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Miljø- og kostholdseksponering for persistente giftige stoffer (PTS) og sporstoffer i svangerskapet og assosiasjoner til fødselsutfall.

Langsomt nedbrytbare organiske miljøgifter (Persistent organic pollutants; POPs) og toksiske metaller har kjente effekter på helse og utvikling hos barn. En viktig samlebetegnelse er persisterende toksiske substanser (PTS). Disse stoffene passerer fra mor til barn gjennom morkakebarriere og navlesnor og senere gjennom brystmelk. På grunn av dette har det ufødte og nyfødte barn risiko for helseeffekter på avgjørende stadier av vekst og utvikling. På den andre side er også ubalanse i viktige mineraler og sporelementer knyttet til helseeffekter. Nivåer av både miljøgifter og sporelementer gir derfor indikasjoner for mulig risiko for fosteret.

Det meste av studier på miljøgifter og effekter for gravide kvinner og deres barn er utført i Europa og Nord-Amerika. En viktig kilde til informasjon er Arctic Monitoring and Assessment Programme (AMAP). AMAP startet i 1991 og inkluderer målinger av PTS-nivåer i humane prøver fra de åtte arktiske land; Canada, Danmark, Finland, Island, Norge, Russland, Sverige og USA. Senere er same protokoll initiert i flere land på den sørlige halvkule, slik som Sør-Afrika, Brasil, Argentina, Bangla Desh og Vietnam. Data for disse substansene og aktuelle helseeffekter er svært mangelfulle, spesielt i afrikanske land. Miljøgifter, også fluorforbindelser, er brukt i Malawi i en årrekke. Derimot er studier på eksponering for sårbare grupper, slik som gravide kvinner og nyfødte barn, svært mangelfulle.

Denne avhandling er basert på data fra en tverrsnittsstudie av gravide kvinner og deres avkom i det sørlige Malawi. Studiens formål var å vurdere prediktorer for nivåer av POPs (inkludert fluorforbindelser PFAS) og sporelementer hos gravide kvinner. Likeledes har vi i studien sett på sammenhenger mellom maternelle serumnivåer av stoffene og svangerskapsutfall. Sosioøkonomiske opplysninger og neonatale svangerskapsutfall ble registrert ved hjelp av spørreskjema administrert av spesielt opplærte helsearbeidere. Venøst blod ble samlet ved bruk av standard vacutainerutstyr for POPs analyser, metaller og sporelementer. For analyse av deltakernes PFAS nivåer ble det brukt væskekromatografi (liquid chromatography tandem-quadrupole mass-spectrometry (UHPLC-MS/MS), og for POPs gasskromatografi (atmospheric pressure ionisation coupled to tandem mass spectrometers (GC-API-MS/MS). Inductively coupled plasma mass-spectrometry (ICP-MS) metodikk ble brukt for elementanalyser.

Mors alder, utdanningsnivå og paritet var hoveddeterminantene for eksponering for PFAS og POPs. Selv om myndighetene i Malawi forbød bruk og distribusjon av DDT for over førti år siden, ble det fortsatt påvist noen spor av DDT og dets metabolitter i noen serumprøver; spesielt med høye konsentrasjoner påvist i mors serumprøver fra urbane områder sammenlignet med landlige omgivelser. Forholdet mellom konsentrasjonene mellom 1-klor-4-[2,2,2-triklor-1-(4-klorfenyl)etyl]benzen (*p, p*'-DDT) og hovedmetabolitten 1-klor-4-[2,2-diklor-1-(4-klorfenyl)etenyl]benzen (*p, p*'-DDE) beregnet i denne studien antyder kosthold som hovedveien for eksponering for disse forbindelsene til gravide kvinner.

Generelt er mors alder og geografisk område de viktigste faktorer som påvirker nivåene av toxiske og essensielle elementer i maternelt blod. Likeledes er nivåer av toxiske metaller svært lave sammenliknet med andre studier globalt. Geometriske eller median nivåer av bly i blod var imidlertid høyere sammenliknet med studier fra Argentina, Colombia, USA, Canada, Spania, Norge og Japan. En invers assosiasjon ble påvist mellom As and Pb mot hodeomkrets og lengde ved fødsel.

De fleste assosiasjoner med fødselsutfall var svært moderate. Høyere konsentrasjoner av *trans*-Nonachlor (*t*-NC) ($p = 0.04$), Oxychlordane (*Oxy*-CD) ($p = 0.01$) og *cis*- Nonachlor (*cis*-NC) ($p = 0.05$) viste sammenheng med mindre hodeomkrets. Tilsvarende var høyere nivåer av Perfluorooctanoate (PFOA) ($p = 0.04$) og Perfluorononanoate (PFNA) ($p = 0.001$) assosierte med mindre hodeomkrets. Høyere nivåer av Perfluorohexane sulfonate (sumPFHxS) ($p = 0.05$) viste en svak sammenheng med større hodeomkrets ved fødsel. Høyere nivåer av PFNA ($p = 0.005$) og Perfluorooctane sulfonate (sumPFOS) ($p <0.001$) var assosierte med kortere gestasjonsalder. Forhøyede nivåer av PFOA ($p <0.001$) og PFNA ($p = 0.005$) viste sammenheng med redusert fødselsvekt. Det samme gjorde SumPFHxS ($p =0.01$). Resultatene for PFAS viser behovet for å undersøke SumPFHxS videre da det følger et mønster som er forskjellig fra tilsvarende studier.

Nivåene av Ni i serum var lave, men viste svak sammenheng med redusert fødselsvekt, lengde, hodeomkrets og gestasjonsalder, p-verdier rundt 0.3-0.5. Økede nivåer av As viste sammenheng med redusert hodeomkrets ($p <0.001$). Tilsvarende var høye nivåer av Pb i maternelt blod assosiert med redusert hodeomkrets og fødselslengde ($p=0.002$ og $p=0.016$).

Resultater fra denne studien kan brukes til å identifisere livsstil og kostholdsfaktorer som påvirker nivåene av giftstoffene POPs og PFAS samt toxiske metaller og sporelementer hos kvinner i reproduktiv alder. Publiserte data fra studier på nivåer av disse stoffene og mistenkte helseffekter fra den nordlige halvkule kan ikke direkte sammenholdes med våre data fra sør på grunn av store forskjeller i levekår, kosthold og andre aktuelle variabler som kan påvirke fødselsutfall og sykdom hos gravide og nyfødte barn. På denne bakgrunn er det svært viktig å analysere nivåer av alle PTS og essensielle elementer i svangerskap og nyfødtperioden både i Malawi og andre land på den sørlige halvkule i et folkehelseperspektiv.

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Dedication

I dedicate this PhD Thesis to my late father, Howard Mwapasa, who was there at the start of my PhD journey but unfortunately could not live long enough to witness my final submission. He used to call me a doctor long before I even obtained my bachelor's degree. How I wish he was here to witness completion of my PhD. However, I believe he is smiling on my achievements from heaven.

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Lastly, I would like to thank my family. My wife Nancy and my daughter Stacy. You put up with my long absences from home. Without your support this would not have been possible.

List of Papers

The thesis is based on the following papers:

Paper I: Mwapasa M, Huber S, Chakhame BM, Maluwa A, Odland ML, Röllin H, Choko A, Xu S, Odland JØ. Serum Concentrations of Selected Poly- and Perfluoroalkyl Substances (PFAS) in Pregnant Women and Associations with Birth Outcomes. A Cross-Sectional Study from Southern Malawi. International Journal of Environmental Research and Public Health. 2023, 20(3), 1689. https://doi.org/10.3390/ ijerph20031689.

Paper II: Mwapasa M, Huber S, Chakhame BM, Maluwa A, Odland ML, Ndhlovu V, Röllin H, Xu S, Odland JØ. Predictors of Maternal Serum Concentrations for Selected Persistent Organic Pollutants (POPs) in Pregnant Women and Associations with Birth Outcomes: A Cross-Sectional Study from Southern Malawi. International Journal of Environmental Research and Public Health. 2023, 20 (7), 5289. http://doi.org/10.3390/ijerph20075289.

Paper III: Mwapasa, M., Xu, S., Chakhame, B. M., Maluwa, A., Odland, M. L., Röllin, H., Choko, A., Huber, S., & Odland, J. Ø. (2023). A cross-sectional study of maternal blood concentrations of toxic and trace elements in pregnant women and association with birth outcomes in southern Malawi (Submitted for publication).

List of abbreviations

AM	Arithmetic means
AMAP	Artic Monitoring and Assessment Programme
As	Arsenic
ATSDR	Agency for Toxic Substances & Disease Registry
CDC	Centre for Disease Control and Prevention
cis-NC	*cis*-Nonachlor
COMREC	College of Medicine Research and Ethics Committee, Malawi
CS	Caesarian Section
CTQ	Centre de Toxicologie du Quebec
Cu	Copper
GC	Gas chromatography
HCB	Hexachlorobenzene
DDD	Dichlorodiphenyl dichloroethylene
DDE	Dichlorodiphenyl dichloroethane
DDT	Dichlorodiphenyl trichloroethane
DF	Detection frequency
MS	Mass spectrometry
OCs	Organochlorines
GC – MS	Gas chromatography – Mass spectrometry
GDP	Gross Domestic Product
GM	Geometric mean
GM	Geometric means

HCB	Hexachlorobenzene
HDLs	High-density-lipoprotein
Hg	Mercury
IARC	International Agency for Research on Cancer
IPC – MS	Inductively coupled plasma mass-spectrometry
KUHeS	Kamuzu University of Health Sciences
LOD	Limit of detection
MeHg	Methyl mercury
MUST	Malawi University of Science and Technology
Ni	Nickel
OCP(s)	Organochlorine pesticide(s)
Oxy-CD	Oxychlordane
Pb	Lead
PFAS	Poly- and Perfluoroalkyl Substances
PFDA	Perfluorodecanoate
PFHxS	Perfluorohexane sulfonate
PFNA	Perfluorononanoate
PFOA	Perfluorooctanoate
PFOS	Perfluorooctane sulfonate
PFUDA	Perfluoroundecanoate
POPs	Persistent Organic Pollutants
PTS	Persistent Toxic Substances
QA/QC	Quality assurance/ quality control

REK	Regional Committees for Medical and Health Research Ethics, Norway
Se	Selenium
SVD	Spontaneous Vaginal Delivery
t-NC	*trans*-Nonachlor
WHO	World Health Organisation
Zn	Zinc

Summary in English

Persistent organic pollutants (POPs) and toxic metals are recognised as having effects on health and development in children. These substances are also known to be transferred from the mother to the foetus and neonates through the umbilical cord and breast feeding respectively. In this regard, the growing foetus and new born child are at a greatest risk of the toxic effects of these substances as they are still in their developmental stages. On the other hand, deficiency or excess levels of some trace elements during pregnancy has also been linked to adverse health effects. In this regard, the levels of these contaminants in maternal blood during pregnancy give an indication of the potential risk to the developing foetus.

Most of the monitoring and studies on effects of persistent toxic substances among pregnant women in relation to reproductive health are conducted in temperate regions, mostly in Europe and America. One important contributor is the Arctic Monitoring and Assessment Programme (AMAP). AMAP started in 1991 and includes monitoring of PTS in eight arctic countries namely Canada, Denmark, Finland, Iceland, Norway, Russia, Sweden and the USA. At the later stage, these studies were also expanded to a few countries in the southern hemisphere. The data on these substances and possible associations with adverse reproductive health effects are scarce, especially in the African settings that include other low- and middle-income countries. POPs (including Poly- and Perfluoroalkyl Substances (PFAS) toxic and trace elements have been used in Malawi for a long time. However, to our knowledge, studies on exposure to these environmental pollutants by susceptible groups like pregnant women and its associations on reproductive health outcomes are limited.

This thesis is based on data from a cross-sectional study of pregnant women and their offspring in southern Malawi. The study aimed at assessing the predictors for concentrations of POPs (including PFAS) toxic as well as trace elements in pregnant women. Furthermore, this study assessed associations between maternal serum concentrations of these substances and birth outcomes. Data on pregnant women socio-demographic characteristics and neonatal birth outcomes were collected using a questionnaire administered by a trained research nurse. Non fasting venus blood samples were collected using a glass red top vacutainer for POPs analyses.

Similarly dark blue vacutainer was used for the collection of a blood sample for toxic and trace elements analyses. Maternal serum PFAS concentrations were analyzed by ultrahigh pressure liquid chromatography tandem-quadrupole mass-spectrometry (UHPLC-MS/MS) and POPs by gas chromatography atmospheric pressure ionisation coupled to tandem mass spectrometers (GC-API-MS/MS). Inductively coupled plasma mass-spectrometry (ICP-MS) technique was used for maternal blood analyses.

Maternal age, level of education and parity were the main determinants of exposure to PFAS and POPs. Although the Malawi government banned the use and distribution of DDT over forty years ago, some traces of DDT and its metabolites were still detected in some serum samples; notably, with high concentrations detected in maternal serum samples from urban areas as compared to rural settings. The *p, p'*-DDE: *p, p'*-DDT concentration ratio found in this study suggests diet as the main route of exposure for these compounds to pregnant women.

In general, Maternal age and area of residence were the main determinants of the concentrations of toxic and trace elements in maternal blood. Furthermore, concentrations of most toxic elements detected in pregnant women were either low or similar if compared to similar studies conducted around the globe. The geometric mean or median concentration of Pb were higher than studies conducted in Ushuaia city in Argentina, Colombia, USA, Canada, Spain, Norway and Japan. Inverse associations were observed between As and Pb versus head circumference and birth length.

Most of the associations with birth outcomes were relatively weak. Higher concentrations of *trans*-Nonachlor (*t*-NC) ($p = 0.04$), Oxychlordane (*Oxy*-CD) ($p = 0.01$) and *cis*- Nonachlor (*cis*-NC) ($p = 0.05$) were associated with smaller neonatal head circumferences. Similarly, increased Perfluorooctanoate (PFOA) ($p = 0.04$) and Perfluorononanoate (PFNA) ($p = 0.001$) concentrations were also associated with smaller neonatal head circumferences. However, high concentrations of Perfluorohexane sulfonate (sumPFHxS) ($p = 0.05$) were associated with bigger neonatal head circumferences. High maternal serum concentrations of PFNA ($p = 0.005$) and Perfluorooctane sulfonate (sumPFOS) ($p < 0.001$) were associated with a shorter gestational age. Increased concentrations of PFOA ($p < 0.001$) and PFNA ($p = 0.005$) were associated with reduced

birth length, as was SumPFHxS (p = 0.01) with birth weight. The results on PFAS highlight the need to investigate SumPFHxS further as it follows a pattern that is different to similar compounds and cohorts. High maternal blood concentrations of Ni showed weak significance for birth weight, birth length, head circumference and gestational age, all p-values around 0.3-0.5. Increased concentrations of As were associated with smaller head circumference (p <0.001). Similarly, higher maternal blood Pb concentrations were associated with smaller head circumference (p =0.002) and birth length (p =0.016).

The results from this study may be used in the identification of specific lifestyles that are associated with increased maternal POPs, PFAS and toxic and trace elements concentrations of women of child-bearing age. Published data from studies on maternal POPs as well as toxic and trace elements concentrations in relation to reproductive health outcomes from the northern hemisphere cannot be directly used in the context of the southern hemisphere. Therefore, assessing the extent of exposure to these substances during pregnancy and its association to reproductive health outcomes in Malawi and other countries in the southern hemisphere is of public health importance.

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1. Introduction

Persistent toxic substances (PTS) are familiar substances such as metals, elements and organic substances that may exert their toxic effects for a long period of time after being released into the environment. This is due to the fact that these substances have long half-lives and detoxify slowly in the environment. Furthermore, PTS have the ability to travel long distances either by air or water currents, and accumulate in the food chain and living organisms including humans (1).

Research in both animals and humans has shown that PTS are linked to adverse health outcomes in reproductive health, neurological deficits and some forms of cancer. There is a growing concern of PTS effects in pregnant women, growing foetuses and newborn children as research has shown that these substances have the ability to move from mother to foetus via the umbilical cord (2-6) and to the child via mother's breast milk (6-12). In this regard, assessing the concentrations of these substances in maternal blood of pregnant women can indicate the potential risk to the developing foetus, infant and growing child until adulthood.

On the other hand, essential trace elements are needed in small amounts for physiological and chemical body processes. Deficiencies, excesses or imbalances of trace elements are also associated with different adverse health conditions. For instance, inadequate levels of trace elements in the human body may hinder normal body biological processes while high concentrations may also be toxic (13, 14). Excess or imbalanced levels of some trace elements have been associated with a number of adverse health conditions including unfavourable pregnancy outcomes. For example, studies have shown that very low levels of Cu and Zn are linked to gestational diabetes mellitus (GDM) (13, 15).

A number of multidisciplinary international projects in the Arctic are in progress monitoring the levels and assessing the effects of selected pollutants in all domains including water, air, flora, fauna and humans (16). However, to fully assess environmental and health consequences of pollutants emissions both in the northern and southern hemispheres, an accurate knowledge about global pathways and distribution of these contaminants is needed.

Malawi and other countries situated in the southern hemisphere are of particular importance to the global research in the science of environmental pollutants and human health outcomes, particularly dealing with reproductive health. There is a lack of comprehensive research in this region as data from the northern hemisphere may not be applicable to Malawi or other countries in the southern hemisphere.

1.1. Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are man-made synthetic compounds highly resistant to biodegradation. These compounds are also known for their high affinity to bioaccumulation and biomagnification in the environment and living organisms, including humans (17, 18). POPs have the potential to undergo a long-range atmospheric transfer, such that they are found in areas that they have never been produced or used (19, 20). The most common POPs route of exposure to humans is through consumption of animal products such as meat, fat, fish, dairy items and eggs (21-24). POPs are lipophilic in nature hence once ingested by humans, they are deposited in adipose tissues and form stable compounds resulting in a lasting toxic body burden (25-28).

1.1.1. Poly- and Perfluoroalkyl Substances (PFAS)

Poly-and perfluoroalkyl substances (PFAS) are highly fluorinated aliphatic man-made chemicals with carbon chains of different lengths and perfluoroalkyl moiety (CnF2n+1-) (14). The unique properties of these compounds like thermal stability, hydrophobicity and lipophobicility makes them ideal for a wide range of uses in both industry and consumer products. The major products from PFAS include surfactants, paper, packaging products, carpet, upholstery and textile (29, 30). PFAS have very strong C–F covalent bonds and hence tend to persist in the environment and accumulate in living organisms including humans. Some PFAS namely PFOS and PFOA are known to be highly persistent in human serum with half-lives ranging from 3.8 to 5.4 years (31). Maternal serum PFAS levels in pregnancy are critical as various research studies have revealed that some PFAS can be transferred from the pregnant woman to the growing foetus through the placenta (32) and cause a number of adverse health effects such as fetal immunosuppression, neurotoxicity and growth restriction (33-35). Furthermore, there is research evidence that PFOS and PFOA are the major sources of total PFAS levels in maternal blood and breast milk (36, 37). Although PFAS concentrations in breast milk are lower as compared to maternal serum, prolonged breastfeeding might increase infants´ burden of PFAS exposure (38). Three PFAS namely PFOS, PFOA and PFHxS as well as their salts and related compounds are listed as industrial POPs in the Stockholm Convention Agreement. Specifically, PFOA and PFHxS were listed in the Annex A (elimination) and PFHxS in the Annex B (restriction). PFAS that are covered and discussed in this thesis include perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanated (PFUDA), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS).

1.1.2. Organochlorine pesticides

Organochlorine pesticides (OCPs) are a subgroup of persistent organic pollutants (POPs) containing multiple chlorine substituents, known for their resistance to degradation in the environment. Although the production and use of most of POPs were restricted following the Stockholm Convention in 2001(39), traces of these compounds are still present in both the environment and humans because of the compounds´ resistance to biodegradation and bioaccumulation (40). In addition, traces of these substances still persist in the environment as few countries where malaria remains endemic were approved to continue the use of DDT in prevention of Malaria following concerns surrounding public health and malaria by the WHO (41). For instance, DDT is still used in the prevention of Malaria in South Africa and India (42). OCPs and their metabolites that were included and discussed in this thesis are Hexachlorobenzene (HCB), 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (*p,p´*-DDT), 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (*o,p´*-DDT), 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene (*p,p´*-DDE), 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl) ethenyl]benzene (*o,p´*-DDE), trans-Nonachlor (t-NC), cis-Nonachlor (cis-NC) and Oxychlordane (*oxy*-CD).

1.1.2.1. HCB

HCB is a compound that does not occur naturally but can be synthesized through chlorination of benzene at temperatures between 150 to 200 ^{0}C. This process of synthesizing HBC is facilitated by a catalyst. However, this compound can also be produced as a byproduct in the production of chlorinated solvents and other chlorinated compounds, including several pesticides (43, 44). This compound exits as a white, crystalline solid compound that is insoluble in water but

is very soluble in fat, oils, and organic solvent. The production and commercialization of HCB was significantly reduced following the signing of the Stockholm Convention in 2001 which limited the production and use of such compounds except for prescribed purposes and only to countries that have registered for exemptions (45). However, HCB is still used for laboratory processes in synthesis of some chloroorganic solvents and pesticides hence releasing carbon tetrachloride and tetrachloroethylene as the main byproducts to the environment. This process also releases small quantities of mono-, di- and tri-chlorobenzene as by products (46).

Chronic intoxication of HCB is associated with porphyria cutanea tarda, skin hyperpigmentation and hirsutism, neurological and orthopedic disorders. There are growing concerns on possible adverse effects of HCB on reproductive health (47, 48) as research has also revealed that this compound has the ability to be transferred to the foetus and neonate through the placenta and breast milk respectively (49).

1.1.2.2. Dichlorodiphenyltrichloroethane (DDT) - group

DDT is a colourless, tasteless organochloride compound that was originally developed as an insecticide. The commercial DDT is compressed isomers (1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl) ethyl]benzene), *p,p´*-DDT, 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (*o,p'*-DDT) as well as their metabolites namely DDE (Dichlorodiphenyldichloroethylene) and DDD (Dichlorodiphenyldichloroethane) with its isomers (50). DDT is known for its persistence once released in the environment. Furthermore, these compounds are recognized for their ability to accumulate in adipose tissues of organisms and are biomagnified in the food chain. The structure of *p,p´*-DDT and *p,p´*-DDE are similar to thyroid

hormones. In this regard, these compounds are considered to be endocrine disrupting compounds (EDCs) as having the ability to interfere with the synthesis, transport and the function of hormones (51).

1.1.2.3. Cis-*nonachlor or* trans-*nonachlor and oxychlordane*

cis-nonachlor (cis-NC) or *trans*-nonachlor (t-NC) are the components of technical chlordane. Research in laboratory animals has revealed that cis-NC or t-NC have more immuno-toxic health effects as compared to technical chlordane (52, 53). On the other hand, oxychlordane (*oxy*-CD) is considered to be a less toxic chlordane compound as compared to cis-NC or t-NC. It is linked to weight loss, reduced feed consumption and thymic atrophy in laboratory animals (54). These studies suggest that these compounds may also have the same adverse health effects to humans.

1.2. Toxic elements

Naturally, toxic elements are present in the soils, water and air at trace and rarely toxic levels due to natural activities like weathering processes. However, sources of toxic elements to the environment may be both from natural and anthropogenic activities. Anthropogenic activities like agricultural and industrial activities, landfilling and mining are the main source of toxic metals to the environment (55, 56). Once these elements enter into the environment, they exist in different forms namely: ions, vapours and salts. Exposure to humans is usually through inhalation, ingestion or dermal absorption. Toxic metals that have been covered in this thesis are Arsenic (As), Mercury (Hg) and Lead (Pb).

1.2.1. Arsenic (As)

Arsenic is present in the environment in both organic and inorganic forms. This metalloid exists naturally in the earth's crust but the sources of pollution to the environment are both natural and anthropogenic. In terms of natural pollution, As may naturally exist in high concentrations in certain areas and contaminate ground water sources (57-60). Arsenic is highly toxic in its inorganic form (arsenite or arsenate) with food, air and water as the main route for human exposure. Organic forms of As such as arsenobetaine are relatively non-toxic and mostly found in seafood (61).

After exposure, As accumulates in tissues such as nails, skin and hair. Prenatal exposure to As is associated with high incidences of adverse reproductive health outcomes namely fetal loss, premature delivery, decreased birth weights and fetal growth (62-64). Furthermore, research evidence suggests that inorganic arsenic accumulates in the placenta and disrupts its normal function (65).

1.2.2. Mercury (Hg)

Mercury (Hg) is a toxic element named after Greco-Roman god and known since ancient times from around 1500 BC (66). This element is widely distributed in the environment through both natural and anthropogenic sources. However, human activity is the main source of distribution of Hg to the environment. Combustion of fossil fuels, wild fires, biomass burning, chlor-alkali industry, batteries and fluorescent lamps and informal gold mining are some of the main source of anthropogenic sources of Hg in the environment (67).

In the environment, Hg exists as elemental vapour, inorganic and organic mercury compounds. Elemental Hg (Hg°) is silver white liquid at room temperature hence easily released

into the atmosphere in form of mercury vapor. On the other hand, inorganic mercury compounds exist in two oxidative states namely mercurous, (Hg^{+}) and mercuric (Hg^{++}). These exists in solid states as mercurous or mercuric salts and mercury compounds with other elements like chlorine, sulfur, or oxygen (68). The most common organic mercury compounds are methylmercury and ethylmercury, with the latter regarded as the most dangerous mercury compound based on its toxicological effects (68-70). Furthermore, methylmercury and ethyl mercury are lipophilic in nature hence can easily bio-accumulate and pass-through cellular membranes. In contrast, inorganic mercury compounds are water soluble and exposure is usually through ingestion then accumulates mainly in the kidneys and result in kidney damage (66).

Blood Hg level below 20 μg/L in humans is considered to be normal (71). However, excessive exposure to Hg may damage the central nervous system and causes tremors, distorted speech, respiratory failure, kidney effects, dizziness, blurred vision, hallucinations, and even death in severely exposed people (72). There are a few inconsistencies regarding evidence on the association between moderate mercury exposure and birth outcomes. However, a number of studies have suggested increased adverse birth outcomes in relation to increased Pb exposure (73-75). The foetus is at a great risk as the placenta does not provide an effective barrier against methylmercury (76).

1.2.3. Lead (Pb)

The use of Lead (Pb) by humans can be traced back to over 7000 years ago (66). It is a blueish-gray metal that is naturally found in the earth´s crust mostly it its ore with Copper, Silver and Zinc. Properties of lead include; malleable, highly resistant to corrosion and fire, low melting

point, able to absorb sound and a poor conductor of electricity (77). The above outlined unique properties have contributed to its increased use to diverse consumer products and leading to a widespread exposure to humans (78). The major sources of lead to the environment are industrial activities, mining, paint, petrol, dust and soil.

Research has suggested that human exposure to Pb is linked to a number of adverse health effects including neurological, renal, cardiovascular, hematological, immunological, reproductive, and developmental effects (79). Lead is usually stored in the bones once it enters the human body and its elimination from this compartment is estimated at 11 to 20 years (79). However, pregnancy and lactation are associated with increased metabolic activity in the bones following an increase demand for calcium from the bones which may also result into the release of Lead (80). Consequently, Pb in the blood stream may in turn be transferred to the foetus and newborn through placenta and breast feeding respectively. There are no safe blood Pb levels as even very low concentrations can also result in adverse health effects especially in children (81).

