right or/ wrong answers so please feel free to give your answer.

1. On how many weekdays do you usually eat breakfast? **Mark one only**

Never	1	
1-2 days	2	
3-4 days	3	
Every weekday (5)	4	☐

2. How often do you usually eat breakfast on a weekend? **Mark one only**

Never	1	
Saturdays only	2	
Sundays only	3	
Saturdays and Sundays	4	☐

3.1 What best describes the way you usually eat during the week? **Mark one only**

3 or more meals a day	1	
2 meals a day	2	
1 meal a day	3	☐

3.2 What best describes the way you usually eat over a weekend? **Mark one only**

3 or more meals a day	1	
2 meals a day	2	
1 meal a day	3	☐

4. How many times do you eat snacks in a day? **Mark one only**

Just once a day	1	
Twice a day	2	
3 or more times a day	3	
Never	4	☐

SECTION B: Fastfoods

1. How often during the **past week** (past 7 days) did you eat any of the following takeaways? **Tick each item**

	0 x last week	1x last week	2x last week	3x last week	4x last week	5+ last week
Hamburger						
Chicken Burger						
Fried fish						
Fried chips						
Pizza						
Vetkoek						
Pies or sausage roll						
Samoosas						
Pita bread						
Hotdog						
Boerewors roll						
Doughnuts						
Sweets						
Cake						
Chocolates						
Chips e.g. nik naks						
Ice cream						
Soft drinks e.g. Coke						
Squash e.g. *Drink-o-pop/Oros*						
Diet drinks						
Other:						

2. How often do you usually eat at a friend's house? (In a week) Tick where applicable.

0 x per week	1x per week	2x per week	3x per week	4x per week	5+ x per week

SECTION C: School lunch box

Think about a typical school week and answer the following questions about your lunch box that you take to school.

1. How often do you generally take a lunch box to school? **Mark one only**

0 x per week	1x per week	2x per week	3x per week	4x per week	5 per week

2. Do you share or exchange what you have in your lunch box with friends?

Yes	No
1	2

3. Which foods do you often have in your lunch box? **Tick each item**

	0 x per week	Less than 2x per week	More than 2x per week
White bread or rolls			
Brown bread or rolls			
Fruit			
Chips			
Pap			
Meat or chicken			
Pie / sausage roll			
Cold drink			
Diet cold drinks			
Fruit juice			
Milk or sour milk			
Yoghurt			
Cheese			
Sweets or chocolates			
Biscuits or cookies			
Peanuts			
Other:			

4. Who prepares your school lunch box (yourself, mother, father etc)

_____

5. Do you get money to spend on food / snacks at school? **Mark one only**

Yes	No	Sometimes
1	2	3

6. How much money do you usually get to spend at school per week on food? **Mark one only**

R1 – R5	1
R6 – R10	2
R11 - R15	3
More than R15	4

7. Which of the following foods did you buy at school (tuck shop)? **Tick each item**

	Did not buy	Bought 1 time	Bought 2 times	Bought 3 times	Bought 4 times	Bought 5 times or more
White bread or rolls						
Brown bread or rolls						
Fresh fruit						
Chips						
Pap and Meat or chicken						
Fried chips						
Pie/sausage roll/samoosa						
Vetkoek						
Cold drink						
Diet cold drinks						
Fruit juice						
Milk or sour milk						
Yoghurt						
Cheese						
Sweets or chocolates						
Cakes/ donuts/ éclairs						
Popcorn						
Peanuts/nuts						
Other:						

1. How often do you snack when you are watching TV? **Mark one only**

Every day	1
More than three days a week	2
Less than 3 days a week	3
Never	4

2. Which snacks did you eat while watching TV last week (past seven days)? And how often? **Tick each item**

	Didn't eat	1 time	2 times	3 times	4 times	5 or more times
Fruit						
Popcorn						
Chocolates						
Bread (any type)						
Crisps e.g. nik-naks						
Biscuits						
Cakes/ donuts/ éclairs						
Drinks e.g. Coke						
Fries						
Other:						

4. Do TV adverts on foods influence you to buy those food items? **Mark one only**

Never	1
Hardly ever	2
Often	3
Very often	4

5. Which food and drinks that you see advertised on TV do you buy?

1.)

2.)

3.)

