Prenatal exposure to DDT and other selected environmental contaminants and their predictors in malaria and non-malaria areas in coastal KwaZulu Natal, South Africa

July 2013

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A dissertation for the degree of Philosophiae Doctor

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SUMMARY

***Background**:* Prenatal exposure to environmental toxicants is of major concern, as foetal development is one of the most sensitive life stages for endogenous and exogenous insults, due to rapid cell division and apoptosis, morphogenesis, and cellular differentiation. Due to limited renal and biliary elimination, and the inability to metabolise toxicants *in utero,* prenatal exposure affects birth outcomes and early childhood development. Permeability of the placenta allows not only for environmental contaminants, but also contaminants released from maternal body stores during pregnancy, to be transferred to the foetus. At present, exposure to multiple chemicals amongst pregnant women has been studied, but more research is required on the subject.

Most of the exposure assessments in pregnant women and birth outcome studies have been performed in developed industrialised countries, mainly in the northern hemisphere. There is a paucity of such assessments from developing countries and countries that are in transition (UNEP, 2011). Populations of the southern hemisphere, where most of these developing countries are situated, may be more susceptible to toxic effects of pollutants, due to their compromised health and economic status, as well as changes in climatic conditions, including a rise in temperatures which may lead to an increase in the number of malaria mosquitoes and changes in their geographic distribution. The same trends may be observed for other insect vectors as well, with the final outcome being an increased use of 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT). It is predicted that coastal populations in the southern hemisphere will be most affected.

The reintroduction of controlled indoor residual spraying (IRS) of DDT for malaria control in 2001 in malaria endemic regions in South Africa (SA), permitted an opportunity to assess prenatal exposure and enhance the current understanding of DDT and other selected contaminants in malaria and non-malaria regions situated along the coast.

This study was initiated as a follow up to a pilot study which evaluated the extent of prenatal exposures to persistent toxic substances (PTS) and birth outcomes in selected geographical regions of SA. The regions under study consisted of three very distinctive rural study sites

situated along the western coast of the Indian Ocean, in the KwaZulu Natal province, namely, site 1 - a malaria endemic site; site 2 - a non-malaria site; and site 3 - an intermittent malaria site.

Results and discussion: In the malaria endemic site, high concentrations of DDT, in particular *p,p'*-1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene (*p,p'*-DDE) and *p,p'*-DDT, were found in the maternal plasma. These levels were significantly higher when compared with the other two sites (i.e. intermittent malaria and non-malaria). In addition, subjects in the malaria endemic site, were not only exposed to elevated levels of DDT, but also exposed to mercury (Hg), γ-Hexachlorocyclohexane (γ-HCH) and endosulfan, although to a lesser extent. In both the intermittent malaria and non-malaria sites, elevated levels of *p,p'*-DDE and *p,p'*-DDT were also found in maternal plasma. A *p,p'*-DDE/*p,p'*-DDT ratio of 5 and 4 was found in the intermittent and non-malaria sites, respectively, indicating recent exposure, and suggesting that food is not the only source of DDT exposure in these two areas. The reason for the elevated levels of DDT in these areas is not clear, considering the long residence time of participants in each site. Overall, maternal age and weight negatively influenced *p,p'*-DDE levels, whereas, having one's home sprayed by the malaria vector control personnel, using wood for cooking and consuming tinned fish significantly increased the *p,p'*-DDE levels.

The γ-HCH and endosulfan 1 and 2 were elevated in all three regions, however, significantly higher levels of endosulfan and γ-HCH were found in the intermittent malaria site, possibly due to the large commercial and subsistence farming activities in the area. The two compounds, endosulfan and γ-HCH correlated strongly with each other, indicating a similar source of exposure. For γ-HCH, drinking borehole water (positive), weight (positive), age (negative) and consumption of processed meat (negative) were strong predictors. Growing one's own food, self-reported poor air quality or exposure to environmental pollution around the home, were all positively associated with endosulfan levels. Consumption of processed meat and dairy products was a negative predictor of endosulfan levels. γ-HCH levels were much higher when compared with some other regions, such as Australia, Mexico and Poland. As γ-HCH and endosulfan are now listed as banned and persistent substances by the Stockholm Convention, efforts must be made to reduce sources of exposure in SA.

The other isomers of Hexachlorocyclohexane (HCH) (α, β- HCH), and the pyrethroid pesticides (*cis*-permethrin, cyfluthrin, cypermethrin and deltamethrin) were detected in less than a fifth of the samples, and Hexachlorobenzene (HCB) was not detectable in any of the samples. This study found low levels of β-HCH compared to those in Russia and Spain.

Very low maternal concentrations of the Polybrominated Diphenyl Ether (PBDE) isomers, 28, 49, 71, 47, 66, 77, 100, 119, 99, 85, 154, 153, 138, were observed across all three sites, although PBDEs have been reported in other studies in breast milk, leachates and catchment areas in South Africa.

Hg was detected in 100% of maternal and cord blood samples in the malaria endemic site, with significantly higher concentrations than the intermittent and non-malaria sites. There was a strong positive correlation ($r^2 = 0.66$) between maternal and cord blood Hg levels. For umbilical cord blood Hg concentrations, the following were strong predictors in the multivariate regression model: maternal blood Hg levels, living in the malaria endemic site, environmental pollution in the home and a household member being involved in fishing.

Conclusion: This thesis evaluated the extent of concomitant exposure to selected organic compounds and Hg *in utero*. Although, as expected, elevated levels of DDT from IRS were found in the malaria endemic site, substantial concentrations of Hg, γ-HCH and endosulfan were also found. In addition, DDT was also found in the intermittent and non-malaria sites, although to a lesser extent. The *p,p'*-DDE/*p,p'*-DDT ratios in the two sites indicate recent on-going use of DDT, possibly illegal use, since DDT use is only allowed in designated areas. Furthermore, high levels of the now banned γ-HCH and endosulfan were found in the intermittent malaria site, with lower levels in the malaria endemic site and non-malaria sites. In the intermittent malaria site, there is an indication of recent use of DDT, as well as significant exposure to γ-HCH and endosulfan. This study has confirmed that pregnant women in these study sites were exposed concurrently to a mixture of chemicals, many classified as endocrine disruptors, indicating the need for the implementation of policies that curtail the use of these chemicals.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

I was fortunate in having three very accomplished scientists as my supervisors; Professor Jon Ø Odland (University of Tromsø), Professor Halina Röllin (South African Medical Research Council and University Pretoria) and Associate Professor Torkjel Sandanger (Norwegian Institute for Air Research and University of Tromsø) who guided and motivated me. I would like to express my sincere gratitude to Professor Jon Ø Odland for his continuous support of my Ph.D study and research, for his patience, motivation, enthusiasm, immense knowledge and guidance. I am deeply grateful to my supervisor Professor Halina Röllin for her influence and scientific support throughout my work on this thesis. You have always been patient and encouraging in times of new ideas and difficulties; discussions with you frequently solved arising research problems and read my numerous revisions and helped to make some sense of the confusion. Above all, you made me feel like a friend, which I appreciate from my heart. The good advice and unsurpassed knowledge of Associate Professor Torkjel Sandanger have been invaluable, for which I am extremely grateful. I learned a great deal from you about analytical research, how to approach new problems and how to develop techniques to solve them. You and your colleagues from National Institute for Air Research (NILU) are highly appreciated.

In addition, I have been very privileged to have known and collaborated with many other exceptional people, who have become my friends over the last several years. To Therese Nøst, Charlotta Rylander and Linda Hanssen – thank you for your analytical input and help during my training and project working visits to NILU. During these visits I was introduced to snow and ice for the first time in my life and learned much about the Norwegian way of life. Your exchange visits to our laboratories in SA are also much appreciated. Thanks to Dr Tahira Kootbodien (SA MRC) and Kerry Wilson (SA NIOH) for helping me to acquire a new understanding of statistics. To Mirriam Mogotsi (SA MRC), thank you for your invaluable contribution to the training of the field work interviewers and overseeing the interview and sample collection processes. I am indebted to Claudina Nogueira (NIOH now Anglo American plc) for reviewing this manuscript, as well as all the publications. Thank you very much.

I would like to thank my colleagues from the SA NIOH; Ina Naik, Bronwyn Adendorff, Penny Theodorou and Halina Tassell for their kindness, friendship and support. In addition, my special thanks go to Penny Theodorou for the Hg analyses and Bronwyn Adendorff for her assistance with the graphics. Many thanks to the KwaZulu Natal Health Department and the hospital staff of the maternity sections. A very special thanks goes to the study participants, without whom the study would not have been possible.

The work for this thesis was funded by the Research Council of Norway and the National Research Foundation, SA (Grant 64528) under the bilateral research collaboration: the Arctic Monitoring and Assessment Programme (AMAP), the Royal Norwegian Ministry for Foreign Affairs, the University of Tromsø and the SA Medical Research Council. My dearest thanks to all.

Lastly, I would like to thank my family for all their love and encouragement: to my parents who raised me with a love of science and supported me in all my pursuits, for the encouragement from my sister Reeta, and my children, Hashil and Ashmeera. And most of all, to my loving, caring, encouraging, and patient husband Bhadresh, whose faithful support during my Ph.D., is so appreciated. I thank you all from the bottom of my heart.

LIST OF PAPERS

The thesis is based on the three papers listed below:

1. Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non-malaria coastal regions of South Africa.
   Kalavati Channa, Halina B. Röllin, Therese H. Nøst, Jon Ø. Odland, Torkjel M. Sandanger.
   Sci Total Environ. 2012; 429:183-190.

2. Regional variation in pesticide concentrations in plasma of delivering women residing in rural Indian Ocean Coastal regions of South Africa.
   Kalavati R. Channa, Halina B. Röllin, Kerry S. Wilson, Therese H. Nøst, Jon Ø. Odland, Inakshi Naik and Torkjel M. Sandanger.
   J. Environ. Monit. 2012; 14:2952-2960.

3. Differences in prenatal exposure to mercury in South African communities along the Indian Ocean.
   Kalavati Channa, Jon Ø. Odland , Tahira Kootbodien, Penny Theodorou, Inakshi Naik, Torkjel M. Sandanger, and Halina B. Röllin.
   Sci Total Environ. 2013; 463-464:11-19

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ABBREVIATIONS

ACT	Artemisinin-based Combination Therapy
AIDS	Auto-Immune Deficiency Syndrome
AMAP	Arctic Monitoring and Assessment Programme
ANOVA	Analysis of Variance
ASTDR	Agency for Toxic Substances and Disease Registry
Au	Gold
BD	Becton Dickinson & Company
BDE	Brominated Diphenyl Ethers
BHC	Benzene Hexachloride
BMI	Body Mass Index
CI	Confidence Interval
COP1	First Conference of the Parties
CUPs	Currently Used Pesticides
DDD	1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene
DDE (or *p,p'*-DDE)	1,1-*bis*-(4-chlorophenyl)-2,2-dichloroethene
DDT (or *p,p'*-DDT)	1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane
DE	Diphenyl Ethers
DOH	Department of Health
EDTA	Ethylene diamine tetra acetic acid
EI	Electron Ion
GC	Gas Chromatography
GFS	Graphite Furnace System
GM	Geometric means
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
Hg	Mercury
Hg^0	Elemental Mercury
HIV	Human immunodeficiency virus
HLB	Hydrophilic-lipophilic-balanced
ICP	Inductively Coupled Plasma

IPEP	International POPs Elimination Project
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Nets
KZN	KwaZulu-Natal Province
LOD	Limit of Detection
LUD	Lower Umfolozi District
MA	Massachusetts
MeHg	Methyl Mercury
Min	Minutes
MO	Missouri
MRC	Medical Research Council
MRM	Multiple reaction monitoring
MS	Mass Spectrometer
n	Number of Samples
NCI	Negative Chemical Ionization
NILU	Norwegian Institute for Air Research
NIOH	The National Institute for Occupational Health
OC(s)	Organochlorine(s)
OCP(s)	Organochlorine pesticide(s)
PAN	Pesticide Action Network
PBDE	Polybrominated Diphenyl Ether
PCB(s)	Polychlorinated Biphenyls
PCDD	Polychlorinated Dibenzo-p-Dioxins
PCDF	Polychlorinated Dibenzo-p-Furans
PFOS	Perfluorooctanesulfonic acid
POPs	Persistent Organic Pollutants
PTS	Persistent Toxic Substances
PTV	Pressure, Temperature, Volume
QA/QC	Quality Assurance and Quality Control
R	Pearson's / Spearman correlation coefficient
RBM	Roll Back Malaria

RSA	Republic of South Africa
SA	South Africa/South African
SD	Standard Deviation
Se	Selenium
SIM	Selected Ion Mode
SPE	Solid Phase Extraction
TB	Tuberculosis
TL	Total Lipids
Tl	Thallium
UNEP	United Nations Environment Programme
USA	United States of America
USEPA	United States Environmental Protection Agency
UT	University of Tromsø
WHO	World Health Organization

1. INTRODUCTION

Environmental contaminants are both naturally occurring substances, such as metals and elements, as well as man-made chemical substances, which includes both organic and metallic products that enter the environment either through weathering processes of natural deposits, or industrial and mining activities, but most frequently as a result of anthropogenic human activities. Most are persistent toxic substances (PTS) that are characterised by very slow degradation rates, their ability to bioaccumulate and biomagnify, and their dispersion into the environment (AMAP, 2004).

Of major concern are the health effects of PTS on humans, fauna and flora. Both toxic elements and persistent organic pollutants (POPs) pose a risk to the health of humans and wildlife (Rodriguez-Dozal et al., 2012). It has been shown that POPs, such as organochlorine pesticides (OCPs), polybrominated diphenyl ether (PBDEs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and furans (PCDDs and PCDFs), can be found in human blood, adipose tissue and breast milk (Doucet et al., 2009; Eskenazi et al., 2003; Guvenius et al., 2003; Hedgeman et al., 2009; Solomon and Weiss, 2002). Toxic metals and elements also accumulate in humans, in their specific target organs. For example, the main target organs for mercury (Hg) are the central nervous system (brain) (Park and Zheng, 2012), kidneys (Barbier et al., 2005; Park and Zheng, 2012) and lungs (USEPA-TTN, 2000).

Another major concern is the exposure of women of reproductive age to PTS, as it has been shown that toxic metals (including Hg), OCPs, PBDEs, PCBs, PCDDs and PCDFs accumulate in the maternal body and are transferred to the foetus via the placenta during pregnancy or to the infants via maternal milk. The pregnant women's body burden of contaminants is directly responsible for the potential health effects in the foetuses and infants (Todaka et al., 2010; Wang et al., 2009).

The time between conception and birth is perhaps one of the most vulnerable life stages. During this time, the environment may have tremendous immediate and lasting effects on foetal health. During pregnancy, the foetus undergoes rapid growth and organ development, and the

maternal environment may directly influence these processes, for better or for worse. In addition, timing of the prenatal exposure to specific contaminants during the pregnancy stage may influence the severity of the detrimental health effects. Early childhood is also a critical period for the continued development and maturation of several biological systems; hence infants are very susceptible to environmental exposures after birth (WECF, 2012).

Numerous studies concerned with the effects of exposure to PTS on reproductive health and birth outcomes have been performed in the northern hemisphere over the last two decades (AMAP, 2011). Regrettably, there is a paucity of similar research conducted in the southern hemisphere, including SA. Under the umbrella of the Arctic Monitoring and Assessment Programme (AMAP), SA was included as a participant from 2004 onwards. A pilot study involved collaboration between the South African Medical Research Council (SA MRC), the University of Tromsø (UT) and the Norwegian Institute for Air Research (NILU). The collaborative study investigated levels of PTS in selected areas of SA. The toxic elements in the blood of delivering women and paired umbilical cord blood samples, the essential elements in maternal serum, as well as selected POPs in maternal plasma, were measured (Hanssen et al., 2010; Röllin et al., 2009a; Röllin HB, 2008; Rylander et al., 2010). Since then, similar studies have been performed or are currently underway in other southern hemisphere regions such as Brazil, Argentina, Malawi, Tanzania and Australia (Rudge et al., 2009). From the pilot study results obtained in SA, it was clear that environmental contamination is region dependent, with evidence of elevated concentrations of some toxic metals, particularly Hg, but also high concentrations of selected POPs. DDT which is applied for malaria vector control, was found in higher concentrations than those found in the northern hemisphere, as expected (Röllin et al., 2009a).

As SA can be considered both a developed and a developing country, it is an ideal study site for the purpose of this project. This country has extensive mining, industrial and agricultural activities, both formal and informal. These activities release both toxic metals and organic pollutants in the living environment (SouthAfrica.Info, 2012a; SouthAfrica.Info, 2012b).

As far as agriculture is concerned, SA has a large sector favouring a highly diverse range of marine (fish farming) and agricultural products, from deciduous, citrus and subtropical fruit to

grain, wool, cut flowers, livestock and game, thereby increasing the use of pesticides. Each individual crop is susceptible to a unique host of pests that in turn require a unique mixture of pesticides. Currently, SA has more than 500 registered pesticides for use (Pesticide Action Network (PAN, 2010)), and is one of the largest importers of pesticides in sub-Saharan Africa (Osibanjo et al., 2003). In addition, pesticides are also used in the management of disease vectors such as malaria. In SA, malaria is currently managed by the use of DDT and pyrethroids via Indoor Residual Spraying (IRS), increasing the risk of exposure to the household members, in particular the most susceptible populations, such as pregnant women, young children, the aged and those in poor health. Past and present mining activities are constantly contributing to the environmental contamination and degradation. Uncontrolled urbanisation, increasing levels of unemployment, poor housing, deprived diet, and inadequate health due to the high rate of communicable diseases (such as HIV/Aids, TB and malaria), further increase the risk of detrimental health effects in the population.

This study was performed in the KwaZulu Natal (KZN) province of SA, which lies along the Indian Ocean coast, where agricultural activities are very prominent. The study was designed under the auspices of Norway-SA Bilateral Research Collaboration, with the SA MRC, UT and NILU being the main collaborators.

2. AIMS OF THE STUDY

To enhance the current understanding of the complex chemical exposures in pregnant women before delivery, in malaria (where DDT is used in IRS), intermittent and non-malaria areas situated along the KwaZulu Natal coast, the following primary objectives were identified:

- Assess the levels of DDT in pregnant women in malaria endemic and non-malarial areas of SA, due to the reintroduction of DDT use for vector management, via controlled IRS.
- Assess the levels of other pesticides that may be used for malaria control, in agriculture, or against household pests.
- Assess the levels of Hg due to industrial activities and possible artisanal mining in the region.
- Compare the levels of pesticides and Hg statistically, and derive an initial risk assessment and possible predictors in pregnant women.

3. BACKGROUND TO THE STUDY

Reproductive health is an area of priority research worldwide. This thesis attempts to quantify levels of selected PTS as an indication of prenatal exposures, by measuring these contaminants in maternal blood compartments of delivering women who reside along the Indian Ocean of the KZN Province in SA.

The regions under study consisted of three very distinctive rural study sites situated along the western coast of the Indian Ocean, namely, site 1 - a malaria endemic site; site 2 - a non-malaria site; and site 3 - an intermittent malaria site. The malaria endemic site was chosen because of the reintroduction of controlled IRS with DDT in 2001.

There is no other comprehensive study that has attempted to quantify the actual exposure to DDT and its metabolites resulting from IRS activities. In addition, other so called currently used pesticides (CUPs) such as some of the pyrethroid (*cis*-permethrin, cyfluthrin, cypermethrin, deltamethrin) pesticides were measured, as well as Hg, which is considered to be the most toxic metal to humans. The study also measured some other selected POPs, such as endosulfan, α-, β-, γ-HCH, HCB and brominated flame retardants. Most (DDT, α-, β-, γ–HCH, endosulfan, some PBDE isomers and HCB) of the contaminants measured are listed by the Stockholm Convention (UNEP, 2008b) as banned chemicals. Results obtained in the malaria endemic site were compared with the results from the other two sites (intermittent malaria and non-malaria area). The findings from this study will identify the extent of prenatal exposure to environmental contaminants in three regions along the coast. In addition, these regions are very rich in other water bodies such as lakes and rivers.

3.1. Malaria in Africa

In Africa, malaria is the biggest killer of children under five years of age, who account for nearly 86% of all malaria deaths. It is estimated that a child dies every 45 seconds from the disease. The effect of malaria on reproductive health is extreme, resulting in high maternal mortality rate, low birth weight and maternal anaemia (WHO, 2011). Moreover, many countries in Africa lack the infrastructure and resources necessary to treat and prevent malaria. As a result, very few countries have benefited from past efforts to eradicate malaria (WHO-RBM, 2010). In the southern part of Africa, malaria remains endemic in the north-eastern border regions of SA and in the adjacent countries of Mozambique, Swaziland and Zimbabwe, where 22 million, 0.5 million and 6 million people respectively, are at risk of contracting the disease (WHO, 2010).