1.3. Trace elements

Trace elements are needed in humans to sustain life hence required in small amounts in the daily diet for vital biochemical body reactions (82). However, deficiency or excess levels of trace elements in the human body is also associated with several adverse health effects (13, 14). The essential trace elements included in this thesis are Copper (Cu), Nickel (Ni), Selenium (Se) and Zinc (Zn).

1.3.1. Copper (Cu)

Copper is a common, malleable and ductile metal in the earth's crust and widely used by humans in industries and agriculture. This element is classified as a transitional metal in the periodic table and is best known for having a high thermal and electrical conductivity. In humans, Cu plays an essential role in the metabolism by allowing critical enzymes to function properly (83). Cu deficiency in the human body is associated with anemia, growth retardation, hypothermia and mental retardation. On the other hand, very high levels of Cu in the human body is linked to nausea, vomiting, diarrhea, profuse sweating, and renal dysfunction (84). In reproductive health, high maternal Cu concentrations have also been associated with increased incidences of preterm births (85). This is due to the reason that Cu together with Se and Zn act as cofactors of antioxidant enzymes responsible for regulating the inflammatory response (86).

1.3.2. Nickel (Ni)

Nickel is a metallic element that is found in the transition series group 10 (VIII b) of the periodic table and has five natural known isotopes. However, the most abundant are Ni^{58} (68.27%) and Ni^{60} (26.10%) (87). This metallic element is naturally distributed on the earth´s crust and slowly released into the atmosphere in small quantities (88). The most common anthropogenic source of Nickel to the environment is combustion of fossil fuels (89). Data on the biological importance of Ni to humans is sparsely available. However, it is suggested that Ni is important for some essential biological processes in both plant and humans (90). In humans, Ni is linked the activation of certain enzymes related to the breakdown or utilization of glucose (91). Although the presence of Ni in the human body has the above benefits, high levels are also related to a number of adverse health effects. For instance, excess human exposure to Ni is known for causing skin

allergies, contact dermatitis, cardiovascular disease, asthma, lung fibrosis, and respiratory tract cancer (92-96). On the other hand, Ni deficiency is known to be linked to reduced iron resorption in the body and hence leading to anaemia and a disturbance in the incorporation of calcium into skeleton (97). The reference levels for Ni in serum is 0.2 μg/L (98).

1.3.3. Selenium (Se)

Se occurs naturally in the earth´s crust, usually combined with other substances and it is commonly found in rocks and soil. However, elemental selenium is commercially produced in manufacturing industries as a by-product of copper refining (99). Se element is considered to be a semi-metal because it possesses intermediate properties between a metal and a non-metal. It is an essential trace element to humans and naturally present in many foods. Intake of Se by humans can have both nutritional or toxic effects depending on the dosage. The biological role of Se in the human body is not well established and still under debate. However, it is suggested that Se has a critical roles in reproduction, DNA synthesis, thyroid hormone metabolism, and protection from oxidative damage and infection (100). On the other hand, excess exposure to Se is linked to cardiovascular, hepatic, nervous, and renal health effects. This trace element is present in nature and humans as organic (selenomethionine and selenocysteine) and/or inorganic forms (selenite, selenide, selenate and the selenium element) (101, 102). Se levels in whole blood only shows a short-term status. However, the whole blood Se concentration of above 100 μg/L in whole-blood (above 80 μg/L plasma concentration) is considered to be adequate for normal body processes(103). A number of research studies have suggested that very low maternal Se concentrations during pregnancy is associated with pregnancy loss, preterm birth and preeclampsia (104-108).

1.3.4. Zinc (Zn)

Zn is one of the common metals in the earth´s crust and it is the bluish-white metal in its elemental (metallic) form. This element is commonly used to coat other metals like iron and steel to avoid corrosion. In addition, Zn can also be combined with other elements namely chlorine, oxygen, and sulfur to form zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide (109). Although Zinc is one of the essential trace elements in the human body, there is evidence that very high or low levels are linked to a number of adverse health effects. For instance, high levels on Zn are associated with hepatotoxicity, Immunological and Lymphoreticular effects. On the other hand, Zinc deficiency has been linked to impaired immune function, dermatitis, hypogonadism with impaired reproductive capacity, and depressed mental function (109, 110). On reproductive health, research has revealed that very high maternal blood Zn concentration is linked to increased incidences of congenital malformations in infants and preterm births (85). Furthermore, animal studies have suggested that very low maternal blood Zn concentrations affects the quality of oocyte. This is because adequate levels of Zn is required for growth and maturation of mammalian oocytes (111-113).

Table 1 give the summary of the sources of toxic and trace elements to the environment, target organs when these elements enter the human body and their known adverse health effects.

Table 1. Summary of the sources, targets and known adverse health effects for individual elements.

Element	Sources	Target	Toxic effects	References
As	Water, soil, and contaminated agricultural and fish products.	The liver, kidneys, muscle, bone, hair, skin and nails.	Carcinogenic (Skin, kidney and lung cancers), irritation of stomach and intestines, type 2 diabetes, spontaneous abortions, small for gestational age, low birth weight, still births and preterm birth.	(62, 114-120)
Hg	Air pollution, dental amalgams, explosives, foods, fresh water fish, insecticides and water.	Cell membranes, kidney and nervous system.	Kidney damage or failure, blindness, hallucinations, tremor and vomiting, reduced placental and fetal growth, Preeclampsia, Preterm births, congenital anomalies	(119, 121, 122)
Pb	Food grown around industrial areas, chemical fertilizer, air pollution, ammunition, paints, pesticides, soil, smocking tobacco.	Bone, brain, heart, kidneys, liver, nervous system and pancreas.	Abdominal pain, anaemia, encephalopathy, neuropathy, hypertension and preterm births, pre-eclampsia, possible carcinogenic.	(123-129)
Cu	Mining, air pollution, and food products.	Heart, nervous system and blood vessels.	*Hypocupremia:* Anemia (microcytic, normocytic, or macrocytic), neutropenia, ataxic myelopathy, hypotension, preterm births, embryonic and fetal abnormalities *Excess Cu:* Indian childhood cirrhosis, and idiopathic copper toxicosis.	(85, 130-132)
Ni	Soils, Nickel – cadmium batteries and electronic equipment, cast coins, jewellery, medical prostheses, industrial waste and fossil fuel combustion.	Kidneys.	*Ni deficiency:* Reduced iron resorption that may leads to anaemia, disturbance in the incorporation of calcium into skeleton *Excess Ni:* Contact dermatitis, cardiovascular disease, asthma, lung fibrosis, and respiratory tract cancer.	(92-96, 133)
Se	Soils, plants, water, air, and food products.	Liver and kidney and thyroid grands.	*Se deficiency;* Cardiovascular disease, infertility, myodegenerative diseases, cognitive decline pregnancy loss, preterm birth and preeclampsia. *Excess Se:* Selenosis, Cancer (for Selenium sulfide exposure), birth defects.	(99, 102, 104-108, 134)
Zn	Soils, air, water, and food products.	Stored throughout the body.	*Zn deficiency:* Dermatitis, anorexia, growth retardation, poor wound healing, hypogonadism with impaired reproductive capacity, impaired immune function, and depressed mental function, increased incidence of congenital malformations in infants, preterm births. *Excess Zn:* Low levels of high-density lipoprotein (HDL), immune function, copper deficiency.	(85, 109, 135)

Abbreviations: As = arsenic; Hg = mercury; Pb = lead; Cu = copper; Ni = nickel; Se = selenium; Zn = zinc

2. Aims

The research aims for Paper I, II and III included in this doctoral thesis were:

1) To examine the determinants of different serum- PFAS concentrations in late pregnancy and investigate their relationship with birth outcomes.

2) To examine predictors of the serum concentrations of different POPs in delivering women and investigate the relationship of the POP concentrations with birth outcomes.

3) To evaluate concentrations of toxic and trace elements in maternal whole blood and investigate the associations with birth outcomes.

3. Materials and methods

All three papers were based on the Malawi study conducted by the PhD candidate titled; *Reproductive health outcomes associated with environmental and dietary exposure to persistent toxic substances (PTS) in Pregnant mothers and infants in Southern Region of Malawi*. This was a cross-sectional study of pregnant women and their offspring conducted in three sites in the southern region of Malawi.

3.1. Study setting

Malawi is a land-locked country located in the south west part of Africa, bordered by Mozambique to the south and east, Zambia to the west and Tanzania to the north with the estimated total population of 19.65 million as of 2021 (136). The country's economy is predominantly agricultural based, with about 90% of the farming conducted in rural areas. According to the World Bank Atlas the country´s GDP per capital was 628.70 USD in 2017 (137). The Malawi health service delivery system is three-tiered. It consists of primary, secondary and tertiary care levels. Primary health care level is consisted of health posts, dispensaries, maternity units, health centres, village health clinics and community and rural hospitals. Secondary health care level is made up of district hospitals and central hospitals are considered to be the tertiary level of health care. These health care delivery levels are linked by a referral system. In this regard, complicated cases at primary health care level are referred to secondary level. Similarly, complicated cases at secondary health care level of care are also escalated to the tertiary level.

The districts in which this study was conducted were Blantyre, Chiradzulu and Thyolo. Blantyre is regarded as the commercial city of Malawi. This district is about 1,039 meters above

the sea level and covers the area of 228 km^2. Thyolo district is situated at the southern end of Blantyre city. It is located at 350 to 3,500 meters above the sea level and covers an area of 1,715 km^2. Chiradzulu is also situated in the southern region of Malawi and covers an area of 767 km^2. Blantyre district represented urban while Thyolo and Chiradzulu districts represented rural settings. **Figure 1** shows the sites in which the study was conducted.

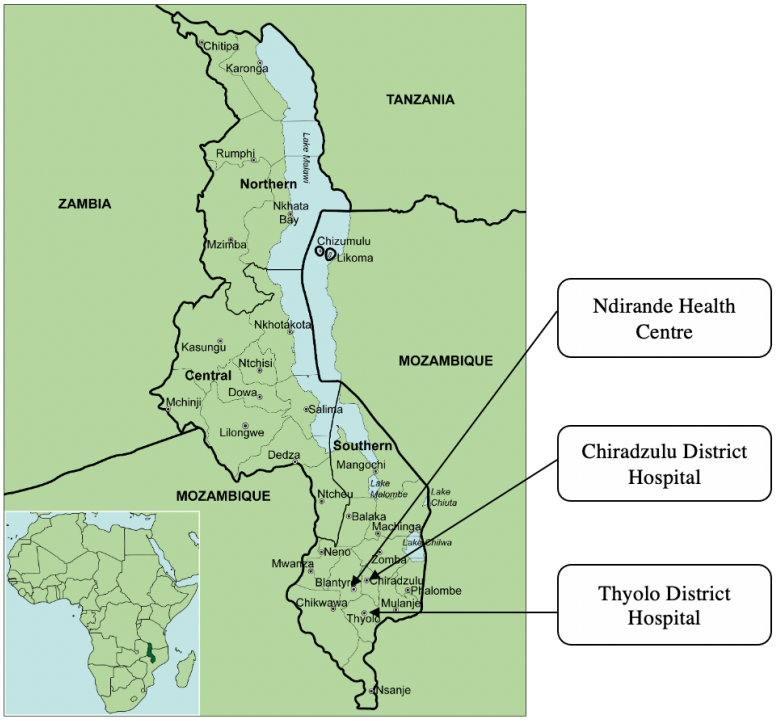

Figure 1. Map showing locations of study sites (reproduced from Paper III)

Source of the map: Adapted from google maps_ https://maps-malawi.com/map-of-malawi-districts

3.2. Study population

The study population constituted all pregnant women attending antenatal care services at the three selected health facilities namely; Ndirande health centre, Chiradzulu and Thyolo district hospitals in the southern region of Malawi.

3.3. Sample size considerations

The main study was powered to detect a linear relationship between maternal blood lead concentration and birth weight (g) based on normal assumptions and a Pearson Correlation coefficient (r). It was assumed that r would be 0.12 based on a study from South Africa (138). Therefore, it was calculated using the formula below. Those 543 participants would be needed to show r of 0.12 with 80% power on a two-sided test at alpha of 0.05. The final sample size was 600 participants, sufficient to also cover for up to 10% refusals.

$$N = \left|\left(Z_{\propto} + Z_{\beta}\right)/C\right|^2 + 3$$ …….…….. Equation 1.

Where:

$$C = 0.5 \times \ln[(1 + r)/(1 - r)]$$

$$Z_{\propto} = 1.960$$

$$Z_{\beta} = 0.842$$

Figure 2. shows the pictorial view of the sample size determination.

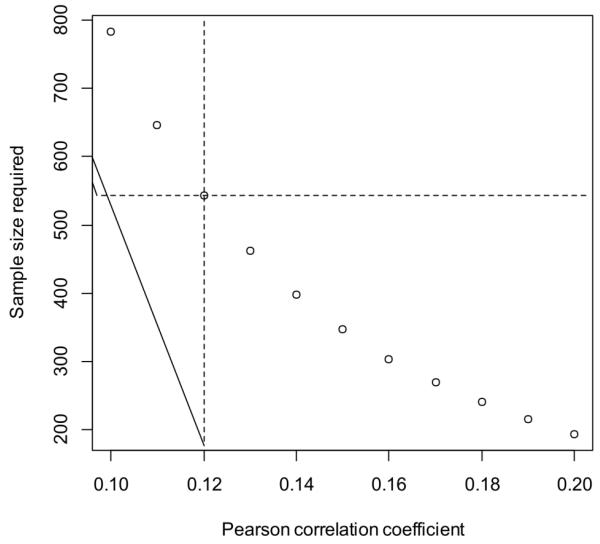

Figure 2. Sample size to detect a given Pearson correlation coefficient

3.4. Recruitment of study participants

A total of 605 Pregnant women in their third trimester were identified from the antenatal clinics at Ndirande Health Centre (Blantyre), Chiradzulu District Hospital (Chiradzulu) and Thyolo District Hospital (Thyolo). Participation in the study was voluntary and all study participants were required to sign or to thumbprint the written informed consent *(appendix 2)* before being recruited into the study. Recruitment of the study participants was strictly upon meeting the inclusion criteria and a written informed consent. Study participants were recruited between August 2020 and July 2021. The study sites were selected using a simple random sampling and consecutive sampling was used to recruit study participants. **Figure 3** illustrates the flow of study participants from assessment to inclusion for chemical analysis.

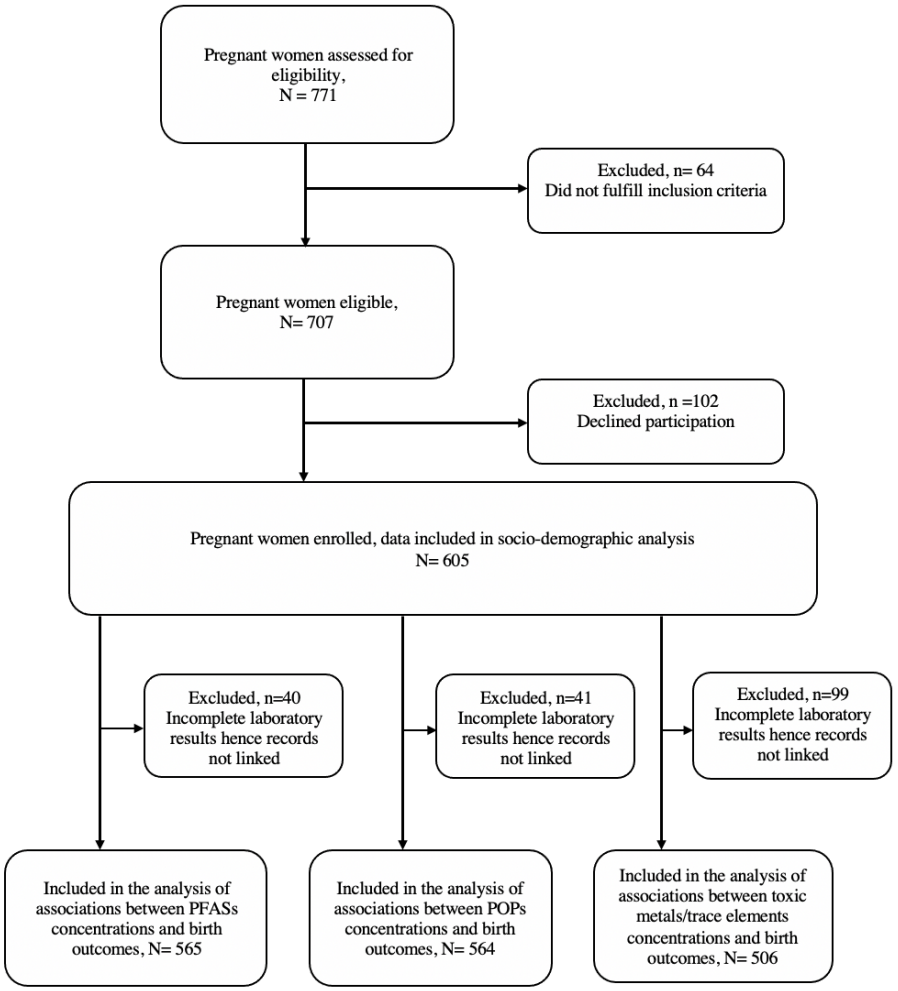

Figure 3. Flow chart illustrating the recruitment of study participants

3.5. Inclusion and exclusion criteria for participants

The inclusion and exclusion criteria that were used in the recruitment of the study participants is outlined below.

Inclusion criteria:

- Pregnant women in their late stages of third trimester of pregnancy.
- 16 years of age and above.
- Permanent resident of Blantyre, Chiradzulu and Thyolo districts.
- Willing to voluntarily sign an informed consent.

Exclusion criteria:

- Any serious medical condition present at the time enrolment.
- High risk Pregnancies with prognosis to be referred to a tertiary health care level.
- Concurrent participation in any other study.
- Not willing to sign the written informed consent.

3.6. Data collection methods

Maternal information was collected through a provider administered questionnaire *(Appendix .3)*. Maternal information collected were:

- Maternal age
- Maternal weight and height
- Parity
- Previous spontaneous abortions 1. Trimester
- Previous spontaneous abortions 2. Trimester
- Previous preterm abortions

- Previous deliveries – birth weight, gestational age, special problems (like delivery complications, malformations of baby, etc.)
- Breast feeding of previous babies, duration (months)
- Occupation of mother (and father, if available)
- Economic situation of mother (if possible)
- Major illnesses and medication of mother (if daily)
- Major food sources in daily life of mother (rice, meat, fish etc) before pregnancy and during pregnancy – self production/local production, supermarket, imported
- Smoking and drinking habits

Information for the neonates were collected using a provider administered questionnaire and the information collected were birth weight, birth length, head circumference, gender and congenital malformations (visible at birth). Ponderal index was calculated using the formula below:

$$\text{ponderal index} = \frac{\text{weight (kg)}}{\text{height}^3\ (\text{m}^3)} \quad \text{..... Equation 2.}$$

Maternal blood samples were collected at the optimal time before or after delivery (about 36 ±12 hours before delivery). Newborn information was also collected by the trained research nurse using a provider administered questionnaire at the time of delivery.

3.7. Clinical sampling collection procedure

All clinical sampling and collection were performed in accordance with the protocols provided by the CTQ Laboratory, Quebec, Canada (139, 140). In this regard, non-fasting maternal venus blood sampling was executed using sterile vacutainer blood collection system based on the protocol. A total of 16 mLs of whole blood sample was collected for blood and serum fractions. A dark blue top vacutainer (BD REF # 368381) was used to collect whole blood sample for the analysis of toxic and trace elements. A glass red top vacutainer (BD REF # 367614) was used to collect serum fraction samples for PFAS and POPs analysis. The serum fraction was obtained by centrifugation of the whole blood at ≤ 1200 g (3,276 rotations per minute) for 10 minutes before being transferred to 4 mLs glass tubes with green lid (27138 sigma Aldrich) for storage. All samples were frozen and stored at temperatures between -35^{0}C to -20^{0}C prior to analysis.

3.8. Chemical analyses

All blood and serum samples were analysed for content of PFAS, POPs as well as toxic and trace elements at the University Hospital of North Norway (UNN), Department of Laboratory Medicine, Norway.

3.8.1. Chemical analysis for PFAS

The detailed procedure for the analysis of Poly- and Perfluoroalkyl substances in maternal serum has been described in the first published paper (paper I) for the Malawi study (141). This technique is based on the methods previously published by Huber S and Brox J (142).

Maternal serum samples were analyzed by an ultrahigh pressure liquid-chromatography tandem-quadrupole mass-spectrometry (UHPLC-MS/MS) technique. Consequently, compounds that were measured include: perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), PFOS, perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorododecane sulfonate (PFDoDS), perfluorooctane sulfonamide (PFOSA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA) and perfluorotetradecanoate (PFTeDA). Furthermore, for some PFAS branched and linear species were also analyzed namely PFHxS, PFHpS, PFOS, PFHxS, PFHpS, PFNS, PFDS and PFOSA. A Waters Acquity UPLC system (Waters, Milford, MA, USA) with an autosampler, binary solvent manager and a column manager coupled to a Xevo TQ-S mass spectrometer (Waters, Milford, MA, USA) through an atmospheric pressure electrospray interface was used in the analysis. To separate the target analytes, an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) (Waters, Milford, MA, USA) with the use of a programmed flow and solvent consisting of 2 mM NH_4OAc in MilliQ-water and 2 mM NH_4OAc in methanol as mobile phase was applied.

The quantification was conducted with the Masslynx and Targetlynx software (Version 4.1, Waters, Milford, MA, USA) and achieved by the internal standard method with isotope-labelled PFAS. Three times of the signal-to-noise was used as the definition for limit of detection (LOD) and three times LOD was used for the method quantification level (MQL). Limit of detection was set as concentrations calculated by the Targetlynx software for each individual sample (LODi) and each individual analyte with a signal-to-noise ratio of 3 divided by the related

sample amount. A blank subtraction was performed batch wise each time a blank contamination (background contribution during sample preparation) was detected. In this regard, blank subtraction was performed batch wise by calculating an average of the blanks added to three times their standard deviation. Concentrations ranging from 0.01 pg/µL to 10 pg/µL were used to plot an eight-point calibration curve which was used for quantification.

Quality Controls

Accuracy and precision of the method was based on the published method article by Huber S and Brox J (142). Quality assurance was determined by running four blank samples, four of each standard reference material samples SRM 1957 and SRM 1958 (NIST, Gaithersburg, MD, USA) and 3 bovine serum samples (Sigma Aldrich, Steinheim, Germany) together with each batch of 81 Malawi study samples. In this regard, the differences from the assigned mean reference concentrations were from 3 to 13% for SRM 1957 and 3 to 9% for SRM 1958 during the Malawi study. Furthermore, the laboratory that performed the analysis of the study samples (University Hospital of North Norway (UNN)), Department of Laboratory Medicine laboratory participates in the quality assurance arrangements with the Arctic Monitoring and Assessment (AMAP) Ring Test for Persistent Organic Pollutants in Human Serum (organized by the Laboratoire de toxicologie, Institut national de santé publique du Quebec, Canada).

Following a chemical analysis above, a total of 24 PFAS compounds were analysed. However, only PFOA, PFNA, PFDA, PFUDA, SumPFHxS and SumPFOS were considered in the final statistical analysis with maternal characteristics and birth outcomes. The selection of the

above listed PFAS for the final analysis was based on the cut off value of ≥ 60% detection frequency and public health significance.

3.8.2. Chemical analysis for POPs

Maternal serum samples were analysed according to the previously published method by Huber *et al.* (143). The procedure in the analysis of persistent organic pollutants has also been described in the published paper II for this study (144). Instrumental analysis was performed by gas-chromatography atmospheric pressure ionisation coupled to tandem mass-spectrometers (GC-API-MS/MS; Waters, Milford, MA, USA). The API was conducted in positive mode under charge transfer conditions. Multiple reaction monitoring mode was applied with two specific transitions for the individual analytes to insure detection on the mass-spectrometer. Quantification was performed using the Masslynx and Targetlynx software (Version 4.1 and 4.2, Waters, Milford, MA, USA) and achieved by the internal-standard method with isotope-labelled compounds.

In total 19 POPs were analysed. However, only eight (HCB, *p,p´*-DDE, t-NC, *p,p´*-DDT, Oxychlordane, cis-NC, *o,p´*-DDT and *o, p´*-DDT) that had a of detection frequency of ≥50%. were selected for final statistical analysis.

3.8.3. Chemical analyses for toxic metals and trace elements

Chemical analyses were performed using the inductively coupled plasma mass-spectrometry (ICP-MS) technique. The detailed chemical analysis for toxic and trace elements has also been described in the paper III of this study. Sample dilution was performed by an automated liquid handler (Tecan Freedom Evo 200, Männedorf, Switzerland) equipped with an 8-channel liquid handler arm for conductive disposable tips, a robotic manipulator arm for transport of

microtiterplates and a shaker (Bioshake, Quantifol instruments GmBH, Jena, Germany). Dilution of 200 μL whole blood was done with a solution consisting of Milli-Q water (Millipore/Merck KGaA, Darmstadt, Germany), 10% v/v ammonia and 2-propanol (both Fluka, Bucharest, Romania), 0.08% v/v Triton X-100 (Sigma/ Merck KGaA, Darmstadt, Germany) and 0.25 μg/L gold solution (Au; Inorganic Ventures, Christiansburg, VA, USA) followed by mixing on the shaker. For instrumental analysis a NexION 300D ICP-MS system (Perkin Elmer, Waltham, Massachusetts, USA) equipped with an ESI-Fast SC2DX auto sampler was used. An internal standard solution was introduced to the nebuliser via a T-piece containing 20 μg/L rhodium and rhenium (Rh and Re; Inorganic Ventures, Christiansburg, VA, USA).

The kinetic-energy-discrimination mode with helium as reaction gas was applied for instrumental analysis and measurements were conducted in triplicates. A matrix matched calibration curve with ClinCal whole blood calibration material from Recipe (Recipe, Munich, Germany) was used for quantitative determination of the elements in the samples. Elements not present in the ClinCal material were spiked during sample preparation. The samples were analysed batchwise with each batch containing 28 samples, three ClinCal calibration samples diluted 1:100, 1:40 and 1:20 respectively, one calibration blank sample, four blank samples and two sets of ClinChek control material level 1, level 2 and level 3 from Recipe (Recipe, Munich, Germany), and Seronorm level 1, level 2 and level 3 (Sero, Billingstad, Norway) for quality assurance and quality control. For control of the background and instrumental carry over diluent blanks were included during the instrumental analysis The laboratory participates successfully in the international quality control programme Quebec Multielement External Quality Assessment

Scheme (QMEQAS) organized by the Centre de Toxicologie du Quebec, Quebec, Canada, which also covers elemental analysis in human whole blood.

Following the above technique, maternal whole blood samples were analysed for 32 elements. However, As, Hg, Pb, Cu, Ni, Se, and Zn were chosen to be included in the final statistical analysis. The selection, was based on previously published literature regarding priority metals that are of public health significance and had a detection frequency of ≥99%.