6. Where do you usually eat your main meal of the day? **Mark one only**

Kitchen at a table/counter	1
Dining room at a table	2
In front of the TV off your lap	3
Other	4

7. How many times do you eat dinner/supper with your family/parents/caregivers?

Never	1
Some Days	2
Most Days	3
Every Day	4

8. How much does your mother/caregiver/father control what you eat?

1.	Not at all	2.	Sometimes	3.	Mostly	4.	Completely

SECTION D: Acculturation

1. What is you favourite "soap opera" on television and why?

2. How many times a week do you watch it?
   time/s a week

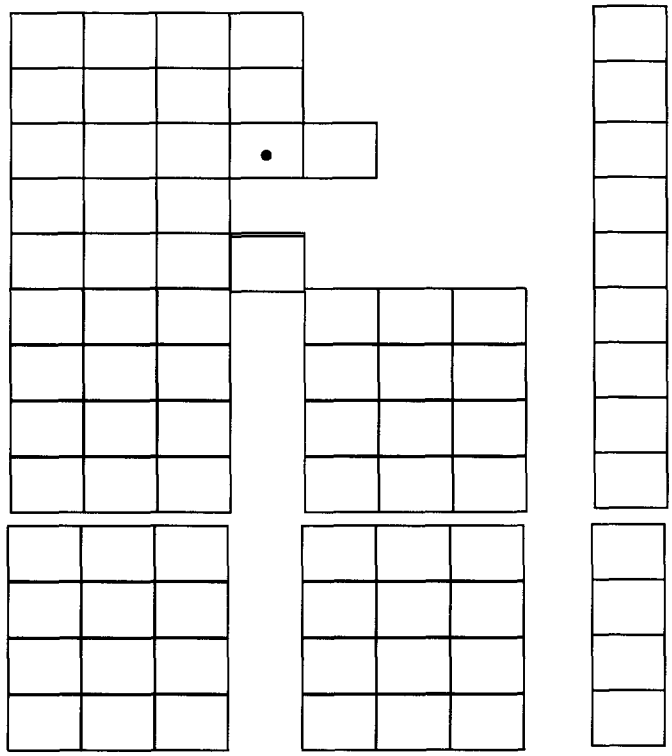
Research Assistant name: **Date:**

Quality checked by: **Date:**

ADOLESCENT MEASUREMENTS

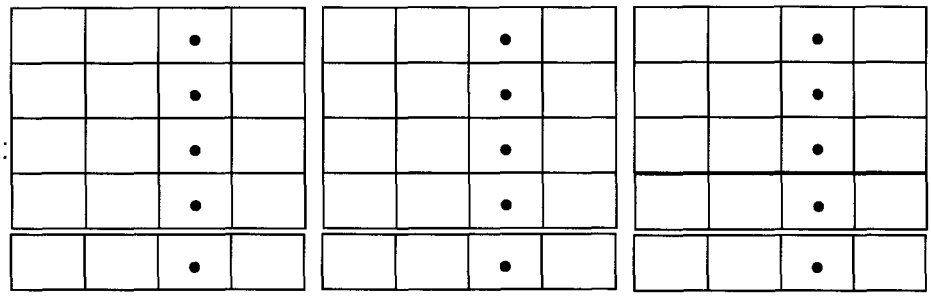
ANTHROPOMETRY

- STANDING HEIGHT: (mm)
- SITTING HEIGHT: (mm)
- WEIGHT: (kg)
- WAIST CIRCUMFERENCE: (mm)
- HIP CIRCUMFERENCE: (mm)
- SHOULDER-ELBOW: (mm)
- ELBOW-WRIST LENGTH: (mm)
- THIGH LENGTH: (mm)
- CALF LENGTH: (mm)
- BICEP GIRTH: (mm)
- BIEPI BREADTH HUMERUS (mm)
- BIEPI BREADTH OF FEMUR (mm)
- CALF GIRTH: (mm)



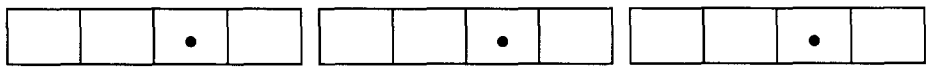
SKINFOLD MEASUREMENTS – LEFT SIDE (mm)

- TRICEP:
- BICEP:
- SUBSCAPULAR:
- SUPRA-ILIAC:
- MEDIAL CALF



FLEXIGRIP

- NON-DOMINANT HAND: L OR R



Research Assistant name: **Date:**

BLOOD PRESSURE

- SYSTOLIC BP
- DIASTOLIC BP
- PULSE
- TIME OF BP

h

Research Assistant name: Date:

BONE SCANS

- DXA scan
- PQCT

Y	N
Y	N

Operator name: Date:

COLLECTION OF SPECIMENS

- Urine 1 and Urine 2
- ULE URINE TEST
- ROUTINE BLOOD SAMPLE

Y	N
Y	N
Y	N

Lab Assistant's name: Date:

PUBERTAL ASSESSMENT

- Pubertal assessment Questionnaire

Y	N

Research Assistant name: Date:

SELF COMPLETION

- Self completion Questionnaire

Y	N

Research Assistant name: Date:

BONE AGE X-RAY

Y	N

NOTES