SA has carried out intensive malaria control activities over many decades and has succeeded in halting transmission in most of the country. At present, approximately 10% (5 million) of the SA population is at risk of contracting malaria by residing in malaria risk areas (Rogan and Chen, 2005). IRS is the primary vector control measure used in SA (WHO, 2010).

In short, IRS involves the treatment of all interior walls and ceilings with insecticides at a prescribed dosage, and is particularly effective against mosquitoes, since many species rest on walls before or after feeding. The main aims of IRS are to reduce the life span and density of vector mosquitoes (WHO, 2006).

3.2. Concerns about DDT use in SA to eradicate malaria

Since the 1940s, the spread of malaria worldwide has been controlled by the use of organochlorines (OCs), mainly DDT [1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane], which has also been used extensively in agriculture. Although DDT has been banned in most countries for the last 30 years, due to its persistent nature, it can still be detected in the environment, not only at the point of origin, but also at remote locales (AMAP, 2004). The major detrimental health effects of exposure to DDT and its breakdown products include breast, liver and other cancers; male infertility; miscarriages and low birth weight; reduced immunity; developmental delay; and nervous system damage (Aneck-Hahn et al., 2007; Cohn et al., 2007; Crinnion, 2009; Hardell et al., 2004; Karmaus et al., 2001; Longnecker et al., 2001; McGlynn et al., 2006; McGlynn et al.,

2008; Narita et al., 2007; Porta et al., 2008a; Quaranta et al., 2006; Ribas-Fito et al., 2003; Sunyer et al., 2005; Vine et al., 2000; Younglai et al., 2002).

In SA, malaria is mostly confined to the low altitude (below 1000 metres above sea level), subtropical, northern border areas of the Limpopo Province, Mpumalanga Province and the north east of the KZN province (Sharp and le Sueur, 1996; Steketee and Campbell, 2010).

In the 1990s, malaria was virtually eradicated in SA. In 1996 the SA government, due to international policy changes, partially replaced DDT with pyrethroid compounds, considered to be less toxic, for its malaria control strategy. This move resulted in the reappearance of severe malaria outbreaks in a very short time. By the year 2000, almost 65 000 cases of malaria had been diagnosed and 424 deaths had occurred countrywide (DOH-RSA, 2010). In addition, the *Anopheles fenestus* mosquitoes, which feed almost exclusively on humans and had not been recorded in SA for many years, had re-emerged in KZN, since they became resistant to pyrethroids within a very short time (Hargreaves et al., 2000; Mouatcho et al., 2007).

A UNEP meeting held in Johannesburg, SA in December 2000, concluded the fifth and final round of negotiations on a treaty to ban POPS [now the Stockholm Convention] (IPEP, 2006). The SA government, experiencing a malaria epidemic at the time, was instrumental in the signing of a treaty that allowed for the re-introduction of DDT usage for malaria vector control. Since then, SA has continued to promote the use of DDT as a necessary intervention for malaria control, and re-expressed its commitment to the continued use of DDT for malaria control during the First Conference of the Parties (COP1) of the Stockholm Convention (UNEP, 2005).

After the reintroduction of DDT use for IRS in 2001, reported malaria cases in SA began to decline almost immediately, with malaria admissions and deaths decreasing by 89%, and outpatient malaria cases by 85% (O'Meara et al., 2010). At the same time, more advanced and modified drug therapy was applied, which replaced the combination of sulphadoxine / pyrimethamine with artemisinin-based combination therapy (ACT). This move further contributed to the decrease in malaria cases in SA (Barnes et al., 2005).

After a long deliberation in 2004, the Stockholm Convention and WHO finally agreed to grant exemption not only to SA, but also other malaria endemic countries, to use DDT for IRS as a major vehicle of malaria vector control, until similarly effective pesticides and methods become available (UNEP, 2008b).

Although permission was granted to use DDT, the Stockholm Convention, WHO and other global initiatives are actively supporting research and development of safe alternative chemical and non-chemical products, methods and strategies (relevant to the specific conditions of countries affected), to reduce the human, environmental and economic burden of malaria. Other additional measures are also introduced such as insecticide treated nets (ITN) which are impregnated with synthetic pyrethroids, which is the only approved class of insecticides for this purpose (Maharaj et al., 2005).

3.3. Pesticide use in South Africa

SA is one of the largest users of pesticides on the African continent. Approximately one fifth of the arable land is used for agriculture, and about one tenth of the economically active population is employed in the agricultural sector. Agriculture is a substantially important income generating activity in SA. This country has a wide variety of registered pesticides; however, detailed information on the proportions of pesticides used is not available (Heeren et al., 2003; PAN, 2010; Quinn et al., 2011).

Although international trends show that many developed countries are adopting policies that promote pesticide reduction, the use of pesticides in SA for agriculture, public health and domestic purposes continues to expand. Due to the banning of the persistent OC compounds, use of the additional pesticides, such as organophosphates, carbamates and pyrethroids has increased. Despite having legal controls that seem to conform to international standards, the present health and environmental impacts of pesticide use in SA are substantial but generally underestimated. From 2000 to 2008, a total of 12 364 pesticide poisoning cases were notified to the Department of Health; however, these figures are a substantial underestimation of the true rates, as many cases go unreported (DOH, 2005).

3.4. Political transition and changes in farming activities in KwaZulu-Natal

After the political transition in SA in 1994, an approximate area of 94 160 hectares of farmland was redistributed to historically disadvantaged people from 1997 to 2000 in the KZN province. During this process, 46% of the land was redistributed to women, either as owners or wives of the owners. This development has led to a change in women's roles in agriculture in SA. There is a definite increase in the number of female-headed households, with women owning their own farms and planting crops, which were traditionally labelled "men's crops". Women started participating in agricultural activities, such as pesticide mixing and application, previously carried out by males, due to the migration of men to the industrial sectors. There is no clear distinction between women's and men's roles in agriculture anymore. In addition, women with lower literacy levels and financial income, as compared to their male counterparts, may be unable to read pesticide information leaflets and purchase protective equipment, thereby increasing their risk of pesticide exposure and adverse health effects (Naidoo et al., 2008). Due to the increase in economic development in the rural areas, agricultural practices and crops grown have changed from mainly subsistence, to a mixture of subsistence, cash crop and commercial farming. The use of pesticides has therefore increased significantly.

Pesticides such as organophosphates, carbamates and pyrethroids are now used to protect the plants, and are considered the major crop protection and veterinary chemicals in SA. Internationally, these pesticides are called CUPs. Studies have measured DDT and CUPs in umbilical cord blood samples in the United States of America (Whyatt et al., 2003), as well as in breast milk samples in SA (Bouwman and Kylin, 2009; Bouwman et al., 2006; Sereda et al., 2009). In the SA study, permethrin was also found at quantifiable levels in the breast milk, followed by cyfluthrin and deltamethrin (Bouwman et al., 2006). Another study performed in Switzerland and USA detected pyrethroids in low concentrations in human milk (Weldon et al., 2011; Zehringer and Herrmann, 2001).

Results from a pilot study in 2006 found high levels of *p,p'*-DDE and *p,p'*-DDT (5177 and 1797 ng/g lipids, respectively) in the two malaria endemic areas of KZN. Other pesticides were also detected, such as HCH and HCB, with γ-HCH being dominant (Röllin et al., 2009a; Röllin HB,

2008). However, since PCB, chlordanes and nanochlors were found in low levels, they were not included in the current study.

3.5. Persistent organic pollutants (POPs)

3.5.1. Overview

POPs have been widely used as pesticides or industrial chemicals, and are known to pose a risk to human health. The following chemicals are currently included in the Stockholm Convention (UNEP, 2008b):

Aldrin	Chlordane
Dieldrin	Endrin
Hexachlorobenzene	Mirex
Polychlorinated biphenyls	Polychlorinated dibenzo-p-dioxins
Pentabromodiphenyl ether	Chlordecone
Heptabromodiphenyl ether	Pentachlorobenzene
Perfluorooctane sulfonic acids	Perfluorooctane sulfonyl fluoride
Polychlorinated dibenzofurans	DDT
Hexabromobiphenyl ether	Heptachlor
Endosulfan and the isomers	Toxaphene
Tetrabromodiphenyl ether	α-, β- and γ–Hexachlorocyclohexane

3.5.2. Health effects of POPs

In humans and animals, there are known adverse health effects of exposure to high levels of POPs. In addition, there is also increasing concern on chronic exposure to low level background exposure to POPs. The most common route of exposure is through contaminated food. Other routes of exposure include contaminated water and direct contact with the chemicals. Many POPs are known to be endocrine disruptors, binding to cellular hormone receptor sites such as estrogen, androgen and thyroid receptors, and have the potential to induce endocrine, neurodevelopmental, immunological and reproductive dysfunctions (Crinnion, 2009).

In SA, adverse reproductive effects of DDT have been reported in some men living in houses sprayed with DDT (compared to men living in houses that were not sprayed) (de Jager et al.,

2009), and increased urogenital malformations have manifested in newborn boys whose mothers were living in DDT treated areas (Bornman et al., 2010). Reproductive effects of POPs exposure have also been reported in other countries (Giordano et al., 2010; Rocheleau et al., 2009). Liver and lung cancer have been detected in the Taiwanese cohort exposed to PCB-contaminated rice oil (Thundiyil et al., 2007). However, there are conflicting results regarding OC exposure and breast cancer risk, even though it is known that some OC compounds act as estrogen agonists or antagonists (Calle et al., 2002). Disruptions of developing immune and respiratory systems from POPs exposure have been shown to result in reduced capacity to fight infections and an increased predisposition to developing allergies, however, in some cases, the exposure-outcome associations are inconclusive (Gascon et al., 2013). Also, the effects on the endocrine system involving changes in thyroid hormone levels were not completely evident (Arisawa et al., 2005; Rogan and Chen, 2005). Nevertheless, more evidence is emerging to substantiate an imbalance in thyroid hormone levels, following exposure to certain OC compounds (Lopez-Espinosa et al., 2010; Meeker et al., 2007). Obesity has also been proposed as another adverse health effect of exposure to endocrine disrupting chemicals during the critical stages of development. Studies suggest that fat cells and mechanisms involved in weight homeostasis may be affected by endocrine disruptors early in life and lend support to the concept that diseases manifesting in adulthood may have their origins in early life (Newbold et al., 2008).

Exposures to low levels of POPs, especially DDT and PCB, have been evaluated in prospective cohort studies in populations which consume fish. Many demonstrated some negative association with mental and psychomotor development with maternal DDT levels (Eskenazi et al., 2008; Torres-Sanchez et al., 2012). However, not all studies support these hypotheses (Jusko et al., 2012). Sajiv *et al* (2012) found higher attention deficit in males compared with females (Sagiv et al., 2012); Rosas and Eskenazi (2008) found a stronger neurological association with DDT compared to DDE (Rosas and Eskenazi, 2008), and Pan *et al* (2009) found that infant neurodevelopment was not impaired at low concentrations of PCBs, DDE and DDT (Pan et al., 2009). Limited studies have been reported for the other POPs; however, exposure to β-HCH (Lopez-Espinosa et al., 2010) and HCB (Ribas-Fito et al., 2007) showed some adverse neurological effects.

Several recent experimental studies suggest that exposure to POPs may cause diabetes in humans. Plasma levels were positively associated with type 2 diabetes, mainly for HCB and PCBs (Rylander et al., 2005). Previously, six POPs (2,2,4,4,5,5-hexachlorobiphenyl, 1,2,3,4,6,7,8-heptachlorodibenzop- dioxin, 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin, oxychlordane, DDT and trans-nanochlor), were strongly and positively associated with diabetes prevalence after adjusting for age, sex, race and ethnicity, poverty-income ratio, BMI, and waist circumference (Lee et al., 2006).

3.5.3. Distribution of POPs

As soon as POPs are released into the environment, they may be transported within a specific region or throughout the world by "global fractionation" process. In this process, chemicals may be latitudinally fractionated according to ambient temperature and their physical-chemical properties (solubility, vapour pressure, molecule size) and the subsequent deposition via rain, fog or snow in the water column, sediment or soil. As vapour, or attached to small particles, POPs move between air, water and soil. They can travel long distances, with south to north the main route, *via* the ocean streams and especially by atmospheric transport. Evaporation and precipitation are regulated by temperature and it accelerates the process (Macdonald et al., 2005; Odland and Nieboer, 2012). The volatile compounds are easily transported to the deposition region. Semi-volatile compounds, such as DDT and γ-HCH, can be washed out via precipitation and temporarily deposited in seawater or soil, and can be absorbed to water, plant and soil surfaces from the gaseous phase. During favourable warm weather conditions, these compounds evaporate again into the atmosphere and undergo further atmospheric transport. SA, having warmer climatic conditions would more likely be the source of POPs, rather than the destination from long range transportation (AMAP, 2004).

3.5.4. Exposure of POPs

Although dietary exposure is considered the most significant route of entry in humans, POPs may be absorbed through inhalation and dermal exposures. A large portion of POPs in the fat of a mother can be transferred to her baby in breast milk, or during pregnancy these substances can be transferred to the unborn child through the placenta. Polychlorinated biphenyls (PCBs), DDT

and its metabolites, dioxins, dibenzofurans and heavy metals are among the toxic chemicals most often found in breast milk (Hooper and McDonald, 2000; Sonawane, 1995).

Studies performed in KwaZulu-Natal found that mothers usually breast-feed their babies for up to two years, which can lead to a significant transfer of toxic chemicals from mother to infant (Bouwman et al., 1990; Bouwman et al., 2006). It has been shown that primiparae mothers had higher concentrations of DDT in their milk than multiparae mothers (Bouwman et al., 1992).

3.5.5. Toxicokinetics of POPs in humans

The uptake of POPs into tissues is a function of the blood flow, lipid content of that tissue, and the partition coefficient for the chemical between the blood and lipids in the specific organs. Once absorbed, POPs are readily distributed via the lymph and circulatory systems to all body tissues and are stored in these tissues generally in proportion to organ tissue lipid content (Morgan and Roan, 1971; Roan et al., 1971). The POPs are then slowly excreted from their stores, through faeces and urine (ASTDR, 2011).

3.6. DDT

1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane (DDT) is an organochlorine pesticide that was once widely used to control insects on agricultural crops and insects that carry diseases like malaria and typhus, but is now used in only a few countries to control malaria. DDT does not occur naturally in the environment. Commercial DDT is a mixture containing mainly 77% of the *p,p'*-DDT and 15% of the *o,p'*-DDT isomer. *p,p'*-DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene; also referred to as dichlorodiphenyl dichloroethylene, DDE) and 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene (DDD) are the metabolites and breakdown products of DDT in the environment (Crinnion, 2009). DDE is the main metabolite of *p,p'*-DDT. It has a longer-half-life, is more toxic, and usually occurs at higher levels than *p,p'*-DDT, but this depends on the time elapsed since exposure. *p,p'*-DDT exposure occurs primarily during its application (Longnecker et al., 1997). The term "total DDT" is often used to refer to the sum of all DDT related compounds (*p,p'*-DDT, *o,p'*-DDT, DDE, and DDD) in a sample. In humans, *p,p'*-DDT is metabolised to *p,p'*-DDE within about six months (Crinnion, 2009). The ratio *p,p'*-DDE/*p,p'*-DDT provides information about how recently exposure took place. *p,p'*-DDE is the most abundant organochlorine pesticide both in the environment and the human body, where it has reproductive, immunological, developmental and carcinogenic effects (AMAP, 2009; Cohn et al., 2007; Crinnion, 2009; de Jager et al., 2009; Ribas-Fito et al., 2003; Rogan and Chen, 2005; Sunyer et al., 2005).

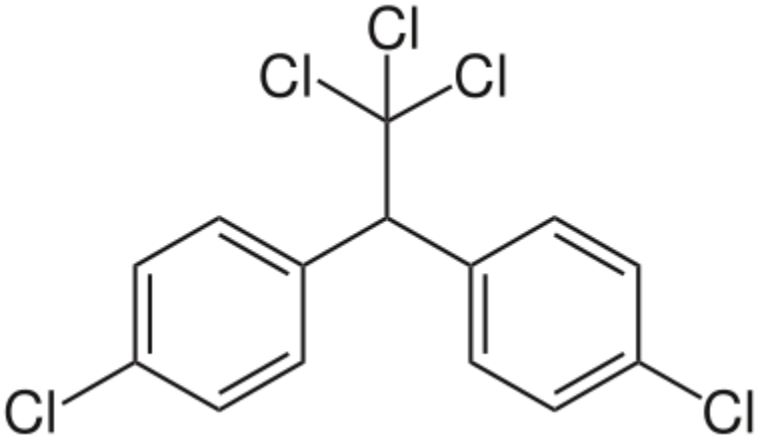


Figure 1: Structure of 1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane.

3.7. Hexachlorobenzene (HCB)

HCB, is a synthetic fully chlorinated hydrocarbon fungicide. HCB is not found naturally in the environment, but is produced as a by-product during the manufacture of, chlorinated hydrocarbons such as tetrachloroethylene and trichloroethylene, and is a contaminant in some pesticides such as pentachloronitrobenzene and pentachlorophenol, therefore, exposure is still possible. This compound also has non-pesticidal industrial uses. HCB exposure in humans results in a liver disease with associated skin lesions. HCB has shown neurological, developmental, endocrine and immunological toxicity in humans (Crinnion, 2009; Ribas-Fito et al., 2007; Sala et al., 2001). The extreme effects of HCB poisoning were reported in a Turkish population which consumed bread contaminated with HCB. Most of the affected people developed a liver condition called *porphyria cutanea tarda*, which disturbs the metabolism of haemoglobin and results in skin lesions. All the children who were breastfed by exposed mothers developed the “pembe yara" or "pink sore" (Gocmen et al., 1989).

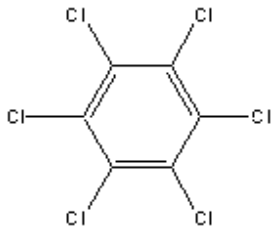
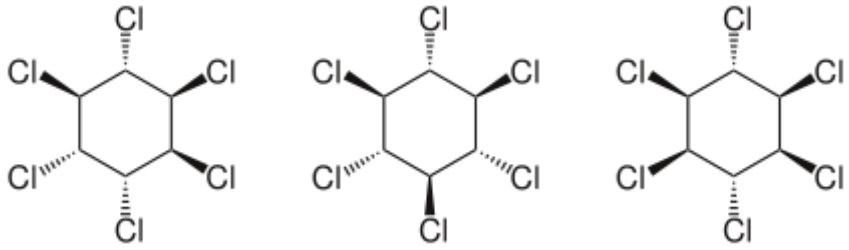


Figure 2: Structure of hexachlorobenzene.

3.8. α, β and γ-HCH

Hexachlorocyclohexane (HCH), formally known as benzene hexachloride (BHC), was produced to be used as an insecticide on fruit, vegetables, forest crops and animals. This chemical is synthetic, and exists in 8 chemical forms called isomers. The different isomers are named according to the position of the hydrogen atoms in the structure of the chemical. The α-, β-, and γ-HCH isomers are widespread environmental pollutants (Schroter et al., 1987), and of the 8 isomers of HCH, only the insecticidal γ isomer (γ-HCH) is of economic use. The α- and β-HCH (by-products of γ-HCH synthesis) are of major concern due to their considerable persistence in

biological systems, where they pollute the environment and certain nutrients (human milk contains the highest levels of β-HCH, as well as considerable amounts of α-HCH) (Yu et al., 2009). The insecticidal γ-HCH, commonly called Lindane, is also available as a prescription medicine (lotion, cream or shampoo) to treat and/or control scabies (mites) and head lice in humans. Workers exposed to γ-HCH are known to show signs of lung irritation, heart and blood disorders, headaches, convulsions, and changes in sex hormones. All isomers can produce liver and kidney effects (ASTDR, 2005).



α-HCH β-HCH γ-HCH

Figure 3: Structure of α, β, and γ-HCH.

3.9. Endosulfan

Endosulfan is a synthetic chlorinated pesticide and was introduced in 1956 as a general use insecticide, to protect food crops such as tea, fruits, vegetables, corn, cereals, oil seeds, potatoes, and grains, as well as wood, from a wide range of sucking and chewing insect pests. Commercially used, endosulfan is composed of its two isomers, the endosulfan 1 and endosulfan 2. Although this pesticide is used in resistance management, it is non-specific, and can therefore negatively impact populations of beneficial insects, such as the honey bees (ExtensionToxicologyNetwork, 1996; Mossler et al., 2012). Endosulfan is a xenoestrogen, a neurotoxin and an endocrine disruptor (Saiyed et al., 2003).