3.9. Ethical consideration

The ethical considerations and approval details are given in the three papers (Papers I, II and III) for this study. Ethics approval to conduct the Malawi PTS study was obtained from College of Medicine Research and Ethics Committee - COMREC (P.11/18/2546) and REK (# 355656 2020). In addition, clearance to collect data at all health centres was obtained from District Health Offices in which the study was conducted. All pregnant women who participated in the study were informed about the aims and procedures of the study before they could consent for enrolment and participation was voluntary, based on signed written consent from the mother. Furthermore, the participants were also informed that samples collected will be analysed in laboratories outside Malawi. Study participants identification was anonymized by assigning unique study numbers to all study participants that could not be traced back to individual women. All study documents were stored in a lockable cabinet accessible by the PhD candidate and the study team only. Study activities including venous whole blood sampling and extraction of information were executed carefully to avoid interference with the normal health service delivery process. Each study participant was given a total sum of $10.00 as compensation for their time and travel expenses as stipulated the COMREC guidelines.

3.10. Statistical analyses

Detailed statistical analyses for selected PFAS, POPs, toxic and trace elements have been described in papers I, II and III respectively. All statistical analyses were conducted with Stata for Mac (SE standard version 17; College Station, Texas, USA). Concentrations of PFAS, POPs, and metallic elements in all three papers included in this thesis were natural log transformed due to non-normal distribution of the concentrations among the participants. Descriptive statistics including arithmetic means, standard deviation (SD), geometric means, median and minimum and maximum or proportion (%) were computed from the data set. Mann–Whitney U and chi-square tests were used to determine the differences in maternal and neonatal characteristics between the urban and rural study participants. Univariable linear regression analysis and stepwise methods were used to determine the maternal characteristics to be included in the maternal and neonates´ characteristics multivariable model. However, maternal characteristics that have already been proven to be related to maternal concentrations of PFAS, POPs, toxic as well as trace elements in the literature automatically included in all multivariable models. Initially, Pearson product moment correlation coefficient (r) and its corresponding 95% confidence intervals was first computed for the main analysis to investigate the linear relationship between birth weight and maternal blood Pb concentration.

The association between natural logarithm transformed maternal PFAS, POPs and toxic as well as trace elements concentrations and birth outcomes namely gestational age (weeks), birth weight (grams), birth length (cm), and head circumference (cm) were assessed by multiple linear

regression models. PFAS models were controlled for maternal age (years), parity, maternal education level (no formal education/primary vs. secondary/tertiary), area of residence (urban vs. rural) and source of drinking water (tap water vs. lake/shallow well and borehole). POPs models were controlled for age (years), parity, maternal education level (no formal education/primary vs. secondary/tertiary), area of residence (urban vs. rural), source of drinking water (tap water vs. lake/shallow well and borehole), previous breast feeding (yes vs. no), beef consumption frequency (< twice a week vs. ≥ twice a week) and goat meat consumption frequency (< twice a week vs. ≥ twice a week).

Toxic and trace elements models were controlled by the covariates as follows: Se Cu and Pb models were adjusted for maternal age (years), parity, maternal education level (no formal education/primary vs. secondary/tertiary), area of residence (urban vs. rural) and source of drinking water (tap water vs. lake/shallow well and borehole). For Zn model, eggs consumption frequency (< twice a week vs. ≥ twice a week) was added to the list of the above covariates. Ni model was adjusted for maternal age (years), parity, maternal education level (no formal education/primary vs. secondary/tertiary), area of residence (urban vs. rural), source of drinking water (tap water vs. lake/shallow well and borehole), beef and eggs consumption frequencies (< twice a week vs. ≥ twice a week). As model was adjusted for maternal age (years), parity, maternal education level (no formal education/primary vs. secondary/tertiary), area of residence (urban vs. rural), source of drinking water (tap water vs. lake/shallow well and borehole), and fresh fish consumption frequency (< twice a week vs. ≥ twice a week) and goat meat consumption frequency (< twice a week vs. ≥ twice a week). Hg model was adjusted for maternal age (years), parity,

maternal education level (no formal education/primary vs. secondary/tertiary), area of residence (urban vs. rural), source of drinking water (tap water vs. lake/shallow well and borehole), fresh and dry fish consumption frequency (< twice a week vs. ≥ twice a week) and green vegetables consumption frequency (< twice a week vs. ≥ twice a week). A significance level of $p < 0.05$ (two-tailed) was set for all analyses.

4. Main results

4.1. Sociodemographic characteristics

Maternal and neonatal characteristics are summarized in **Table 2.** Demographic characteristics reported in this thesis have also been described in papers I, II and III. The mean age (SD) for the women recruited in this study was 24.8 (6.22) years. Mothers recruited from urban areas had a mean age of 2 years older than those from rural settings ($p < 0.001$) and had attained higher levels of education ($p < 0.001$). About 33% of all women were multiparous just over 90% were married. The majority of urban study participants (96.8) reported tap water as their main drinking water source as just 8.2 % from the rural setting. Neonates from urban areas weighed on average 180 g more ($p < 0.001$) and had higher birth weight ($p = 0.002$). There was no statistically significant difference between the urban and rural setting on the women´s marital status ($p = 0.081$), mean gestational age ($p = 0.075$ and newborn head circumference ($p = 0.845$). The mean gestational age and newborn head circumference did not differ significantly between the urban and rural neonates.

Table 2. Sociodemographic characteristics of women recruited and their neonates´ birth measures (reproduced and modified from Paper I)

			Place of residence			
Variable	**Characteristic**	**Total**	**Urban**	**Rural**	***p*-value**	**Missing data**
Total participants (n)		605	308	297		*(count)*
Age (Years) [a]	Mean (SD)	24.8 (6.22)	25.6 (5.68)	23.9 (6.67)	<0.001	1
Mode of delivery (%)[b]	CS	91 (16.0)	53 (18.6)	38 (13.4)	0.111	37
	SVD	474 (83.5)	229 (80.4)	245(86.6)		
	Breech	2 (0.4)	2 (0.7)	0 (0.0)		
	Vac extr	1 (0.2)	1 (0.4)	0 (0.0)		
Gravidity (%)[b]	1	220 (36.4)	78 (25.3)	142 (47.7)	<0.001	0
	2	157 (25.9)	106 (34.4)	51 (17.1)		
	3	117 (19.3)	73 (23.8)	44 (14.8)		
	4	111 (18.4)	51 (16.5)	60 (20.4)		
Parity (%)[b]	0	207 (34.5)	61 (20.2)	146 (49.2)	<0.001	6
	1	137 (22.8)	86 (28.5)	51 (17.2)		
	2	256 (42.7)	155 (51.3)	100 (33.6)		
Maternal education (%)[b]	None / primary	325 (53.9)	118 (38.6)	207 (69.7)	<0.001	2
	Secondary/ Tertiary	278 (46.1)	188 (61.4)	90 (30.3)		
Marital Status (%)[b]	Married	544 (90.0)	283 (91.9)	260 (87.6)	0.081	0
	Single	61 (10.0)	25 (8.1)	37 (12.4)		
Breast Feeding [b]	Yes	355 (59.1)	208 (68.0)	147 (49.8)	<0.001	4
	No	246 (40.9)	98 (32.0)	148 (50.2)		
Drinking water source (%)[b]	Tap	322 (53.5)	298 (96.8)	24 (8.2)	<0.001	3
	Lake/ s-well	137 (22.8)	2 (0.7)	135 (45.9)		
	Borehole	143 (23.8)	8 (2.6)	135 (45.9)		
Gestational age (weeks) [a]	Mean (SD)	37.58 (1.53)	37.47(1.4)	37.71(1.6)	0.075	64
Birth weight (Kg) [a]	Mean (SD)	3.09 (0.47)	3.18 (0.46)	3.00(0.46)	<0.001	33
Birth length (cm) [a]	Mean (SD)	45.04 (4.28)	45.66(5.5)	44.51(2.6)	0.002	64
Head circumference (cm) [a]	Mean (SD)	33.17 (1.83)	33.14(1.94)	33.19(1.7)	0.845	64

[a] Mann–Whitney U test (urban vs rural).

[b] Chi-square test (urban vs rural).

Abbreviations: SD = standard deviation of mean, CS = Caesarian Section, SVD = Spontaneous Vaginal Delivery.

4.2. Maternal factors associated with serum PFAS concentrations and association with birth outcomes.

Overall, living in rural areas was associated with low maternal blood PFAS concentrations. Specifically living in rural setting was associated with decreased maternal serum concentrations for PFOA (β = −0.581; 95% CI: −0.957 to −0.204; p = 0.003), PFUDA (β = −0.412; 95% CI −0.812 to −0.013; p = 0.043) and SumPFOS (β = −1.535; 95% CI: −2.943 to −0.127; p = 0.003 in maternal serum. However, increased concentrations of SumPFHxS were associated with living in rural areas (β = 1.715; 95% CI: 0.067 to 3.363; p = 0.041). The use of lake and shallow wells as the drinking water source was associated with high maternal serum PFOA concentrations as compared to tap water (β = 0.645; 95% CI: 0.252 to 1.0385; p = 0.001).

Table 3 shows the univariate and multivariable analyses for maternal characteristics versus POPs.

Table 3. Univariate and multivariable linear regression analyses of PFAS concentrations in serum and maternal characteristics.

Maternal Characteristics	n	Poly- and Perfluoroalkyl Substances (PFAS) [a]					
		PFOA	PFNA	PFDA	PFUDA	SumPFHxS	SumPFOS
Univariate linear regression							
Maternal age (years)	549	0.003	0.007	0.003	0.009	-0.007	0.014
Gravidity [b]	549						
Gravida 2		0.17	0.125	0.157	**0.295***	-0.199	**0.246***
Multi gravida		0.099	0.044	0.063	0.112	-0.039	0.173
Parity [c]	544						
Para 1		**0.38***	**0.203***	0.141	0.2	-0.141	**0.38***
Multiparity		0.178	0.135	0.119	0.178	-0.104	**0.279***
Education of mothers [d]	548	**0.188***	0.052	**0.192***	**0.256***	**-0.362****	**0.316****
Area of residence [e]	550	**-0.408****	**-0.212***	**-0.3****	**-0.519****	**0.515****	**-0.907****
Source of drinking water [f]	546						
Lake/shallow well		0.076	-0.015	**-0.185***	**-0.505****	**0.408****	**-0.663****
Borehole		**-0.684****	**-0.284***	**-0.363****	**-0.43****	**0.666****	**-0.909****
Multivariable linear regression							
Maternal age (years)	537	−0.005	0.005	−0.004	0.007	−0.057	0.037
Parity [c]							
Para 1		**0.278***	0.117	0.086	0.022	−0.127	−0.701
Multiparity		0.105	0.02	0.099	−0.036	0.386	−0.866
Education of mothers [d]	537	0.093	0.02	0.14	0.128	−1.073	−0.441
Area of residence [e]	537	**−0.581***	−0.316	−0.170	**−0.412***	**1.715***	**−1.535***
Source of drinking water [f]	537						
Lake/shallow well		**0.645****	0.311	0.058	−0.068	−0.577	−0.145
Borehole		−0.124	0.022	−0.155	−0.024	1.077	−0.348

Values shown are linear regression analyses coefficients. [a] Maternal serum PFAS concentrations were natural log transformed before analysis. [b] Gravida 1 used as reference. [c] Para 0 is used as a reference category. [d] None and primary educational level are the reference categories. [e] Urban is the reference category. [f] Tap water is the reference category. Statistically significant coefficients printed in board. * $p < 0.05$; ** $p < 0.001$.

Abbreviations: PFDA = Perfluorodecanoate, PFHxS = Perfluorohexane sulfonate, PFNA = Perfluorononanoate, PFOA, Perfluorooctanoate, PFOS = Perfluorooctane sulfonate, PFUDA = Perfluoroundecanoate

In general, maternal serum PFAS concentrations were inversely associated with a number of birth outcomes. For instance, maternal PFOA serum concentrations were inversely associated with head circumference (β = −0.056; 95% CI: −0.109 to −0.002; p = 0.043) and birth length (β = −0.049; 95% CI: −0.077 to −0.022; p < 0.001). Furthermore, increased maternal serum PFNA concentrations were associated with decreased neonatal head circumference (β = −0.080; 95% CI: −0.125 to −0.035; p = 0.001), birth length (β = −0.033; 95 CI: −0.057 to −0.010; p = 0.005) and gestational age (β = −0.083; 95% CI: −0.141 to −0.023; p = 0.005). Maternal serum SumPFHxS (SumPFHxS) concentrations were inversely associated birth weight (β = −0.189; 95% CI: −0.371 to −0.006; p = 0.043) and ponderal index (β = −0.090; 95% CI: −0.175 to −0.005; p = 0.037). Another inverse association was observed for SumPFOS (In_SumPFOS) with birth weight (β = −0.261; 95% CI: −0.457 to −0.064; p = 0.009 and gestational age (β = −0.119; 95% CI: −0.183 to −0.055; p < 0.001).

Few positive associations were observed for PFOA with ponderal index (β = 0.136; 95% CI: 0.040 to 0.233; p = 0.005) and SumPFHxS with head circumference (β = 0.048; 95% CI: 0.001 to 0.095; p = 0.045). **Figure 4** shows the results of for the multivariable linear regression analyses associations between maternal serum PFAS concentrations in the overall sample versus birth weight, birth length, head circumference and gestational age.

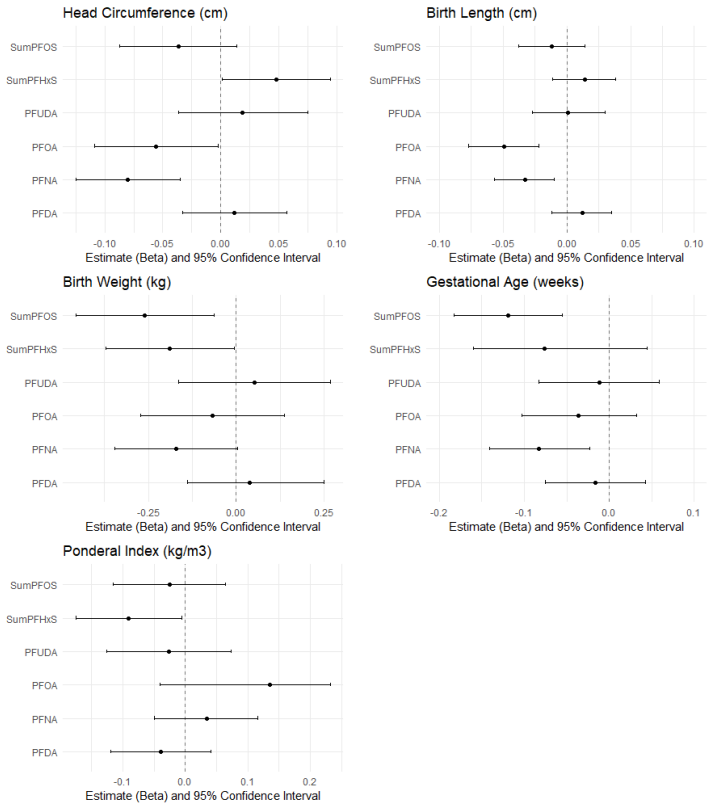

Figure 4. Changes in the birth outcomes associated with maternal serum concentrations of PFAS (n=565).

Maternal blood PFAS concentrations were natural log-transformed.

Multiple linear regression coefficients are displayed with 95% confidence interval.

All associations between birth outcomes and PFAS levels in model A were adjusted for maternal age, area of residence (urban vs. rural), maternal educational level, parity and source of drinking water.

Abbreviations: SumPFOS = perfluorooctane sulfonate; SumPFHxS = perfluorohexane sulfonate; PFUDA = perfluoroundecanoic acid; PFOA = perfluorooctanoate; PFNA = perfluorononanoate; PFDA = perfluorodecanoate.

4.3. Maternal factors associated with serum POP concentrations and association with birth outcomes.

Higher levels of education was positively associated with increased concentration of *p,p´*-DDE ($p = 0.008$), *p,p´*-DDT ($p < 0.001$), cis-NC ($p = 0.014$), *o,p´*-DDT ($p = 0.007$) and *o,p'*-DDE ($p = 0.019$). Similarly, increased maternal age was also positively associated with higher *p,p´*-DDT ($p = 0.013$), *o,p´*-DDT ($p = 0.017$) and *o,p´*-DDE ($p = 0.045$) serum concentrations. Furthermore, the use of lake/shallow well as drinking water source was associated with high maternal serum concentration of t-NC ($\beta = 0.929$; 95% CI: 0.430 to 1.427; $p < 0.001$) and cis-NC ($\beta = 1.311$; 95% CI: 0.642 to 1.980; $p < 0.001$) in reference to the use of tap water. Similarly, women who reported borehole as their main drinking water source had high serum t-NC ($\beta = 0.742$; 95% CI: 0.260 to 1.224; $p = 0.003$) and cis-NC ($\beta = 1.026$; 95% CI: 0.379 to 1.672; $p = 0.002$) concentrations as compared to tap water users. Women who reported previous breast-feeding had statistically significant lower *p,p´*-DDE ($\beta = -0.643$; 95% CI: −1.174 to −0.113; $p = 0.018$) serum concentrations as compared to those who had no such history.

Univariate analysis revealed that increased beef consumption was associated with high HCB ($\beta = 0.900$; 95% CI: 0.035 to 0.145; $p < 0.001$), *p,p´*-DDT ($\beta = 0.544$; 95% CI: 0.247 to 0.841 ; $p < 0.001$) and *o,p'*-DDE ($\beta = 0.209$; 95% CI: 0.038 to 0.379; ; $p = 0.017$). Similarly, goat meat consumption was associated with high levels of HCB ($\beta = 0.076$; 95% CI: 0.024 to 0.129; $p = 0.004$), *p,p´*-DDT ($\beta = 0.351$; 95% CI: 0.066 to 0.636 ; $p = 0.016$) and *o,p'*-DDE ($\beta = 0.206$; 95% CI: 0047 to 0.369; $p = 0.013$).

However, the above reported associations vanished when multivariable linear regression was applied. The univariate and multivariable analyses results for maternal characteristics versus POPs are shown in **Table 4.**

Table 4. Univariate and multivariable linear regression analyses of POPs concentrations in serum and maternal characteristics.

Maternal Characteristics	n	Persistent Organic Pollutants (POPs)							
		HCB [a]	*p´,p´*-DDE [a]	t-NC [a]	*p´,p´*-DDT [a]	*Oxy*-CD [a]	Cis-NC [a]	*o´,p´*-DDT [a]	*o´,p´*-DDE [a]
Univariate linear regression									
Maternal age (years)	558	0.001	**0.06** **	**−0.043** **	**0.026** *	**0.039** **	**−0.037** *	0.001	0.008
Parity [b]	554								
Para 1		**0.068** *	**−0.289**	**−0.353** *	0.355	**−0.377** *	−0.295	0.047	0.152
Multiparity		0.041	**−1.068** **	**0.695** **	0.276	**−0.724** **	**−0.701** **	−0.131	0.023
Education of mothers [c]	556	**0.058** *	**0.600** **	0.154	**0.835** **	0.136	0.273	**0.446** *	**0.291** **
Area of residence [d]	559	**−0.12** **	−0.21	**0.343** *	**−1.129** **	**0.429** **	**0.508** *	−0.25	**−0.308** **
Source of drinking water [e]	555								
Lake/ shallow well		**−0.12** **	−0.113	**0.533** **	**−1.174** **	**0.486** *	**0.790** **	−0.28	**−0.248** *
Borehole		**−0.080** **	−0.27	**0.399** *	**−0.816** **	**0.433** *	**0.561** *	−0.139	**−0.391** **
Previous breast-feeding [f]	554	0.027	**−0.643** **	**−0.575** **	0.164	**−0.620** **	**−0.568** **	-0.035	0.044
Beef consumption [g]	559	**0.090** *	0.111	−0.181	**0.544** *	−0.231	−0.211	−0.03	**0.209** *
Egg consumption [h]	552	0.003	0.115	0.018	**0.284**	−0.025	−0.017	0.022	**0.218** *
Fresh fish consumption [i]	556	0.004	0.186	0.178	0.157	0.127	**0.313** *	−0.027	**0.221** *
Dry fish consumption [j]	558	0.024	−0.25	0.206	−0.341	−0.009	0.032	−0.342	−0.102
Goat consumption [k]	556	**0.076** *	0.212	−0.124	**0.351** *	−0.173	−0.165	0.041	**0.206** *
Green veg consumption [l]	545	0.167	1.288	1.356	1.012	1.079	**2.439** *	0.562	0.183
Multivariable linear regression									
Maternal age (years)	539	−0.003	−0.005	−0.017	0.037 *	−0.057	−0.003	**0.042** *	**0.009** *
Parity [b]	539								
Para 1		0.048	0.119	−0.057	−1.103	0.054	0.046	−0.429	−0.112
Multiparity		0.051	−0.497	−0.225	−0.356	−0.144	−0.229	**−0.927** *	−0.375
Education of mothers [c]	539	0.023	**0.341** *	0.181	**0.578** **	0.178	0.397 *	**0.436** *	**0.196** *
Area of residence [d]	539	−0.101	−0.391	−0.47	**−1.302** **	−0.036	−0.499	−0.591	−0.206
Source of drinking water [e]	539								
Lake/ shallow well		0.003	0.182	**0.929** **	0.11	0.475	**1.311** **	0.205	−0.038
Borehole		0.028	−0.03	**0.742** *	0.397	0.388	**1.026** *	0.324	−0.145
Previous breast-feeding [f]	539	−0.013	**−0.643** **	−0.2	0.026	−0.404	−0.225	0.342	0.16
Beef consumption [g]	539	0.017	−0.189	−0.071	−0.103	−0.077	−0.016	−0.211	−0.017
Goat consumption [h]	539	0.031	0.189	0.048	−0.164	−0.01	−0.007	−0.172	0.052

Values shown are linear regression analyses coefficients. [a] Maternal POPs concentrations were natural log transformed before analysis. [b] Para 0 is used as a reference category. [c] None and primary educational level are the reference categories. [d] Urban is the reference category. [e] Tap water is the reference category. [f] No previous breast-feeding is the reference category. [g–l] Less than twice a week is used as the reference category. Statistically significant coefficients printed in board. * $p < 0.05$; ** $p < 0.001$.

Associations between the selected POPs and birth outcomes were assessed in multivariable linear regression models with maternal age, area of residence (urban versus rural), educational level, parity and source of drinking water as covariates. In this regard, increased gestational age was associated with high concentrations of *p,p′*-DDE (β = 0.087; 95% CI: 0.008 to 0.166; p = 0.031), *p,p′*-DDT (β = 0.110; 95% CI: 0.193 to 0.166; p = 0.01) and *o,p′*-DDT (β = 0. 0.115; 95% CI: 0.016 to 0.213); p = 0.022). However, increased concentrations of t-NC (β = −0.053; 95% CI: −0.105 to −0.0015; p = 0.044), Oxychlordane (β = −0.071; 95% CI: −0.123 to −0.017; p = 0.010) and cis-NC (β = −0.070; 95% CI: −0.140 to −0.006; p = 0.048) in maternal serum were associated with decreased neonatal head circumference. **Figure 5** shows the results of the multivariable linear regression analyses associations between maternal serum POPs concentrations in the overall sample versus birth weight, birth length, head circumference and gestational age.

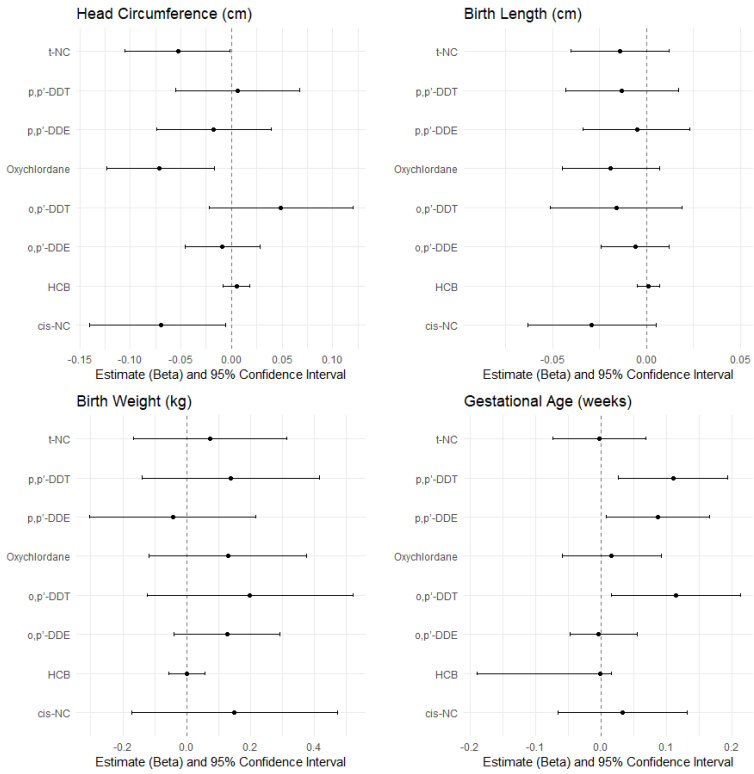

Figure 5. Changes in the birth outcomes associated with maternal serum concentrations of POPs (n=564).

Maternal serum POP concentrations were natural log transformed before analyses.

Multiple linear regression coefficients are displayed with 95% confidence interval.

All association between birth outcomes and POPs were adjusted for: maternal age, are a of residence (urban vs. rural), maternal educational level, parity and source of drinking water.

Abbreviations: t-NC = trans-Nonachlor; *p,p´*-DDT = 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene ; *p,p´*-DDE= 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene ; *o,p´*-DDT= 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene ; *o,p´*-DDE = 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene ; HCB = Hexachlorobenzene ; cis-NC= cis-Nonachlor.

4.4. Maternal factors associated with blood toxic and trace elements concentrations and their association with birth outcomes.

Univariable linear regression analysis and stepwise methods were used to determine the maternal characteristics to be included in the maternal and neonates´ characteristics multivariable model. However, maternal characteristics that are already known to be associated with birth outcomes from the literature were automatically considered as covariates in the multivariable analysis. Multivariable linear regression analysis showed positive associations between maternal age and blood concentrations of Cu (β = 0.005; 95% CI: 0.001 to 0.009; p = 0.023), As (β = 0.014; 95% CI: 0.002 to 0.026; p = 0.034) and Hg (β = 0.015; 95% CI: 0.003 to 0.027; p = 0.013). Furthermore, primiparity was associated with high concentrations of Hg (β = 0.166; 95% CI: 0.013 to 0.320; p = 0.034). Living in rural areas was significantly associated with high maternal blood Ni (β = 0.140; 95% CI: 0.019 to 0.262; p = 0.024) and As (β = 0.498; 95% CI: 0.236 to 0.759; p <0.001) concentrations. The use of lakes and shallow wells as drinking water source was positively associated with increased concentrations of Pb (β = 0.223; 95% CI: 0.023 to 0.359; p = 0.032) as compared to tap water.

The univariate and multivariable analyses for maternal characteristics versus toxic and trace elements versus maternal characteristics are shown in **Table 5.**

Table 5. Univariate and multivariable linear regression analyses of toxic and trace element concentrations in blood versus maternal characteristics.

Maternal Characteristics	n	Univariate linear regression: Toxic metals and trace elements						
		Ni	Se	Cu	Zn	As	Hg	Pb
Age (years)	501	-0.004	**0.005***	**0.003***	-0.001	0.009	**0.016****	-0.001
Parity [b]	498							
Para 1		-0.041	0.01	-0.015	0.011	-0.082	**0.276****	-0.026
Multiparity		-0.036	0.05	0.014	-0.014	-0.001	**-0.193***	-0.056
Education of mothers [c]	499	-0.033	0.042	0.001	-0.002	**-0.163***	0.086	-0.056
Area of residence [d]	502	**0.113 ****	**-0.072***	-0.006	0.03	**0.488****	**0.228****	0.147*
Source of drinking water [e]	498							
Lake/ shallow well		0.062	**-0.086***	-0.042	0.033	**0.654****	**-0.218****	**0.235****
Borehole		**0.118 ****	**-0.076***	0.029	0.016	**0.225***	**-0.142***	0.067
Beef Consumption [f]	502	**-0.069***	0.012	0.017	0.024	**0.309****	0.074	-0.06
Eggs consumption [g]	496	**-0.061***	0.055	0.013	**0.068***	0.116	0.103	0.084
Fresh fish Consumption [h]	499	**-0.061***	0.023	-0.03	-0.007	**0.146***	**0.215****	0.076
Dry fish Consumption [i]	501	0.024	-0.034	-0.001	0.003	0.038	**0.226***	-0.008
Goat Consumption [i]	500	**-0.082***	0.015	-0.006	0.199	**-0.217***	0.056	0.032
Green Veg Consumption [k]	488	-0.096	-0.239	-0.059	-0.237	-0.003	**-0.630***	-0.059
		Multivariable linear regression						
		(n=481)	(n=490)	(n=490)	(n=484)	(n=485)	(n=473)	(n=490)
Maternal age (years)		0.004	0.004	**0.005***	-0.004	**0.014***	**0.015***	0.007
Parity [b]								
Para 1		-0.024	-0.025	-0.034	0.03	0.037	**0.166***	-0.07
Multiparity		-0.054	-0.011	-0.035	0.005	0.01	-0.02	-0.108
Education of mothers [c]		-0.005	0.026	-0.002	0.002	0.013	0.024	-0.03
Area of residence [d]		**0.140***	0.003	-0.001	0.054	**0.498****	**-0.223***	-0.091
Source of drinking water [e]								
Lake/ shallow well		-0.079	-0.071	-0.039	-0.006	0.143	-0.01	**0.223***
Borehole		-0.027	-0.062	0.028	-0.014	-0.166	0.17	0.119
Beef Consumption [f]		-0.006	-	-		-0.063	-	-
Eggs consumption [g]		-0.035	-	-	**0.078***	-	-	-
Fresh fish Consumption [i]		-0.026	-	-	-	**0.100****	0.183	-
Dry fish Consumption [j]		-	-	-	-	-	0.172	-
Goat Consumption [k]		-	-	-	-	-1.079		-
Green Veg Consumption [l]		-	-	-	-	-	**-0.720***	-

Values shown are standard multiple regression analyses coefficient. [a] Metal and trace element concentrations were natural log transformed before analysis. [b] Para 0 as a reference category. [c] None and primary educational level as reference category. [d] Urban as reference category. **e** Tap water as reference category. [f,g,h,i j & k] Less than twice a week as reference categories. Ni model was adjusted for maternal age, education, area of residence, source of drinking water and beef/eggs diet. Se, Cu and Pb models were adjusted for maternal age, education, area of residence and source of drinking water. Zn model was adjusted for maternal age,

education, area of residence and source of drinking water and egg diet. As model was adjusted for maternal age, education, area of residence and source of drinking water and fresh fish/goat meat diet. Hg model was adjusted for maternal age, education, area of residence and source of drinking water and fresh/dry fish and green vegetables diet. Statistically significant coefficients printed in board. *$p < 0.05$; ** $p < 0.001$.

Abbreviations: Ni = nickel; Se = selenium; Cu = copper; Zn = zinc; As = arsenic; Hg = mercury; Pb = lead.

Maternal blood Ni concentrations were positively associated with birth weight ($\beta = 0.063$; 95% CI: 0.003 to 0.124; $p = 0.047$), birth length ($\beta = 0.009$; 95% CI: 0.001 to 0.018 $p = 0.026$), head circumference ($\beta = 0.018$; 95% CI: 0.002 to 0.034; $p = 0.029$) and gestational age ($\beta = 0.023$; 95% CI: 0.002 to 0.044; $p = 0.035$). Inverse association were observed between maternal whole blood As concentrations and neonatal birth length (β = -0.018; 95% CI: -0.035 to -0.0001; p =0.048), head circumferences (β = -0.067; 95% CI: -0.101 to -0.034; p <0.001). Furthermore, another inverse association was observed for maternal blood Pb concentrations with head circumference ($\beta = -0.048$; 95% CI: -0.079 to -0.017; $p = 0.002$) and birth weight ($\beta = -0.020$; 95% CI: -0.036 to -0.004; $p = 0.016$). **Figure 6** shows the results of the multivariable linear regression analyses associations between whole blood toxic and trace elements concentrations in the overall sample versus birth weight, birth length, head circumference and gestational age.

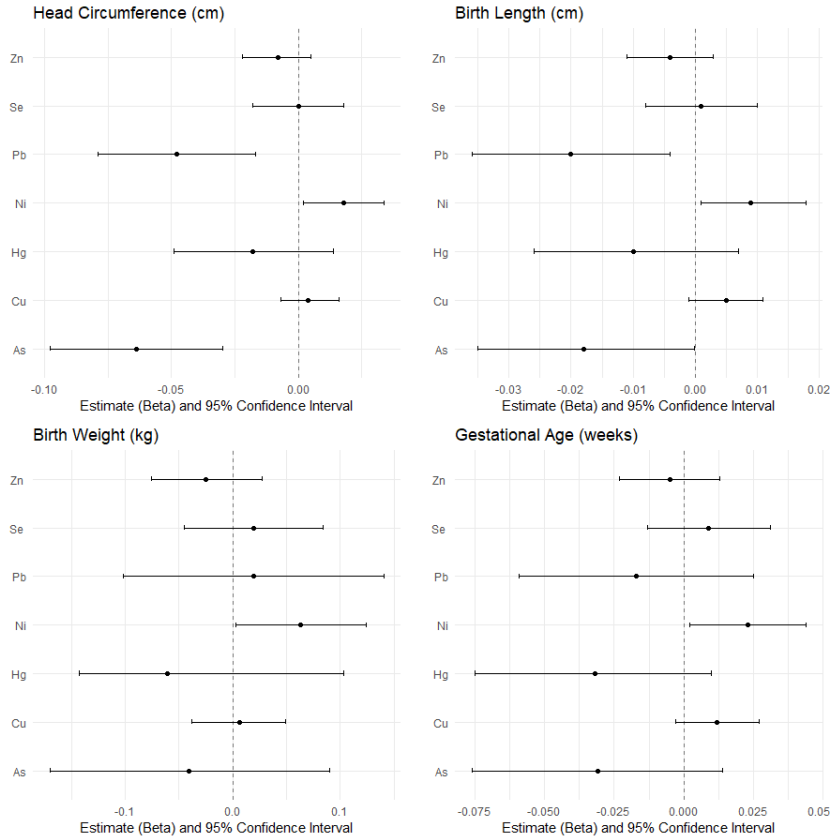

Figure 6. Changes in the birth outcomes associated with maternal blood concentrations of toxic and trace elements (n=506)

The elements concentrations were natural log transformed before analyses.

Multiple linear regression coefficients are displayed with 95% confidence interval.

Ni model was adjusted for maternal age, education, area of residence, source of drinking water and beef/eggs diet. Se Cu and Pb models were adjusted for maternal age, education, area of residence and source of drinking water. Zn model was adjusted for maternal age, education, area of residence and source of drinking water and eggs diet. As model was adjusted for maternal age, education, area of residence and source of drinking water and fresh fish/goat meat diet. Hg model was adjusted for maternal age, education, area of residence and source of drinking water, fresh and dry fish and green vegetables diet.

Abbreviations: Ni = nickel; Se = selenium; Cu = copper; Zn = zinc; As = arsenic; Hg = mercury; Pb = lead.

5. Discussion

5.1. Summary of main results

Paper I: All PFAS (PFOA, PFNA, PFDA, PFUDA and SumPFOS) concentrations assessed in this study, except for SumPFHxS were low as compared to similar studies around the globe. The majority of PFAS observed were either negatively associated with birth outcomes. Only one PFAS namely PFOA showed a positive association with some birth outcomes. Thus, PFOA versus birth length. Maternal PFHxS concentration in relation to birth outcomes followed a different pattern as compared to similar compounds and many other cohorts.

Paper II: Maternal level of education, age and parity were the main predictors of the concentrations of POPs in the maternal serum in this study. In general, the concentrations of POPs observed were in the middle to lower range as compared to other studies conducted in South Africa (145), Cambodia (146), Finland (147) and Brazil (148) except for HCB which registered higher maternal serum concentrations as compared to Cambodia study (146). The ratio between *p,p´*-DDE and *p,p´*-DDT concentrations suggested weak dietary exposure of DDT through the food chain

Paper III: Maternal age and area of residence were the main determinants of the concentrations of toxic and trace elements in maternal blood. Maternal blood toxic and trace elements concentrations observed in the present study were generally low or comparable to the majority of similar studies conducted globally except for Pd that had a geometric mean or median higher than

studies conducted in Ushuaia city in Argentina (149), Colombia (150), Puerto Rico (151), USA(152), Canada (153), Spain (154), Norway (155) and Japan (156).

5.2. Strengths of the results

This study had a very large sample size as compared to similar studies in the field of PFAS, POPs, toxic as well as trace elements among pregnant women and their association with birth outcomes. This increased the chances of precise estimates. Furthermore, detailed information on maternal sociodemographic characteristics, lifestyle, diet, environmental characteristics, obstetric history and the newborn child birth outcomes were collected. Our study reported on POPs wet weight concentrations while most published studies report in lipid adjusted concentrations. Only few epidemiological studies have reported on maternal wet weight POP concentrations. In addition, our study measured a total of 24 PFASs, including PFDA, PFNA and PFHxS. The literature shows that PFDA, PFNA and PFHxS have not been as extensively studied as compared to PFOA and PFOS.

The study questionnaires were administered by well-trained research nurses and blood sample collected by qualified and experienced laboratory technicians with full supervision by PhD candidate. The present study was conducted in both urban and rural settings. In this regard, the results may be generalised to both settings (urban and rural settings).

5.3. Methodological considerations

5.3.1. Sampled body compartment and instrumentation analyses

Whole blood samples were used in the analysis of trace elements and serum for PFAS as well as POPs. This decision was made because detection of these PFAS and POPs in whole blood and serum can be used to assess the current physiological burden of a human body as resent exposures can also be detected. Furthermore, these body compartments have been used in the diagnosis of several compounds and trace elements in the clinical arena for a long time.

Instrumental analysis for the detection of PFAS was performed by UHPLC-MS/MS as this instrumental analysis method is proven for high sensitivity, accuracy, repeatability and robustness. This method was optimized and has the ability to detect PFAS in very low volumes (50µL) of serum samples (142), hence making it suitable for populations with very low PFAS exposure. Similarly, the use of the GC-API-MS/MS as the instrumentation method in the analysis of POPs was selected based on the high sensitivity, accuracy, repeatability and robustness (143).

The use of ICP-MS technique in the detection of toxic and trace elements in the blood samples was selected based the method´s high sensitivity, good precision and accuracy. Furthermore, ICP-MS method has the ability to analyse sequential measurement and quantification of toxic and trace elements, consequently reducing the cost and analyses time. This method of quantification is very sensitive that it can detect toxic or trace elements in whole blood or serum volumes as low as 200 µL, thereby making this approach ideal for identification and quantification of toxic as well as trace elements (133) in populations with different levels of exposure.

In addition, all instrumentation methods used in the analysis of PFAS, POPs and toxic as well as trace elements described above were selected to be used based on the reason that they have been proven to be suitable for analyses of studies with high number of samples (133, 142, 143).

5.3.2. Precision (random error)

The sample size for this study was relatively large as compared to similar studies across the globe hence the change for unprecise estimates were minimal. However, the sample size power calculation was based on the linear association between maternal lead concentrations (representing all PTS, Toxic and essential metals) and birth weight (representing all birth outcomes). In this regard, interpretation of subgroup analysis was taken with caution.

5.3.3. Internal validity (systematic error)

5.3.3.1. Selection bias

Selection bias occurs when the characteristics of the participants recruited in the study are significantly different to those who did not take part. Consequently, the association between exposure and outcome of interest is different between those who participated and did not participate in the study (157). In this study, 104 declined to take part out of the 707 pregnant women that were eligible for the study, representing a decline rate of 14.7%. Furthermore, 565, 564 and 506 pairs of maternal data and blood samples were analyzed for PFAS, POPs and toxic and trace elements respectively out of the planned 600. In this regard, selection bias might have

also occurred because of the either unavailability or insufficient sample volume from some women. However, the proportion numbers of missing or insufficient sample volume were very minimal, thus 40 (6.6%) for PFAS, 41(6.8%) for POPs and 99 (16.4 %) for the toxic and essential elements.

5.3.3.2. Information bias

Data on maternal diet and lifestyle were self-reported by the pregnant women. Consequently, it may not be very accurate as the study participants may have chosen to report on what they considered acceptable or desirable rather than their actual diet. However, the same questionnaire was used for all pregnant women and executed by well-trained research nurses to minimise information bias.

5.3.3.3. Confounding

Some differences were observed in sociodemographic characteristics of the pregnant women who were recruited for this study. Thus, participants recruited from urban versus those recruited from rural settings. Except for mode of delivery, statistically significant differences were observed for maternal age, gravidity, parity, maternal education and source of drinking water between urban and rural study participants (**Table 2**). In this regard, maternal mean age for pregnant women recruited from the urban setting was slightly higher as compared to their rural counterparts. In addition, a significant high proportion of women recruited from the urban had

attained higher education as compared to those recruited from the rural. Notably, tap water was the main source of drinking water among the urban study participants while shallow wells and boreholes came out as the key source for participant from rural settings. However, these covariates were adjusted for in the multivariable linear regression analysis to ensure that they did not influence the results.

Despite the fact that several explanatory factors were looked at, it is still possible that a few explanatory factors and unidentified confounders exists as this was not a randomized controlled trial. However, evidence suggests that this might not be a major problem in other study designs as long as the sample size is large enough which was the case with the present study.

5.3.4. External validity (generalizability)

The results presented in papers I, II and III are from the data collected from both urban and rural areas, and the sample size was large enough as compared to similar studies conducted around the globe. In this regard, the present results can be applied to both settings. In addition, to representativeness of the sample to both urban and rural settings, simple random sampling was used to select study sites while consecutive sampling in the selection of study participants increased the generalizability of the results. However, the samples were collected from health centres situated in the southern part of Malawi only. Consequently, they may not be generalisable to the whole country.

5.4. Comparison with other studies and interpretation of findings

5.4.1. Poly- and Perfluoroalkyl Substances

Compared to similar studies conducted in Europe and Africa, the maternal serum PFAS concentration detected in this study were generally low. Specifically, maternal serum concentrations observed for PFOA, PFDA and PFUDA in the present study were low as compared to a similar published studies from Sweden, Norway, Canada, Spain, Russia, Uzbekistan and Denmark (158-164). Similarly, the concentrations observed for PFOA and PFNA were low as compared to a study by Hanssen *et al.* in South African study (165). The above differences could be explained by the effects of year of sampling and different lifestyles between Malawi and the other areas that were used for comparison. On year of sampling, it was noted that the most recent studies that were used in comparing the results with this study were conducted at least three years ago. In this regard, there is a possibility that the exposure to PFAS is decreasing due to the international restrictions of both the production and use of international PFOS and PFOA by the Stockholm Convention. Furthermore, differences in lifestyles may also play a role in the observed differences as most women recruited in this study reported that their main source of their food was from local production or local markets (99.3%) versus only 0.7% reporting depending on supermarket and imported foods. This is supported by separate studies conducted in North America and Europe which suggested that imported food are the main source of PFAS exposure (166-168).

However, the maternal serum PFNA concentrations detected in this study among pregnant women were comparable to EMASAR study conducted in two regions in Argentina namely

Ushuaia and Salta (169). However, the concentrations were slightly low as compared to the results from a Norwegian study conducted by Berg *et al*. (159). The median maternal PFOS concentrations detected in the current study (0.53 ng/mL) were slightly lower than those found in the Argentinean regions of Salta and Ushuaia (0.84 and 0.70 ng/mL, respectively), (169) but were very low compared to those found in a study by Starling *et al*. (162) that was conducted out in Norway (12.9 ng/mL) (162).

5.4.2. Persistent Organic Pollutants

Published studies on maternal POP analysis as wet weight concentration of the POP/unit serum are scarce. However, the maternal blood concentrations of most POPs observed in the present study were either similar or low as compared to a number of similar published studies. In this regard, the concentration of *p,p´*-DDE were lower than studies conducted in Finland and Cambodia (146, 147). In addition, the median (min–max) concentrations of *p,p´*-DDE and *o,p´*-DDT observed in Malawi study were low (448 (0.10–23,600) vs. 720.0 (20–8047) pg/mL, and 5.10 (0.11–163) vs. 20.0 (20–20) pg/mL, respectively). However, the median concentration (min–max) of maternal serum *o,p´*-DDE in this study was high (0.34 (0.04–8.9) vs. 0.008 (0-008–0.010) pg/mL) as compared to the Cambodian study.

Among all DDT group compounds, *p,p´*-DDE was the most abundant compound detected in the maternal serum and these results are consistent with previous studies from Cambodia and Vietnam (146, 170). However, the results for maternal serum *p,p´*-DDT concentrations were similar to Cambodian study conducted by Steinholt et al. (146). Thus the median (min–max)

maternal serum *p,p´*-DDT concentrations observed in these two studies were almost the same (35.5 (0.43–1700) pg/mL ww for this study and 33.0 (3–519) pg/mL ww for the Cambodian study).

p,p´-DDE was the most abundant compound detected in the maternal serum of pregnant women in Malawi. These findings are comparable to results from previous published studies from Cambodia and Vietnam (146, 170). This was expected as *p,p´*-DDE is the most common and stable metabolite of DDT. In this regard, *p,p´*-DDE is more likely to be detected at a higher level as compared to other metabolites in both human and environmental samples. In addition, despite the restrictions on the production and use of DDT over 40 year ago, various studies have found detection frequency of *p,p´*-DDE to be close to 100% (171-173). Data from the present study showed no indication of a fresh *p,p'*-DDT -exposure as the mean ratio for *p,p´*-DDE: *p,p´*-DDT concentrations was low. This is due to the fact that the ratio of *p,p´*-DDE and *p,p´*- DDT can be used to determine an individual´s history of *p,p´*-DDT exposure. Specifically, the ratio of <5 indicates recent exposure, usually due to the use of pesticides. On the other hand a ratio of >30 is interpreted as a strong indication for a dietary source of the compound (170, 174). In this regard, the *p,p´*-DDE versus *p,p´*-DDT ratio found in this study was about 13.5 hence suggesting no recent but a weak dietary exposure through the food chain.

5.4.3. Toxic and trace elements

The maternal blood concentrations for toxic and trace elements detected in the present study were generally lower than or comparable to levels reported in similar studies conducted across the globe **(Table 6).**

Table 6. Global Comparisons of toxic and trace elements concentrations among pregnant women (μg/L).

Location of the Study		Sample Collection Period	Sample size	Toxic and trace metallic elements						
				As	Hg	Pb	Cu	Ni	Se	Zn
Africa	Malawi (Present Study) [a]	2020 -2021	605	0.32	0.24	14.23	1610	0.86	85.29	5590
	Benin (175) [a]	2015	60	-	-	38	1544	-	-	5215
	South Africa (176) [b]	2005 - 2006	62	0.57	0.65	23	1730	-	104	6290
Aisia-Pacific	China [a] (177)	2016	915	3.88		9.96			133.52	
	Australia (178) [b]	2008 - 2011	173	1.26	-	-	1252	<2.0	88.2	2330
	China (179)[a]	-	209	-	-	39.5	-	-	143.53	-
	China (180) [b]	2010	215	0.52	0.26	24.48	-	-	-	-
	China (181) [b]	2006 - 2007	-	3.81	-	64.32	-	-	-	6312.5
	Japan (156) [b]	2001 - 2006	649	4.06		10.83	1289.2	-	176.4	-
Europe	Spain (154) [b]	2016 - 2017	40	1.8	1.8	12	1664	-	107	6708
	Norway (155) [a]	2007 - 2009	211	1.8	1	9.2	1780	-	72	5480
	Bolivia (182)[a]	2007 - 2008	419	6.14	-	-	-	-	113.7	-
North America	Puerto Rico (151) [a]	2011 - 2017	1183	0.34	1.2	3.3	1552	1	-	4682
	Mexico (63) [a]	2007 - 2008	299	-	-	23.8	-	-	-	-
	USA (152) [a]	2009 - 2011	211	0.45	0.45	8.9	-	-	-	-
	Costa Rica (183) [a]	2010 - 2011	418	-	-	-	-	-	-	-
South America	Suriname (184) [b]	2016	76	-	3.88	47.3	-	-	-	-
	French Guiana (185) [a]	2013	531	-	-	32.6	-	-	-	-
	San Antonio de los Cobres, Argentina (186) [b]	2012 - 2013	169	2.2	-	21	-	-	86	6100
	Salta city, Argentina (*Smokers)* (149) [a]	2011-2012	498	0.55	0.62	15.83	1782	-	129.25	6682
	(Nonsmokers) [a]		-	-	0.6	14.96	1766	-	128.83	6720
	Ushuaia, Argentina *(Smokers)* (149) [a]	2011 - 2012	198	0.63	0.35	10.08	1688	-	80.06	7633
	(Nonsmokers) [a]		-	-	0.34	9.81	1781	-	80.56	7815
	Colombia (150) [b]	2009 - 2010	381	-		9.5	-	-	-	-
	Brazil (187) [b]	2007 - 2008	155	0.6	0.6	16.2	1735	-	64	6420
	Canada (153) [b]	2008 - 2011	1673	-	0.56	5.6	-	-	-	-
	Peru (188) [a]	2004 -2005	204	-	-	-	-	-	-	-

a Values were expressed as geometric mean. **b** Values were expressed as median in the study.

Abbreviations: As = arsenic; Hg = mercury; Pb = lead; Cu = copper; Ni = Nickel; Se = selenium and Zn = zinc.

Details on the comparisons of the maternal blood toxic and trace elements concentration between this study and other similar studies conducted elsewhere are given below:

5.4.3.1. Arsenic

The maternal blood concentrations of As detected in the present study were comparatively low in reference to published studies from Australia (178), Argentina (149, 186), Bolivia (182), Brazil (187), China (177, 181), Spain (154), Norway (155), South Africa (176) and Japan (156). However, the concentrations for As detected in this study were comparable to a study conducted by Ashrap P *et al*. in Puerto Rico by (151).

5.4.3.2. Mercury

The geometric mean or median concentrations of Pb in the present study were low as compared to similar studies conducted in Argentina (149, 186), Brazil (187), Bolivia (182), Canada (153), Peru (188), Suriname (184), Spain (154), Norway (155), South Africa (176), Australia (178) and Puerto Rico (151). However, the concentrations were comparable to a study conducted in China by Jin, *et al* (180).

5.4.3.3. Lead

The maternal blood Pb concentration observed in the present study were low as compared to similar studies conducted in Suriname (184), Benin (175), China (179-181), French Guiana (185), Argentina (186), Brazil (187), Mexico (63), China (179-181) and South Africa (176). However, the concentrations observed were comparable to results from EMASAR Study conducted in Argentina(149) and higher than results from Spain (154), Puerto Rico (151), Ushuaia

city in Argentina (149), USA (152), Canada (153), Colombia (150) , Norway (155) and Japan (156).

5.4.3.4. Nickel

Studies on the detection of Ni in whole blood samples are scarce. However, in the present study whole blood samples were chosen for analysis as this body compartment gives the physiological burden to Ni exposure. Maternal blood Ni concentrations observed in the present study were similar to other similar studies from Puerto Rico (151) and Australia (178).

A couple of other studies used urine samples since they are easier to collect as compared to whole blood samples (189-192). Furthermore, there is a greater increase in urinary excretion in Ni exposed humans; therefore urine is often the preferred measurement of Ni exposure. However, after oral exposure, it takes considerably shorter time (2.5 versus 8 hours) to detect Ni in blood as compared to urine samples (193).

5.4.3.5. Selenium

Maternal blood Se concentrations detected in the present study were generally low as compared to similar studies conducted in Spain (154), Argentina (149), South Africa (176), Japan (156) and Peru (188) but higher than studies from Brazil (187) and Norway (155). However, the current maternal whole blood Se were similar to a study conducted by Callan *et al*, in Australia (178).

5.4.3.6. Copper

The geometric mean or median Cu concentrations in the maternal blood detected in Malawi study were low as compared to all similar studies conducted worldwide. On this, the concentrations were higher than studies conducted in Spain (154), Argentina(149), Norway (155), Brazil (187) and South Africa (176) but higher as compared to results obtained from Puerto Rico (151), Benin (175), Australia (178) and Peru (188).

5.4.3.7. Zinc

The maternal blood concentrations for Zn detected in Malawi study very was low as compared to a number of similar studies conducted elsewhere around the globe (149, 151, 154-156, 175, 176, 178, 186, 187).

5.5. Maternal sociodemographic characteristics and associations with blood or serum PFAS, POP, toxic and trace elements concentrations

5.5.1. Maternal characteristics and serum PFAS concentrations

Area of residence was the main determinant of the levels of most PFAS in maternal serum. In this regard, increased levels of PFOA, PFUDA and SumPFOS in maternal serum was associated with living in urban areas. The main reason for the differences in the concentrations of the above substances between the urban and rural settings can be attributed to differences in socioeconomic conditions and lifestyles, including dietary habits. PFFA are usually used in industrial and consumer products like in the textile impregnation, electroplating, lubricants production and firefighting foam (29) which are more likely to be produced and used in urban settings. In this regard, the probability of being exposed to these substances would be higher in urban areas

compared to rural settings. Furthermore, previous research has revealed that drinking water is the main route of PFAS exposure to humans (194-197). In this study, the majority of pregnant women recruited from the urban areas reported tap water as the main source of their drinking water. On the other hand, the majority of rural study participants depended on borehole as a source of drinking. This finding calls for a follow up study to assess the levels of PFAS in tap versus borehole water.

5.5.2. Maternal characteristics and serum POP concentrations

Maternal level of education, age and parity were the main predictors of maternal serum´s POP concentrations. In this regard, high levels *p´,p´*-DDT, *o´,p´*-DDT and *o´,p´*-DDE in maternal blood were associated with higher levels of maternal age. This was expected as advanced age women may have more exposure to these compounds over the years compared to young women. The finding on positive association between maternal age and the concentrations in POPs in their serum in consistent with studies conducted in the USA, Norway and Sweden (198, 199).

Different lifestyles and diet would be a one of the contributing factors to the difference in the levels of some POPs (HCB and *p,p´*-DDT) in maternal serum between the urban and rural settings. In this study higher concentrations of HCB and *p,p´*-DDT were detected in pregnant women from the urban areas as compared to their counterparts from the rural. There is a possibility that women living in urban areas were exposed to a diet that predisposed them to a high exposure to these substances. For instance, a very large proportion of urban pregnant women reported super markets and imported food as their main source of their daily food. Furthermore, most of the foods that are consumed in the Malawian urban settings are usually sourced from supermarkets and imported from South Africa where DDT is still used for Malaria control. On the other hand, the

diet for women from the rural settings is largely based on home grown and local markets foods. However, further research is needed to find out if indeed there is a difference in the levels of POPs in foods from the two settings.