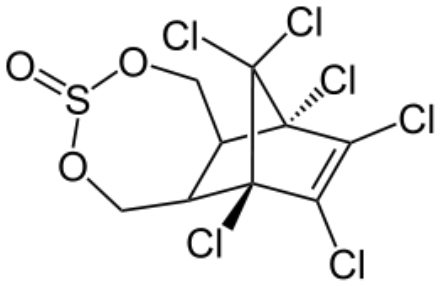


Figure 4: Structure of endosulfan.

3.10. Pyrethroid pesticides

In contrast, pyrethroids are predominantly synthetic forms of pyrethrins and are among the most widely used pesticides globally. These compounds are extensively used in agriculture currently, in horticulture, by exterminators and for indoor application (as an insect repellent), but they are also used for the treatment of head lice and fleas. The different pyrethroids biological half-lives vary between 2.5 and 12 hours in blood plasma (Leng and Gries, 1997). Although pyrethroids are considered the least toxic among pesticides today, they have been shown to be neurotoxic to humans. In mammals, it has been shown that pyrethroids at high doses affect nerve impulse transmission, by interacting with the sodium channels (Couture et al., 2009).

3.11. Polybrominated diphenyl ethers (PBDE)

Polybrominated diphenyl ethers (PBDE) are well known flame retardants which are widely used in the industrial and consumer market. They are chemically similar to PCBs, with 209 possible types of PBDE congeners and are numbered using the same system as PCBs (Costa et al., 2008). Three mixtures of PBDEs, namely, pentabrominated DE, octabrominated DE, decabrominated DE have been marketed (Costa et al., 2008). Only decabrominated DE is still produced in the USA and still widely used globally, because the European Union and several other states in the US have banned pentaBDE and octaBDE (Costa et al., 2008; Tu et al., 2012; van der Ven et al., 2009). PBDEs are also persistent organic pollutants (Gill et al., 2004; Johnson-Restrepo and Kannan, 2009; Odusanya et al., 2009). In the environment they have been detected in outdoor air, sediments, and leachates in landfills, sludge, soil, indoor air and house dust (Costa et al., 2008). In humans, PBDEs have been detected in human adipose tissue, serum and breast milk (Costa et al., 2008). The following PBDEs are found in high amounts in most cases: BDE-47, BDE-99 and BDE-153 (Costa et al., 2008). In the general population, the main sources of exposure are diet (fish, meats and dairy products that are found to contain the highest concentrations of PBDEs) and the indoor environment. There are currently no studies in SA that have measured PBDEs in maternal plasma. The measurements of PBDEs were however found in certain bird species in SA (Polder et al., 2008), in breast milk in the Limpopo Province (Darnerud et al., 2011), and in certain landfill sites around SA (Odusanya et al., 2009). The health effects from exposure to PBDE include thyroid hormone disruption, neurodevelopmental effects and, for some congeners, cancer (McDonald, 2002).

3.12. Mercury

Hg is a highly neurotoxic metal that has various physical and chemical forms presenting with different toxicities. The most important forms of Hg are the metallic form (elemental mercury, Hg^0) and the organic form (methyl mercury, [MeHg]). Hg^0 is released from the earth's crust by volcanic and other geothermal activities, thus contributing to the natural background levels (Hansen and Gilman, 2005). Anthropogenic sources of atmospheric Hg emissions include fossil fuel combustion, mining and smelting, and solid waste incineration. To a lesser extent, Hg may also be released from the soil and from industrial wastewater (UNEP, 2002).

In the environment, Hg is transformed through complex biogeochemical interactions and can be transported long distances through the air or via water-courses ending up in soil, water bodies or snow. Hg is often re-emitted into the environment. This repeated re-emission is called the "grasshopper effect" (EnvironmentCanada, 2010).

Microorganisms (bacteria, phytoplankton in the ocean, and fungi) convert inorganic Hg to MeHg, which after release can enter the water bodies or soil where it remains for a long time, particularly if attached to small particles. Of major concern is MeHg deposited into the aquatic environment, because it is ingested by biota. Since MeHg has a high affinity for sulphur ligands, it binds to the sulphur-containing amino acid cysteine and enters the protein pool. Due to the long half life of MeHg (72 days), this chemical bio-accumulates and biomagnifies in marine and fresh water organisms (Hansen and Gilman, 2005).

Most of the MeHg originally present in small organisms will eventually be stored in the larger and older fish. Saltwater fish (especially sharks and swordfish) that have a long life and can grow to a very large size tend to have the highest levels of MeHg in their bodies. As a result, populations who consume top-of-the-food-chain fish species and marine mammals will have the highest exposure levels. In humans, about 95% of MeHg ingested from fish is absorbed (Aberg et al., 1969). Plants (such as corn, wheat, and peas) have very low levels of Hg, even if grown in soils containing Hg at significantly higher than background levels. Mushrooms, however, can accumulate high levels of MeHg if grown in contaminated soils (EncyclopediaOfEarth, 2012).

3.12.1. Mercury sources in SA

It was reported in 2006 that Hg emissions in SA were second only to China, contributing more than 10% of global Hg emissions (Pacyna et al., 2006). Coal combustion, past formal gold mining and current extensive informal gold mining were identified as the main contributors. However, there is some doubt about the validity of these figures, and some sort of verification is required. The nature and extent of Hg pollution, and its impacts in SA have not been extensively studied, and most studies are being initiated from emergency incidents (i.e., the effluent spill from the Hg plant into the Mngcewni River in KwaZulu-Natal during the late 1990's). As a result of that pollution, it was recommended that the consumption of fish by local communities be significantly reduced, therefore mercury exposure needed to be evaluated. In SA, coal combustion in many poorer households (for cooking and/or heating) and in the informal artisanal mining industry are common and therefore may also contribute significantly to Hg emissions (UNEP, 2008a).

3.12.2. Mercury uptake and excretion in humans

The main routes of exposure to Hg are inhalation, ingestion and dermal absorption. After inhalation of Hg vapours, most (about 80%) of the Hg enters the bloodstream directly from the lungs, and is then rapidly distributed to other parts of the body. Most of the metallic form will accumulate in the kidneys. Some metallic Hg enters the brain, where it is readily converted to an inorganic form and remains "trapped" indefinitely (Bernhoft, 2012). The metallic Hg absorbed into the body is eventually excreted through urine and faeces, while smaller amounts leave the body in exhaled breath (USEPA, 1997).

After human consumption of fish and mammals, or other foods that are contaminated, the MeHg enters the bloodstream easily and moves rapidly to most tissues, and readily enters the brain. The foetus is much more susceptible to the toxic effect of MeHg than the mature adult. MeHg present in the blood of a pregnant woman will move across the placental barrier effortlessly and enter the foetal system. The excretory half life of methyl mercury in man is about 70 days (Bernhoft, 2012). When MeHg does leave the body following exposure, it is lost slowly over a period of several months, mostly in the inorganic form in the faeces (Aberg et al., 1969; USEPA, 1997).

3.12.3. Toxic effects of mercury

The nervous system is very sensitive to all forms of Hg. Both MeHg and metallic Hg vapours are more harmful than other forms, because more Hg in these forms reaches the brain. Exposure to high levels of metallic, inorganic, or organic Hg can permanently damage the brain, kidneys, and the developing foetus. The developmental neurotoxicity of MeHg became evident in the 1950s in Minimata Bay, Japan, after industrial effluent heavily contaminated with Hg entered the bay (Harada, 1995).

The primary health effect of exposure to MeHg for foetuses, infants, and children (PANNA, 2012), even at low doses is impaired neurological development to both the sensory and central nervous system (Harada, 1995). MeHg exposure in the womb can adversely affect foetal brain development, which continues after birth. Congenital MeHg poisoning can cause cerebral palsy syndrome (Davis et al., 1994). Impacts on cognitive thinking, memory, attention, language, fine motor and visual spatial skills, and decrease in IQ have been seen in children exposed to MeHg in the womb (Grandjean et al., 1997; Inskip and Piotrowski, 1985).

Hg present in the mother's body passes to the foetus, where it accumulates. It can also pass to a nursing infant through breast milk. Hg concentrations are generally found to be higher in the cord blood compared to the paired maternal blood, because the MeHg fraction (usually >98% of total Hg) binds to haemoglobin and has an especially high affinity for foetal haemoglobin. Thus, the cord blood Hg in its methylated form passes easily through the placenta (Rudge et al., 2009). Massive Hg exposure may result in brain damage, mental retardation, incoordination, blindness, seizures, inability to speak, as well as other nervous, digestive and urinary system damage (ASTDR, 1999). Lesser prenatal doses have been associated with neurodevelopmental delays and cognitive deficits.

3.12.4. Interaction of mercury and selenium

It has been shown that the trace element selenium (Se) can have a protective effect against Hg. High levels of Hg exposure deplete the amount of cellular Se available for the biosynthesis of thioredoxin reductase and other selenoenzymes that prevent and reverse oxidative damage. If the Se depletion is severe and long lasting, it results in brain cell dysfunctions that can ultimately

cause death (Ralston and Raymond, 2010). The content of Se in foods depends on the concentration of Se in the soil where the crops were grown. The following foods are generally considered good sources of Se: Brazil nuts, sunflower seeds, fish (tuna, halibut, sardines, flounder, salmon), shellfish (oysters, mussels, shrimp, clams, scallops)**,** meat (beef, liver, lamb, pork), poultry (chicken, turkey), eggs, mushrooms (button, crimini, shiitake), grains (wheat germ, barley, brown rice, oats), and onions. It is important to note that in most places, including Africa, there is very little Se in the soil, and therefore only sparse amounts are available to plants (Frank, 2008).

4. MATERIALS AND METHODS

4.1. Study populations

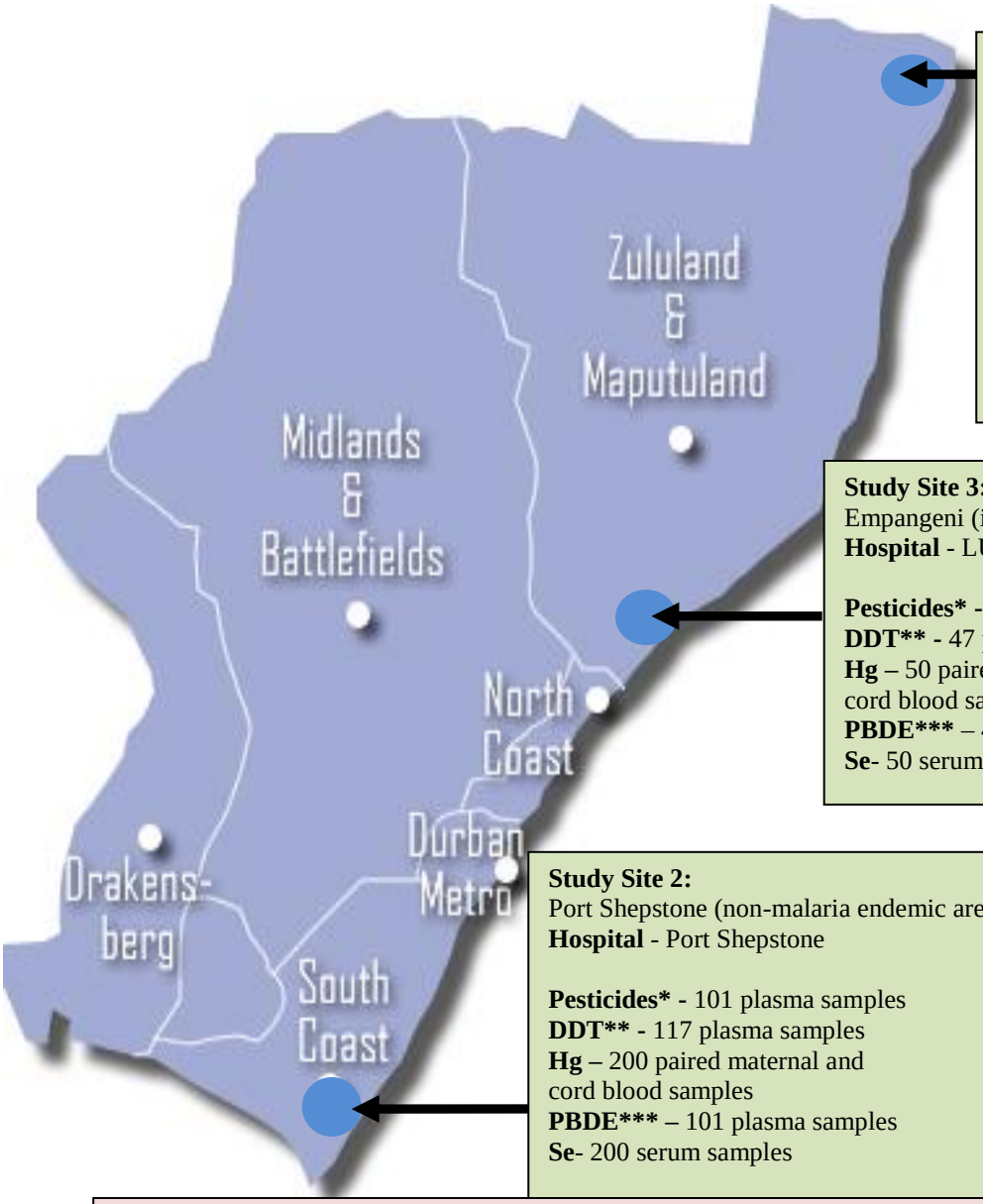
This study investigated 3 different regional mother-and-child-cohorts namely: Site 1 – Manguzi (malaria endemic), Site 2 – Port Shepstone (non-malaria), and Site 3 – Empangeni (intermittent malaria). Samples from all three sites were collected in the summer months from February to May 2008. See Figure 6.

4.2. Compounds measured in this study

The following POPs, as well as Hg were measured in blood components in this study: *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, α, β, γ-HCH, HCB, endosulfan 1and endosulfan 2. The following pyrethroid pesticides were measured: *cis*-permethrin, cyfluthrin, cypermethrin, deltamethrin. PBDE isomer levels were also measured.

4.3. Biological fluids used for the measurement of internal dose

Measuring the internal dose of POPs in human blood has many advantages over measuring the same variables in urine. In blood, the parent compound is measured and no detailed information on the metabolism of the toxicants in the body is required. In addition, detailed information is not required on the metabolites. Blood is also a regulated fluid, which means that the volume does not vary with water intake or other factors (unlike urine where corrections for dilution are always necessary). In addition, blood concentrations of the toxicant are often at a maximum directly after exposure, so the preferred time range for sampling may be clearer than with urine. Furthermore, blood measurements are more likely to reflect the dose available at the target site (Needham et al., 1995). However, when using blood or plasma, a clean-up procedure is required to eliminate interfering substances, such as lipids that co-elute with the analytes, resulting in inaccurate results. In addition, the establishment of an analytical laboratory at currently acceptable international standards is a relatively expensive undertaking. The use of isotope-labelled analytical standards and high-resolution mass spectrometry for routine POPs analysis is particularly expensive. For pyrethroids, urine is a better medium for monitoring in terms of limit of detection. In this study, all the pesticides were analysed in one medium (plasma), and under one preparation so as to lower costs and time.



Study Site 1:
Manguzi (malaria endemic area)
Hospital - Manguzi

Pesticides* - 91 plasma samples
DDT** - 91 plasma samples
Hg – 100 paired maternal and cord blood samples
PBDE*** – 91 plasma samples
Se - 100 serum samples

Study Site 3:
Empangeni (intermittent malaria endemic area)
Hospital - LUD Memorial War

Pesticides* - 49 plasma samples
DDT** - 47 plasma samples
Hg – 50 paired maternal and cord blood samples
PBDE*** – 49 plasma samples
Se- 50 serum samples

Study Site 2:
Port Shepstone (non-malaria endemic area)
Hospital - Port Shepstone

Pesticides* - 101 plasma samples
DDT** - 117 plasma samples
Hg – 200 paired maternal and cord blood samples
PBDE*** – 101 plasma samples
Se- 200 serum samples

Notes:
*Pesticide analysis included the following:

- α-, β-, and γ-HCH, Endosulfan 1 and 2, HCB,
- Pyrethroids (*cis*-permethrin, cyfluthrin, cypermethrin and deltamethrin).

**DDT & metabolites analysis included the following:

- *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *o,p'*-DDD and *p,p'*-DDD.

***PBDE isomers included the following:

- PBDE28, PBDE49, PBDE71, PBDE47, PBDE66, PBDE77, PBDE110, PBDE119, PBDE99, PBDE85, PBDE154, PBDE153 and PBDE138.

Figure 5: Map of the study sites, number of participants and blood components used for specific analyses.

4.4. Recruitment of participants and informed consent

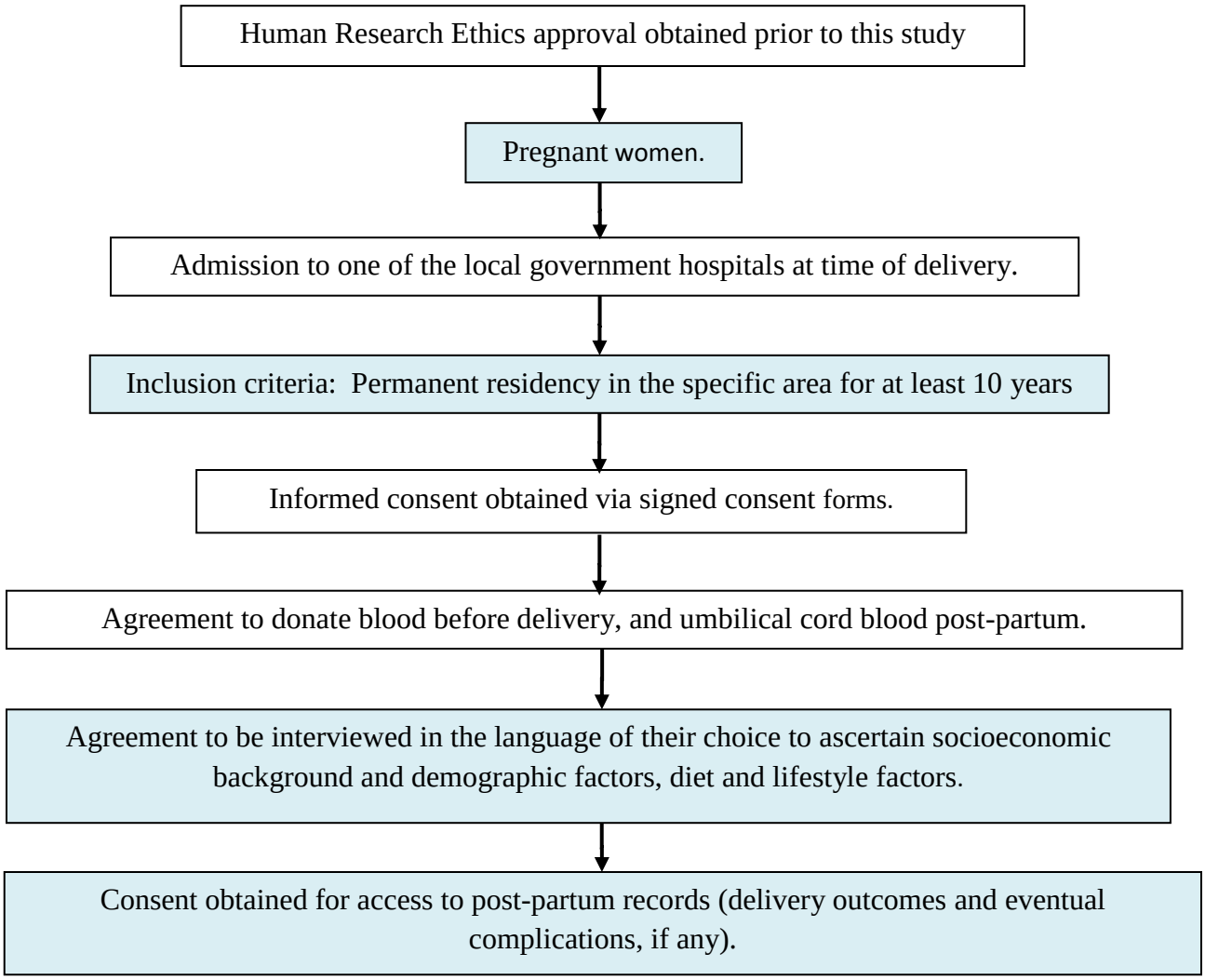


Figure 6: Procedure followed for the recruitment of participants.