Similarly, the above explanation may also hold for positive associations between increased levels of maternal education and *p,p´* DDE, *p,p´*-DDT, *o,p´*-DDT and *o,p´*-DDE as women with advanced education are more likely to consume imported foods from supermarkets due to their improved economic status.

5.5.3. Maternal characteristics and toxic and trace elements

Maternal age and area of residence were the main determinants of the levels of toxic and trace elements in maternal blood. Specifically, increased maternal blood levels of Cu was detected on order women as compared to young women. This was rather expected for Cu as previous studies have also revealed positive association between maternal age and serum Cu concentrations (149). Similarly, increased maternal blood As and Hg concentrations were also associated with increased maternal age. This may be attributed to the fact that their advanced age predisposes them to increased exposure to these elements over the years compared to young women.

Higher concentrations of Pb were detected in pregnant women recruited from the rural setting as compared to their counterparts from the urban settings. These results in this study are not consistent with other similar studies. However, the results may be explained by the differences in the economic status between Malawi and other countries in which similar studies were conducted. Malawi is not a highly industrialized country and most of the goods that are used are

imported from other countries. In this regard, the potential of As contamination due to manufacturing industries is very low. However, the country´s economy is highly dependent on agriculture and most of the farming land is situated in rural areas. Consequently, high proportion of rural land is continuously dosed with both organic and inorganic fertilizers. This postulation is supported by a number of research studies that have suggested the use of phosphate fertilizer as the main source of As contamination to the soils and drinking water sources (200-202). Arsenic in the rural soils may then find its way to either drinking water sources or foods as these have been suggested as the main route of As exposure to humans (203, 204). However, there is a need for a follow up research on water (including tap versus borehole), soil and food samples collected from both urban and rural settings to ascertain this hypothesis.

Increased levels of Ni were detected in blood sampled collected from the rural as compared to urban settings. Notably, one of the rural sites from which the study participants were recruited was close to a large landfill. Just like what other studies conducted in Cameroon, Nigeria, Bangladesh and India have revealed (205-209), it is very likely the landfill was the main contributing factor to high levels of Ni in the blood of women recruited from rural setting in this study.

6. Dissemination of findings

The results have been published in peer-reviewed journals (papers I and II). In addition, abstracts for paper I and paper II were also submitted to international Norwegian Research School of Global Health PhD conferences conducted in 2022 and 2023 respectively. Furthermore, the

summary of the results will be presented to the health facilities which participated in this study and the Helse Nord RHF in Norway.

7. Conclusion and future direction

Malawi study made a comprehensive effort to examine the determinants of selected PFAS, POPs, toxic and trace elements concentrations during pregnancy and their associations with social demographic characteristics and birth outcomes. Maternal age, level of education and parity were the common main determinants of exposure to PFAS and POPs. In addition, maternal age and area of residence are the main determinants of exposure to toxic and trace elements concentration. In this regard, the results from this study can be used as a benchmark upon which knowledge of the main predictors for the maternal levels of these compounds and elements among pregnant women at the delivery stage and from low–middle income African settings can be built.

Maternal serum PFAS concentrations detected in this study, except for SumPFHxS, were low as compared to similar studies conducted in other parts of the world. This is a good finding as it may imply that there is very low environmental contamination of PFAS in Malawi and hence low exposure of these substances to pregnant women. However, there is a need for more studies to explore the reason behind a different pattern followed for PFHxS compared to similar compounds and many other cohorts.

Generally, maternal POPs, toxic and trace elements concentrations detected in this study were low or comparable to concentrations detected in similar studies across the globe. This is a good outcome. Notably, traces of DDT and its metabolites were also detected in maternal serum

although the Malawi government banned the use and distribution of DDT in 1985. The *p,p´*-DDE versus *p,p´*-DDT concentrations ratio revealed weak dietary exposure of DDT through the food chain. In addition, higher levels of DDT and its metabolites were detected in urban pregnant women as compared to their counterparts from the rural setting. In this regard, there is a need for more research to explore the reason behind the observed differences. On this, future studies can explore the levels of these compounds in maternal serum between the urban and rural settings in relation to the sources of food.

Although the concentrations of most toxic elements detected among pregnant women were low as compared to similar studies conducted around the globe, some inverse associations were observed between As versus neonatal head circumference and birth length. Similarly, Pb versus neonatal head circumference and birth length. These findings support the revised 2021 CDC guidelines on the recommended limit of blood Pb which stipulates that there is no safe Pb levels in children as even low levels can cause adverse health effects (81). However, since some traces of Pb were detected in the maternal blood in this study, there is a need for continued human biomonitoring surveys and more studies to establish the sources of both environmental and dietary Pb exposure to vulnerable populations including pregnant women.

The results from this study may be used in the identification of specific lifestyles that are associated with increased maternal POP, PFAS and toxic elements concentrations as well as too low or high trace elements levels in women of child-bearing age.

We recommend policy makers in Malawi to consider setting up a national wide POPs, PFAS and toxic metals biomonitoring program to assess levels of these substances at a national level especially in vulnerable populations. This human biomonitoring program on persistent toxic substances could be established in collaboration with partners from the global north where more biomonitoring programs on these substances are at advanced stages. Establishment of such a program could be a significant step towards long-term mapping of the levels of these POP, PFAS and toxic metals in the Malawian population and identification of specific lifestyles that are associated with increased concentrations of these substances. However, there is still a lot more work to be done in Malawi in relation to the levels of human exposure to POP, PFAS and toxic elements in relation to adverse health effects examined and described in this study.

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PAPERS I, II & III

PAPER I

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Article

Serum Concentrations of Selected Poly- and Perfluoroalkyl Substances (PFASs) in Pregnant Women and Associations with Birth Outcomes. A Cross-Sectional Study from Southern Malawi

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Abstract: Pervasive exposure to per-and polyfluoroalkyl substances (PFASs) shows associations with adverse pregnancy outcomes. The aim of the present study was to examine the determinants of different serum PFAS concentrations in late pregnancy and their relationship with birth outcomes in southern Malawi. The sample included 605 pregnant women with a mean age of 24.8 years and their offspring from three districts in the southern region of Malawi. Six PFAS were measured in serum from third-trimester women. The serum PFAS concentrations were assessed with head circumference, birth length, birth weight, gestational age and ponderal index. Participants living in urban areas had significantly higher serum levels of PFOA, PFNA and SumPFOS, while SumPFHxS concentrations were higher in women from rural settings. High PFOA, PFNA and SumPFHxS concentrations were generally inversely associated with head circumference. Birth length was negatively associated with PFOA and PFNA while SumPFHxS was negatively associated with birth weight. SumPFOS was inversely associated with gestational age. Urban area of residence was the strongest predictor for high PFAS concentrations in the maternal serum and was generally associated with adverse birth outcomes. The results highlight the need to investigate SumPFHxS further as it follows a pattern that is different to similar compounds and cohorts.

Keywords: poly- and perfluoroalkyl substances; birth outcomes; southern Malawi

1. Introduction

Per-and polyfluoroalkyl substances (PFASs) are highly fluorinated aliphatic substances that consist of carbon (C) chains of different length with a perfluoroalkyl moiety (CnF2n+1-) [1]. These compounds are man-made synthetic chemicals that are highly resistant to biodegradation and show high affinity for bioaccumulation (due to more intake than excretion of the chemicals) and biomagnification in the environment and living organisms, including humans. Although the production and usage of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in particular has been gradually reduced in several countries since the year 2000, human exposure continues mainly due to persistence in the environment and use. PFASs

have a wide range of applications in industry as well as consumer products [2,3] and are also known to have long half-lives. For instance, it is estimated that the arithmetic and geometric mean half-lives of serum elimination in human beings for PFOS, perfluorohexane sulfonate (PFHxS) and PFOA are as follows: 5.4 years and 4.8 years for PFOS; 8.5 years and 7.3 years for PFHxS; and 3.8 years and 3.5 years for PFOA, respectively [4]. Furthermore, PFASs are able to undergo atmospheric and marine long-range transport and are hence found in remote areas such as the Antarctic and Arctic, far away from their areas of production and use [5–7].

Globally, there are growing concerns about the links between exposure to PFAS compounds and adverse health effects [8]. A wide range of adverse health effects associated with different single PFASs but also sum concentration of PFASs were observed in previously published studies [9]. The health effects of concern related to PFASs include altered metabolism and fertility [10], increased risk of being overweight or obese [11] and reduced ability of the immune system to fight infections [12]. New research indicates that PFASs have the ability to transfer from mother to child through the placenta. In this regard, there is growing concern over possible adverse impacts on development and health later in life due to early exposure to these chemicals [13]. Of particular concern are both short-term and long-term subtle effects that might influence reproductive health, pregnancy outcomes, reduce defense against diseases and increase the risk of cancer [14].

A number of studies have suggested an association between higher concentrations of some PFAS and low birth weight [15–19], preterm birth [17,18], birth length [20] and gestational age [21]. Most of the monitoring and research on PFASs has been conducted in developed countries, especially in Europe and North America. Only a few studies were published from developing countries and countries situated in the southern hemisphere. Biomonitoring data from the northern hemisphere may not be applicable to the southern hemisphere. In this regard, results and findings from Malawi and other countries situated in the southern hemisphere are of particular importance and needed in order to evaluate the current exposure situation to PFASs in these regions together with associations related to health outcomes in general. Furthermore, although the WHO developed PFOA and PFOS guidelines for drinking water standards, there are recommendations from public health advocates, scientists and organizations for a revision or withdrawal of the guidelines. The call for the revision or withdrawal of the guidelines follows an argument that they are neither health protective nor based on the best available scientific evidence, and hence, they are more likely to promote global health inequities [22]. In this regard, data from this study may provide a foundation to be used in the process of revision of the above-stated guidelines. The present study on pregnant women from three different locations in Malawi was conducted with the aim to examine the determinants of different serum PFAS concentrations in late pregnancy and investigate their relationship with birth outcomes.

2. Materials and Methods

2.1. Study Design and Study Population

This is a cross-sectional study of delivering women giving birth and their offspring. The study was conducted in the southern region of Malawi, in antenatal clinics/wards and labor wards of three government health facilities. The health facilities that were randomly selected for the study include Ndirande Health Centre, Chiradzulu District Hospital (CDH) and Thyolo District Hospital (TDH). Ndirande Health Centre is located in Blantyre, which is the commercial city of Malawi, while Chiradzulu and Thyolo district hospitals are located in Thyolo and Chiradzulu districts, respectively. All study sites are located in the southern region of Malawi. Ndirande Health Centre is situated in an urban setting while the other district hospitals represent the rural setting.

2.2. Data Collection and Management

Study participants were recruited between August 2020 and July 2021 using data collection tools that were pretested in a pilot survey conducted soon before the main survey. Personal characteristics, socioeconomic status, lifestyle, infant information and

environmental characteristics were collected through a questionnaire administered by a trained research nurse. A total of 605 women and neonate pairs were recruited into the study. However, 40 were excluded from the serum PFAS analysis due to a lack of biological samples, yielding a final study population of 565. Figure 1 gives details on the numbers in the recruitment process.

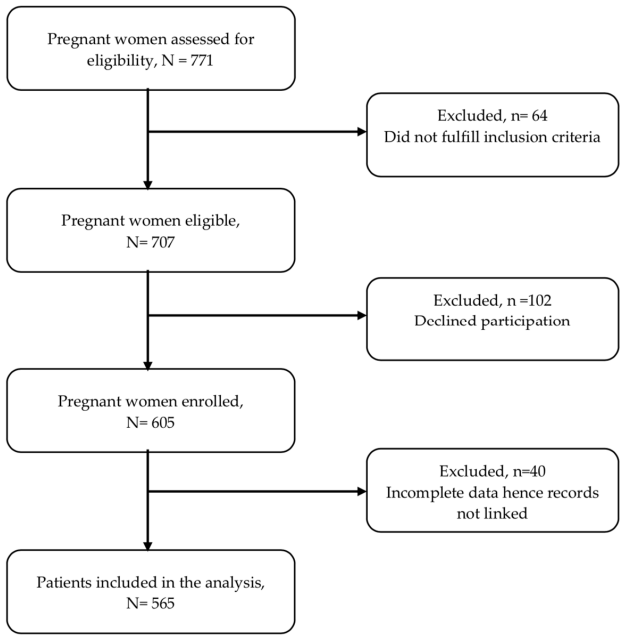

Figure 1. Flow chart of included and excluded participants.

2.3. Serum Blood Sample Collection and Preliminary Analysis

Blood samples were collected from the mothers at an optimal time before delivery (36 ± 12 h prior to delivery). Methods for collection and transportation of whole blood and serum samples were adapted from CTQ Laboratory guidelines, Quebec, Canada [23]. In brief, venous blood was collected from the mother using a 5 mL red-top BD Vacutainers® (REF # 367614). The red-top vacutainer containing the whole blood was then left to stand at room temperature for about 60 min for complete clot formation before centrifugation at $\leq 1200 \times g$ (3276 rotations per minute) for 10 min at room temperature (18–25 °C). After centrifugation, the serum was transferred to two glass tubes with green lids (27138 Sigma Aldrich, St. Louis, MO, USA) using a disposable glass pipette. Transfer of serum to the green-lid tubes was performed with caution to avoid pipetting the red cells along with the serum. All collected biological samples were stored in a freezer at a temperature between −35 °C and −20 °C before being shipped to University Hospital of North Norway (UNN), Department of Laboratory Medicine, for analysis.

2.4. Serum Sample Analysis

Sample preparation, instrumental analysis, quantification and quality controls have been described in detail elsewhere [24]. Briefly, extracts were prepared by an automated liquid handler Tecan Freedom Evo 200 (Männedorf, Switzerland). Perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), PFOS, perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorododecane sulfonate (PFDoDS), perfluorooctane sulfonamide (PFOSA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA),

perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA) and perfluorotetradecanoate (PFTeDA) were analyzed by ultrahigh pressure liquid chromatography tandem-quadrupole mass-spectrometry (UHPLC-MS/MS). Sum of branched and linear species (Σ) was quantified for PFHxS, PFHpS, PFOS, PFHxS, PFHpS, PFNS, PFDS and PFOSA. Analyses were performed with a Waters Acquity UPLC system (Waters, Milford, MA, USA) consisting of a binary solvent manager, an autosampler and a column manager coupled to a Xevo TQ-S mass spectrometer (Waters, Milford, MA, USA) through an atmospheric pressure electrospray interface. Separation of the target analytes was achieved on an Acquity UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm) (Waters, Milford, MA, USA) by using a programmed flow and solvent gradient of 2 mM NH_4OAc in MilliQ-water and 2 mM NH_4OAc in methanol as mobile phase. Quantification was conducted by applying the Masslynx and Targetlynx software (Version 4.1, Waters, Milford, MA, USA) and achieved by the internal standard method with isotope-labelled PFASs. Method quantification level (MQL) was defined as ten times a signal-to-noise ratio or three times the limit of detection (LOD). LODs (minimum limit of detection) were set as concentrations calculated by the Targetlynx software for each individual sample (LODi) and each individual analyte with a signal-to-noise ratio of 3 divided by the related sample amount. Where blank contamination was detected (background contribution during sample preparation), a blank subtraction was performed batch wise by calculating an average of the blanks added to three times their standard deviation. An eight-point calibration curve with concentrations ranging from 0.01 pg/µL to 10 pg/µL was applied for quantification.

Quality Controls

Accuracy and precision of the method was described in detail in the associated method article [24]. For quality assurance, 4 blank samples, 4 SRM 1957 and SRM 1958 (NIST, Gaithersburg, MD, USA) samples and 3 bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of 96 samples. Differences from the assigned mean reference concentrations were from 3 to 13% for SRM 1957 and 3 to 9% for SRM 1958 during the Malawi study. Additionally, our laboratory participates successfully in the international quality control program: the Arctic Monitoring and Assessment (AMAP) Ring Test for Persistent Organic Pollutants in Human Serum (organized by the Laboratoire de toxicologie, Institut national de santé publique du Quebec, Canada). Solvent injections were performed regularly during instrumental analysis in order to monitor instrument background and carryover effects.

2.5. Measurement of Birth Outcomes

Birth outcome variables measured in this study were gestational age (weeks), birth weight (kilograms), birth length (cm), head circumference (cm) and ponderal index (kg/m^3). Ponderal index was calculated using the following formula: ponderal index = weight(kg)/height^3 (m^3).

2.6. Statistical Analysis

Statistical analyses were carried out using the Stata for Mac (SE standard version 17; College Station, TX, USA). A total of 24 compounds were analyzed, but most of the compounds were under the limit of detection. However, 6 out of the 24 PFASs analyzed compounds—namely PFOA, PFNA, PFDA, PFUDA, SumPFHxS and SumPFOS—had detection limits of over 60% and hence were used for the statistical analysis. Data were given as arithmetic means, standard deviation (SD), median, and minimum and maximum or proportion (%) for describing sociodemographic characteristics of the study population. A significance level of $p < 0.05$ (two-tailed) was set for all analyses.

PFAS concentrations were log-transformed before assessing linear associations due to non-normal distribution of the concentrations among participants.

2.7. Ethical Considerations

The study was carried out following ethical rules and guidelines. Ethical clearance was obtained from the College of Medicine Research and Ethics Committee (COMREC)- Malawi (P.11/18/2546) and REK-Norway (#355656 2020). Permission to conduct the study at the selected sites was sought from Blantyre (for Ndirande Health Centre), Chiradzulu (for CDH) and Thyolo (for TDH) district health offices. Participation in the study was voluntary, based on signed written consent from the mother. Confidentiality was maintained by assigning pseudo-anonymized identification numbers to all study participants. The sampling of blood and extraction of information did not interfere with the health service delivery process and took place either before or after delivery, depending on the individual circumstances of each study participant.

3. Results

3.1. Maternal Sociodemographic Data and Neonate Birth Characteristics

The selected maternal sociodemographic characteristics of the n = 605 individuals are presented in Table 1.

Table 1. Sociodemographic characteristics of the mothers and neonates.

			Place of Residence		
Variable	**Characteristic**	**Total**	**Urban**	**Rural**	***p*-Value**
Total participants (n)		605	308	297	
Age (years)	Mean (SD)	24.80(6.22)	25.63 (5.65)	23.93 (6.66)	<0.001
Gravidity (%)	1	220 (36.4)	78 (25.4)	142 (47.7)	<0.001
	2	157(26.0)	106 (34.5)	51 (17.1)	
	3	117 (19.3)	73 (23.8)	44 (14.8)	
	4	111 (18.4)	50 (16.3)	61 (20.5)	
Parity (%)	0	207 (34.5)	61 (20.2)	146 (49.0)	<0.001
	1	137 (22.8)	86 (28.5)	51 (17.1)	
	2	256 (42.7)	155 (51.3)	101 (33.9)	
Education level of mother (%)	None/primary	325 (53.9)	118 (38.6)	207 (69.7)	<0.001
	Secondary/tertiary	278 (46.1)	188 (61.4)	90 (30.3)	
Marital status (%)	Married	544 (89.8)	283 (91.9)	261 (87.6)	0.081
	Single	62 (10.2)	25 (8.1)	37 (12.4)	
Breast feeding (%)	No	246 (40.9)	98 (32.0)	148 (50.2)	<0.001
	Yes	355 (59.1)	208 (68.0)	147 (49.8)	
Source of drinking water, count (%)	Tap	322 (53.5)	298 (96.8)	24 (8.2)	<0.001
	Lake/shallow well	137 (22.8)	2 (0.7)	135 (45.9)	
	Borehole	143 (23.8)	8 (2.6)	135 (45.9)	
Use of pesticides at home (%)	Do not use pesticides	480 (79.5)	288 (93.5)	192 (64.9)	<0.001
	Pesticides	124 (20.5)	20 (6.5)	104 (35.1)	
Fishing (%)	Do not Fish	596 (98.5)	308 (100.0)	288 (97.0)	0.002
	Fish	9 (1.5)	0 (0.0)	9 (3.0)	
Gestational age (weeks)	Mean (SD)	37.59 (1.53)	37.47 (1.43)	37.71 (1.62)	0.075
Birth weight (kg)	Mean (SD)	3.09 (0.46)	3.18 (0.45)	3.00 (0.46)	<0.001
Birth length (cm)	Mean (SD)	45.19 (3.46)	45.95 (4.08)	44.53 (2.66)	<0.001
Head circumference (cm)	Mean (SD)	33.17 (1.83)	33.14 (1.94)	33.19 (1.72)	0.753
Ponderal index (kg/m^3)	Mean (SD)	3.43 (0.97)	3.44 (1.23)	3.43 (0.66)	0.874

SD: standard deviation of mean.

Participants ages ranged from 16 to 45 years, with a mean (SD) age of 24.8 (6.2) years. Out of the 605 participants recruited, 308 pregnant women were recruited from urban (Ndirande Health Centre) and 297 from rural (Chiradzulu and Thyolo district hospitals) settings. In this regard, mean age of the pregnant women recruited from the urban setting was almost 2 years older than those from rural areas, with a mean of 25.6 (SD = 6.7) and 23.9 (SD = 5.7) years of age, respectively. Similarly, the mean age of spouses from the urban setting was 30.7 (SD = 6.4) versus 28.1 (SD = 7.8) for rural.

The data showed a significant difference in gravidity, parity and educational levels between the two groups. In this regard, nulliparity was significantly high in rural areas. Para 1 and multiparty were statistically high in urban areas as compared to rural.

Over half (69.7%) of the women from the rural areas either did not attend any formal school or were educated up to primary level only as compared to only 38.6% from the urban area. Furthermore, a vast proportion (61.4%) of those recruited from the urban area attained education up to secondary or tertiary level, while only 30.3% from the rural area attained such levels. A high percentage of women (96.8%) residing in the urban areas used tap water as their source of drinking water in comparison to only 8.1% from the rural locations. Shallow wells and boreholes were the most common (44.1% and 45.5%, respectively) sources of drinking water for the rural study participants.

A total of 572 neonates were recruited in this study. Out of this, 296 were boys (51.8%). The mean birth weight, length and head circumference were 3.09 kg, 45.28 cm and 33.15 cm, respectively, in the overall sample. Detailed information about neonates according to area of residence (urban versus rural) is also outlined in Table 1.

3.2. Maternal PFASs Serum Concentrations

Six out of the twenty-four PFASs analyzed compounds—PFOA, PFNA, PFDA, PFUDA, SumPFHxS and SumPFOS—had detection limits of over 60% and hence were used for the statistical analyses. Table 2 outlines the serum concentration in ng/mL of the above listed 6 PFASs. The highest PFAS median concentrations found in this study were 3.09 ng/mL and 0.533 ng/mL for SumPFHxS and SumPFOS, with detection rates of 99.8% and 99.5%, respectively. The lowest median concentration levels of all the PFASs assessed was 0.018 ng/mL for PFUDA. The measured maternal serum PFASs in descending order of median concentration were SumPFHxS > SumPFOS > PFOA> PFDA> PFNA > PFUDA.

Table 2. Maternal serum concentrations (ng/mL) of PFASs.

Maternal Serum Concentrations (ng/mL) of PFASs (n = 565)			
PFASs	**% > LOD**	**Mean (SD) [a]**	**Median (Min–Max)**
PFOA	95.2	0.18 (0.31)	0.12 (0.002–2.66)
PFNA	96.3	0.05 (0.09)	0.04 (0.001–1.94)
PFDA	93.8	0.07 (0.05)	0.06 (0.002–0.490)
PFUDA	60.2	0.02 (0.02)	0.02 (0.002–0.160)
SumPFHxS	99.8	4.68 (4.59)	3.09 (0.001–28.3)
SumPFOS	99.5	1.40 (4.32)	0.53 (0.002–56.7)

[a] Arithmetic mean with standard deviation (SD). The limit of detection (LOD) > 60% of the samples.

3.3. PFASs in Serum and Maternal Characteristics

Multiple linear regression analysis of maternal sociodemographic/lifestyle characteristics versus the concentrations of different PFASs provided a relatively comprehensive description of the main maternal risk factors related to serum PFAS levels (Table 3).

Adjusted for maternal age, parity, maternal educational level, area of residence (urban vs. rural) and source of drinking water, living in the rural setting was associated with decreased maternal PFOA ($\beta = -0.581$; 95% CI: -0.957 to -0.204; $p = 0.003$), PFUDA ($\beta = -0.412$; 95% CI -0.812 to -0.013; $p = 0.043$) and SumPFOS ($\beta = -1.535$; 95% CI: -2.943 to -0.127; $p = 0.003$) serum concentrations. Conversely, high concentrations of SumPFHxS were associated with living in rural areas ($\beta = 1.715$; 95% CI: 0.067 to 3.363; $p = 0.041$). However, there was no statistically significant inverse association between serum PFNA and PFDA concentrations and area of residence.

Table 3. Multivariable linear regression of PFAS concentrations in blood serum and maternal characteristics.

	Maternal Characteristics	Maternal Serum PFAS Concentrations [a]		
		n	β (95% CI)	*p*-Value
PFOA	Maternal age (years)	537	−0.005 (−0.025 to −0.015)	0.649
	Parity	537		
	Para 0		Reference category	
	Para 1		0.278 (0.026 to 0.531)	0.031
	Multiparity		0.105 (−0.190 to 0.401)	0.484
	Education of mothers	537		
	None/primary		Reference category	
	Secondary/tertiary		0.093 (−0.091 to 0.277)	0.32
	Area of residence	537		
	Urban		Reference category	
	Rural		−0.581 (−0.957 to −0.204)	0.003
	Source of drinking water	537		
	Tap		Reference category	
	Lake/shallow well		0.645 (0.252 to 1.039)	0.001
	Borehole		−0.124 (−0.503 to 0.225)	0.521
PFNA	Maternal age (years)	537	0.005 (−0.012 to 0.022)	0.562
	Parity	537		
	Para 0		Reference category	
	Para 1		0.117 (−0.098 to 0.331)	0.286
	Multiparity		0.020 (−0.231 to 0.271)	0.874
	Education of mothers	537		
	None/primary		Reference category	
	Secondary/tertiary		0.020 (−0.136 to 0.176)	0.801
	Area of residence	537		
	Urban		Reference category	
	Rural		−0.316 (−0.636 to 0.003)	0.052
	Source of drinking water	537		
	Tap		Reference category	
	Lake/shallow well		0.311 (−0.023 to 0.645)	0.068
	Borehole		0.022 (−0.300 to 0.343)	0.895
PFDA	Maternal age (years)	537	−0.004 (−0.022 to 0.013)	0.65
	Parity	537		
	Para 0		Reference category	
	Para 1		0.086 (−0.135 to 0.306)	0.445
	Multiparity		0.099 (−0.160 to 0.358)	0.452
	Education of mothers	537		
	None/primary		Reference category	
	Secondary/tertiary		0.140 (−0.021 to 0.301)	0.089
	Area of residence	537		
	Urban		Reference category	
	Rural		−0.170 (−0.500 to 0.159)	0.311
	Source of drinking water	537		
	Tap		Reference category	
	Lake/shallow well		0.058 (−0.286 to 0.402)	0.741
	Borehole		−0.155 (−0.487 to 0.177)	0.359
PFUDA	Maternal age (years)	537	0.007 (−0.014 to 0.028)	0.531
	Parity	537		
	Para 0		Reference category	
	Para 1		0.022 (−0.246 to 0.290)	0.873
	Multiparity		−0.036 (−0.350 to 0.278)	0.822
	Education of mothers	537		
	None/primary		Reference category	
	Secondary/tertiary		0.128 (−0.067 to 0.323)	0.199
	Area of residence	537		
	Urban		Reference category	
	Rural		−0.412 (−0.812 to −0.013)	0.043
	Source of drinking water	537		
	Tap		Reference category	
	Lake/shallow well		−0.068 (−0.485 to 0.350)	0.749
	Borehole		−0.024 (−0.426 to 0.379)	0.908

Table 3. *Cont.*

	Maternal Characteristics	Maternal Serum PFAS Concentrations [a]		
		n	β (95% CI)	*p*-Value
SumPFHxS	Maternal age (years)	537	−0.057 (−0.145 to 0.031)	0.202
	Parity	537		
	Para 0		Reference category	
	Para 1		−0.127 (−1.233 to 0.979)	0.822
	Multiparity		0.386 (−0.908 to 1.680)	0.558
	Education of mothers	537		
	None/primary		Reference category	
	Secondary/tertiary		−1.073 (−1.878 to −0.268)	0.009
	Area of residence	537		
	Urban		Reference category	
	Rural		1.715 (0.067 to 3.363)	0.041
	Source of drinking water	537		
	Tap		Reference category	
	Lake/shallow well		−0.577 (−2.299 to 1.145)	0.511
	Borehole		1.077 (−0.583 to 2.737)	0.203
SumPFOS	Maternal age (years)	537	0.037 (−0.038 to 0.112)	0.331
	Parity	537		
	Para 0		Reference category	
	Para 1		−0.701 (−1.646 to 0.243)	0.145
	Multiparity		−0.866 (−1.972 to 0.240)	0.125
	Education of mothers	537		
	None/primary		Reference category	
	Secondary/tertiary		−0.441 (−1.129 to 0.246)	0.209
	Area of residence	537		
	Urban		Reference category	
	Rural		−1.535 (−2.943 to −0.127)	0.033
	Source of drinking water	537		
	Tap		Reference category	
	Lake/shallow well		−0.145 (−1.616 to 1.326)	0.847
	Borehole		−0.348 (−1.767 to 0.071)	0.63

[a] Maternal blood PFAS concentrations were natural log-transformed. All association between PFAS concentrations in serum and maternal characteristics were adjusted for maternal age, parity, maternal educational level, area of residence (urban vs. rural) and source of drinking water.

3.4. PFAS Concentrations and Birth Outcomes

Multiple linear regression analysis of birth outcomes and maternal serum PFAS concentrations shows the possible associations between different maternal serum PFAS concentrations and birth weight, head circumference, birth length, gestational age and ponderal index. These results were examined by multiple regression analysis while adjusting for maternal age, area of residence (urban vs. rural), maternal educational level, parity and source of drinking water (Table 4). A statistically significant inverse association was observed between the natural log-transformed maternal serum of PFOA concentrations (In_PFOA) and head circumference (β = −0.056; 95% CI: −0.109 to −0.002; $p = 0.043$). Similarly, a negative association was also observed between In_PFOA and birth length (β = −0.049; 95% CI: −0.077 to −0.022; $p < 0.001$). Conversely, a positive association was observed between In_PFOA and ponderal index (β = 0.136; 95% CI: 0.040 to 0.233; $p = 0.005$). No associations were observed between In_PFOA and birth weight or gestational age in both models.

There was a statistically significant negative relationship between natural log-transformed maternal serum PFNA concentrations (In_PFNA) and head circumference (β = −0.080; 95% CI: −0.125 to −0.035; $p = 0.001$). Similarly, negative associations were also observed between In_PFNA and birth length (β = −0.033; 95 CI: −0.057 to −0.010; $p = 0.005$) and gestational age (β = −0.083; 95% CI: −0.141 to −0.023; $p = 0.005$). However, there was no statistically significant association between In_PFNA and birth weight and ponderal index. Furthermore, a statistically significant negative association was observed between natural log of maternal serum SumPFHxS (In_SumPFHxS) concentrations and birth weight (β = −0.189; 95% CI: −0.371 to −0.006; $p = 0.043$). Similarly, an inverse statistically significant association was

observed between In_SumPFHxS and ponderal index (β = −0.090; 95% CI: −0.175 to −0.005; $p = 0.037$).

Table 4. Multivariable analysis results of linear regression analysis measuring effects of maternal serum PFAS concentrations on birth outcomes.

	Outcomes	Maternal Serum PFAS Concentrations [a]		
		n	β (95% CI)	*p*-Value
PFOA	Head Circumference (cm)	478	−0.056 (−0.109 to −0.002)	0.043
	Birth Length(cm)	478	−0.049 (−0.077 to −0.022)	<0.001
	Birth Weight (kg)	508	−0.067 (−0.272 to 0.138)	0.523
	Gestational Age (weeks)	480	−0.036 (−0.103 to 0.032)	0.298
	Ponderal Index (kg/m^3)	477	0.136 (0.040 to 0.233)	0.005
PFNA	Head Circumference (cm)	478	−0.080 (−0.125 to −0.035)	0.001
	Birth Length(cm)	478	−0.033 (−0.057 to −0.010)	0.005
	Birth Weight (kg)	508	−0.171 (−0.346 to 0.003)	0.054
	Gestational Age (weeks)	480	−0.083 (−0.141 to −0.023)	0.005
	Ponderal Index (kg/m^3)	477	0.035 (−0.050 to 0.116)	0.404
PFDA	Head Circumference (cm)	478	0.012 (−0.033 to 0.057)	0.597
	Birth Length(cm)	478	0.012 (−0.012 to 0.035)	0.33
	Birth Weight (kg)	508	0.038 (−0.139 to 0.25)	0.673
	Gestational Age (weeks)	480	−0.016 (−0.075 to 0.043)	0.589
	Ponderal Index (kg/m^3)	489	−0.39 (−0.119 to 0.041)	0.340
PFUDA	Head Circumference (cm)	478	0.019 (−0.036 to 0.075)	0.494
	Birth Length(cm)	478	0.001 (−0.027 to 0.030)	0.924
	Birth Weight (kg)	508	0.052 (−0.165 to 0.269)	0.636
	Gestational Age (weeks)	480	−0.0122 (−0.083 to 0.059)	0.735
	Ponderal Index (kg/m^3)	477	−0.026 (−0.125 to 0.074)	0.608
SumPFHxS	Head Circumference (cm)	478	0.048 (0.001 to 0.095)	0.045
	Birth Length(cm)	478	0.014 (−0.011 to 0.038)	0.265
	Birth Weight (kg)	508	−0.189 (−0.371 to −0.006)	0.043
	Gestational Age (weeks)	480	−0.160 (−0.076 to 0.044)	0.600
	Ponderal Index (kg/m^3)	477	−0.090 (−0.175 to −0.005)	0.037
SumPFOS	Head Circumference (cm)	478	−0.036 (−0.087 to 0.014)	0.153
	Birth Length(cm)	478	−0.012 (−0.038 to 0.014)	0.348
	Birth Weight (kg)	508	−0.261 (−0.457 to −0.064)	0.009
	Gestational Age (weeks)	490	−0.119 (−0.183 to −0.055)	<0.001
	Ponderal Index (kg/m^3)	477	−0.245 (−0.115 to 0.065)	0.591

[a] Maternal blood PFAS concentrations were natural log-transformed. All associations between birth outcomes and PFAS levels in model A were adjusted for maternal age, area of residence (urban vs. rural), maternal educational level, parity and source of drinking water.

Another statistically significant negative association was observed between the natural log of SumPFOS (In_SumPFOS) serum concentrations and birth weight (β = −0.261; 95% CI: −0.457 to −0.064; $p = 0.009$). An inverse association was also observed between In_SumPFOS and gestational age (β = −0.119; 95% CI: −0.183 to −0.055; $p < 0.001$). Conversely, statistically significant positive associations were observed between In_PFOA and ponderal index (β = 0.136; 95% CI: 0.040 to 0.233; $p = 0.005$). A similar trend was also detected between In_SumPFHxS and head circumference (β = 0.048; 95% CI: 0.001 to 0.095; $p = 0.045$).

4. Discussion

Area of residence was the main determinant of maternal serum PFAS, with higher concentrations registered from urban settings than from rural areas. Increased concentrations of some maternal serum PFASs (i.e., PFOA, PFNA, SumPFHxS and SumPFOS) were mostly inversely associated with some, but not all, birth outcomes. There were very few notable

positive associations between PFASs and birth outcomes as follows: SumPFHxS with head circumference (cm) and PFOA with ponderal index.

Maternal serum PFOA, PFDA and PFUDA concentrations observed in this study were lower compared to other studies from Sweden, Norway, Canada, Spain, Russia, Uzbekistan and Denmark [25–31]. In addition, PFOA and PFNA concentrations were also lower than those observed in a South African study [32]. In this regard, the results from the present study are suggestive of the effects of year of sampling and different lifestyles between Malawi and the other areas that were used for comparison. For instance, most women recruited in this study reported either local production or local markets (99.3%) as their main source of food for consumption, with only 0.7% of women reporting consumption of supermarket and imported foods. The above result adds weight to suggestions from other studies conducted in North America and Europe which concluded that imported food, fast food, and preprepared food packed in food packaging material are the main source of PFAS exposure. However, the maternal serum PFNA concentrations observed in our study were close to the results found in the Estudio del Medio Ambiente y la Salud Reproductiva (EMASAR) study that compared PFAS concentrations in maternal serum between two different regions in Argentina, Ushuaia and Salta [33]. Nevertheless, they were slightly low as compared to the results from a Norwegian study conducted by Berg et al. [26].

The median for maternal SumPFOS concentrations observed in the present study (0.533 ng/mL) was slightly lower that the levels observed in Ushuaia and Salta (0.84 and 0.70 ng/mL, respectively) regions in Argentina [33]. However, the levels detected are very low as compared to results from a study conducted by Starling et al. (12.9 ng/mL) in Norway [29]. The underlying reasons for such a difference could be due to the different lifestyles between the areas. As already discussed above, our sample constituted women that predominantly use local production and local markets as their main source of food as compared to both Argentina and Norway, hence the lower probability of exposure to SumPFOS. In contrast, the maternal serum SumPFHxS concentrations observed in our study were very high as compared to other studies conducted elsewhere. For instance, the median SumPFHxS in our study was 3.09 ng/mL against 0.18 ng/mL and 0.22 ng/mL for Ushuaia and Salta, respectively, in Argentina.

Area of residence was one of the main determinants for the blood serum PFAS concentrations, with higher blood serum PFOA and PFNA concentrations detected in those living in urban areas than those living in rural areas. This difference could be explained by differences in socioeconomic conditions and lifestyles, including dietary habits. PFASs are used in many industrial and consumer applications such as textile impregnation, paper production, fire-fighting foam, lubricants production and electroplating [2]. These compounds are more likely to be produced and used in urban settings. In this regard, the odds of exposure to these compounds to mothers residing in the urban areas is expected to be high as compared to rural settings.

Drinking water is known to be one of the main sources of PFASs exposure for humans [34–37]. In this regard, the source of drinking water could also be the reason for elevated serum PFAS concentrations in urban study participants. The majority of urban study participants recruited use tap water (surface water) as a source of drinking water. Boreholes (ground water) are the main source of drinking water for their counterparts from the rural setting. However, there is a need for further studies to assess the level of PFASs between tap and borehole water to evaluate this hypothesis.

Various research studies have shown that both PFOA and PFOS can cross the placental barrier [38–44] and affect neonate birth weight, birth length and gestational age. In this regard, high PFAS concentration in the placenta may presumably restrict fetal growth. The results of the present study did not show a clear association between PFOA and birth weight ($p > 0.05$). These results are consistent with the Brazilian Ribeirão Preto (BRISA) study and a systematic review that included results from nine different studies that found no significant association between PFOA and birth weight [45,46]. In contrast, we observed an inverse association between SumPFOS and birth weight ($p = 0.009$). Similarly, the

meta-analysis of 23 papers conducted by Yang et al. [47] also found a negative association between PFOS and birth weight. SumPFOS was also indicated to be inversely associated with gestational age ($p < 0.001$). However, the results showed a statistically significant inverse association between maternal serum PFOA concentrations and head circumference. Furthermore, there was also an inverse association between PFOA concentrations and length at birth.

Increased maternal serum PFNA concentrations in the present study were statistically associated with low birth weight. This finding was similar to the study conducted in Spain by Manzano-Salgado et al. [19]. However, our findings become statistically nonsignificant after adding source of drinking water to the line of covariates. This could be due to confounding of the association by source of drinking water.

Maternal serum SumPFHxS concentrations showed a statistically significant association with low level of education. This association may be reflective of behavior and lifestyle factors associated with socioeconomic status. Unexpectedly, a statistically significant positive association was observed between maternal SumPFHxS concentrations and head circumference. This is in contrast to a study by Xiao et al. [48] that observed an inverse association between PFHxS and head circumference. Furthermore, another study conducted in the Spanish cohort did not find any association between the two variables [19]. The differences in the results between the current study and the other two could be explained by different sample sizes and the methodologies that were used. In this regard, there is a need for more research assessing the relationship between individual PFASs and head circumference. No statistically significant associations were observed between parity and each of the PFASs assessed in the present study. These findings are in contrast to other studies that observed significantly higher PFOA concentrations in nulliparous compared to multiparous women [49,50].

Our study has several important strengths. To our knowledge, this is the first study assessing PFAS concentrations in delivering women from Malawi. In this regard, our study provides highly valuable benchmark information on PFAS exposure status of delivering women from Malawi. Furthermore, our study contributes to a body of knowledge on the rather underrepresented research area on PFAS from the African continent. Different socioeconomic status and lifestyle sites were included in the present study, hence providing a representative sample for all settings. Furthermore, we measured a panel of 24 PFASs, including PFDA, PFNA and PFHxS, which have not been as extensively studied as PFOA and PFOS. We also acknowledge several limitations of our study. The number of patients assessed for eligibility matched the sample size calculation. However, some participants refused to have their blood sample collected, which reduced the total number of data analyzed in the final data set. However, we assumed that this would not have a significant effect on the power of the current study.

5. Conclusions

The present study explores and presents PFAS concentrations among delivering women in Malawi. Area of residence was the predictor for high concentrations of PFASs detected in serum of women from urban settings. Maternal serum PFAS concentrations were associated with some but not all birth outcomes. PFAS concentrations assessed in the present study, except SumPFHxS, are lower as compared to other parts of the world. Follow-up studies are needed to evaluate the association between the source of drinking water and maternal serum PFAS concentrations. There is a need to conduct further investigations on PFHxS as it follows a totally different pattern compared to similar compounds and many other cohorts.

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PAPER II

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Article

Predictors of Maternal Serum Concentrations for Selected Persistent Organic Pollutants (POPs) in Pregnant Women and Associations with Birth Outcomes: A Cross-Sectional Study from Southern Malawi

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Abstract: Population exposure to persistent organic pollutants (POPs) may result in detrimental health effects, especially to pregnant women, developing foetuses and young children. We are reporting the findings of a cross-sectional study of 605 mothers in their late pregnancy, recruited between August 2020 and July 2021 in southern Malawi, and their offspring. The aim was to measure the concentrations of selected POPs in their maternal serum and indicate associations with social demographic characteristics and birth outcomes. A high level of education was the main predictor of *p,p′*-DDE ($p = 0.008$), *p,p′*-DDT ($p < 0.001$), cis-NC ($p = 0.014$), *o,p′*-DDT ($p = 0.019$) and *o,p′*-DDE ($p = 0.019$) concentrations in maternal serum. Multiparity was negatively associated with *o,p′*-DDE ($p = 0.021$) concentrations. Maternal age was also positively associated (*p,p′*-DDE ($p = 0.013$), *o,p′*-DDT ($p = 0.017$) and *o,p′*-DDE ($p = 0.045$) concentrations. Living in rural areas was inversely associated with high maternal serum concentrations of *p,p′*-DDT ($p < 0.001$). Gestational age was positively associated with *p,p′*-DDE ($p = 0.031$), *p,p′*-DDT ($p = 0.010$) and *o,p′*-DDT ($p = 0.022$) concentrations. Lastly, an inverse association was observed between head circumference and t-NC ($p = 0.044$), Oxychlordane ($p = 0.01$) and cis-NC ($p = 0.048$). These results highlight the need to continue monitoring levels of POPs among vulnerable populations in the southern hemisphere.

Keywords: persistent organic pollutants; birth outcomes; Southern Malawi

1. Introduction

Persistent organic pollutants (POPs) are carbon-based, man-made synthetic chemical substances produced mainly for commercial purposes, such as for pests and disease control, crop production and industrial use [1]. These compounds have very long half-lives when released into the environment. They accumulate in the environment and adversely affect the food chain and living organisms, including humans, because of their lipid solubility

and resistance to biodegradation [2]. However, it was not until 1962, when Rachel Carson, through her book entitled *Silent Spring*, revealed that these substances could be both uncontrollable and unexpectedly toxic [3]. Globally, there is growing evidence suggesting the link between these compounds and the occurrence of various noncancerous and cancerous health outcomes. Among many adverse health effects, there is growing concern about the possible association between serum POPs and reproductive health, wheezing in infants or children, Type 2 diabetes mellitus (DMT2), liver cancer, breast cancer and more recently, Parkinson's disease [4–7].

Although the use and production of most POPs were restricted following the Stockholm Convention in 2001, some are still present in different environments and in living organisms, including humans, due to their bio-accumulative and long-persistent natures [8]. Twelve key compounds that were included in the Stockholm Convention were aldrin, chlordane, dichlorodiphenyl trichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (dioxins) and polychlorinated dibenzofurans (furans) [9].

Since Malawi´s economy is largely dependent on agriculture, with about 80% of the population employed in this sector [10], there is an increasing demand for the use of pesticides. It is estimated that, in general, Malawi uses at least 2000 metric tons of pesticides annually, of which 70% are used for agriculture [11]. While there is extensive use of pesticides for agriculture in Malawi, there are limited data on the determinants and concentrations of POPs in the maternal serum. Various research studies have established that POPs are permeable through the placenta, and the findings have suggested that they can impair the growth and development of the foetus [12–14] and that exposure to POPs in the early stages of life (in utero) may pose a critical risk to health.

Most of the monitoring and research on POPs has been carried out in countries situated in the northern hemisphere. On the other hand, in the southern hemisphere, there has been limited research on predictors for POP concentrations in maternal serum and their associations with reproductive health outcomes. Research on POPs and human health outcomes, particularly concerning reproductive health in Malawi and other countries in the southern hemisphere, is of importance for global health [15]. This study, therefore, aimed to examine predictors of the serum concentrations of different POPs in delivering women and investigate the relationship of the POP concentrations with birth outcomes in Malawi.

2. Materials and Methods

2.1. Study Design, Population and Sites

Details on the study design, population and sites were described in detail in the previous publication of our study [16]. In brief, this cross-sectional study of pregnant women and their offspring was conducted in the southern region of Malawi, and study participants were recruited between August 2020 and July 2021.

The inclusion criteria included all pregnant women who were in the late stages of their pregnancy; 16 years of age and above; permanent residents of the Blantyre, Chiradzulu and Thyolo districts; and willing to voluntarily sign an informed consent statement. All women with serious medical conditions present at the time of enrolment, high risk pregnancies with prognoses to be referred to a tertiary health care level, or were also participating in another study were excluded. In this regard, a total of 771 pregnant women were assessed for eligibility and 605 were recruited for the study. However, only 564 women–neonate pairs were included in the final study population for statistical analysis on the associations between POP concentrations, as 41 either declined to have their biological sample collected, or the volume of the sample was insufficient. Figure 1 describes the details of the number of participants assessed for inclusion and recruited.

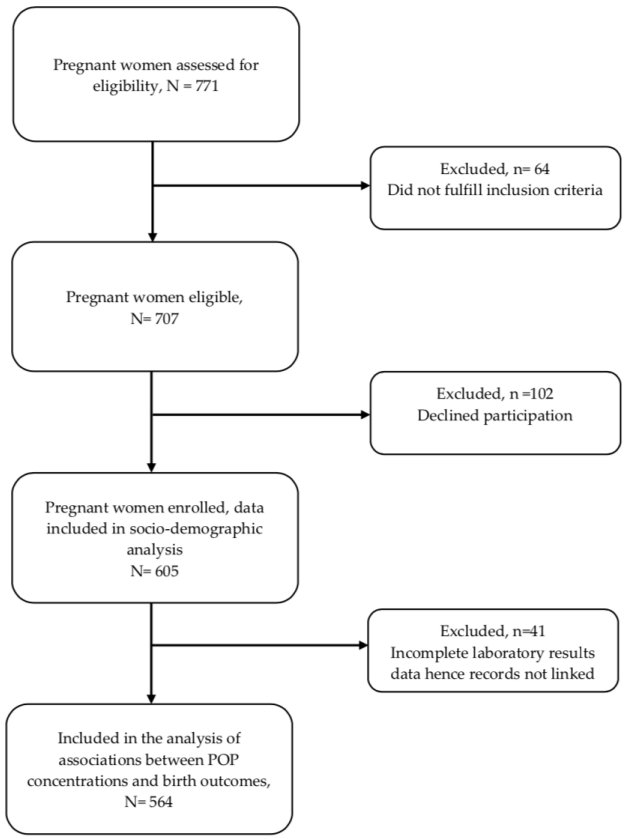

Figure 1. Flow chart of included and excluded participants.

2.2. Study Questionnaire

The questionnaire that was used for the collection of information about mothers and neonates was already described in the first publication of our study [16]. Briefly, pregnant women´s personal characteristics, reproductive history, socioeconomic status, lifestyle, environmental characteristics, diet and neonatal data were collected just before delivery using a provider-administered questionnaire.

2.3. Sample Collection and Preliminary Analysis

Methods for the whole blood sample collection and preliminary analysis used in the present study were adapted from the international quality control system QA/QC established by the Centre de Toxicologie du Quebec [17]. The detailed process of the sample collection and preliminary analysis were described in our first publication for the study [16].

2.4. Serum Sample Analysis

Serum samples were analysed at the Environmental Pollutant Laboratory at the University Hospital of North Norway according to the previously published method by Huber et al. [18]. In short, a Tecan Freedom Evo 200 (Männedorf, Switzerland) liquid-handling workstation was applied for automated sample preparation. First, 150 µL aliquots of serum samples were diluted prior to extraction on reversed phase 96-well plates, which was followed by clean-up on normal phase extraction columns. Gas chromatography atmospheric pressure ionisation coupled to tandem mass spectrometers (GC-API-MS/MS;

Waters, Milford, MA, USA) were used for instrumental analysis. The API was conducted in positive mode under charge transfer conditions. For detection on the mass spectrometer, the multiple reaction monitoring mode was applied with two specific transitions for the individual analytes. Quantification was performed using the Masslynx and Targetlynx software (Version 4.1 and 4.2, Waters, Milford, MA, USA) and achieved by the internal-standard method with isotope-labelled compounds.

2.5. Measurement of Birth Outcomes

The following birth outcome variables were measured soon after delivery: gestational age (weeks), birth weight (kg), birth length (cm) and head circumference (cm) [16]. Gestational age was estimated at delivery by referring to the antenatal health records and the ponderal index was used to estimate the nutritional status of the newborns and calculated using the following formula [16,19]:

$$\text{ponderal index} = \frac{\text{weight (kg)}}{\text{height}^3\ (\text{m}^3)} \quad (1)$$

2.6. Statistical Analysis

Data analysis was performed using the statistical software Stata for Mac (SE standard version 17; College Station, TX, USA). Our analysis focused on compounds with the detection frequency of ≥50%. With this criterion, the compounds that qualified for the analyses were: HCB, *p,p′*-DDE, t-NC, *p,p′*-DDT, Oxychlordane, cis-NC, *o,p′*-DDT and *o,p′*-DDT (Table 1). To control for missing data, concentrations of selected compounds below the limit of detection (LOD) were replaced by LOD/2. LODs were automatically calculated by the Masslynx software for each individual analyte and sample. Detailed information on the LODs is given in Supplementary Table S1. Descriptive statistics including arithmetic means, standard deviation (SD), median and minimum and maximum or proportion (%) were computed from the data set (Table 1). A significance level of $p < 0.05$ (two-tailed) was set for all analyses. The data were fitted into a multivariable regression model with maternal characteristics as the response variable and the significant POP concentrations as predictors. All POP concentrations were log-transformed before inclusion in the multivariable regression models due to non-normal distribution of the concentrations among the participants.

Table 1. Maternal serum concentrations (wet weight pg/mL) of POPs (n = 564).

POPs	% > DF	AM (SD) [a]	GM (95% CI) [b]	Median (Min–Max)
HCB	99.8	102 (20.7)	99.8 (97.4–104.19)	102 (0.57–203)
p,p′-DDE	99.3	878 (1575)	405 (359.5–456.7)	478 (0.10–23,600)
t-NC	98.1	73.7 (126)	35.3 (31.7–39.3)	34.5 (0.01–1240)
p,p′-DDT	86.9	81.3 (136)	30.9 (27.1–35.2)	35.5 (0.43–1700)
Oxychlordane	84.5	63.7 (102)	29.7 (26.6–33.1)	34.4 (0.05–1050)
cis-NC	84	13.1 (26.6)	4.3 (3.8–5.0)	5.4 (0.02–203)
o,p′-DDT	73.4	9.51 (16.0)	3.3 (2.8–3.8)	5.10 (0.11–163)
o,p′-DDE	58.4	0.55 (0.8)	0.34 (0.32–0.37)	0.34 (0.04–8.9)

[a] Arithmetic mean (AM) with standard mean deviation (SD). [b] Geometric mean (GM) with 95% CI. The detection frequency (DF) >50% of the samples. Missing data have been inputted as LOD/2.

3. Results

3.1. Maternal Socio-Demographic and Neonates' Anthropogenic Data

The socio-demographic and neonates' anthropogenic data are described in our previously published article from this Malawi study [16]. The age of women recruited in the study ranged from 16 to 45 years, with a mean (SD) age of 24.8 (6.2) years. Just over half of the women (308) were recruited from the urban setting, and 297 were from rural areas. Women recruited from the urban setting were almost two years older than their counter-

parts recruited from rural areas. In this regard, the mean ages (SD) for pregnant women recruited from urban and rural areas were 25.6 (6.7) and 23.9 (5.7) years, respectively.

The data showed differences in gravidity, parity and educational levels between the urban and rural pregnant women. On this, nulliparity was significantly high in rural areas. In contrast, Para 1 and multiparity were statistically high among women recruited from urban areas, as compared to their counterparts from rural sites.

A small (38.6%) proportion of women recruited from urban areas either did not attend any formal school or were educated just up to the primary level. However, the majority (61.4%) attained an education up to the secondary or tertiary level. In contrast, the majority of pregnant women from rural areas did not attain higher educational levels (69.7% attained up to the primary level versus only 30.3% attaining the secondary/tertiary level). The use of tap water as the source of drinking water was very common (96.8%) among urban study participants. As for the rural setting, shallow wells and boreholes were collectively the common source of drinking water, representing 44.1% and 45.5%, respectively.