4.5. Sampling procedure

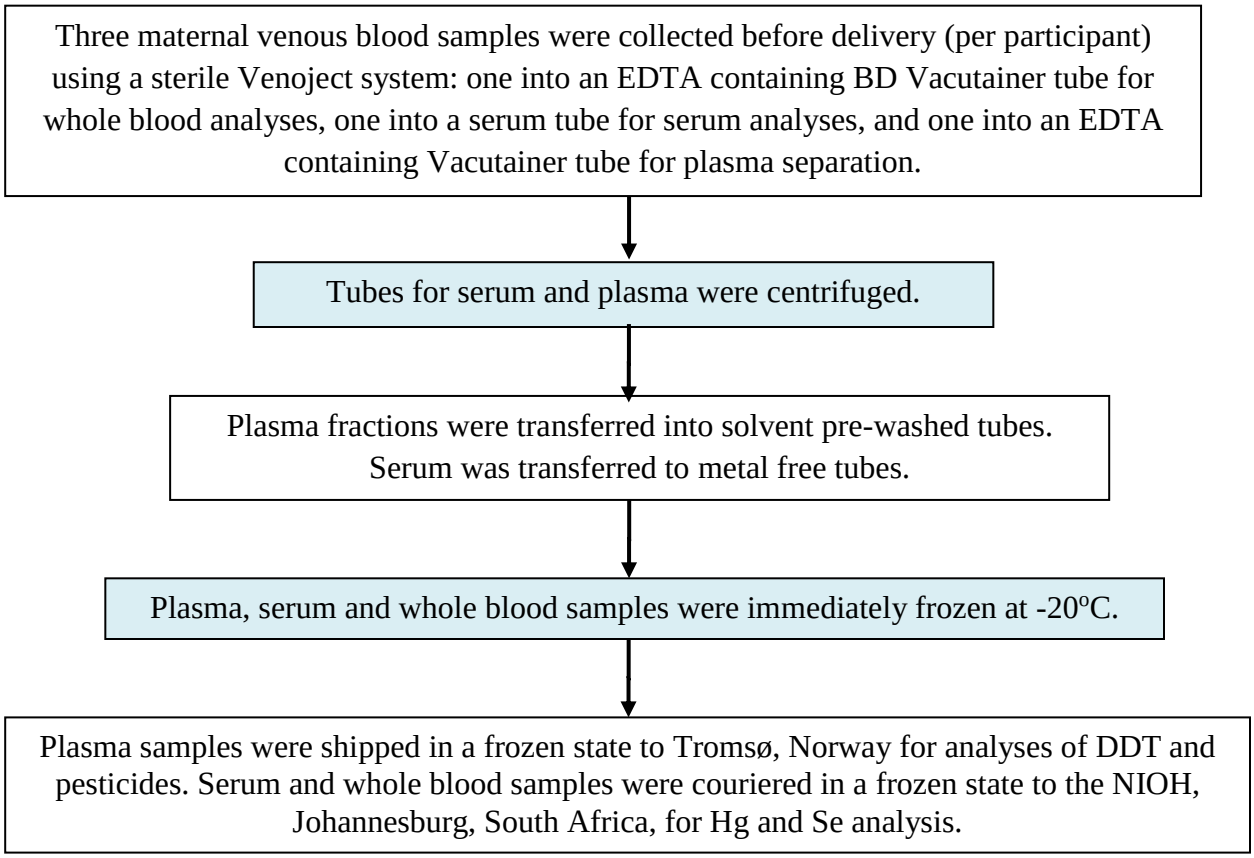


Figure 7: Procedure for collection of samples.

4.6. Analytical procedures

POPs and the pyrethroids were extracted from plasma with dichloromethane using solid phase extraction with slight modifications (Sandanger et al., 2007). The extracted samples were cleaned on a column containing 1 g of deactivated silica, concentrated and injected onto the GC-MS.

4.6.1. DDT & Pesticides

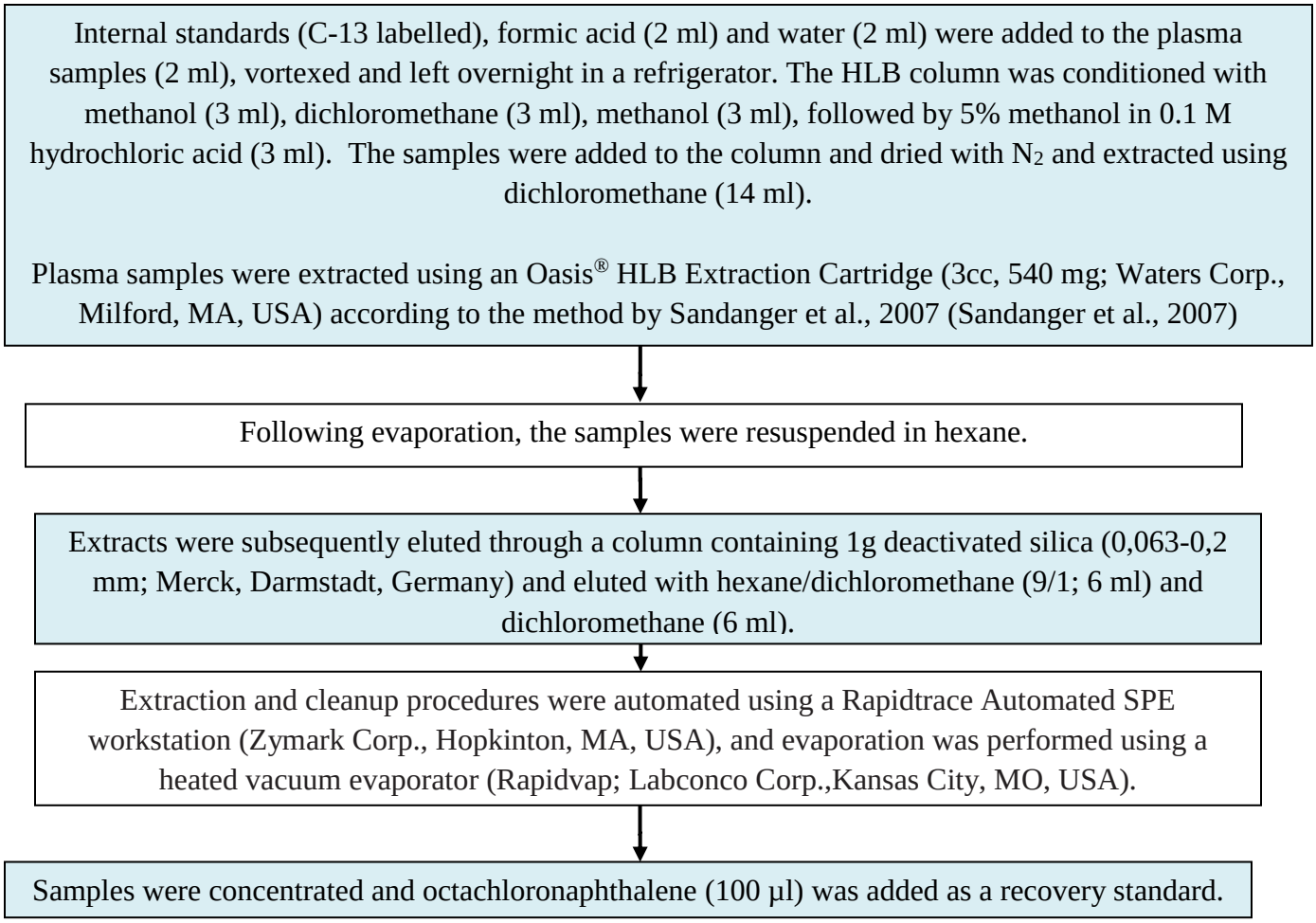


Figure 8: Analytical procedure for DDT and Pesticides.

4.6.2. Mercury

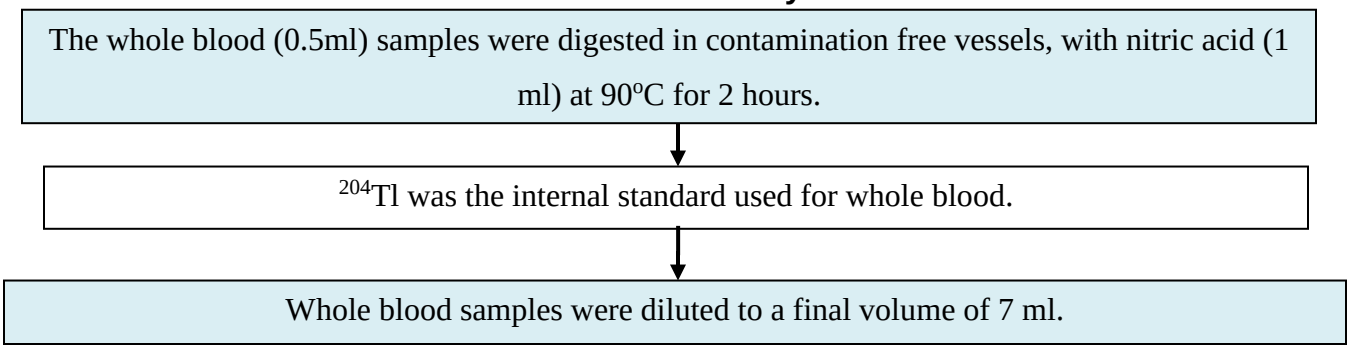


Figure 9: Analytical procedure for Mercury.

4.7. Instrumental measurements

4.7.1. DDT & Pesticides

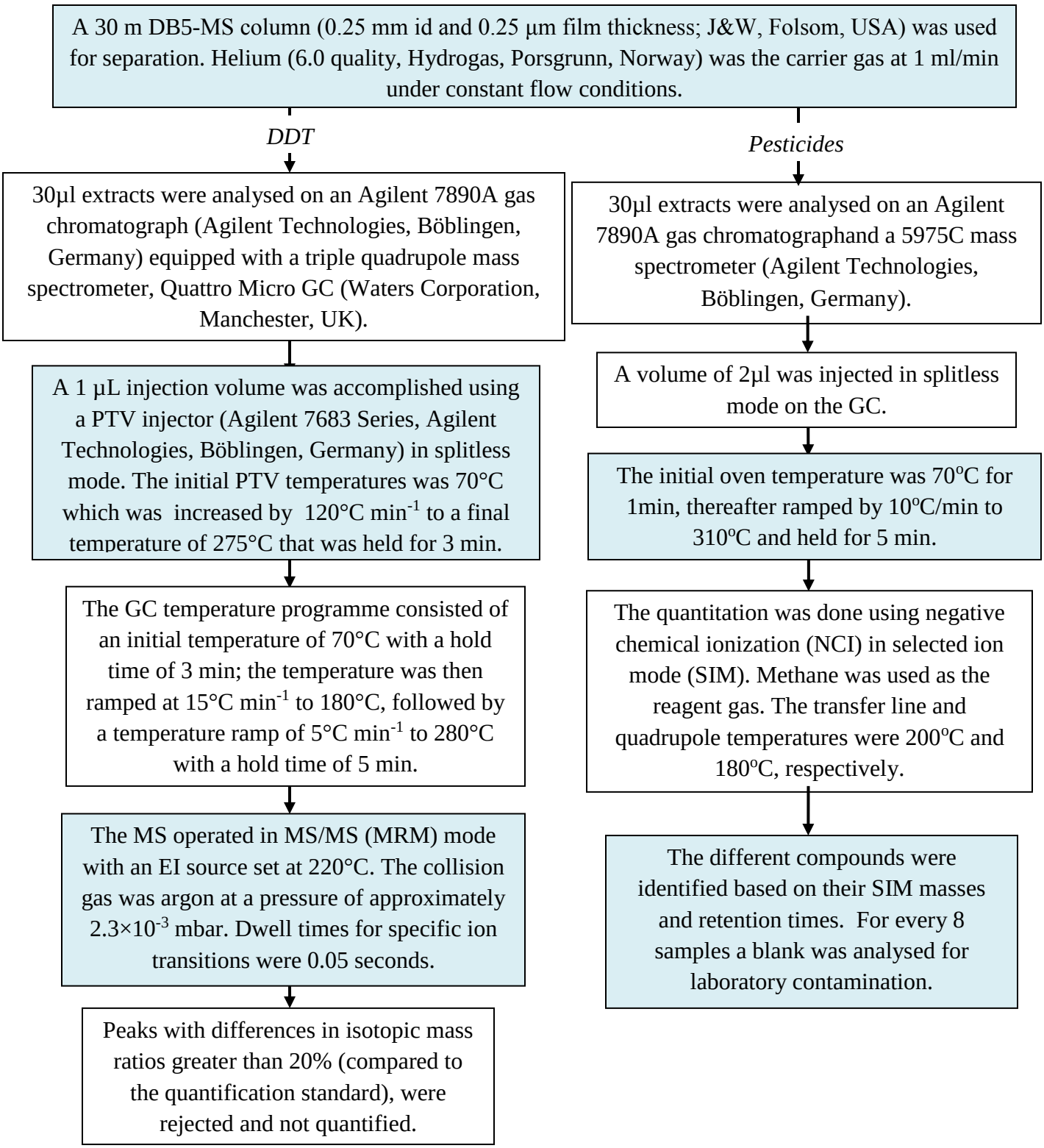


Figure 10: Instrumental procedure for DDT and pesticides.

4.7.2. Mercury

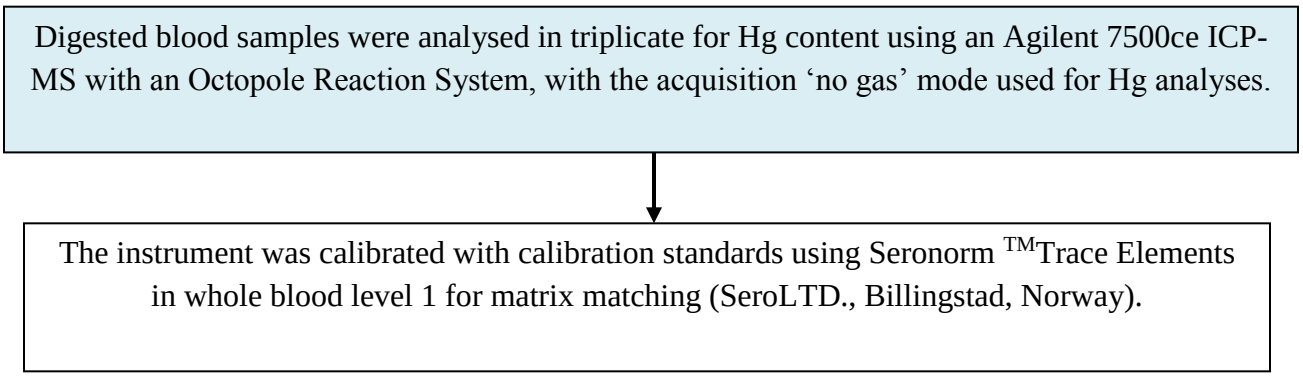


Figure 11: Instrumental procedure for Mercury.

4.7.3. Selenium

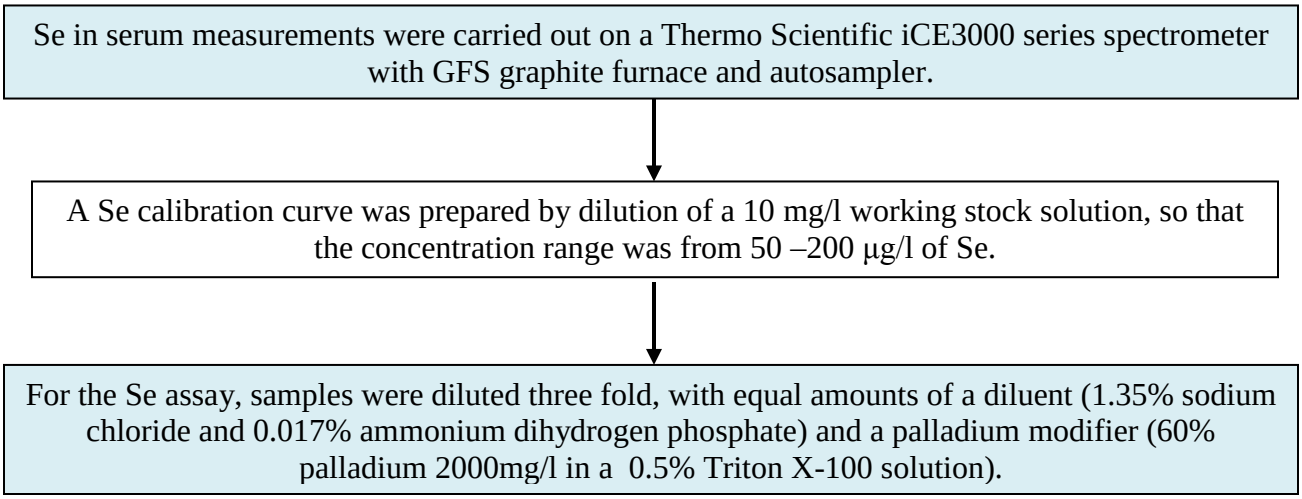


Figure 12: Analytical procedure for Selenium.

4.8. Quality assurance and quality control

4.8.1. DDT & Pesticides

DDT: The inclusion of certified reference materials and an internal QAQC pool in the analyses, assured the accuracy.

Pesticides: Spiked bovine serum samples were analysed after every 12 samples and Standard Reference Material 1957 from the National Institute of Standards and Technology were analysed after every 24 samples.

↓

The NILU laboratory participates in international inter-laboratory comparison programmes (AMAP Human Ringtest for plasma samples with +/- 20% deviation from result as best performance, according to AMAP Ringtest protocol).

↓

The limits of detection (LODs) were calculated using the signal to noise ratio calculations in serum samples, and corresponded to 3 times the area of the noise or 3 times the average concentrations found in blank samples.

Figure 13: Quality assurance for DDT and pesticides.

4.8.2. Mercury

Certified reference controls, Seronorm ^{TM}Trace Elements in whole blood, levels 1 and 2 were analysed after every 10 samples.

↓

The detection limit for Hg in blood was 0.08 µg/l.

↓

The NIOH laboratory participates in the 'New York State Department of Health, External Quality Assurance Programme, three times a year.

↓

The percentage recovery for the mercury controls in blood ranged from 83.2 -104%, with a coefficient of variation of 5%.

Figure 14: Quality assurance for Mercury.

4.8.3. Selenium

The detection limit for Se in serum was 6.5 µg/l.

Figure 15: Quality assurance for Selenium.

4.9. Lipid correction

Lipids were determined enzymatically for DDT and pesticides and the total lipids were calculated according to the formula used by (Sandanger et al., 2003b).

4.9.1. Justification for lipid correction

The concentration of lipid soluble compounds is available in two ways, either as whole or wet weight basis (i.e., weight per volume of serum), or lipid weight basis (i.e., per gram of total lipid). The whole wet weight concentrations reflect recent exposure and the steady state circulating levels (Sandanger et al., 2003b). For lipid soluble chemicals, the lipid adjusted measurement is recommended, because of the lipid soluble nature of these chemical compounds and their concentrations in adipose tissue (Bernert et al., 2007). Adjusting for lipid content provides standardised body burden estimation and allows for comparisons between studies. Total lipids (TL) in mg/dL were estimated by using the summation of lipid values of individuals: TLs = (2.27 * Total Cholesterol) + Triglycerides + 62.3l; the serum specific gravity was also taken into account in the adjustment.

4.10. Statistical analyses

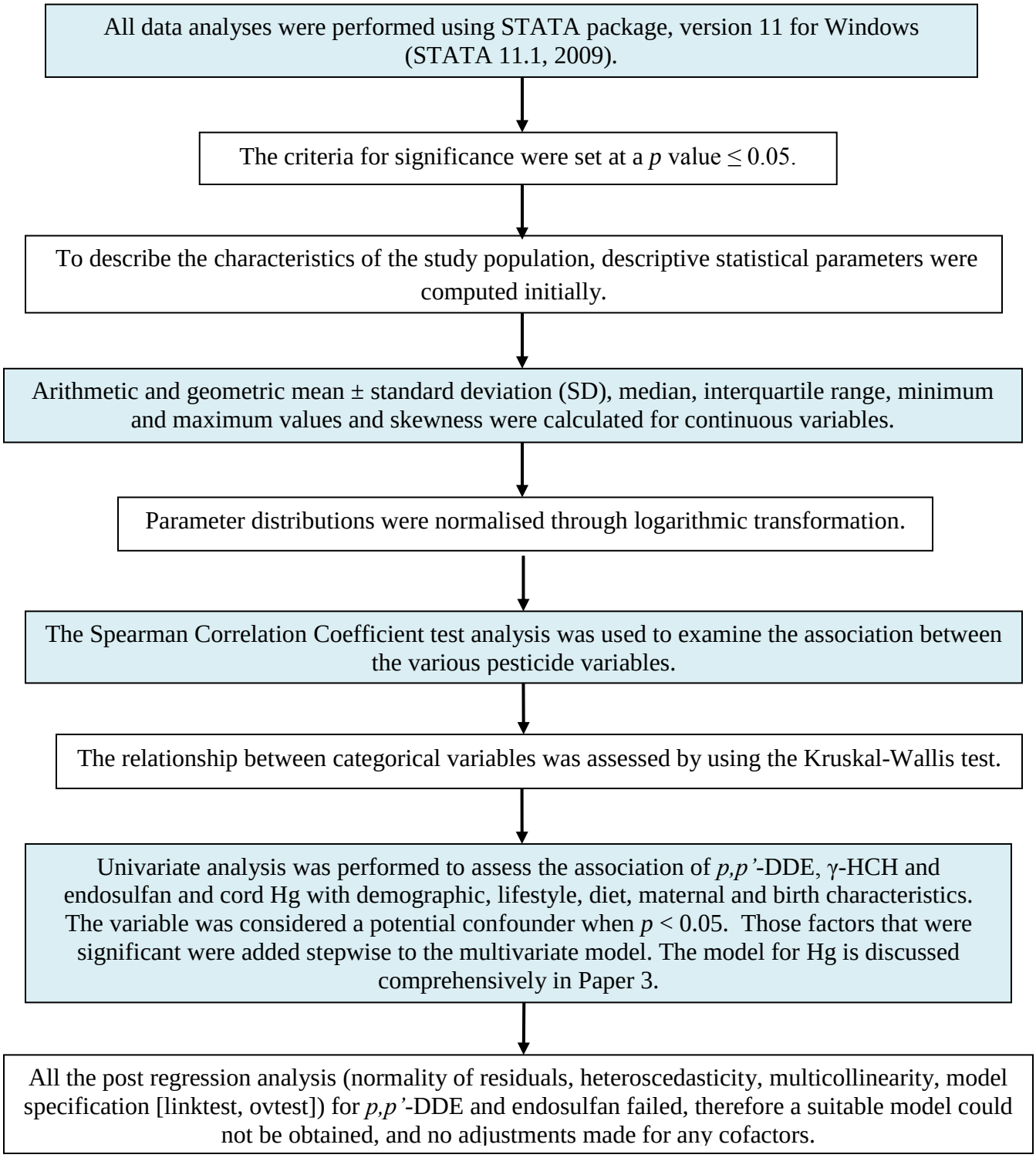


Figure 16: Statistical analysis procedure.