In total, 572 neonates (51.8% boys) were recruited in the present study. The mean birth weight, length and head circumference for the neonates recruited were 3.09 kg, 45.28 cm and 33.15 cm, respectively, in the overall sample. Detailed information about maternal sociodemographics and the neonates´ anthropogenic data according to their area of residence (urban versus rural) is also outlined in the Supplementary Table S2.

3.2. Maternal POP Serum Concentrations

Eight out of the 19 POPs with detection frequencies (DF) $\geq$50% were included in the statistical analysis. The eight compounds included in the statistical analysis were Hexachlorobenzene (HCB), 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene (*p,p′*-DDE), trans-Chlordane (t-NC), 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (*p,p′*-DDT), Oxychlordane, cis-Nonachlor (cis-NC), 1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene (*o,p′*-DDT) and 1-chloro-2-[2,2-dichloro-1-(4-chlorophenyl)ethenyl]benzene (*o,p′*-DDE). Table 1 provides descriptive statistics for the selected eight POPs in pg/mL (as wet weight concentration of the POP/unit serum). The two POPs with the highest median concentrations observed in this study were 478 pg/mL and 102 pg/mL for *p,p′*-DDE and HCB with detection frequencies of 99.3% and 99.8%, respectively. On the other end, the lowest median concentration assessed was 0.34 pg/mL for *o,p′*-DDE. Detailed information of the POPs that were included in the in the statistical analysis is presented in Table 1.

3.3. Concentrations of POPs in Maternal Serum and Associations with Maternal Characteristics

The POP concentrations followed an approximately normal distribution after logarithmic transformation. Maternal characteristics, namely age and parity, were automatically considered to be included in the multivariate analysis, as they are already known to be associated with POP concentrations in maternal serum based on previous studies. Furthermore, univariable linear regression analysis was used to determine the covariates that needed to be included in the multivariable linear regression models. In this regard, the following factors were selected to be included in the multivariable analysis: maternal educational level, previous breast-feeding and beef and goat meat consumption frequencies. Tables 2 and 3 show the detailed results of the univariate and multivariable analyses, respectively, between maternal characteristics and different POP concentrations in maternal serum.

Multivariable linear regression models of the aforementioned variables provided an overall description of the main factors influencing the concentrations of the selected POPs. Comprehensive descriptions of the main maternal risk factors related to the serum POP levels are given in Tables 2 and 3.

Table 2. Univariate linear regression of POP concentrations in blood serum and maternal characteristics.

Maternal Characteristics	HBC [a]	*p′,p′*-DDE [a]	t-NC [a]	*p′,p′*-DDT [a]	Oxychlordane [a]	Cis-NC [a]	*o′,p′*-DDT [a]	*o′,p′*-DDE [a]
Maternal age (years)	0.001	**0.06** **	**−0.043** **	**0.026** *	**0.039** **	**−0.037** *	0.001	0.008
Parity [b]								
Para 1	**0.068** *	**−0.289**	**−0.353** *	0.355	**−0.377** *	−0.295	0.047	0.152
Multiparity	0.041	**−1.068** **	**0.695** **	0.276	**−0.724** **	**−0.701** **	−0.131	0.023
Education of mothers [c]	**0.058** *	**0.600** **	0.154	**0.835** **	0.136	0.273	**0.446** *	**0.291** **
Area of residence [d] (urban vs. rural)	**−0.12** **	−0.21	**0.343** *	**−1.129** **	**0.429** **	**0.508** *	−0.25	**−0.308** **
Source of drinking water [e]								
Lake/shallow well	**−0.12** **	−0.113	**0.533** **	**−1.174** **	**0.486** *	**0.790** **	−0.28	**−0.248** *
Borehole	**−0.080** **	−0.27	**0.399** *	**−0.816** **	**0.433** *	**0.561** *	−0.139	**−0.391** **
Previous breast-feeding [f]	0.102	**−0.643** **	**−0.575** **	0.164	**−0.620** **	**−0.568** **	-0.035	0.044
Beef consumption frequency [g]	**0.090** *	0.111	−0.181	**0.544** *	−0.231	−0.211	−0.03	**0.209** *
Egg consumption frequency [h]	0.003	0.115	0.018	**0.284**	−0.025	−0.017	0.022	**0.218** *
Fresh fish consumption frequency [i]	0.004	0.186	0.178	0.157	0.127	**0.313** *	−0.027	**0.221** *
Dry fish consumption frequency [j]	0.024	−0.25	0.206	−0.341	−0.009	0.032	−0.342	−0.102
Goat consumption frequency [k]	**0.076** *	0.212	−0.124	**0.351** *	−0.173	−0.165	0.041	**0.206** *
Green vegetables consumption frequency [l]	0.167	1.288	1.356	1.012	1.079	**2.439** *	0.562	0.183

Values shown were simple linear regression analyses coefficients. [a] All POP concentrations were log 10 transformed before analysis. [b] Para 0 is used as a reference category. [c] None and primary educational level are the reference categories. [d] Urban is the reference category. [e] Tap water is the reference category. [f] No previous breast-feeding is the reference category. [g–l] Less than twice a week is used as the reference category. * $p < 0.05$; ** $p < 0.001$. Significant findings are printed in bold.

Table 3. Multivariable linear regression of POP concentrations in blood serum and maternal characteristics.

Maternal Characteristics	HBC [a]	*p′,p′*-DDE [a]	t-NC [a]	*p′,p′*-DDT [a]	Oxychlordane [a]	Cis-NC [a]	*o′,p′*-DDT [a]	*o′,p′*-DDE [a]
Maternal age (years)	−0.003	−0.005	−0.017	0.037 *	−0.057	−0.003	**0.042** *	**0.009** *
Parity [b]								
Para 1	0.048	0.119	−0.057	−1.103	0.054	0.046	−0.429	−0.112
Multiparity	0.051	−0.497	−0.225	−0.356	−0.144	−0.229	**−0.927** *	−0.375
Education of mothers [c]	0.023	**0.341** *	0.181	**0.578** **	0.178	0.397 *	**0.436** *	**0.196** *
Area of residence [d] (urban vs rural)	−0.101	−0.391	−0.47	**−1.302** **	−0.036	−0.499	−0.591	−0.206
Source of drinking water [e]								
Lake/shallow well	0.003	0.182	**0.929** **	0.11	0.475	**1.311** **	0.205	−0.038
Borehole	0.028	−0.03	**0.742** *	0.397	0.388	**1.026** *	0.324	−0.145
Previous breast-feeding [f]	−0.013	**−0.643** **	−0.2	0.026	−0.404	−0.225	0.342	0.16
Beef consumption frequency [g]	0.017	−0.189	−0.071	−0.103	−0.077	−0.016	−0.211	−0.017
Goat consumption frequency [h]	0.031	0.189	0.048	−0.164	−0.01	−0.007	−0.172	0.052

Values shown were simple linear regression analyses coefficients. [a] All POP concentrations were log 10 transformed before analysis. [b] Para 0 is the reference category. [c] None and primary educational level are the reference category. [d] Urban is the reference category. [e] Tap water is the reference category. [f] No previous breast-feeding is the reference category. [g,h] Less than twice a week is the reference category. * $p < 0.05$; ** $p < 0.001$. Significant findings are printed in bold.

After adjusting for maternal age, parity, area of residence (urban versus rural), source of drinking water and beef and goat meat consumption, participants educated up to either secondary or tertiary levels had significantly higher concentrations of *p,p′*-DDE ($p = 0.008$), *p,p′*-DDT ($p < 0.001$), cis-NC ($p = 0.014$), *o,p′*-DDT ($p = 0.007$) and *o,p′*-DDE ($p = 0.019$) than those who were only educated up to the primary level. Similarly, greater maternal ages were statistically associated with higher maternal serum concentrations of *p,p′*-DDT ($p = 0.013$), *o,p′*-DDT ($p = 0.017$) and *o,p′*-DDE ($p = 0.045$).

Multiparity was significantly associated with decreased maternal serum concentrations of some POPs. This was observed for *o,p′*-DDT ($\beta = -0.927$; 95% CI: −1.713 to −0.141; $p = 0.021$). Living in rural areas was inversely associated with maternal serum concentrations of *p,p′*-DDT ($\beta = -1.302$; 95% CI: −1.871 to −0.733; $p < 0.001$).

Increased maternal t-NC serum concentrations were positively associated with the use of tap lake/shallow well ($\beta = 0.929$; 95% CI: 0.430 to 1.427; $p < 0.001$) and borehole water ($\beta = 0.742$; 95% CI: 0.260 to 1.224; $p = 0.003$) as sources of drinking water, as opposed to the use of tap water. Similarly, high levels cis-NC were also positively associated with the use of lake/shallow well ($\beta = 1.311$; 95% CI: 0.642 to 1.980; $p < 0.001$) and borehole ($\beta = 1.026$; 95% CI: 0.379 to 1.672; $p = 0.002$) water. Lastly, women who reported previous

breast-feeding had statistically lower *p,p′*-DDE serum concentrations (β = −0.643; 95% CI: −1.174 to −0.113; p = 0.018) in reference to those who without such history.

3.4. Maternal Dietary Habits and Level of Education

Human exposure to POPs is mainly through the ingestion of foods of animal origin, such as meat, fish, dairy items and eggs [20]. In this regard, data on diet and other maternal characteristics, such as the maternal educational level, were also explored. Univariate analysis results showed that the consumption of beef more than twice a week was statistically significantly associated with higher maternal serum concentrations of HCB (β = 0.900; 95% CI: 0.035 to 0.145; $p < 0.001$), *p,p′*-DDT (β = 0.544; 95% CI: 0.247 to 0.841; $p < 0.001$) and *o,p′*-DDE (β = 0.209; 95% CI: 0.038 to 0.379; p = 0.017). A similar trend of associations was also observed for the consumption of goat meat as follows: HCB (β = 0.076; 95% CI: 0.024 to 0.129; p = 0.004), *p,p′*-DDT (β = 0.351; 95% CI: 0.066 to 0.636; p = 0.016) and *o,p′*-DDE (β = 0.206; 95% CI: 0047 to 0.369; p = 0.013). However, these associations were not observed in the multivariable linear regression model.

3.5. POP Concentrations in Maternal Serum and Their Associations with Birth Outcomes

In order to investigate the possible associations between the selected POPs and birth outcomes, multivariable linear regression models were applied and adjusted for maternal age, area of residence (urban versus rural), maternal educational level, parity and source of drinking water. As shown in Table 4, multivariable linear regression analysis showed positive associations between the natural log-transformed maternal serum concentrations of *p,p′*-DDE (β = 0.087; 95% CI: 0.008 to 0.166; p = 0.031), *p,p′*-DDT (β = 0.110; 95% CI: 0.193 to 0.166; p = 0.01), *o,p′*-DDT (β = 0. 0.115; 95% CI: 0.016 to 0.213); p = 0.022) and gestational age. Furthermore, statistically significant inverse associations were observed between natural log-transformed maternal serum concentrations of t-NC (β = −0.053; 95% CI: −0.105 to −0.0015; p = 0.044), Oxychlordane (β = −0.071; 95% CI: −0.123 to −0.017; p = 0.010), cis-NC (β = −0.070; 95% CI: −0.140 to −0.006; p = 0.048) and head circumference of the neonates. No significant associations were observed between the selected POPs and birth length, birth weight or ponderal index ($p > 0.05$).

Table 4. Results of linear regression analysis measuring associations between maternal serum POP concentrations on birth outcomes.

POP	Outcomes	Maternal Serum POP Concentrations [a]		
		n	β (95% CI)	*p*-Value
HCB	Head circumference (cm)	492	0.005 (−0.008 to 0.018)	0.447
	Birth length (cm)	492	0.001 (−0.005 to 0.007)	0.824
	Birth weight (kg)	522	0.001 (−0.055 to 0.057)	0.975
	Gestational age (weeks)	492	−0.001 (−0.189 to 0.016)	0.871
	Ponderal index (kg/m^3)	491	−0.00007 (−0.0002 to −0.00007)	0.32
p,p′-DDE	Head circumference (cm)	492	−0.018 (−0.074 to 0.039)	0.542
	Birth length (cm)	492	−0.005 (−0.034 to 0.023)	0.708
	Birth weight (kg)	522	−0.043 (−0.303 to 0.217)	0.744
	Gestational age (weeks)	492	0.087 (0.008 to 0.166)	0.031
	Ponderal index (kg/m^3)	491	0.00029 (−0.00034 to 0.0009)	0.367
t-NC	Head circumference (cm)	492	−0.053 (−0.105 to −0.0015)	0.044
	Birth length (cm)	492	−0.014 (−0.040 to 0.01199)	0.288
	Birth weight (kg)	522	0.073 (−0.167 to 0.313)	0.548
	Gestational age (weeks)	492	−0.002 (−0.074 to 0.069)	0.949
	Ponderal index (kg/m^3)	491	0.0000151 (−0.001 to 0.001)	0.96

Table 4. *Cont.*

POP	Outcomes	Maternal Serum POP Concentrations [a]		
		n	*β* (95% CI)	*p*-Value
p,p′-DDT	Head circumference (cm)	492	0.006 (−0.055 to 0.067)	0.848
	Birth length (cm)	492	−0.013 (−0.043 to 0.017)	0.401
	Birth weight (kg)	522	0.139 (−0.139 to 0.417)	0.325
	Gestational age (weeks)	492	0.110 (0.026 to 0.193)	0.01
	Ponderal index (kg/m^3)	491	0.0003 (−0.0004 to 0.0009)	0.45
Oxychlordane	Head circumference (cm)	492	−0.071 (−0.123 to −0.017)	0.01
	Birth length (cm)	492	−0.0187 (−0.045 to 0.007)	0.159
	Birth weight (kg)	522	0.1288 (−0.119 to 0.376)	0.307
	Gestational age (weeks)	492	0.016 (−0.059 to 0.092)	0.668
	Ponderal index (kg/m^3)	491	0.0002 (−0.0004 to 0.0008)	0.575
cis-NC	Head circumference (cm)	492	−0.070 (−0.140 to −0.006)	0.048
	Birth length (cm)	492	−0.029 (−0.063 to 0.005)	0.108
	Birth weight (kg)	522	0.150 (−0.172 to 0.472)	0.36
	Gestational age (weeks)	492	0.033 (−0.066 to 0.131)	0.515
	Ponderal index (kg/m^3)	491	0.0002 (−0.001 to 0.001)	0.578
o,p′-DDT	Head circumference (cm)	492	0.049 (−0.022 to 0.120)	0.173
	Birth length (cm)	492	−0.016 (−0.051 to 0.0187)	0.363
	Birth weight (kg)	522	0.199 (−0.123 to 0.521)	0.225
	Gestational age (weeks)	492	0.115 (0.016 to 0.213)	0.022
	Ponderal index (kg/m^3)	491	−0.0001 (−0.001 to 0.001)	0.723
o,p′-DDE	Head circumference (cm)	492	−0.009 (−0.046 to 0.028)	0.617
	Birth length (cm)	492	−0.006 (−0.0238 to 0.012)	0.572
	Birth weight (kg)	522	0.127 (−0.040 to 0.293)	0.136
	Gestational age (weeks)	492	−0.004 (−0.047 to 0.055)	0.88
	Ponderal index (kg/m^3)	491	0.000001 (−0.0004 to 0.0004)	0.995

[a] Maternal serum POP concentrations were log 10-transformed. All association between birth outcomes and POPs were adjusted for: maternal age, area of residence (urban vs. rural), maternal educational level, parity and source of drinking water.

4. Discussion

Maternal level of education, age and parity were the main predictors of concentrations of POPs in the maternal serum. In this regard, increased maternal serum POP concentrations were found to be associated with increased levels of maternal education, age and parity. Living in urban settings was associated with increased maternal serum concentrations of HCB and *p,p′*-DDT. Some POPs (*p,p′*-DDE, *p,p′*-DDT and *o,p′*-DDT) were negatively associated with gestational age. Furthermore, t-NC, cis-NC and Oxychlordane were inversely associated with head circumference. Notably, *o,p′*-DDT was positively associated with gestational age.

The maternal serum POP concentrations observed in this study were generally low, as compared to other published studies conducted elsewhere [15,21,22] but comparable to the study conducted by Steinholt et al. in Cambodia [19]. In this regard, the median (min–max) maternal serum *p,p′*-DDT concentrations observed in these two studies were almost consistent (35.5 (0.43–1700) pg/mL ww for the current study and 33.0 (3–519) pg/mL ww for the Cambodian study). It was also noted that the median HCB concentration in the maternal serum observed in the present study was high (102 (0.57–203) pg/mL) compared to the Cambodian study (44.0 (10–196) pg/mL). In contrast, the median (min–max) concentrations of *p,p′*-DDE and *o,p′*-DDT observed in the present study were on the lower side (448 (0.10–23,600) vs. 720.0 (20–8047) pg/mL, and 5.10 (0.11–163) vs. 20.0 (20–20) pg/mL, respectively) and *o,p′*-DDE was on the higher side (8.00 (8–10) vs. 0.34 (0.04–8.9) pg/mL) compared to the Cambodian study.

For DDT and its metabolites, *p,p′*-DDE was the most abundant compound detected in the maternal serum. These findings are similar to previous studies from Cambodia and

Vietnam [19,23]. This is because *p,p′*-DDE is the most common and stable metabolite of *p,p′*-DDT; hence, it is the most likely to be detected in both environmental and human samples. Furthermore, various studies worldwide have shown that the detection frequency of *p,p′*-DDE is 100% [24–26] despite restrictions on its production and use over 40 years ago. In addition, our dataset showed no indication of a fresh DDT-exposure source, since the concentrations of *p,p′*-DDT were rather low, as compared to *p,p′*-DDE.

The level of education was the main predictor of POP concentrations in the maternal serum. Generally, high educational levels were significantly associated with increased maternal serum concentrations of most of the POPs. This was the case with *p,p′*-DDE ($p = 0.008$), *p,p′*-DDT ($p < 0.001$), cis-NC ($p = 0.014$), *o,p′*-DDT ($p = 0.007$) and *o,p′*-DDE ($p = 0.019$). The positive statistically significant associations between the compounds *p,p′*-DDE and *p,p′*-DDT and educational level were consistent with the results found by Steinholt et al. in another study conducted in Cambodia [19]. This could be explained by differences in diets between the two groups. Human exposure to POPs is mainly through ingestion of foods of animal origin, such as meat, fish, dairy items and eggs [20]. Although the univariate analysis showed statistically significant positive associations between consumption of beef and goat and the concentrations a number of POPs (HBC, *p,p′*-DDT and *o,p′*-DDE), similar associations were not observed in the multivariable linear regression analysis.

Just as with maternal educational level, higher maternal age was also statistically significantly associated with higher maternal serum concentrations of *p,p′*-DDT, *o,p′*-DDT and *o,p′*-DDE. Concentrations of the above POPs in their serum were higher in older women than in younger women. This was expected, as high levels of most persistent organic compounds in the maternal serum increase with age, as previously reported in published literature [23,27–30]. It was also observed that living in urban areas was significantly associated with high concentrations of *p,p′*-DDT in the maternal serum. This could be attributed to different lifestyles, especially related to diet. For instance, pregnant women living in urban setting are more likely to consume meat-based foods frequently, as compared to rural residents. Furthermore, women residing in urban areas have high chances of consuming food that is contaminated with DDT and its metabolites, as most food stuffs in supermarkets are imported from South Africa, where DDT is still used for malaria prevention. However, there is a need for a large study on the effect of diet on the concentrations of maternal POPs to ascertain the above hypotheses.

Multiparity was associated with decreased maternal serum concentrations of *o,p′*-DDT. These results are similar to other studies that have also revealed that parity is negatively associated with maternal serum concentrations of some POPs [23,30] due to the fact that these compounds are permeable through the placenta and are transferred to the growing foetus [18,29,31–33]. Thus, a negative association between *p,p′*-DDT and parity is attributed to mother-to-foetus transfer of these compounds through the placenta. Furthermore, a large proportion of women (58.9%) recruited in this study reported previous breast-feeding. Since POPs are lipophilic in nature, they tend to be secreted into breast milk [34–38], and the inverse association observed can also be explained by depletion of these compounds in the maternal serum due to the transfer from mother to the baby through breast-feeding.

In the present study, positive associations were observed between gestational age and each of the following POPs: *p,p′*-DDE, *p,p′*-DDT and *o,p′*-DDT. These findings concur with results from a study by Jusko et al., in which a positive association was observed between *p,p′*-DDT and *o,p′*-DDT with gestational age [39]. However, the findings in the present study are in contrast to the results in a study by B J Wojtyniac et al. in the Kharkiv cohort, which found that lower gestational ages were associated with an increase in log *p,p′*-DDE exposure [40]. The difference in results between these two studies could be due to the sample size, regional distribution, data analysis methods and selection of confounding factors. In addition, we also observed a statistically significant inverse association between some POPs (t-NC, Oxychlordane and cis-NC) and the head circumferences of the neonates, as reported in other studies. There are limited published data on studies on the association between t-NC, Oxychlordane and cis-NC with head circumference. However, the previ-

ously listed substances are endocrine disruptors; hence, they are more likely to affect foetal growth, which may result in smaller head circumferences of the neonates.

The main limitation of this study is its cross-sectional design that prevents causality associations and, therefore, causality associations cannot be shown. However, to our knowledge, this is the first study to explore concentrations of selected POPs in the maternal serum of pregnant women in Malawi and their associated pregnancy outcomes. The study revealed predictors of POP levels in maternal serum and their associations with birth outcomes. Another strength is that samples were drawn from different settings (urban and rural), which makes the results more representative of Malawian women.

5. Conclusions

Our study made a comprehensive effort to examine the determinants of selected persistent organic pollutant concentrations in maternal serum during pregnancy and their associations with social demographic characteristics and birth outcomes. This study gives a foundation upon which knowledge of the main predictors for POP levels in maternal serum among pregnant women at the delivery stage and from low–middle income African settings can be built. In this regard, maternal level of education, age and parity were the main predictors of the concentrations of POPs in the maternal serum. Although the concentrations of the POPs observed were generally low, there is still a need for more research to periodically monitor the concentrations of these compounds and the associated lifestyles during pregnancy. In addition, the results from the present study may be used to facilitate the identification of specific lifestyles that are associated with high concentrations of POPs in the maternal serum of women of child-bearing age.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ijerph20075289/s1: Table S1: Method detection limits (MDL) concentrations (wet weight pg/mL) for all POPs analysed at the laboratory; Table S2: Socio-demographic characteristics of recruited women and their neonates.

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Informed Consent Statement: Written informed consent was obtained from all participants involved in this study.

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PAPER III

A cross-sectional study of maternal blood concentrations of toxic and trace elements in pregnant women and association with birth outcomes in southern Malawi

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Appendices

Appendix 1: Participant Information Sheet – English.

Appendix 2: Informed consent form for enrolment (English).

Appendix 3: Study Questionnaire

Appendix 4: Supplementary Table 1. Multivariable analysis results of linear regression analysis measuring effects of maternal serum PFAS concentrations on birth outcomes.

Appendix 5: Supplementary Table 2. Multivariable analysis results of linear regression analysis on maternal serum POPs concentrations and birth outcomes.

Appendix 6: Supplementary Table 3. Method detection limits (MDL) concentrations (wet weight pg/mL) for all POPs analysed at the laboratory.

Appendix 7: College of Medicine Research and Ethics Committee (COMREC) Ethics approval.

Appendix 8: Regional Committees for Medical and Health Research Ethics (REK) Ethics approval.

Appendix 1: Participant Information Sheet – English

NTNU
Norwegian University of
Science and Technology

PTS Study

Participant Information Sheet – English

Reproductive health effects associated with environmental and dietary exposure to persistent toxic substances (PTS) in Pregnant mothers and infants in Malawi

Purpose of the study

You are being asked to take part in a study being done by the College of Medicine in partnership with Norwegian University of Science and Technology (NTNU) Norway and the University of Pretoria. The purpose of the study is to help us know if persistent toxic organic substances and metals have any effect on the pregnancy outcome and the growth of the babies.

Persistent toxic substance can be found anywhere in our environment. High blood levels of these substances in a pregnant woman can have an effect on the foetus and even the growing baby. We are trying to find out if we can find these substances in the blood during pregnancy and to see any effect on the pregnancy outcomes as well as the growth of the child. This is important to help to try and prevent mother and their babies from getting problems from these substances and plan for improved health services in Malawi.

Procedure

If you decide to take part in this study, you will receive the normal antenatal care, delivery and postnatal services from the local health facility. Additionally, here is what will happen:

1) We will ask you questions about yourself and your household.
2) We will also collect information about where you live that may be used to locate you and your child in the future for further research.
3) We will measure your weight and height. None of these measurements will harm or be painful to you.
4) During delivery we also take a small amount of blood from your arm. The blood volume that will be collected is 16 mls. The blood will be used to study the persistent toxic substances and the essential elements status. This sample collection will not harm you. When the baby is born we will measure the weight, length, head circumference of baby in addition we will look for any congenital malformations
5) Other information will be gathered from your hospital records.
6) After we have completed our laboratory analyses, there will be some left-over blood samples from you and your child and we ask your permission to keep those samples for future research. If we do any further analyses in the future from these samples, you would not be contacted again, but we would seek permission from the College of Medicine Research and Ethics Committee, COMREC:
7) During the study period all your children will receive normal health care at this hospital.

Benefits

The results from this study will provide useful information on safe motherhood in Malawi

We recommend that you deliver at a health facility and to facilitate that a total sum of MK 7,350.00 is given to you to compensate your time and travel expenses.

If we find out during the study that you or your baby has any illness, we will give free treatment or help you obtain free treatment at the health centre.

Risks

Drawing blood may cause some pain or discomfort. It may also result in tenderness and bruising at the puncture site. However, these will be short-lived.

Apart from these, the study has no known risks.

Confidentiality

The records of this study will be kept securely at the Universities of Malawi, Norwegian University of Science and Technology (NTNU), Norway and the University of Pretoria and your or your child's personal details will not be known by or released to anybody other than the study personnel. Neither your or your child's name nor that of any member of your family will be used in any of the publications that will come out of the study.

Cost

If you take part in this study, it will not cost you or any member of your family any money.

Right to refuse to participate of withdraw from the study

Taking part in this study is completely voluntary. You may also change your mind about taking part in the study, and withdraw your own or your child´s participation in the study at any time. You do not need to give any reason for withdrawing your consent to participate.

Questions

If you want any details or have any concerns about any aspect of this study, please speak to the research team or get in contact with the Principal Investigator, Mr Mphatso Mwapasa. If you want to raise any complaits or concerns formally, you can do so through the Secretariat of the College of Medicine Research and Ethics Committee.

Principal Investigator

Mr Mphatso Mwapasa

College of Medicine

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Chichiri, Blantyre

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College of Medicine Research Ethics Committee Secretariat

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Tel #: 01 989 766

Appendix 2: Informed consent form for enrolment (English)

NTNU
Norwegian University of
Science and Technology

PTS Study_Informed consent form for enrolment (English)

Date of visit: |___|___| |___|___| 20 |___|___|

Enrolment identification code: |___|___|___|___|___|___| - |___|___|___|

Name of Participant: _____

Address: _____

_____ has informed me about the purpose and implementation of this study. I have been given a possibility to ask further questions and all unclear issues have been explained to me. I know that participation or non-participation in the study does not influence in any way the health services I or my children are getting from this Hospital.