4.11. Ethical considerations

The study protocol was approved unconditionally by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (Protocol: M040314). Approval was also granted by the Provincial Health Research Committee, KwaZulu-Natal Department of Health (Reference: HRKM001/08), and the Regional Committee for Medical Research Ethics, REK Nord.

5. RESULTS

5.1. *Paper 1: Prenatal exposure to DDT in malaria endemic region following indoor residual spraying and in non-malaria coastal regions of South Africa*

DDT and its metabolites were measured in the plasma of pregnant women before delivery at three sites along the Indian Ocean coast. In total, 255 women participated in the study at the following sites: malaria endemic (n = 91; Site 1), intermittent malaria (n = 47; Site 3) and non-malaria sites (n = 117; Site 2). Indoor residual spraying (IRS) with DDT increased the concentrations of DDT and its metabolites in the plasma of delivering women. *p,p'*-DDT and *o,p'*-DDT, and their metabolites *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDE and *o,p'*-DDD, were significantly higher in Site 1 (malaria endemic), compared to Site 2 (non-malaria) and Site 3 (low risk/intermittent-malaria). In Site 1, the dominant metabolite *p,p'*-DDE was detected in 100% of the samples, while *p,p'*-DDT was detected in 99% of samples. The low *p,p*'-DDE/ *p,p*'-DDT ratio of 1.75 is expected in Site 1, as this site is a malaria endemic site and IRS takes place as part of the malaria vector control programme.

In the intermittent and non-malaria sites >80% of the samples had detectable levels of *p,p*'-DDE. The low *p,p*'-DDE/ *p,p*'-DDT ratio of 4 and 5 in Site 2 and Site 3, respectively, where no IRS occurs, also reflects recent-ongoing exposure.

The Kruskal-Wallis test showed that in the malaria endemic Site 1, the women whose homes were sprayed by the malaria control programme, the younger mothers, as well those that had no children or had never breastfed, had elevated DDT levels.

5.2. Paper 2: *Regional variation in pesticide concentrations in plasma of delivering women residing in rural Indian Ocean Coastal regions of South Africa*

Of the pesticides measured (α-, β- and γ-HCH, endosulfan, HCB and the pyrethroids: *cis*-permethrin, cyfluthrin, cypermethrin, and deltamethrin), results indicated that subjects were mainly exposed to γ-HCH (detected in 100%), endosulfan 1 (95%) and endosulfan 2 (34%). The rest of the pesticides were detected in < 31% of the samples. Endosulfan and γ-HCH levels were significantly higher in Site 3 (Empangeni-low malaria endemic), compared to Site 1 (Manguzi-malaria endemic) and 2 (Port Shepstone – non-malaria endemic).

Endosulfan 1 and 2 levels correlated strongly in all three sites ($r > 0.7$), and both endosulfans correlated strongly with γ-HCH in only Sites 2 and 3 ($r > 0.63$).

Using the Kruskal-Wallis test, a significant increase of γ-HCH levels were seen in women in Site 1 (malaria endemic) who reported growing one's own food ($p = 0.0005$), who drank water from the tap ($p = 0.0040$) and using pesticides in the garden ($p = 0.0161$). In Site 3 (low malaria endemic), a significant increase in γ-HCH levels was seen in women who used pesticides in the garden ($p = 0.0145$). No significant relationships were found for endosulfan, except that the levels decreased with the number of the children in site 2 (non-malaria endemic) ($p = 0.0070$).

5.3. Paper 3: *Differences in prenatal exposure to mercury in South African communities along the Indian Ocean*

The overall results for maternal Hg levels ranged from 0.2-13 µg/l, while the corresponding cord blood levels were 0.2-18 µg/l. Site 1 participants (n = 100) had significantly higher maternal blood geometric mean (GM) Hg (0.93 µg/l) compared with Site 2 (n = 200; 0.49 µg/l) and Site 3 (n = 50; 0.56 µg/l). The same pattern was found in the paired cord blood Hg levels. Cord blood Hg levels (GM) in Site 1 was 1.45 µg/l, Site 2 was 0.7 µg/l and Site 3 was 0.73 µg/l. There was a strong positive correlation (r^2 = 0.66) between maternal and cord blood Hg levels. No correlation was seen between maternal Hg and Se levels.

The percentage of subjects where the largest Hg levels in maternal blood were found to be above the 90th percentile were as follows: 86% residing in Site 1; 37% in the age group 20-29; 92% with no reported environmental pollution around the home; 57% using the outdoor tap as a source of drinking water; 74% using wood for cooking; 57% consuming fish once per week; 53% consuming tinned fish and 83% having their home sprayed as part of the malaria vector control programme.

As expected, maternal Hg levels had a significant influence on cord blood Hg levels. The univariate analysis showed that living together with one's partner ($p < 0.001$), residing in Site 1 ($p < 0.001$), living in an informal house ($p = 0.050$), using wood ($p < 0.001$) or gas ($p = 0.040$) as a fuel for cooking, using borehole water ($p = 0.001$) instead of municipal water, having one's home sprayed by the malaria control personnel ($p < 0.001$), having a household member involved in fishing ($p < 0.001$) and the consumption of that fish ($p = 0.002$), using pesticides in the garden ($p < 0.001$), or consuming fresh ($p = 0.031$) or tinned fish ($p = 0.005$) had a positive influence on the log of Hg levels in cord blood. In contrast, education ($p = 0.009$), consumption of fruit ($p = 0.033$), or dairy products ($p < 0.001$), increasing parity ($p = 0.036$) or BMI ($p = 0.003$) had a negative influence on cord blood Hg levels.

The multivariate regression model found that the following were strong predictors of elevated umbilical cord blood Hg concentrations: maternal blood Hg levels ($p < 0.001$), living in Site 1 (p

< 0.001), environmental pollution in the home (p = 0.004) and having a household member involved in fishing (p = 0.002).

5.4. Additional analytical and statistical results not discussed in the papers

5.4.1. PBDE

A large range of PBDE isomers was also measured along with the pesticides on the GC-MS instrument, following the same methodology as for the pesticides. The isomers measured included PBDE28, PBDE49, PBDE71, PBDE47, PBDE66, PBDE77, PBDE110, PBDE119, PBDE99, PBDE85, PBDE154, PBDE153 and PBDE138. These results were not published, as more than 90% of the results were below the detection limit (Table 1). The low levels were found in all three sites with no regional differences, indicating a low exposure to PBDEs in SA women from this study group.

Table 1: List of PBDE isomers measured with % of samples above the detection limit.

Name of isomer	% above detection limit	Detection limit (pg/ml)
PBDE 28	0	4
PBDE 49	5	6
PBDE 71	0	6
PBDE 47	9	6
PBDE 66	0	6
PBDE 77	0	4
PBDE 100	0	6
PBDE 119	0	6
PBDE 99	2	6
PBDE 85	0	6
PBDE 154	0	6
PBDE 153	2	4
PBDE 138	0	6

5.4.2. Predictors of DDT exposure

In Paper 1, no regression analysis was performed on the DDT data. From the univariate analysis, the positive predictors of *p,p*'-DDE include, using wood as a fuel for cooking (p = 0.042), having

one's home sprayed by the malaria vector control programme ($p = 0.008$), consuming tinned fish ($p < 0.001$), Hg in cord blood ($p = 0.018$) or Hg in maternal blood ($p = 0.026$). The age ($p = 0.025$) and weight ($p = 0.001$) of the mother, or consuming dairy products ($p = 0.018$) or drinking bottled water ($p < 0.001$), all negatively influenced the *p,p*'-DDE levels (Table 2). No multivariate regression model could be formulated because none of the post regression tests (normality of residuals, heteroscedasticity, multicolllinearity, model specification [linktest, ovtest]) passed. This implies that no adjustment was made for any cofactors.

Table 2: Overall univariate analyses with log *p,p'*-DDE.

Log *p,p'*-DDE	**Univariate**		
	β	**t**	***p***
*Age group: <20**			
20 – 29	-0.365	-3.14	0.002
30 – 39	-0.296	-2.03	0.046
≥ 40	-0.586	-2.28	0.025
*Fuel used for cooking: Electricity**			
Gas	0.233	1.12	0.266
Wood	0.304	2.06	0.042
*Indoor Residual Spraying for Malaria: No**			
Yes	0.688	2.73	0.008
*Consume Fresh fish: Seldom**			
Once/week	0.079	0.80	0.426
Everyday	0.439	1.73	0.087
*Consume Tin fish: Seldom**			
Once/week	1.130	4.82	0.000
Everyday	1.045	4.55	0.000
*Consume Leafy vegetables: Seldom**			
Once/week	0.668	1.83	0.071
Everyday	0.710	1.95	0.054
*Consume Dairy productsSeldom**			
Once/week	0.152	1.28	0.205
Everyday	-0.514	-2.43	0.018
*Consume Bottled water: Seldom**			
Once/week	-0.089	-0.66	0.510
Everyday	-1.733	-4.19	0.000
Maternal weight (kg)	-0.016	-3.43	0.001
Mercury in cord blood	0.128	2.43	0.018
Mercury in maternal blood	0.228	2.26	0.026

* reference

5.4.3. Evaluation of concomitant exposure to mercury and *p,p'*-DDE

The common positive predictors for both Hg and DDT exposure in the univariate analysis include: residential area, using wood for heating, having one's home sprayed by the malaria vector control programme, or consuming tinned fish. The age of the mother or consuming dairy products were negatively associated with both mercury and DDT exposure. The combination of Hg and *p,p*'-DDE gave a multivariate regression model explaining 30% of the model, that included, log *p,p*'-DDE, maternal Hg, maternal age and weight, and spraying the home by the malaria vector control programme. However, none of the post regression tests (normality of residuals, heteroscedasticity, multicollinearity, model specification [linktest, ovtest]) passed and therefore the model is not presented.

It is well known that during breastfeeding, PTS are transferred to the baby. However, breastfeeding did not show any association with any of the pesticides or Hg, possibly because the exposure is continuous. In contrast, increasing parity was associated with a decrease in pesticide levels. However, this should be taken with caution as more than 50% of the women in the study were nulliparous.

5.4.4. Predictors of γ-HCH and endosulfan

In Paper 2, the only relationships shown were those investigated using the Kruskal-Wallis test. The only association from the Kruskal-Wallis that remained in the multivariate model was an increase in γ-HCH levels with drinking borehole water. Table 3 below shows the multivariate regression analysis model of the log of γ-HCH. From the different study areas, mothers in Site 3 ($p < 0.001$) had significantly higher levels of γ-HCH compared to Site 1, whereas no significance was found with mothers from Site 2. Drinking borehole water as a source of drinking water ($p = 0.005$), significantly increased the levels of γ-HCH, but not when rain / river water was consumed. γ-HCH levels were significantly lower in women who consumed processed meat at least once a week ($p = 0.016$). With an increase in maternal weight ($p = 0.025$) or a decrease in maternal age ($p = 0.026$), the levels of γ-HCH increased significantly. This could be due to those women who had not breastfed being significantly younger ($p = 0.0000$, T-Test). The model explained 37% variation in the γ-HCH levels.

Table 3: Linear regression model of log γ-HCH.

Covariate	**Std. β**	***t***	***p***	**95% CI**	
				Lower	**Upper**
Study site: Manguzi (Site 1)					
Port Shepstone (Site 2)	0.36	0.22	0.111	-0.08	0.80
Empangeni (Site 3)	2.13	0.25	0.000	1.63	2.63
Source of drinking water: Tap					
Rain/River	0.06	0.30	0.828	-0.53	0.66
Borehole	0.83	0.29	0.005	0.26	1.40
Consumption of processed meat: Seldom					
Once a week	-0.46	0.19	0.016	-0.84	-0.09
Daily	-0.29	0.26	0.259	-0.79	0.22
Weight	0.02	0.01	0.025	0.00	0.04
Age	-0.04	0.02	0.026	-0.07	-0.00
r^2	37%				

Table 4 shows the univariate regression analysis of the log of endosulfan. No multivariate regression model could be formulated. Living in Site 3 ($p < 0.001$), environmental pollution around the home ($p < 0.001$), or growing one's own food ($p = 0.008$), significantly increased the endosulfan levels, whereas consuming meat ($p = 0.001$) or dairy ($p = 0.017$) had a protective effect on endosulfan levels.

Table 4: Overall univariate analyses with log endosulfan.

Log endosulfan	**Univariate**		
	β	**t**	***p***
*Residential Area: Study site 3**			
Study site 1	-0.726	-7.37	0.000
Study site 2	-1.063	-11.40	0.000
*Air quality around home: Good**			
Bad	0.712	5.92	0.000
*Environmental pollution around home: No**			
Yes	0.582	4.60	0.000
*Grow own food in garden: No**			
Yes	0.240	2.68	0.008
*Consume Processed meat: Seldom**			
Once/week	-0.310	-3.06	0.003
Everyday	-0.406	-3.36	0.001
*Consume Dairy products: Seldom**			
Once/week	-0.082	-0.67	0.504
Everyday	-0.279	-2.40	0.017

The results below (Table 5) clearly show a good correlation between endosulfan and γ-HCH, being more prominent in Site 2 and Site 3. The good correlation indicates a possibility of a common source of exposure. Some correlation exists between α-HCH and endosulfan 2, especially in Site 2.

Table 5: Spearman correlation (r) results of γ, α-HCH and endosulfan.

	α-HCH			**γ-HCH**			**Endosulfan 1**		
	Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
γ-HCH	0.43	0.44	0.69						
Endosulfan 1	0.49	0.48	0.48	0.57	0.85	0.75			
Endosulfan 2	0.55	0.78	0.47	0.48	0.64	0.71	0.83	0.72	0.95

6. DISCUSSION

6.1. Main findings

6.1.1. PTS levels in blood of the participating women

High levels of DDT metabolites, particularly *p,p'*-DDE and *p,p'*-DDT, were found in maternal plasma in the malaria endemic site (Site 1), where IRS is taking place. The direct and recent activity of IRS is confirmed by the low *p,p'*-DDE / *p,p'*-DDT ratio of 1.75, confirming that the malaria prevention programme is applied during the summer months when sample collection for this study took place. Environmental studies that were performed in two districts of SA (Venda) have shown that using DDT for IRS has resulted in indoor air contamination in the ranges of 750 - 6000 ng/m^3 with a mean of 2200 ng/m^3, in comparison with non-IRS dwellings, where DDT concentration was found to be much lower (range: 1.5 - 2.8 ng/m^3, mean 7.2 ng/m^3 (Van Dyk et al., 2010). The authors suggested that inhalation or contact with DDT particles falling from the treated walls and roofs of the dwellings is a probable mechanism of continuous exposure, as is ingestion through contaminated food or water. Similar findings were reported from Mozambique (Manaca et al., 2012a). In the malaria area of Mozambique, before IRS was restarted in 2006, the *p,p'*-DDE concentration measured in cord blood samples was around 0.6 ng/ml, which was much lower than that found in the maternal blood in Site 1 of the current study (20 ng/ml). It is evident that there has been a steady decline in *p,p'*-DDE levels from 2003 to 2006, before the reintroduction of IRS, in Mozambique (Manaca et al., 2012b).

Recent studies performed in other malaria prone regions, such as Saudi Arabia and India, have found lower concentrations of DDT in maternal serum samples (550 pg/ml and 2300 pg/ml, respectively; compared to this study (20279 pg/ml) (Al-Saleh et al., 2012; Dewan et al., 2013). However, *p,p'*-DDE concentrations higher than this study's results (3840 ng/g lipid) were found in agricultural workers in Bolivia (median 4788.7 ng/g lipid) (Mercado et al., 2013) and in Mexican inhabitants (15800 ng/g lipid) (Waliszewski et al., 2012). In Australia, where no IRS with DDT is taking place, the concentrations measured in maternal serum samples (mean 1050 pg/ml) (Reid et al., 2013) were similar to the levels found in the intermittent malaria site (Site 3; 1167 pg/ml) of this study (Reid et al., 2013).

This study found that in Site 1, where DDT levels were high, Hg levels in maternal plasma and paired cord whole blood were also elevated. Although the Hg results for pregnant women in this study were found to be lower than those reported from Brazil, Korea, Greenland and Canada, the continuous low level exposure to Hg in combination with DDT remains a concern. The hepatotoxicity of the combination of DDT and MeHg in Amazonian fish tissues, has been demonstrated by its greater effect on glucose-6-phosphate dehydrogenase (G6PDH) and glutathione-S-transferase activities and lipid damage, when compared to the effects of the individual chemicals (Filipak Neto et al., 2008). This study's findings suggest that efforts should therefore be made to reduce the concomitant exposure to DDT and the continuous low level of Hg in pregnant women. It may not be easy to reduce DDT through IRS until suitable alternatives are found to eradicate malaria, but surely efforts can be made to reduce the Hg exposure. It has been reported that fish-eating communities in the Amazon are at a high risk of exposure to both Hg and DDT (Rabitto Ida et al., 2011). This study showed in its univariate regression analysis that *p,p'*-DDE levels in maternal plasma ($p < 0.001$) and Hg levels in cord blood ($p = 0.005$) were significantly increased when the subjects consumed tinned fish on a daily basis. However, consumption of fresh fish only significantly increased cord blood Hg levels ($p = 0.031$) and not *p,p'*-DDE levels ($p = 0.087$). This study consisted of a higher percentage of subjects that consumed tinned fish (25%) when compared to fresh fish (8%) on a daily basis. More than 50% of the study subjects ate fresh or tinned fish less than once a week. This study's demographics substantiate the general understanding that fresh fish in not highly consumed in SA, and that tinned fish is a more economically viable option, especially for low income communities.

DDT and its metabolites were also found in mothers residing in the intermittent (Site 3) and non-malaria (Site 2) study areas, but to a lesser extent than in Site 1. The non-malaria and low-risk malaria sites suggest recent, on-going exposure to parent DDT as shown by the low *p,p'*-DDE / *p,p'*-DDT ratio of 4 and 5, respectively. However, these low *p,p'*-DDE / *p,p'*-DDT ratios (<10) cannot be attributed to food as being the only source of exposure to DDT (Kang et al., 2008). A previous pilot study conducted in 2006 in various sites throughout SA (Röllin et al., 2009b), found *p,p'*-DDE / *p,p'*-DDT ratios ranging from 3 to 22. The two DDT sites (inland and coastal) had a ratio of 3 (similar to the current study; whereas the mining site close to Mozambique (also

a malaria endemic area) had a ratio of 8, indicating recent exposure. However, the area in the Western Cape of SA, which is very far from any malaria endemic region, had a ratio of 22, suggesting high environmental persistence of DDT and ongoing bioaccumulation or past limited use of DDT (Jaga and Dharmani, 2003). This suggests that there are no gradients of DDT from the spraying area, and that the subjects in Site 2 and Site 3 were exposed to DDT in the recent past, thus indicating limited, scattered, and illegal use of DDT in both sites. Efforts must be made to find the source of DDT in non-malaria endemic areas in SA, so as to stop its use. Evidence of illegal use of DDT has also been reported in Bolivia (Mercado et al., 2013).

This study found that in Site 1, the participants were not only exposed to DDT and Hg, but also to γ-HCH and endosulfan. Endosulfan was detected in > 97% of the subjects and γ-HCH was detected in all subjects in Site 1. The health effects of the synergistic exposure to various organochlorine (OC) pesticides and Hg is of great concern. In Site 1, it is anticipated that subjects will continue to be exposed to DDT used for malaria eradication, until safer and more effective substitutes are developed. Hence, it is very important that the public should be informed to not use additional OC pesticides in households and in agricultural activities, but to use the safer alternatives instead, such as the pyrethroids.

In the present study, γ-HCH levels were significantly higher in Site 3 (Empangeni), compared to Sites 1 (Manguzi) and 2 (Port Shepstone). This can be attributed to extensive commercial and subsistence farming taking place there, with women being the active workers on the farms. It has been reported in France that areas of more intensive agricultural activity show larger concentrations of γ-HCH in the soil (Orton et al., 2013). The results of the pilot study of 2006, as well as the outcomes from the current study, clearly show that γ-HCH use and exposure is not evenly distributed throughout SA (Röllin et al., 2009b). Delivering women residing in areas with high agricultural activity have higher levels of γ-HCH compared to women living in urban, mining and industrialised regions. Interestingly, studies done in South Africa before 1997 have found γ-HCH to be the most dominant OC pesticide in soil and sedimentation (Quinn et al., 2009).

No detectable concentrations of γ-HCH were found in Australian maternal samples (Reid et al., 2013), or in other similar studies in Poland and Mexico (Jaraczewska et al., 2006; Rodriguez-Dozal et al., 2012). Hoferkamp reported that γ-HCH is unable to biomagnify in the arctic food webs, accounting for the low detection for countries in the Arctic Circle (Hoferkamp et al., 2010). This may apply to other food webs across the globe.

In India, a recent study reported γ-HCH concentrations of 6.60 ng/ml in maternal blood which are very similar to levels found in the present study, in Site 3, i.e. 6.14 ng/ml (Dewan et al., 2013). Furthermore, in healthy children (aged 6-12 years) in Mexico, very high levels of γ-HCH were reported in 3 communities (mean 1639.6 ng/g lipid; (Antonio et al., 2013), compared to Site 3 of this study (956 ng/g lipid). These high γ-HCH levels are not consistent throughout Mexico, as shown by Waliszewski, where γ-HCH was not detected in the serum of the inhabitants (Waliszewski et al., 2012). Furthermore, lower levels of γ-HCH were seen in male and female residents in Hong Kong (161 ng/g lipid) and in Romania (127 ng/g lipid) (Dirtu et al., 2006; Wang et al., 2013).

In SA, Mexico and India, where agriculture is an important sector, higher levels of γ-HCH have been found in the population, indicating that agriculture plays a role in γ-HCH persistence in exposed individuals. In SA, γ-HCH was produced until the 1980s, and only banned for use in 2009. The samples for the current study were collected in 2008, therefore a follow up study of this cohort is recommended to ascertain if the banning of γ-HCH use would have decreased the γ-HCH concentrations in the study population.

Similar to γ-HCH, endosulfan concentrations in maternal plasma were significantly higher in women residing in Site 3, compared to those residing in Sites 1 and 2. The high maternal levels of endosulfan in Site 3 are most probably the result of endosulfan usage in extensive commercial and subsistence farming in the area, and are indicative of on-going exposure. In addition, strong positive correlations were found between γ-HCH and endosulfan in maternal plasma, indicating a similar source of exposure, and most probably the use of a pesticide formulation, containing both γ-HCH and endosulfan. The OC pesticide, endosulfan, continues to be widely used as an

insecticide across the globe, including SA, with technical formulations dominated by endosulfan 1.

In SA (Vaal Triangle region), endosulfan has been found in soil and sediment media (Quinn et al., 2009). Additionally, in the Western Cape province, endosulfan has been found in the Lourens River (Schulz, 2001), in rural surface and ground drinking water sources (Dalvie et al., 2003), and in farm workers (Dalvie et al., 2009). SA is a signatory to the Rotterdam Convention and therefore is legally obliged to implement the local banning of endosulfan. Since all sales and use of endosulfan were terminated on the 31st April 2012, it is anticipated that levels of endosulfan will decrease in the future.

Globally, levels of endosulfan 1 do not show a declining trend in atmospheric monitoring data, reflecting on-going use of this pesticide (Weber et al., 2010). However, a recent New Delhi – India study has shown a decrease in endosulfan concentrations in maternal blood, between the years 2008 and 2012, from 3700 to 2200 pg/ml (Pathak et al., 2008; Sharma et al., 2012). These endosulfan concentrations are still much higher than the mean concentration observed in this SA study, i.e. 837 pg/ml. In Brazil, lower concentrations of endosulfan (Sarcinelli et al., 2003) were reported in delivering mothers in 2003 (108 pg/ml); however, current levels reported on endosulfan in the general popuation were far higher (10400 pg/ml) (Freire et al., 2012).

Researchers in Spain reported a very wide range in endosulfan levels. Extremely high levels of endosulfan 2 (76380 pg/ml) were found in maternal serum (Jimenez Torres et al., 2006), compared to the levels reported (1310 pg/ml) in cord serum (Jimenez Torres et al., 2006; Mariscal-Arcas et al., 2010); levels of 4.02 ng/g were found in placenta (Freire et al., 2011); and one study reported non-dectable levels in serum in the general population (Aurrekoetxea Agirre et al., 2011). This current SA study found higher endosulfan concentrations (mean of 837 pg/ml) when compared to studies carried out in Mexico, in pregnant women before delivery (153 pg/ml) (Alvarado-Hernandez et al., 2013), and in children aged 6-12 years (250 pg/ml) (Meza-Montenegro et al., 2013).

All other pesticides investigated in this SA study (HCB; α, β-HCH; *cis*-permethrin; cyfluthrin; cypermethrin; deltamethrin) showed low levels in blood components. In contrast, reports from

Mexico, Spain and Brazil found β-HCH to be the dominant HCH isomer, in higher concentrations (Alvarado-Hernandez et al., 2013; Aurrekoetxea Agirre et al., 2011; Freire et al., 2012; Wang et al., 2013). β -HCH is one of the five stable isomers of technical HCH and due to its persistence, it can still be detected at low background levels in all environmental media.

Pyrethroids are used for malaria control in the SA study sites, nevertheless, very low concentrations were detected in all three sites. This may be due to the fact that the samples chosen were plasma instead of urine, even though urine has been found to be more suited for biological monitoring to detect the pyrethroids. Pyrethroid metabolites are more stable in urine, whereas in plasma they are more susceptible to further bio-degeneration (Leng et al., 1997). Furthermore, due to the short half-life of pyrethroids (2.5 – 12 hours), samples have to be collected directly at the end of exposure.

The concentrations of Polybrominated Diphenyl Ether (PBDE) isomers, 28, 49, 71, 47, 66, 77, 100, 119, 99, 85, 154, 153, 138 were also found to be low in maternal plasma samples across all three sites in this study. As these sites are predominantly rural, lower levels are expected, as the concentrations of PBDEs in air samples have been shown to be higher in urban and industrialised locations across Europe, when compared with rural or remote regions (Jaward et al., 2004). However, Australia reported no difference between the rural and urban residential dust samples, except for PBDE 209 (Stasinska et al., 2013). Relatively high concentrations of PBDEs (28, 47, 71 and 75) in SA have been detected in leachates, in landfill sites in an industrialised region (Odusanya et al., 2009), but low levels were detected in a river catchment area in Gauteng (Olukunle et al., 2012). In Limpopo province, which is situated in the north, bordering Zimbabwe, the presence of PBDEs was reported in breast milk samples, with PBDE 183 having the highest mean value (Darnerud et al., 2011). The authors suggested that PBDE 183 concentrations were elevated due to either specific PBDE usage or contamination. Although low levels of PBDE were found in maternal plasma in the current study, the afore-mentioned reports indicate higher levels of PBDE in certain study areas. In Canada, a greater percentage detection of PBDEs was found in pregnant women (33 - 86 %) before delivery (Foster et al., 2012), and in children; PBDE 47 was detected in all samples, compared to this current study (0 – 9%) (Turgeon O'Brien et al., 2012).

6.1.2. Main predictors of exposure

6.1.2.1. Study Site

Subjects in study Site 1 were not only exposed to elevated concentrations of DDT from IRS, but also to γ-HCH, endosulfan and low levels of Hg. The concomitant prenatal exposure of some POPs and Hg has deleterious effects on neurodevelopment and immune system function (Donaldson et al., 2010). In this region, historically, DDT has been used to curtail and stop malaria infections and death. However, in terms of other contaminants, concerted efforts should be made to reduce exposure to γ-HCH, endosulfan and Hg. Subjects are exposed to γ-HCH and endosulfan from agricultural activity, and as both chemicals have been banned since the collection of the samples for the current study, a reduction in the levels is anticipated in the future, assuming no illegal use of the chemicals. Exposure to Hg is suspected to come from environmental conditions, probably due to the influx of pollutants from surrounding mining, water bodies, as well as other industrial and farming activities, as well as the consumption of fish.

In study Site 3 (Empangeni), significantly higher levels of γ-HCH and endosulfan were found when compared with the other two areas, most probably due to the large number of commercial farms, as well as extensive subsistence farming taking place in this area. A media statement in South Africa, in 2010, reported that insecticides that contain the active ingredient γ-HCH were sold in many nurseries and other retail outlets, despite a national ban on the use of such products one year previously (AVCASA, 2010). Educating the farm workers and owners on the environmental and health effects of the compounds may play an important role in stopping the use of these chemicals. The authorities also need to play a stronger role in the discontinuation of these compounds.

6.1.2.2. Age

In the current study, DDT and γ-HCH levels significantly decreased as the maternal age increased. These findings are contradictory, as many reports show positive and statistically significant associations between age and concentrations of DDT and other POPs (Arrebola et al., 2009; Llop et al., 2010; Valera et al., 2013; Wolff et al., 2005). In the scientific literature, the phenomenon is explained as, the older the subject, the greater the accumulation time of the

compounds due to the relatively long half-lives of many of the OCs (Grandjean et al., 2008; Jakszyn et al., 2009; Koppen et al., 2009; Llop et al., 2010). Pharmacokinetic modelling suggests that accumulation with age requires a continuous supply. From placental transfer, through childhood to adulthood, the body burden (and thus the serum / plasma levels) can reflect the time since peak exposure (past, recent and current), the year born, and the body type (lean versus obese). It is possible that the elevated levels of DDT (increased body burden) in this study may have an effect on the half-life of this chemical. Although suggested in the literature, no studies have been done to show the effect of high / low body burden on the half-life of OCs, or possible changes in half-life for different age groups (Bates et al., 2004). Also, in pregnant women, additional factors may contribute to the variation in pesticide levels, such as potential dilution effect as a consequence of weight gain, different sources of exposure, age, physical activity, diet and toxicokinetics (Kotlyar and Carson, 1999; Wolff et al., 2005).

6.1.2.3. Drinking Water

Drinking borehole water instead of municipal water resulted in an increase in γ-HCH levels, indicating that the water may be contaminated by the surrounding environment. A major contribution to chemical contamination is wastewater discharges that negatively impact on water quality, due to both the organic and inorganic constituents of wastewater. Additional contamination may come from agricultural activities in which fertilisers and pesticides are used throughout the year. Globally, the more agricultural activity in an area, the more contamination of pesticides is possible in the wastewater. Since Site 3 has larger areas of commercial and subsistence farming, the water is more likely to be contaminated, compared to Sites 1 and 2.

6.1.2.4. Dietary Predictors

Fish is important in a healthy diet, in that it is a lean and low-calorie source of protein. However, some fish may contain MeHg or other OC chemicals, at sufficiently high levels to be of concern. Unless there are direct sources present, the main source of OC chemicals is the diet, with fish and marine mammals being the main contributors.

In this study, DDT as well as Hg levels increased as the consumption of fish increased, but fish consumption had no effect on γ-HCH and endosulfan levels. Only tinned fish was positively

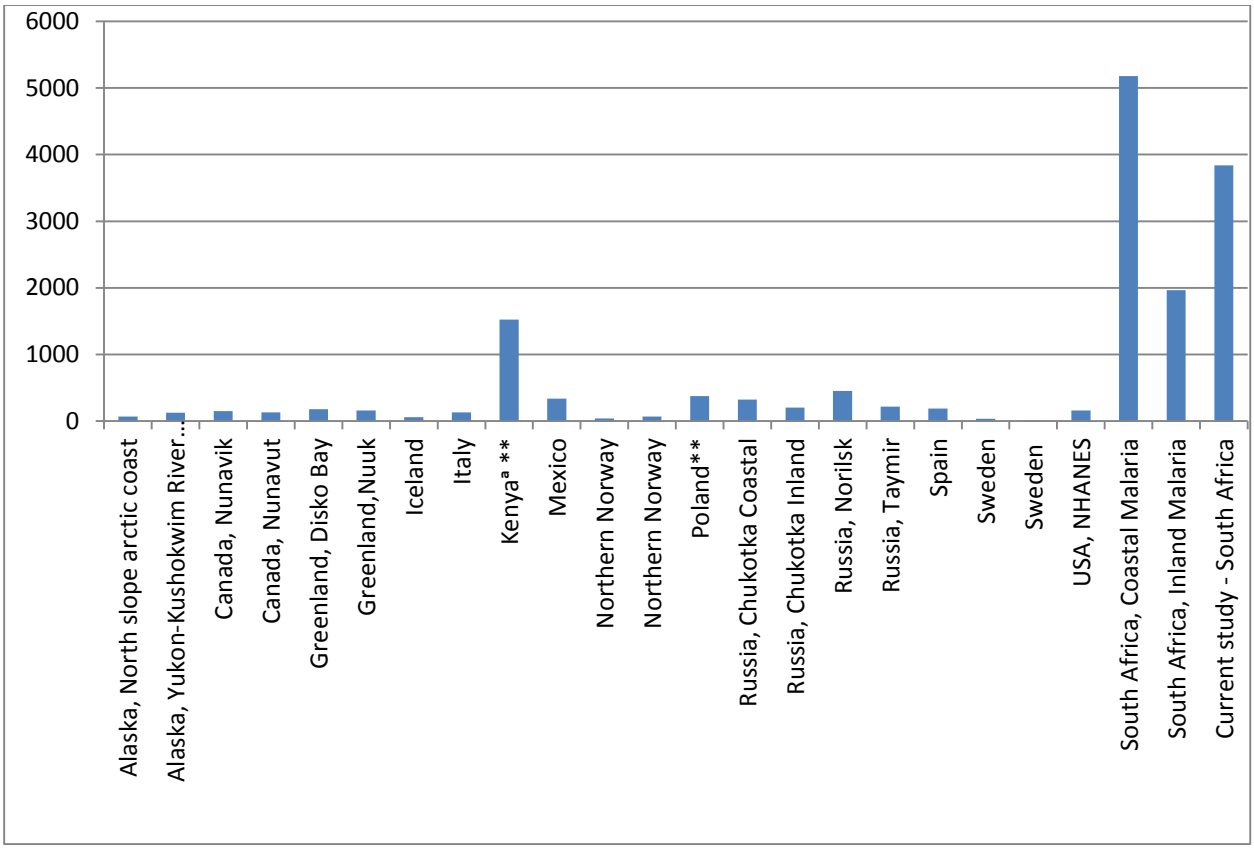
associated with DDT levels, indicating that not only the amount of fish, but also the type of fish is important. Tinned fish is an economically viable option which is more readily available than fresh fish. The KwaZulu Natal coast has warm water fish species, whereas most studies have been done with cold water fish species, and therefore not easily comparable. The consumption of fish in this study did not influence the levels of DDT or Hg to a large extent, unlike some studies done in the northern hemisphere (Furberg et al., 2002; Kvalem et al., 2009; Sandanger et al., 2003a). In Norway (Kvalem et al., 2009) it was found that fish and seafood were closely associated with coastal living, with fatty fish being the main source of protein. One study reported that boiled fresh cod-liver oil contained significant levels of *p,p'*-DDE and PCBs (Sandanger et al., 2003a). A study along the coast of North Norway, with a high lean fish / seafood intake, observed a significant association between OC plasma levels and seagull egg intake (Furberg et al., 2002). Another study showed that fatty fish contributes more strongly to the intake of OCs, when compared to lean fish (Hansen et al., 2010). In Vietnam it was found that the concentrations of contaminants were dependent on the size, age, species and amount of fish, as well as whether contaminants were from local sources and / or environmental deposition by long-range transport (Kannan et al., 1992).

This SA study found that eating processed meat had a protective effect, reducing γ-HCH and endosulfan levels. This is most probably due to the increased consumption of bought processed meat, when compared with local meat which may be contaminated with pesticides. In SA, consumption of processed meat is a lifestyle indicator and may also point toward a decreased consumption of fruits and vegetables. In general, consumption of fish, meat, dairy products and fats as well as fruits and vegetables are considered a source of exposure through the diet (Gasull et al., 2011). These dietary indicators were included in the linear regression models, but have been shown to not play a significant role, except for the processed meat. This may be due to changes in diet of individuals and differences in pesticide levels in the same food item, depending on where and when it was consumed, as well as the dietary habits before pregnancy.

6.2. Global comparison of pesticides and mercury levels

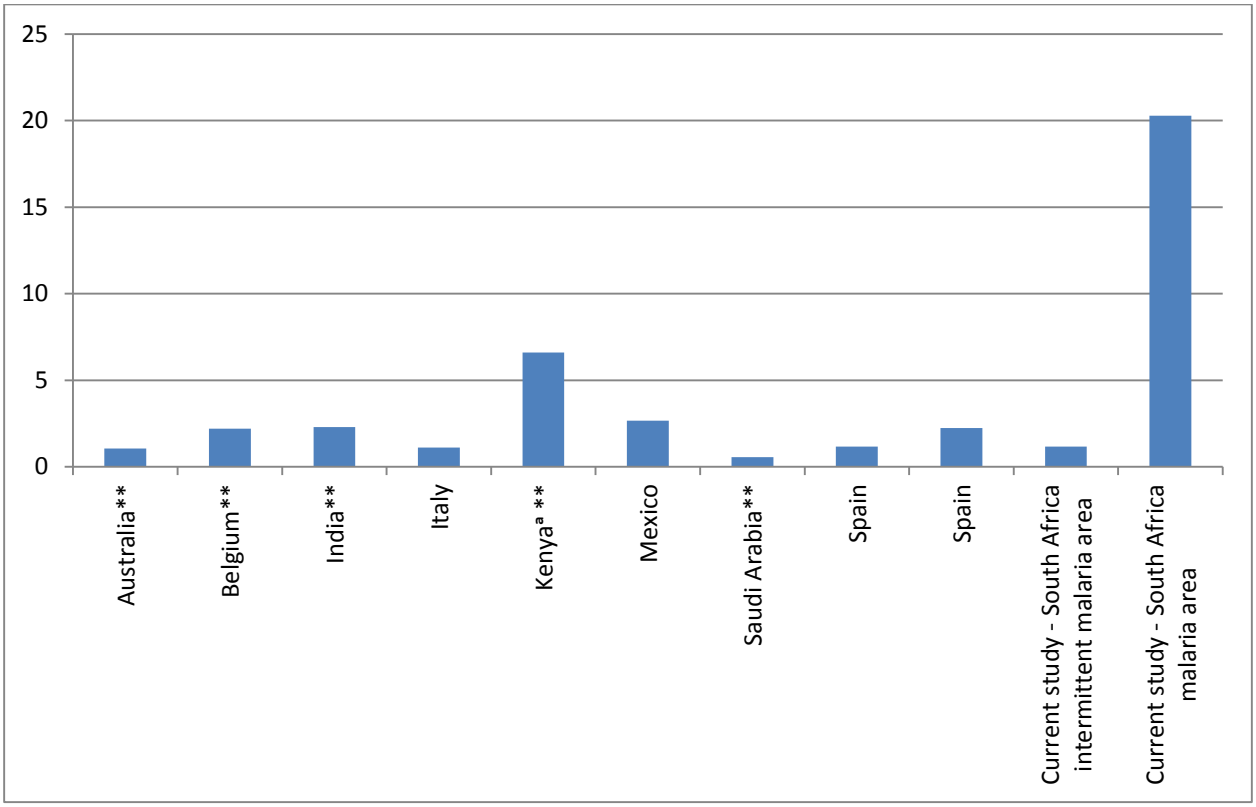
This study clearly shows higher concentration of DDE in SA, when compared with other countries (Figure 17). High levels of DDT were also found in blood samples in agricultural workers in Bolivia and Mexican inhabitants. For γ-HCH, South Africa has similar levels in maternal blood samples (Figure 20), to those found in India (Dewan et al., 2013; Pathak et al., 2008), but higher levels than those found in Australia (Reid et al., 2013), Brazil (Sarcinelli et al., 2003), Poland (Jaraczewska et al., 2006), Mexico (Rodriguez-Dozal et al., 2012) and Kenya (Kanja et al., 1992) (Figure 20). The distribution of relatively volatile OC compounds (such as hexachlorobenzene) is dependent on latitude and demonstrates the global distillation effect (geochemical process where POPs are known to be transported from warmer to cooler regions of the Earth, particularly to the Arctic and mountain tops). Less volatile OC compounds (such as endosulfan) are not as effectively distilled and tend to remain in the region of use (Simonich and Hites, 1995). DDT and γ-HCH are semi-volatile, i.e. they evaporate, but very slowly, and therefore the effect of global distillation is slower. Thus, these chemicals occur initially in high levels in the environment, close to the region of use, before they are globally distributed. β-HCH concentrations were found to be very low in maternal samples from SA, compared to those found in Russia, Spain, Ireland and Mexico (Figure 19). Similarly, Hg levels, although detected in 100% of the maternal samples in SA, were lower than most countries (Figure 21). The different exposure patterns for the various compounds indicate geographical variations on the human body fluid concentrations. Both global emissions with long-range transport and local sources are significant, in terms of individual and population exposures. It is important to note that when comparing population concentrations between studies, a number of factors have to be taken into consideration, such as: year of sampling, and characteristics of the study group such as age, gender, area of residence, diet and obstetrical history (Porta et al., 2008b; Sandanger et al., 2009). Special attention should be given to the analytical methods and quality assurance measures used; taking into account the matrices in which the concentrations are measured (e.g. whole blood, serum or plasma) and the units used to express the concentrations e.g., wet-weight or lipid-adjusted for OCs, in plasma or serum.