After getting and considering all relevant information, I voluntarily give my consent that I and my children can participate in the indicated study. I also giving consent to the following:

☐ 1. Specimen will be exported for analysis

☐ 2. Specimen will be kept for future research

☐ 3. The study team may contact and visit my home for future research

☐ 4. Probable home visit and domestic sample collection

I do, however, retain the right to withdraw from the study at any point, without giving any reason for my decision.

Name: _____

Signature / Thumbprint of the client: _____

Date: |___|___| |___|___| 20 |___|___|.

Name of the person obtaining the consent: _____

Signature of the person taking the consent.: _____

Date: |___|___| |___|___| 20 |___|___|.

Name of the facility: _____

Appendix 3: Study Questionnaire

NTNU
Norwegian University of
Science and Technology

Reproductive health effects associated with environmental and dietary exposure to persistent toxic substances (PTS) in Pregnant mothers and infants in Southern Region of Malawi

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 Mr Mphatso Mwapasa on 0999 280 538

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

Clinic Name: ……Clinic address: …….....

SECTION A: MATERNAL SOCIOECONOMIC INFORMATION

1. Patient's Study Number…….....
2. Addresss.....
3. Age ..... Gravidity..... Parity …… .....
4. Educational level …… Occupation ……

5. Marital status.....Single/Single/Widowed/Divorced

6. If married
    a. Age of the husband.....
    b. Education.....
    c. Occupation.....
7. Major illnesses of mother, specify……
8. Medication of mother if daily, specify……
9. How many children do you already have?.....
10. How many daughters: Number……Ages……
11. How many sons: Number……Ages……
12. Did you breastfeed your previous babies, if yes, for how long……?
13. How long have you lived at present address: Years.....?
14. How do you describe your area of residence?
    a. Urban
    b. Rural
15. Sources of drinking water
    a. Tap
    b. Lake
    c. Borehole
16. Do you use pesticides Y/N if yes where?
    a. Inside home

b. On farm/garden

17. Do you or member of family fish? Y/N

a. Where ……

SECTION B: DIET AND LIFESTYLE

Daily food sources of mother

Food item	Description				Number / week								
	Medium serving size	S	M	L	A	B	C	D	E	F	G	H	I
MEATS	2 pieces												
Beef	2 pieces												
Pork	2 pieces												
Goat	2 pieces												
Lamb	2 pieces												
Chicken	1 pieces												
FISH													
Fresh fish	2 pieces												
Dry fish	2 pieces												
VEGETABLES AND GRAINS													
Green vegetable	1 cup												
Bean	1 cup												
Peas	1 cup												
Eggs	2 eggs												
OTHERS													
Rice	1 cup												
Nsima	1 portion												
Cassava	2 pieces												
Milk	1 cup												
Irish potatoes	2 pieces												
Sweet potatoes	2 pieces												
Bananas	1 banana												
Mangoes	1 mango												
pineapples	4 slices												
Avocado peas													

KEY

A	never
B	1 per month
C	2-3 per month
D	1per week
E	2 per week
F	3-4 per week
G	5-6 per week
H	1 per day
I	2+ per week
M	Medium serving
L	Large serving
S	Small serving

Note: Small serving size is half (0.5) medium serving size or less

Large serving size is one and half (1.5) medium serving size or more

18. Sources of food

    a. Own/local production

    b. Local market

    c. Supermarket

    d. Imported

Lifestyle of mother

1. Do you smoke

    a. Yes/No

    b. If yes how many cigarettes daily……

2. Do you drink alcohol

    a. Yes/No

    b. If yes what kind……how many tots daily……

Lifestyle of the spouse

3. Does your spouse smoke

    c. Yes/No

    d. If yes how many cigarettes daily……

4. Does your spouse drink alcohol

    c. Yes/No

    d. If yes what kind……how many tots daily……

SECTON C: MATERNAL INFORMATION

POST DELIVERY MEDICAL INFORMATION

To be completed by attending medical personnel or designated field worker.

Maternal weight before delivery: …… Kilograms

Maternal height: ……cm

Previous spontaneous abortions in the first trimester: (if any) ……

1 at how many months……

2 at how many months……

3 at how many months……

Previous spontaneous abortions in second trimester (if any) ……..

1 at how many months……

2 at how many months……

3 at how many months……

Previous still births < week 37 (if any) ……

1 at how many months……

2 at how many months……

3 at how many months……

Any infertility problems, if any:

……

Any complications/problems during pregnancy (hypertension, pre-eclampsia, infections)

……

……..

SECTION D: INFORMATION ABOUT THE NEWBORN CHILD

1. Mode of delivery: SVD/vac extr/forceps/CS/other
1. Birth weight of baby: ……kg
2. Length of baby at birth: ……cm
3. Head circumference of baby: ……cm
4. Gestation age of baby (based on Naegele term):
5. APGAR score 1min…… 5min……
6. Gender of baby……

7. Congenital malformations (visible at birth) ……

8. Any delivery complications? if yes, what kind

……..

9. Any other medical/obstetrical observations or conditions

.....…….

END OF QUESTIONNAIRE

THANK YOU

Appendix 4: Supplementary Table 1. Multivariable analysis results of linear regression analysis measuring effects of maternal serum PFAS concentrations on birth outcomes.

PFAS	Birth Outcomes	Maternal Serum PFAS Concentrations [a]		
		n	β (95% CI)	*p*-Value
PFOA	Head Circumference (cm)	478	- 0.056 (- 0.109 to - 0.002)	0.043
	Birth Length(cm)	478	-0.049 (- 0.077 to - 0.022)	<0.001
	Birth Weight (kg)	508	- 0.067 (- 0.272 to 0.138)	0.523
	Gestational Age (weeks)	480	- 0.036 (- 0.103 to 0.032)	0.298
	Ponderal Index (kg/m^3)	477	0.136 (0.040 to 0.233)	0.005
PFNA	Head Circumference (cm)	478	- 0.080 (- 0.125 to - 0.035)	0.001
	Birth Length(cm)	478	- 0.033 (- 0.057 to - 0.010)	0.005
	Birth Weight (kg)	508	- 0.171 (- 0.346 to 0.003)	0.054
	Gestational Age (weeks)	480	- 0.083 (- 0.141 to - 0.023)	0.005
	Ponderal Index (kg/m^3)	477	0.035 (- 0.050 to 0.116)	0.404
PFDA	Head Circumference (cm)	478	0.012 (- 0.033 to 0.057)	0.597
	Birth Length(cm)	478	0.012 (- 0.012 to 0.035)	0.33
	Birth Weight (kg)	508	0.038 (- 0.139 to 0.25)	0.673
	Gestational Age (weeks)	480	- 0.016 (- 0.075 to 0.043)	0.589
	Ponderal Index (kg/m^3)	489	- 0.39 (- 0.119 to 0.041)	0.340
PFUDA	Head Circumference (cm)	478	0.019 (- 0.036 to 0.075)	0.494
	Birth Length(cm)	478	0.001 (- 0.027 to 0.030)	0.924
	Birth Weight (kg)	508	0.052 (- 0.165 to 0.269)	0.636
	Gestational Age (weeks)	480	- 0.0122 (- 0.083 to 0.059)	0.735
	Ponderal Index (kg/m^3)	477	- 0.026 (- 0.125 to 0.074)	0.608
SumPFHxS	Head Circumference (cm)	478	0.048 (0.001 to 0.095)	0.045
	Birth Length(cm)	478	0.014 (- 0.011 to 0.038)	0.265
	Birth Weight (kg)	508	- 0.189 (- 0.371 to - 0.006)	0.043
	Gestational Age (weeks)	480	- 0.160 (- 0.076 to 0.044)	0.600
	Ponderal Index (kg/m^3)	477	- 0.090 (- 0.175 to - 0.005)	0.037
SumPFOS	Head Circumference (cm)	478	- 0.036 (- 0.087 to 0.014)	0.153
	Birth Length(cm)	478	- 0.012 (- 0.038 to 0.014)	0.348
	Birth Weight (kg)	508	- 0.261 (- 0.457 to - 0.064)	0.009
	Gestational Age (weeks)	490	-0.119 (- 0.183 to - 0.055)	<0.001
	Ponderal Index (kg/m^3)	477	- 0.245 (- 0.115 to 0.065)	0.591

[a] Maternal blood PFAS concentrations were natural log-transformed. All associations between birth outcomes and PFAS levels in model A were adjusted for maternal age, area of residence (urban vs. rural), maternal educational level, parity and source of drinking water.

Appendix 5: Supplementary Table 2. Multivariable analysis results of linear regression analysis on maternal serum POPs concentrations and birth outcomes.

POPs	Birth Outcomes	Maternal serum POPs concetrations [a]		
		n	β (95% CI)	*p*-Value
HCB	Head Circumference (cm)	492	0.005 (- 0.008 to 0.018)	0.447
	Birth Height (cm)	492	0.001 (- 0.005 to 0.007)	0.824
	Birth Weight (kg)	522	0.001 (- 0.055 to 0.057)	0.975
	Gestational Age (weeks)	492	- 0.001 (- 0.189 to 0.016)	0.871
p, p′-DDE	Head Circumference (cm)	492	- 0.018 (-0.074 to 0.039)	0.542
	Birth Height (cm)	492	- 0.005 (- 0.034 to 0.023)	0.708
	Birth Weight (kg)	522	- 0.043 (- 0.303 to 0.217)	0.744
	Gestational Age (weeks)	492	0.087 (0.008 to 0.166)	0.031
t-NC	Head Circumference (cm)	492	- 0.053 (- 0.105 to - 0.015)	0.044
	Birth Height (cm)	492	-0.014 (- 0.040 to 0.012)	0.288
	Birth Weight (kg)	522	0.073 (- 0.167 to 0.313)	0.548
	Gestational Age (weeks)	492	- 0.002 (- 0.074 to 0.069)	0.949
p, p′-DDT	Head Circumference (cm)	492	0.006 (- 0.055 to 0.067)	0.848
	Birth Height (cm)	492	-0.013 (-0.043 to 0.017)	0.401
	Birth Weight (kg)	522	0.139 (-0.139 to 0.417)	0.325
	Gestational Age (weeks)	492	0.110 (0.026 to 0.193)	0.01
Oxy-CD	Head Circumference (cm)	492	- 0.071 (- 0.123 to - 0.017)	0.01
	Birth Height (cm)	492	- 0.019 (- 0.045 to 0.007)	0.159
	Birth Weight (kg)	522	0.129 (- 0.119 to 0.376)	0.307
	Gestational Age (weeks)	492	0.016 (-0.059 to 0.092)	0.668
Cis-NC	Head Circumference (cm)	492	- 0.070 (- 0.140 to -0.006)	0.048
	Birth Height (cm)	492	- 0.029 (- 0.063 to 0.005)	0.108
	Birth Weight (kg)	522	0.150 (- 0.172 to 0.472)	0.36
	Gestational Age (weeks)	492	0.033 (- 0.066 to 0.131)	0.515
o, p′-DDT	Head Circumference (cm)	492	0.049 (- 0.022 to 0.120)	0.173
	Birth Height (cm)	492	- 0.016 (- 0.051 to 0.0187)	0.363
	Birth Weight (kg)	522	0.199 (- 0.123 to 0.521)	0.225
	Gestational Age (weeks)	492	0.115 (0.016 to 0.213)	0.022
o, p′-DDE	Head Circumference (cm)	492	- 0.009 (- 0.046 to 0.028)	0.617
	Birth Height (cm)	492	- 0.006 (- 0.0238 to 0.012)	0.572
	Birth Weight (kg)	522	0.127 (- 0.040 to 0.293)	0.136
	Gestational Age (weeks)	492	- 0.004 (- 0.047 to 0.055)	0.88

[a] Maternal serum POP concetrations were natural log transformed.
All association between Birth outcomes and POPs were adjusted for: maternal age, area of resedence (urban vs rural), maternal educational level, parity and source of drinking water.

Appendix 6: Supplementary Table 3. Method detection limits (MDL) concentrations (wet weight pg/mL) for all POPs analysed at the laboratory.

POPs	Mean MDL Concentrations	Median MDL Concentrations	Min - Max MDL Concentrations
o, p´-DDE	0.3	0.3	0.07 to 1.53
p, p´--DDE	0.4	0.3	0.09 to 4.26
o, p´-DDD	0.6	0.5	0.20 to 1.44
p, p´-DDD	1.8	1.5	0.34 to 7.14
o, p´-DDT	0.7	0.6	0.09to 2.99
p, p´-DDT	2.0	1.8	0.46 to 6.73
HCB	1.1	0.9	0.02 to 12.74
Mirex	6.2	5.8	0.76 to 23.85
a-HCH	2.1	1.8	0.85 to 9.67
g-HCH	2.1	2.0	0.79 to 7.59
b-HCH	3.6	3.5	1.69 to 7.85
Heptachlor	0.3	0.2	0.002 to 2.89
t-CD	0.5	0.3	0.01 to 8.51
c-CD	0.5	0.3	0.02 to 8.37
t-NC	1.8	0.6	0.02 to 24.44
cis-NC	0.9	0.3	0.02 to 28.94
Oxychlordane	6.6	5.9	0.05 to 35.33
cis-Heptachlorepoxide	2.6	2.3	0.06 to 44.24
Dieldrin	6.3	5.1	0.07 to 41.35

Appendix 6: Supplementary Table 4. Multivariable analysis results of linear regression analysis on maternal blood toxic and trace elemets concentrations and birth outcomes.

Toxic metals/ Trace elements	Birth Outcomes	Maternal blood metal concetrations [a]		
		n	*β* (95% CI)	*P* Value
Ni	Birth weight (kg)	458	0.063 (0.003 to 0.124)	0.047
	Birth length (cm)	433	0.009 (0.001 to 0.018)	0.026
	Head Circumference (cm)	433	0.018 (0.002 to 0.034)	0.029
	Gestational age (Weeks)	435	0.023 (0.002 to 0.044)	0.035
Se	Birth weight (kg)	467	0.019 (- 0.045 to 0.084)	0.558
	Birth length (cm)	442	0.001 (- 0.008 to 0.010)	0.773
	Head Circumference (cm)	442	0.0003 (- 0.017 to 0.018)	0.969
	Gestational age (Weeks)	443	0.009 (- 0.013 to 0.031)	0.426
Cu	Birth weight (kg)	467	0.006 (- 0.038 to 0.049)	0.795
	Birth length (cm)	442	0.005 (- 0.001 to 0.011)	0.117
	Head Circumference (cm)	442	0.004 (- 0.007 to 0.016)	0.439
	Gestational age (Weeks)	443	0.012 (- 0.003 to 0.027)	0.118
Zn	Birth weight (kg)	461	- 0.025 (- 0.076 to 0.027)	0.343
	Birth length (cm)	436	- 0.004 (- 0.011 to 0.003)	0.289
	Head Circumference (cm)	436	- 0.008 (- 0.022 to 0.005)	0.214
	Gestational age (Weeks)	437	- 0.005 (- 0.023 to 0.013)	0.588
As	Birth weight (kg)	462	- 0.041 (- 0.170 to 0.090)	0.532
	Birth length (cm)	437	- 0.018 (- 0.035 to -0.0001)	0.048
	Head Circumference (cm)	437	- 0.064 (- 0.098 to -0.030)	<0.001
	Gestational age (Weeks)	439	- 0.031 (- 0.076 to 0.014)	0.173
Hg	Birth weight (kg)	450	- 0.020 (- 0.143 to 0.103)	0.75
	Birth length (cm)	426	- 0.010 (- 0.026 to 0.007)	0.249
	Head Circumference (cm)	426	- 0.018 (- 0.049 to 0.014)	0.276
	Gestational age (Weeks)	443	- 0.032 (- 0.075 to 0.010)	0.134
Pb	Birth weight (kg)	467	0.019 (-0.102 to 0.141)	0.756
	Birth length (cm)	442	- 0.020 (- 0.036 to - 0.004)	0.016
	Head Circumference (cm)	442	- 0.048 (- 0.079 to - 0.017)	0.002
	Gestational age (Weeks)	443	- 0.017 (- 0.059 to 0.025)	0.428

[a] elements concentrations were natural log transformed before analyses.
Ni model was adjusted for maternal age, education, area of residence, source of drinking water and beef/eggs diet. Se Cu and Pb models were adjusted for maternal age, education, area of residence and source of drinking water. Zn model was adjusted for maternal age, education, area of residence and source of drinking water and eggs diet. As model was adjusted for maternal age, education, area of residence and source of drinking water and fresh fish/goat meat diet. Hg model was adjusted for maternal age, education, area of residence and source of drinking water, fresh and dry fish and green vegetables diet.
Abbreviations: Ni = nickel; Se = selenium; Cu = copper; Zn = zinc; As = arsenic; Hg = mercury; Pb = lead.

CERTIFICATE OF ETHICS APPROVAL

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

P.11/18/2546 - Reproductive health effects associated with environmental and dietary exposureto persistent toxic substances (PTS) in Pregnant mothers and infants in Malawi. Version 2.0 by Mphatso Mwapasa

On 28-Feb-19

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

28-Feb-19

Dr. YB. Mlombe - Chairperson (COMREC)

Date

Appendix 7: College of Medicine Research and Ethics Committee (COMREC) Ethics approval.

Appendix 8: Regional Committees for Medical and Health Research Ethics (REK) Ethics approval.

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK midt	Linda Tømmerdal Roten	73597506	10.03.2022	355696

Jon Øyvind Odland

Prosjektsøknad: Mijøgiftnivåer hos gravide kvinner i Malawi. En tverrsnittsstudie knyttet til kosthold og miljøgifteksponering.
Søknadsnummer: 355696
Forskningsansvarlig institusjon: Norges teknisk-naturvitenskapelige universitet

Prosjektsøknad godkjennes med vilkår

Søkers beskrivelse

Malawiske helsemyndigheter har sammen med Malawi University of Science and Technology (MUST) planlagt en tverrsnittsstudie av gravide og fødende kvinner på tre utvalgte steder i det sørlige Malawi. Stedene er valgt for å skaffe et bilde av situasjonen i urbane og rurale strøk med forskjellig disponering for sprøytemidler i landbruket og miljøforurensing i et bymiljø. Designet på studien er adaptert til tilsvarende miljø og helse og helse prosjekter i over 20 land, ledet av prosjektleder. Det hentes informasjon om mors helse, ernæring og svangerskapsutfall. Det samles blodprøver på gitte tidspunkter i svangerskapet. Aktuelle stoffer som skal undersøkes er sprøytemidler, slik som DDT, uorganiske stoffer, slik som bly og kvikksølv, samt organiske stoffer knyttet til global forurensing, slik som PCB. De kjemiske analyser er planlagt ved Universitetssykehuset i Nord Norge (UNN) og ved Universitetet i Pretoria, etter avtaler med begge universiteter. Prosjektleder har bistilling som professor begge steder. Undersøkelser fra arktiske strøk har vist at disse stoffene kommer inn i kroppen gjennom kostholdet. De kan ha hormonelle virkninger og det ufødte barn er derfor spesielt sårbart. Vi har ingen informasjon om situasjonen i det sørlige Afrika og det er derfor svært viktig å skaffe kunnskap til bruk i folkehelsesammenheng. Det planlegges å innrullere inntil 600 gravide basert på informert samtykke. Befolkningen snakker kun chichewa og 30 % kan ikke lese og skrive. Derfor er inkludering basert på muntlig informasjon sammen med skriftlig materiale. Resultater gis tilbake via helsemyndighetene som kan bruke dataene for både regulering av utslipp og kostholdsråd for gravide kvinner. Vitenskapelig planlegges å bruke forskjellige statistiske modeller, feks multipel regresjon, tilsvarende det som er utprøvd i de andre land som har gjort tilsvarende studier. Utfall og resultater meldes tilbake til helsemyndighetene som også har ansvar for den informasjon som tilfaller de enkelte pasienter. PhD kandidaten skal analysere dataene for vitenskapelige publikasjoner. De pågående klimaendringene medfører også at de aktuelle stoffer endrer sin nedbrytingsprosess med påfølgende endring i biologisk virkning. En oppfølgingsstudie av barna i småbarnsalder er aktuell. Det vil i så fall bli en ny studie med nye godkjenninger.

Innledning

REK midt **Telefon**:73 59 75 11 | **E-post**:rek-midt@mh.ntnu.no
Besøksadresse: Øya Helsehus, 3. etasje, Mauritz Hansens gate 2, Trondheim **Web**:https://rekportalen.no

Viviser til dinsøknadom forhåndsgodkjenningavovennevnteforskningsprosjekt.Søknaden og tilbakemeldingen dinble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikkMidt-Norge (REK midt)i møtet16.02.2022.Vurderingen er gjort med hjemmel i helseforskningsloven § 10.

REKs vurdering

Komiteens opprinnelige prosjektsammendrag

Dette er en søknad om videreføring av et prosjekt (REKs saksnummer 126264) som ble påbegynt før vilkår for godkjenning var oppfylt. Formålet med studien er å undersøke helseutfall knyttet til eksponering av spesifikke sprøytemidler og miljøgifter hos gravide kvinner i Malawi og deres nyfødte. Utvalget består av 600 gravide kvinner over 18 år fra tre ulike områder som representerer både by og land. Helseopplysninger er innhentet fra pasientjournal og via spørreskjema. Blodprøver er innhentet på flere tidspunkter i svangerskapet, samt etter fødsel, og disse skal lagres i en spesifikk forskningsbiobank. Analyser av blodprøvene vil utføres ved Universitetet i Tromsø - Norges Arktiske Universitet og Universitetet i Pretoria, Sør-Afrika. Studien er samtykkebasert, og skal danne grunnlag for en doktorgrad ved NTNU. NTNU er forskningsansvarlig institusjon.

Saksgang

Søknaden ble første gang behandlet i REK midts møte01.12.2021. Endelig vedtak ble utsatt fordi prosjektet hadde noen uklarheter knyttet til hvilken informasjon deltakerne har fått, og hvor de biologiske prøvene befinner seg. Vi ba om at instituttledelsen må fremskaffe etterspurt informasjon ettersom du ved flere anledninger tidligere ikke har besvart våre spørsmål eller oppklart uklarheter. Vi ba også instituttledelsen vurdere å utpeke en ny prosjektleder. Vi mottok tilbakemelding fra instituttledelsen 14.01.2022.

Oppsummering av tilbakemeldingen

- Fra tilbakemeldingen fremgår det at det eksisterer et informasjonsskriv knyttet til samtykkeerklæringen. Dette finnes både på det lokale språket (chichewa) og på engelsk, og er vedlagt tilbakemeldingen. I tilfeller der deltakere hadde mangelfulle lese- og skriveegenskaper, ble informasjonsskrivet brukt som basis for muntlig informasjon.
- De biologiske prøvene befinner seg i Tromsø, men er ikke analysert.
- Instituttledelsen ønsker ikke å erstatte deg som prosjektleder fordi det vil sette prosjektet i en kritisk situasjon. Men instituttleder vil følge prosjektet nøye, og jobber med løsninger for å utvide prosjektgruppen.

Forsvarlighet

Komiteen har vurdert tilbakemelding og prosjektsøknad. Vi finner at instituttledelsen har besvart våre spørsmål, og oppklart uklarhetene på en tilfredsstillende måte. Vi vurderer at de avgitte samtykkene er dekkende for overføringen av biologiske prøver fra Malawi til Norge kun dersom kvinnene har krysset i samtykkeerklæringen på at «specimen will be exported for analysis». Vi forutsetter derfor at kun prøver fra kvinner som har samtykket til overføringen blir analysert i Tromsø. Vi oppfatter at lokal etisk komité i Malawi (COMREC) gir etisk godkjenning et år av gangen. Vi ber deg derfor om å sende oss kopi av forlenget lokal etisk godkjenning ut over 28.02.2022 dersom COMREC krever forlenget etisk godkjenning etter at innsamlingsperioden er avsluttet. Ellers har komiteen ingen forskningsetiske innvendinger til prosjektet. Under forutsetning av at vilkårene nedenfor tas til følge vurderer REK at prosjektet er forsvarlig, og at hensynet til deltakernes velferd og integritet er ivaretatt.

Godkjenner opprettelse av spesifikk forskningsbiobank

Du søker om godkjenning for opprettelsen av en spesifikk biobank som du er ansvarshavende for. Forskningsbiobanken vil bestå av blodprøver (fullblod og serum) tatt ved ulike tidspunkter i svangerskapet og etter fødsel fra ca. 600 kvinner. Komiteen godkjenner opprettelsen. Det er ikke angitt noen tidsbegrensning på forskningsbiobanken. Komiteen setter derfor en tidsavgrensning i tråd med sluttdatoen for prosjektet, til og med 31.12.2025. Du er ansvarlig for at materialet destrueres senest ved sluttdato.

Vilkår for godkjenning av spesifikk forskningsbiobank

1. Du kan kun bruke materialet i biobanken i dette konkrete prosjektet.Annen brukvil kreve søknad tiloss, og vil normalt sett kreve innhenting av nytt samtykke.
2. Materiale i forskningsbiobanker skal oppbevares og behandles forsvarlig. Oppbevaring og behandling skal skje med respekt for giveren av materialet, jf. helseforskningsloven § 27.

Vilkår for godkjenning

1. Komiteen forutsetter at kun prøver fra kvinner som har samtykket til overføringen av biologisk materiale fra Malawi blir analysert i Tromsø.
2. Komiteen minner om at koblingsnøkkel skal bli værende i det landet materialet er samlet inn i ved internasjonalt samarbeid. Materialet kan ikke bli benyttet til studier utover hva det foreligger REK-godkjenning for.
3. Komiteen forutsetter at ingen personidentifiserbare opplysninger kan framkomme ved publisering eller annen offentliggjøring.
4. Komiteen forutsetter at du og alle prosjektmedarbeiderne følger institusjonens bestemmelser for å ivareta informasjonssikkerhet og personvern ved innsamling, bruk, oppbevaring, deling og utlevering av personopplysninger. Bestemmelsene må være i samsvar med REKs vilkår for godkjenning.
5. Av dokumentasjonshensyn skal opplysningene oppbevares i 5 år etter prosjektslutt. Du og forskningsansvarlig institusjon er ansvarlig for at opplysningene oppbevares

avidentifisert, det vil si atskilt i en koblingsnøkkelfil og en datafil. Opplysningene skal deretter slettes eller anonymiseres.

Vedtak

Godkjent på vilkår

Sluttmelding
Prosjektleder skal sende sluttmelding til REK på eget skjema via REK-portalen senest 6 måneder etter sluttdato 31.12.2025, jf. helseforskningsloven § 12. Dersom prosjektet ikke starter opp eller gjennomføres meldes dette også via skjemaet for sluttmelding.

Søknad om endring
Dersom man ønsker å foreta vesentlige endringer i formål, metode, tidsløp eller organisering må prosjektleder sende søknad om endring via portalen på eget skjema til REK, jf. helseforskningsloven § 11.

Klageadgang
Du kan klage på REKs vedtak, jf. forvaltningsloven § 28 flg. Klagen sendes på eget skjema via REK portalen. Klagefristen er tre uker fra du mottar dette brevet. Dersom REK opprettholder vedtaket, sender REK klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag (NEM) for endelig vurdering, jf. forskningsetikkloven § 10 og helseforskningsloven § 10.

Med vennlig hilsen

Vibeke Videm

Professor, dr.med./ overlege

Leder, REK midt

Linda Tømmerdal Roten

Seniorrådgiver, REK midt

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