Figure 17: Comparison between countries, of maternal *p,p'*-DDE (GM) ng/g lipids.



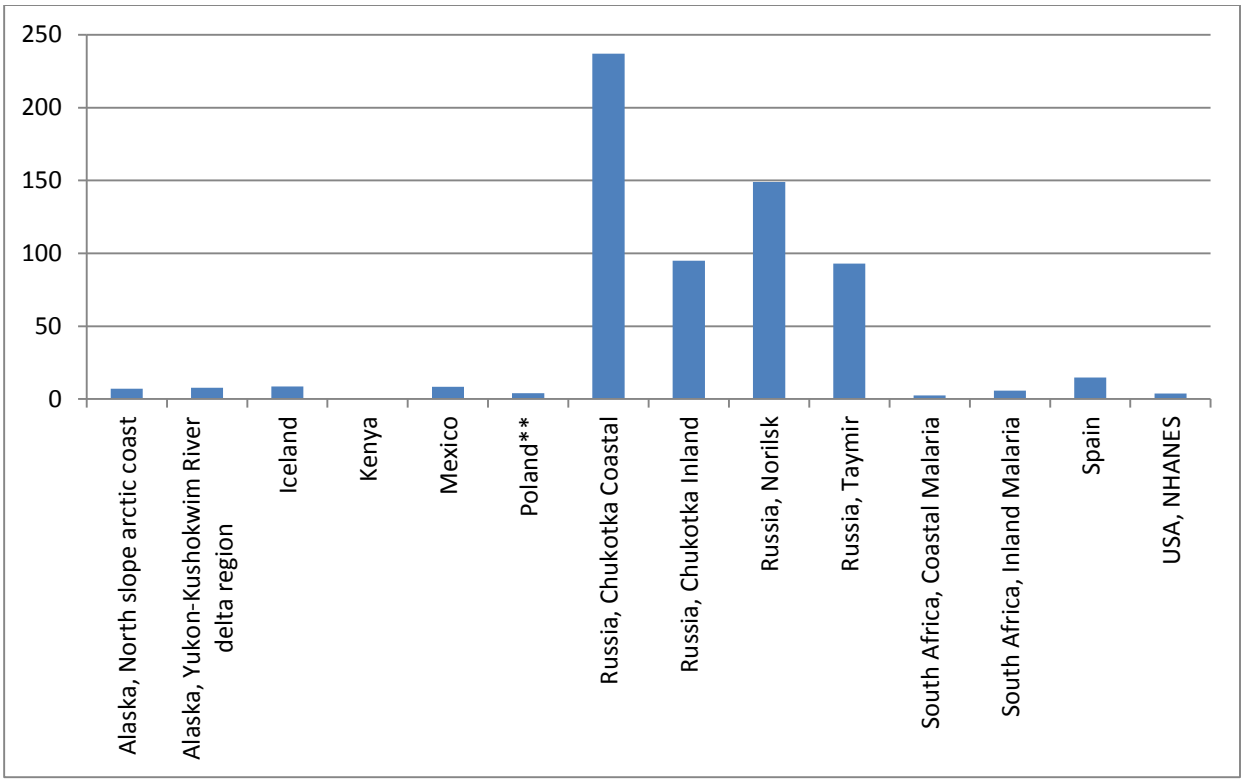
Location of study (n) year of study: Alaska, North slope arctic coast (43) 1999-2003 (AMAP, 2009); Alaska, Yukon-Kushokwim River (206) 2004-2006 (AMAP, 2009); Canada, Nunavik (42) 2004-2007(AMAP, 2009); Canada, Nunavut (99) 2005-2007(AMAP, 2009); Greenland, Disko Bay (20) 2006(AMAP, 2009); Greenland, Nuuk (10) 2005(AMAP, 2009); Iceland (40)2004(AMAP, 2009); Italy (70) 2006(Bergonzi et al., 2009); Kenya (11) 1986(Kanja et al., 1992); Mexico (240) 2005-2006(Rodriguez-Dozal et al., 2012); Northern Norway (50) 2007-2008(Hansen et al., 2010); Northern Norway (10) 2004(AMAP, 2009); Poland (18) 2004 (Jaraczewska et al., 2006); Russia, Chukotka Coastal (68) 2001-2003(AMAP, 2009); Russia, Chukotka Inland (58) 2001-2003(AMAP, 2009); Russia, Norilsk (59) 2001-2003(AMAP, 2009); Russia, Taymir (69) 2001-2003(AMAP, 2009); Spain (541) 2003-2005(Llop et al., 2010); Sweden (25) 2007(AMAP, 2009); Sweden (323) 1996-1999(Glynn et al., 2007); USA, NHANES (179) 1999-2002(Wang et al., 2009); South Africa, Coastal Malaria (11) 2005-2006(Röllin et al., 2009b); South Africa, Inland Malaria (12) 2005-2006(Röllin et al., 2009b); Current study - South Africa (91).

Figure 18: Comparison between countries, of maternal *p,p'*-DDE (GM) ng/ml.



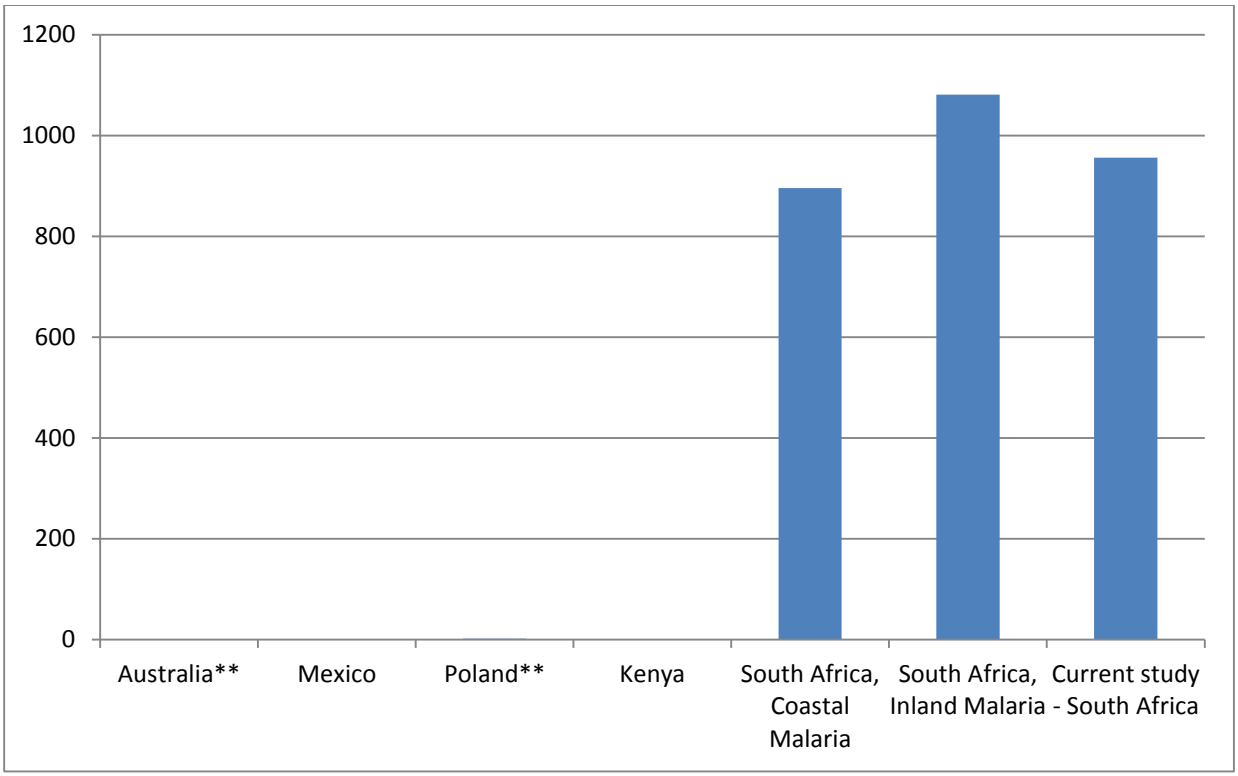
Location of study (n) year of study: Australia (167) 2009-2011 (Reid et al., 2013); Belgium (44) (Covaci et al., 2002); India (60) 2009-2010 (Dewan et al., 2013); Italy (70) 2006 (Bergonzi et al., 2009); Kenya (11) 1986 (Kanja et al., 1992); Mexico (240) 2005-2006 (Rodriguez-Dozal et al., 2012); Saudi Arabia (1518) 2005-2006 (Al-Saleh et al., 2012); Spain (541) 2003-2005(Llop et al., 2010); Spain (72) 1997-1999(Sala et al., 2001); Current study - South Africa intermittent malaria area (47); Current study – South Africa Malaria area (91).

Figure 19: Comparison between countries, of maternal β-HCH (GM) ng/g lipids.



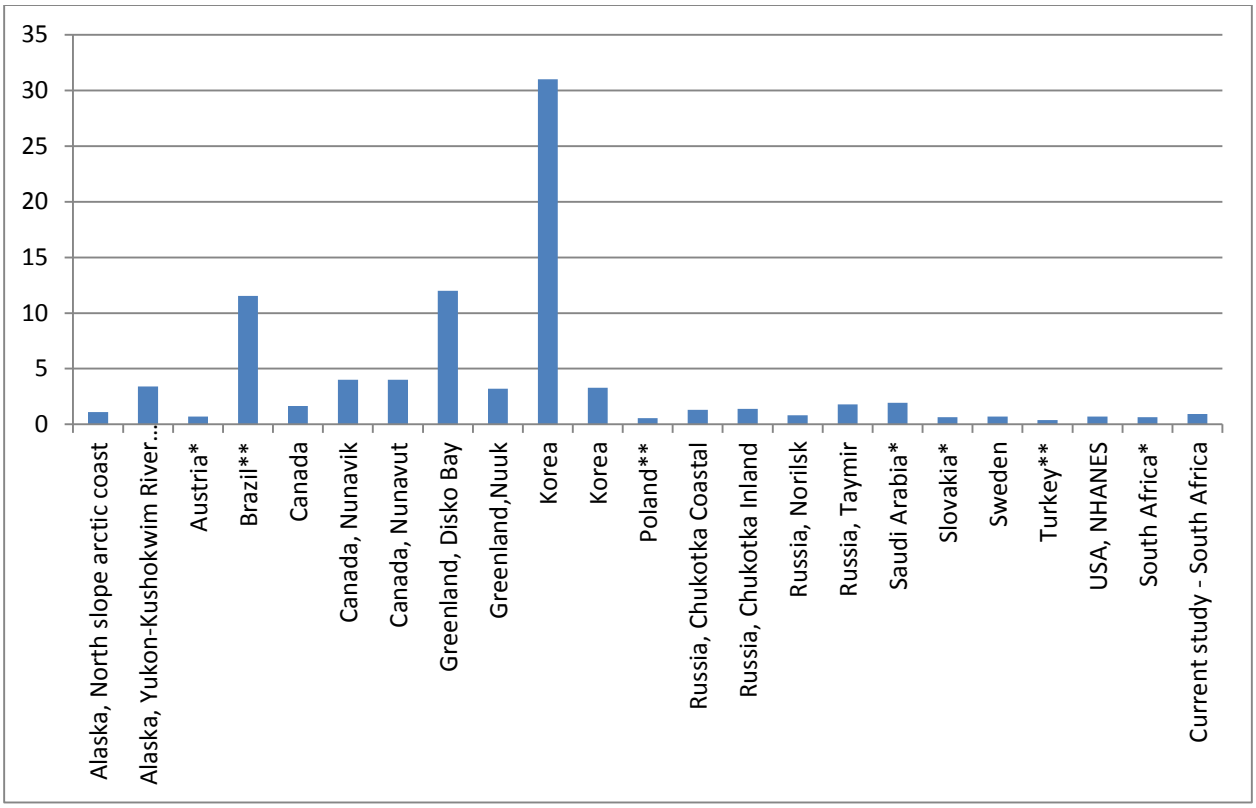
Location of study (n) year of study: Alaska, North slope arctic coast (43) 1999-2003 (AMAP, 2009); Alaska, Yukon-Kushokwim River (206) 2004-2006 (AMAP, 2009); Iceland (40) 2004 (AMAP, 2009); Kenya (11) 1986(Kanja et al., 1992); Mexico (240) 2005-2006 (Rodriguez-Dozal et al., 2012); Poland (18) 2004 (Jaraczewska et al., 2006); Russia, Chukotka Coastal (68) 2001-2003 (AMAP, 2009); Russia, Chukotka Inland (58) 2001-2003 (AMAP, 2009); Russia, Norilsk (59) 2001-2003 (AMAP, 2009); Russia, Taymir (69) 2001-2003 (AMAP, 2009); South Africa, Coastal Malaria (11) 2005-2006 (Röllin et al., 2009b); South Africa, Inland Malaria (12) 2005-2006(Röllin et al., 2009b) Spain (541) 2003-2005 (Llop et al., 2010); USA, NHANES (179) 1999-2002 (Wang et al., 2009).

Figure 20: Comparison between countries, of maternal γ-HCH (GM) ng/g lipids.



Location of study (n) year of study: Australia (167) 2009-2011 (Reid et al., 2013); Mexico (240) 2005-2006 (Rodriguez-Dozal et al., 2012); Poland (18) 2004 (Jaraczewska et al., 2006; Sandanger et al., 2007); Kenya (11) 1986 (Kanja et al., 1992); South Africa, Coastal Malaria (11) 2005-2006 (Röllin et al., 2009b); South Africa, Inland Malaria (12) 2005-2006 (Röllin et al., 2009b); Current study - South Africa (49).

Figure 21: Comparison between countries, of maternal mercury (GM) µg/l.



Location of study (n) year of study: Alaska, North slope arctic coast (43) 1999-2003 (AMAP, 2009); Alaska, Yukon-Kushokwim River (75) 2004-2006 (AMAP, 2009); Austria (87) 2005(Gundacker et al., 2010); Brazil (1510) 2000-2002(Santos et al., 2007); Canada (351) 1994-1999(Butler Walker et al., 2006); Canada, Nunavik (42) 2004-2007(AMAP, 2009); Canada, Nunavut (99) 2005-2007(AMAP, 2009); Greenland, Disko Bay (20) 2006(AMAP, 2009); Greenland, Nuuk (10) 2005(AMAP, 2009); Korea (797) 2006-2010(Kim et al., 2011); Korea (417) 2006-2008(Lee et al., 2010); Poland (231) 2001-2003(Jedrychowski et al., 2006); Russia, Chukotka Coastal (68) 2001-2003(AMAP, 2009); Russia, Chukotka Inland (58) 2001-2003(AMAP, 2009); Russia, Norilsk (59) 2001-2003(AMAP, 2009); Russia, Taymir (69) 2001-2003(AMAP, 2009); Saudi Arabia (1574) 2005-2006(Al-Saleh et al., 2011); Slovakia (99)(Palkovicova et al., 2008); Sweden (23) 2007(AMAP, 2009); Turkey (143) 2004-2006(Unuvar et al., 2007); USA, NHANES (622) 2003-2006(Jones et al., 2010); South Africa (62)(Rudge et al., 2009); Current study - South Africa (99).

Key:

* = Median

** = Mean

a = µg/kg

6.3. Gaps in data

Pesticide use in SA is an integral part of every-day life. The farmer, consumer, exporter and end-user of natural resources, such as water, are exposed to pesticides. In addition, pesticide exposure could occur from malaria prevention programmes. Pesticide research is well established in SA; however, limitations exist in terms of technical expertise (laboratory competency and equipment). Although pesticides have been detected in most media, there is still a lack of knowledge of pesticide background levels. These levels are required so as to make realistic impact and health assessments when studying highly contaminated areas.

There is a problem in some areas with low literacy, and in many cases proper training on the use of pesticides is not provided. In addition, obsolete pesticide stockpiles can leach into the environment and have disastrous effects on ground water. Although a stockpile control programme is in place to remove banned pesticides, and agricultural users and retailers have to declare the use of pesticides, very little control mechanism is actually implemented.

Although pesticides are required, the advantages must be weighed against the negative side effects. Serious consideration must be given to promoting and expanding the correct use of less harmful substances.

There is a lack of data on prenatal exposure to POPs in the southern hemisphere, and especially in Africa. Due to good climatic conditions, the southern hemisphere is ideal for extensive farming and the use of not only the 'old' POPs, but also the new emerging POPs, such as PFOS and flame retardants. More studies are required in the southern hemisphere.

6.4. Limitations

This investigation was based on a cross-sectional study, i.e. simultaneous analyses of exposures and effects. The exposure component is the focus of the scientific papers.

To obtain high validity, and thus dependable results, the challenge is to minimise the sources of random and systematic errors. Random errors were reduced by quality control measures being in place at both the NILU and NIOH (e.g. use of reference and control samples, and the laboratories

participation in external-proficiency testing), but could have been increased by the lower sample size in the intermittent malaria site. Method errors were considered minimal, as all protocol procedures and measurements were standardised, both in the field and in the laboratory according to the AMAP protocol.

In order to have increased validity, an absence of bias and generalisation of the population are required.

For DDT, the intermittent study site was included between the malaria and non-malaria sites as a form of a second control, and therefore only a small number of women participated. The sample size was also limited by costs. The power of analyses was reduced due to the small sample size in the intermittent malaria area. The unequal sample sizes could have added to the sampling bias.

The recruitment of subjects only from government hospitals may have caused a selection bias in terms of socioeconomic status. Including mothers from the private hospitals in this investigation could potentially have resulted in a more accurate representation of the women residing in all three study sites. However private clinics are very scarce in deeply rural areas and even if present, these clinics care for a very small percentage of the population. Participating women were predominantly African Blacks. A better representation of population groups/ethnicity within the samples chosen for this study would have increased the validity of the results for the population as a whole. If too many traits are similar, then selection bias is a strong possibility.

Although every effort was made to acquire accurate information from the pregnant women, there is the possibility that some of the information could be biased, due to cultural misunderstandings or imprecise recall. However, the language barrier was eradicated by asking the interview questions in the women's preferred language.

The type of pesticides used in and around the residences was requested in the questionnaire, but many of the subjects were not able to name the type of pesticide used, which could have added value to the findings and more accurate predictors could have been determined.

6.5. Policy formulation

The Stockholm Convention is a global treaty which aims to protect humans, animals and the environment from exposure to POPs. The convention was adopted in 2001. SA has accepted the convention as 23rd May 2004. SA has also accepted the Rotterdam Convention as of June 2006. A decision has to be made by SA to eliminate or restrict the use of chemicals (DDT, endosulfan) under the Stockholm Convention, and implement an import control response for chemicals under the Rotterdam Convention. These thesis research findings will affect the way that authorities assess pesticide control, in terms of protecting the population from negative health effects. The high concentrations of DDT, endosulfan and γ-HCH levels found in this study indicate a need to assess the source of exposure and eliminate these sources as far as possible, so as to reduce the local and global burden. The results will help the policy makers on contaminants not only in SA but also globally.

6.6. Concluding remarks

This is one of the first studies to measure prenatal exposure to DDT resulting from controlled IRS with DDT in coastal rural settings. As expected, this study confirms that the levels of DDT and its metabolites from IRS, as measured in pregnant women, were significantly higher than in regions where IRS does not occur. In addition, the subjects from the intermittent and non-malaria areas were also exposed to DDT, and the observed differences in concentrations were significant. The reason for the elevated levels in the non-malaria area are not clear, and considering the long residence time in each place, the migration of people from contaminated regions is not a likely reason. The low *p,p'*-DDE / *p,p'*-DDT ratios in the two sites confirm that the exposure is not only due to food alone, and suggests recent, scattered on-going use of DDT in the surrounding areas. Further efforts must be made to identify the source of DDT exposure in these two sites.

The malaria endemic site showed the highest levels of DDT and Hg in maternal blood, compared to the other two study sites. Although Hg was detected in 100 % of maternal and cord blood samples analysed, it was found to be in lower concentrations than in some other countries. In addition, the women who participated in the study are also exposed to γ-HCH and endosulfan. Although low levels of Hg and the other pesticides were detected, the negative parallel

neurotoxic effects for vulnerable groups such as the foetus, newborns and growing children, remain a concern. In addition, the combination of high *p,p'*-DDE and *p,p'*-DDT levels and some Hg exposure, requires extra attention in preventing possible health effects, especially to the foetus and during breastfeeding. Education should be provided as a priority to household members on ways to restrict exposure following IRS.

The other pesticides measured, viz γ-HCH and endosulfan 1 and 2, were found to be elevated in all three regions, with significantly higher concentrations in Site 3. Agricultural spraying may be a common source, but considering the elevated levels in Site 3, there is reason to believe there are other sources. Thus, the exposure patterns are unique for each site. Furthermore, these concentrations of γ-HCH and endosulfan were higher than those found in other countries. With γ-HCH being on the Stockholm POP list, and endosulfan being on the 9 new POP lists, efforts must be made to identify and remove sources of exposure, to curtail the use of these pesticides.

The results presented are of particular value to policy makers and regulatory toxicology organisations, as they characterise the extent of controlled exposure to DDT, used exclusively for IRS purposes. The findings of this study will form a basis for further investigation into foetal exposure to pollutants.

Future studies are required to assess the health effects on the children of this study cohort, the sources of DDT in non-IRS sites, as well as γ-HCH and endosulfan sources, so as to reduce exposure.

In addition to exposure to a variety of pesticides, the effect of climate change will exacerbate the situation, leading to catastrophic outcomes such as accelerating sea level rise, droughts, floods, storms and heat waves. These phenomena will have broad based negative impacts, especially for the poorest and most vulnerable people living along the South African coastline, by increasing the prevalence of malaria, as well as other vector-borne and diarrhoeal diseases; decreasing agricultural yields; and having many more detrimental effects on health, habitats and the economy.

The period from the embryonic stages to the growth of the child is very sensitive to environmental impact and changes in the climate. The foetus is prone to exposure to different metals and POPs through the placenta. Substances such as Hg and lead, can affect the developing brain before birth and during childhood. Thus, the monitoring of certain compounds in mother-child cohorts is highly recommended.

Mankind will have to endeavor to set policies, and enforce practices and behavior norms that will provide long-term economic opportunities and improved quality of life around the world, while maintaining a sustainable climate and viable ecosystems.

6.7. Future perspectives

As evident from this study, pregnant women in SA are exposed to numerous pollutants (DDT, γ-HCH and endosulfan) at concentrations exceeding benchmark levels, indicating serious prenatal exposures in the three sites. The sources of exposure in the various sites require more in-depth investigation. Further studies should concentrate on other geographical regions with special attention given to simultaneous exposure to mixtures of contaminants. To assess the developmental risks associated with exposure to selected contaminants, follow-up cohort studies should be initiated. A concerted effort is necessary to increase data quality and availability, and to develop new environmental monitoring and surveillance databases, including a traceable birth register. One recommendation is to establish a multidisciplinary scientific network between the northern and southern hemispheres, in an effort to gain a better understanding of the dynamics of the contaminants that are having negative impacts on populations and environments, across the globe. The added dimension of climate change and its detrimental outcomes presents a unique opportunity to investigate the transport, trends and potential health impacts of the contaminants that are less likely to remain in the Arctic, due to the global distillation effect. Higher temperatures, coupled with the decimation of sea ice, can result in constant re-emissions of contaminants to the atmosphere and their transportation out of the Arctic region, mostly in a southward direction. Since it is predicted that the southern hemisphere will be affected the most by climatic changes, especially the coastal regions, more assessments and targeted investigations into the state and impacts of contaminants are required in this region, with some urgency.

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SUBJECT INFORMATION SHEET

Dear Mother

We are researchers from the Medical Research Council (my name is…..) and we are studying levels of pollutants in the environment and maternal health in selected areas of South Africa. Specifically, the study will be looking at the levels of pollutants in the environment and in the blood and urine of expectant mothers admitted for delivery in clinics in these areas. Studies conducted in other parts of the world showed that in certain areas environmental pollution may have an effect on the foetus and newborns. By conducting this research in South Africa we will be able to determine the current situation in our country.

We would appreciate it very much if you agree to participate in our study. If you agree, we will be asking you to answer series of questions, which should take 35 minutes. The interview will focus on general information about yourself, including your age, health and diet, your house type, and your occupation. A trained research assistant will be interviewing you.

We also ask that you allow us to take a blood sample of approximately 20ml from you (4 teaspoons) once only, and to donate about 20ml of urine. A professional nursing sister will take the blood sample at the clinic. Sterile, disposable equipment will be used and disposed of immediately, so there is no chance of transfer of any infection from one subject to another. The technique is safe and there is only a slight prick as the needle passes through the skin. Over the years we have sampled blood from many hundreds of people in this way without any problems. We will measure the concentration of some metals and organic chemicals in your blood and urine.

We also ask you to allow us to take 10 ml of blood sample from the cord (umbilical cord/khujwana/nkaba) after your baby is delivered. This is done only after the cord is cut so there is no interference with delivery and your baby. Doctor attending to you will do it. Cord blood will also be analyzed for chemicals.

Finally we also ask your permission to obtain information from hospital records about your baby's weight and length, sex and gestational age, and health.

We do not know at this stage if this study will have a direct benefit to you, but if we find anything wrong with your results, we will come back to you. The results of this study will be published, but the names of you or your child will not be mentioned. You may request the results in relation to yourself and your child.

You are free to withdraw from the study at any time without having to give a reason. Remember that your participation in the study is completely voluntary and not taking part in it, or withdrawing from it, carries no penalty of any sort. Your health care will not be influenced.

If you would like to discuss the study further, or have any questions, please do not hesitate to contact, Dr Halina Röllin, telephone: 011 274 6064 during office hours.

SUBJECT INFORMED CONSENT

Name: Surname:	House/Plot Number:
Street:	Town:
Contact telephone number:	Age:
Clinic address:	
Subject Study Number:	Area Study Code:

I……

(Full name(s) and surname of participant)

hereby agree to participate in the Environment and Reproductive Health Study being undertaken by the Medical Research Council.

The research objectives have been explained to me and I understand that I will be required to complete a questionnaire about my health and diet, living conditions and occupation.

I also understand that I will be expected to donate approximately 20 ml of blood (4 teaspoons) and 20 ml of urine on my last visit to clinic before delivery or at delivery. Medical personnel will take blood under sterile conditions.

I understand that 10 ml of the blood from the cord (umbilical cord/khujwana/nkaba) will be collected after delivery by the doctor.

I give permission to hospital to disclose medical information regarding myself (age, weight before delivery, height, health status and medication) and details about baby (health at delivery, weight and length).

I understand that ethical approval for this investigation has been obtained.

I understand that there is no cost involved.

I understand that all results will be treated with strict confidentiality.

I acknowledge that the results of this research project may be published in medical and scientific journals; however, my name, and the names of my family, will not be mentioned. The results will be reported only as a group.

I understand my participation is voluntary, and that I am free to withdraw from the project at any time without prejudice. I further understand that should I request it, the results in relation to my child and myself will be made available to me.

Signature……date…….

MEDICAL RESEARCH COUNCIL

ENVIRONMENT & REPRODUCTIVE HEALTH STUDY

This questionnaire is part of the Medical Research Council environment and reproductive health study. We would like to request that you take the time to answer the questionnaire with the assistance of trained interviewer. We thank you in advance for your participation.

If you have questions or need more information, please do not hesitate to call Dr Halina Röllin at 011 274 6064(office hours).

SUBJECT STUDY NUMBER …… **AREA STUDY CODE…….**

Clinic Name: ......

Clinic address: …….....

Town/Province: ……

Interview date (date/month/year): .....

Interviewer: …….....

SECTION A: BACKGROUND DETAILS

In this section we would like to obtain a few background details about yourself.

1. What is your first name? .....……

2. What is your surname? .....…….

3. What is your home address?.....

   .....

   .....

4. What is your present contact telephone number
   ……

5. When were you born? (please give day, month and year)

   Where……

   Day ……

   Month ……

   Year …….

6. How many children do you already have:

   ……

7. How many daughters do you have: number: …… ages: …….

8. How many sons do you have: number: ……..ages ……..

9. What language do you usually speak at home (**please circle!**)

   1. English
   2. Afrikaans
   3. Xhosa
   4. Sotho
   5. Zulu
   6. Other (please specify) …….

10. What is your race/population group? (**please circle!**)

(This question is being asked because in South Africa population group is still closely linked to economic status, which in turn is closely linked to certain environmental factors.)

1. African black
2. Coloured
3. Asian
4. White

11. What is your nationality? …….

12. How would you describe your place of residence **(please circle one answer only)**

1. Urban (city)
2. Rural (farming community)
3. Peri urban (close to the city)
4. Informal settlement
5. Close to industrial site: (please specify) ……
6. Don't Know

13. How long have you lived at your present home address?

Years ……

Months ……

SECTION B: HOUSING

In this section we would like to have some information about the household you presently live in

1. Is this home: **(please circle)**

    1. Owned
    2. Rented

2. How would you describe your home? **(please circle)**

    1. House
    2. Flat
    3. Backyard dwelling
    4. Informal house (shack)
    5. Other (please specify) .....

3. How many rooms, not counting the kitchen, bathroom or toilet, does this home have? ……

4. What fuel is used most of the time for cooking? **(please circle)**

    1. Electricity
    2. Paraffin
    3. Gas
    4. Wood
    5. Coal
    6. Car batteries
    7. Other (please specify) .....……..

5. What fuel is used **most** of the time for heating the home? **(please circle)**

    1. Electricity

2. Paraffin

3. Gas

4. Wood

5. Coal

6. Car batteries

7. None

Other (please specify) .....

6. Does anyone regularly smoke at home? **(please circle!)**

1. Yes

2. No

7. How many people regularly smoke cigarettes in the home? (At least one cigarette per day at home)
.....……

8. Did you smoke: **(please circle)**

1. Before pregnancy

2 During pregnancy

3. Both

4. Number of cigarettes daily

9. Where do you get your drinking water from most of the time? **(please circle)**

1. Indoor tap

2. Outdoor tap

3. Rainwater tank

4. Borehole

5. River/stream

6. Other (please specify)…….

SECTION C: SOCIAL AND ENVIRONMENTAL ASPECTS

In this section we will ask some questions relating to you and other people living in your home.

1. Marital status: **(please circle)**

    1. Married
    2. Divorced
    3. Single
    4. Living together
    5. Widowed

2. How many people live in this house?

    1. Males older than 15 years
    2. Women (including yourself) older than 15 years
    3. Children aged 15 years or younger

3. What is your highest educational qualification?

    …….. .....

4. Do you have permanent job: **(please circle)**

    1. YES
    2. NO

    If yes, what type (seasonal or pemament) **please underline**

    Employer……..

    Occupation/Position……..

5. For how many years have you held your current job?

    …….

6. What does your husband/partner do at work?
……..
……
……

7. Where does he work?
……..
…….

8. What is the highest education qualification of your husband/partner?
…….. .....

9. How many years has he held his current job?

…….

10. How many people, living with you, have permanent jobs?
…….

11. Does anyone living in the house, work from home? (please circle)

    1. Yes
    2. No
    3. Don't know

    If yes, what do they do?

.....…….
…….

12. If maintaining or repairing your home, do you or handyman use lead-containing materials (paints, solders etc).? **(please circle)**

    1. Yes
    2. No
    3. Don't know

13. What is the total monthly income in your family?

.....

14. Describe the hobbies of people living in the house (for instance car repairs, pottery, welding, etc).

……

……
……

15. What is your opinion on air quality in your area? **(please circle)**

    1. Good
    2. Bad
    3. I don't know

16. Are there any sources of environmental pollution around your home? **(please circle)**

    1. Yes
    2. No
    3. If yes specify source ,……

    …….

17. How far is your home from the nearest the highway? …. .**Km**

18 Do you use pesticides for insect control (flies, bugs, cockroaches, mosquitoes, in your home?) **(please circle)**

    1. Yes
    2. No

19. If yes, are these pesticides used in: **(please circle)**

    1. Kitchen
    2. Living room
    3. Bedroom
    4. Others, please specify

    ……

20. What are the names of the pesticides do you use?

    ……..

21. How often do you use the pesticides?

    1. In a week

2. In a month

22. Where do you store these pesticides? ……

23. Do you grow your own food? (vegetables, fruits, others) **(please circle)**

1. Yes
2. No

If yes specify……

24. Do you use pesticides in you garden? **(please circle)**

1. Yes
2. No

If yes please specify which pesticide?
…….

25. Do you or a member of your household fish? **(please circle)**

1. Yes
2. No

Where do you fish? Please name the location …….

Please name the fish type ……

26. If yes do you consume this fish? **(please circle)**

1. Yes
2. No

27. Is your house sprayed regularly by Malaria Control Programme

1. Yes……
2. No

If yes how often…….

28. Is the crop at farms near your house sprayed.

1. Yes
2. No

SECTION D: INFORMATION ABOUT YOUR JOB

Please list in chronological order all the jobs you were engaged in for the period of more than 6 months, over the past 10 years. Also mention whether you were exposed to any chemicals during your work (which):

JOB DESCRIPTION AND WHERE	FROM	TO	DO YOU KNOW OF ANY CHEMICALS USED AT YOUR WORK PLACE

Chemicals check list (please circle Y/N)

1	Solvents (turpentine, spirits, paraffin,	Yes	No
2	Paints	Yes	No
3	Metals (in foundry, mine	Yes	No
4	Cleaning fluids (floor, windows	Yes	No
5	Polish (for floor, car polish	Yes	No
6	Paint removers	Yes	No
7	Oils/lubricants (grease….)	Yes	No
8	Spray paints	Yes	No
9	Spray oils	Yes	No
10	Others, please specify	Yes	No

SECTION E: HEALTH

In this section some information about your health status is requested.

1. Are you well at present?

    1. Yes
    2. No
    3. Don't know

2. If you are not well, what are the problems?

    ….....……

    ….....……

3. Do you suffer from any of the following? (**circle correct answers please)**

    1. Diabetes

        Since when / How long ……..

        Are you on medication for this condition? YES NO

    2. Thyroid gland

        Since when / How long ……..

        Are you on medication for this condition? YES NO

    3. Liver disease

        Since when / How long

        Are you on medication for this condition? YES NO

    4. Heart disease

        Since when / How long

        Are you on medication for this condition? YES NO

    5. High blood pressure

        Since when / How long

        Are you on medication for this condition? YES NO

    6. Infectious/parasite disease, **if yes please tick:**

1. TB (tuberculosis)

2. Pneumonia

3. Virus hepatitis

If other please specify ……..

7. Cancer YES NO

If yes, please specify …….

If yes, did you receive treatment: ……..

Please specify type of treatment…….

8. Are there any hereditary diseases in family (for example high blood pressure, lung disease, etc.?)

If yes, please specify ……..

9. Do you suffer from any other illnesses (for example skin condition etc):

1. Yes

2. No

If yes, please specify)….....

10. Have you ever been given any home remedies for illnesses or to improve your health (please circle)?

1. Yes

If yes, please specify……..

2. No

3. Don't know

11. Are you taking any prescription medication at present? (please circle)

    1. Yes:

    If yes, what medication are you taking, please specify:

    ……..

    ……

    2. No

    3. Don't know

12. Are you taking any special remedies during your pregnancy?

    If yes, please specify ……

    ……

13. How long did you breastfed your other children:

    1. Child 1 (oldest)……..

    2. Child 2…….

    3. Child 3…….

    4. Child 4……

    5. Child 5…….

14. Do you plan to breastfeed this child:

    1. Yes

    2. No

SECTION F: DIET AND LIFESTYLE

Please answer questions about your usual diet before and during pregnancy:

Table: Frequency of consumption of food / before / during pregnancy,
1 =seldom; 2= at least once a week; 3-= almost every day

TYPE OF FOODSTUFF	**BEFORE PREGNANCY**	**DURING PREGNANCY**	**FOOD LOCALLY PRODUCED (by respondent or in the area) Y/N**
PROTEINS			
Meat			
Poultry			
Processed meet (smoked sausage, ham etc)			
Tinned meat			
Eggs			
Fish fresh			
Fish tinned			
Fish smoked			
Sea food			
VEGETABLES AND FRUITS			
Vegetables root (potatoes, carrots, beetroot, onion etc)			
Vegetables leafy/ground (spinach, cabbage, lettuce, cucumbers, pumpkin, water melon etc)			
Vegetables vine/tree (mielie, beans, tomatoes, garlic)			
Fruits			
DAIRY PRODUCTS AND FATS			
Dairy products (milk)			
Butter and cheese			
Fats (oil, margarine)			
CARBOHYDRATES			
Cereals (mieliepap,rice,noodles)			
Bread			
Sugar			
FLUIDS			
Fresh fruit juices			
Soft drinks			
Bottled water			
NON FOOD			
Non food items specify			

2. How many cups of coffee do you drink?

    1. Daily

    2. Weekly

3. How many cups of tea do you drink?

    1. Daily

    2 . Weekly

4. How many bottles of beer do you drink?

    1. Weekly

    2. Monthly

    3. None

5. How many bottles of wine do you drink?

    1. Weekly

    2. Monthly

    3. None

6. How many glasses of vodka or other strong alcohols do you drink?

    1. Weekly

    2. Monthly

    3. None

7. Do you smoke?

    1. Yes

    2. No

8. If yes, for how many years have you smoked regularly? ……**years**

9. At what age did you start to smoke regularly? …….

10. What do you smoke? **(please circle)**

    cigarettes self-rolled cigarettes pipe cigars

11. How many cigarettes do you smoke daily?.....

12. If you do not smoke, did you smoke earlier? Yes No

13. At what age did you start to smoke regularly?.....

14. At what age did you quit smoking? …….

15. Within the last 6 months, did you take any drugs that influenced on your mood?

    1. Yes
    2. No
    3. Refused to answer

16. Do you have any hobby?

    1. Yes
    2. No
    3. If yes, what?

17 How many times you visited this clinic during pregnancy:……

END OF QUESTIONNAIRE,
THANK YOU for answering the questions, your assistance is highly appreciated.

MEDICAL RESEARCH COUNCIL

ENVIRONMENT & REPRODUCTIVE HEALTH STUDY

POST DELIVERY MEDICAL INFORMATION
To be completed by attending medical personnel or designated field worker.

This questionnaire is part of the Medical Research Council environment and maternal health study being conducted on women attending your prenatal and delivery clinic. The subject volunteered to participate in this study and agreed that we extract necessary information from their hospital records after delivery.

If you have questions or need more information, please do not hesitate to call
Dr Halina Röllin at 011 274 6064 (office hours).

SUBJECT STUDY NUMBER …… **AREA STUDY CODE…….**

Clinic Name: .....

Clinic address: …….....

Patient name: ……

Patient Hospital Code ……..

Delivery date: …….....

Name of Doctor or Sister attending: …….....

Was cord blood collected: ……Yes……No……

If no, please explain ……

SECTON A: MATERNAL INFORMATION

1. Maternal age: ……
2. Maternal weight before delivery: …… Kilograms
3. Maternal height: ……cm…
4. Parity: …….
5. Previous spontaneous abortions 1. trimester: (if available)
6. Previous spontaneous abortions 2. trimester (if available)
7. Previous preterm abortions <week 37 (if available)
8. Major illnesses of mother – if any:

   ……

   ……
9. Any complication during pregnancy (hypertension, pre-eclampsia, infections)

   ……..

   ……..
10. Medication of mother – if daily:

    ……

    ……..

SECTION B: INFORMATION ABOUT THE NEWBORN CHILD

1. Birth weight of baby: ……kg…..
2. Birth length of baby: ……cm
3. Caput circumference of baby: ……cm
4. Gestation age of baby (based on Naegele term):
5. APGAR score (any sign of asphyxia?) ……
6. Gender of baby ……
7. Congenital malformations (visible at birth) ……
8. Any delivery complications, if yes, what kind (section Caesarean, forceps, vacuum, retentio placenta)

   ……..

   ……
9. Any other medical observations or conditions

   .....……..

   ……..

END OF QUESTIONNAIRE
THANK YOU

ISBN xxx-xx-xxxx-xxx-x