ISBN 978-82-326-3092-9 (printed ver.)
ISBN 978-82-326-3093-6 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2018:151

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The Scandinavian SGA Study, 1986-94

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Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
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Printed by NTNU Grafisk senter

Persistente organiske miljøgifter i graviditeten, pre- og postnatal vekst og overvekt/fedme i barndommen

I løpet av det 20. århundret har mennesker blitt stadig høyere eksponert for persistente organiske miljøgifter. To viktige grupper av disse miljøgiftene er perfluorerte og klororganiske forbindelser. Nivåene av perfluorerte forbindelser i miljøet økte betydelig fra 1960 til 1990-tallet, og ble deretter gradvis redusert fra rundt år 2000 etter restriksjoner om bruk. Siden 1980-tallet har de klororganiske forbindelsene blitt kraftig redusert i luft og biota, særlig i vestlige land, pga. verdensomspennende forbud og restriksjoner rundt bruken av dem. Alle disse miljøgiftene har persistente egenskaper, de akkumuleres i næringskjeden, og i en bakgrunnspopulasjon skjer eksponeringen hovedsakelig gjennom maten. Under graviditeten transporteres miljøgiftene fra mor til foster via morkaken, og etter fødselen fra mor til spedbarn via brystmelken. Det har vært spekulert i om disse stoffene kan virke «hormonforstyrrende», og at de dermed potensielt kan forstyrre utviklingen hos fosteret, som kan føre til bl.a. føtal veksthemming og/eller senere overvekt. Likevel har man foreløpig ikke hatt tilstrekkelig evidens fra epidemiologiske studier til å konkludere rundt dette.

Denne avhandlingen består av tre delstudier og bruker data fra den skandinaviske SGA-studien (*Scandinavian Successive Small-for-Gestational Age (SGA) Births Study*). Dette er en stor, prospektiv, populasjonsbasert multisenterstudie, som ble utført i Trondheim og Bergen (Norge), samt i Uppsala (Sverige) i perioden 1986-94. De gravide kvinnene ble fulgt fra tidlig svangerskap og undersøkt ved deres studiesenter i svangerskapsuke 17, 25, 33 og 37, samt ved fødsel. Etter fødselen ble utvalgte barn og mødre fulgt opp gjennom det første leveåret og fram til fem års alder. Serumprøver fra mor rundt svangerskapsuke 17 ble analysert for flere perfluorerte og klororganiske forbindelser.

I den første studien rapporterte vi kvinnenes konsentrasjoner av perfluorerte og klororganiske forbindelser, samt undersøkte faktorer som kunne være assosiert med nivået av forbindelsene. Vi fant at de svenske kvinnene hadde gjennomsnittlig høyere serumnivå av perfluorerte forbindelser og polyklorert bifenyl (PCB) 153, sammenlignet med de norske kvinnene. Alle forbindelsene var negativt assosiert med tidligere ammevarighet. Gjennom hele inklusjonsperioden (1986-88) økte nivået av perfluorerte forbindelser, mens nivået av klororganiske forbindelser gikk ned. Nivået av klororganiske forbindelser økte med økende alder, mens nivået av perfluorerte forbindelser økte med tiden siden forrige ammeperiode. Andre faktorer, som mors BMI, røykestatus, utdanningsnivå og alkoholforbruk var også assosiert med noen av miljøgiftene.

I den andre studien fant vi at mors nivå av perfluorert oktansyre (PFOA), PCB 153 og heksaklorbenzen (HCB) var assosiert med indikasjoner på føtal veksthemming, inkludert høyere odds for SGA-fødsel, men bare blant de *svenske* mor-barn-parene. Vi fant også indikasjoner på sterkere assosiasjoner blant svenske guttebarn sammenlignet med jentebarn.

I den tredje studien fant vi at mors nivå av perfluoroktylsulfonat (PFOS) og PFOA var positivt assosiert med barnets BMI, tykkelse av tricepshudfold og høyere odds for overvekt/fedme ved fem års alder. Ved stratifisering i land var det kun blant de *norske* mor-barn-parene at disse assosiasjonene besto. Vi fant også indikasjoner på at ikke-monotone dose-respons forhold kunne være involvert.

Dette arbeidet belyser vanskelighetene med å etablere kausale sammenhenger mellom persistente organiske miljøgifter og pre- og postnatale vekstmønster pga. komplekse utviklingsprosesser, i tillegg til korrelerte eksponeringer, mangel på monotone dose-respons forhold, samt flere ulike eksponeringsveier og miljøgiftsnivå mellom ulike regioner. Likevel kan resultatene indikere en mulig sammenheng mellom enkelte miljøgifter og vekst og utvikling hos barn opp til fem års alder. Flere veldesignede prospektive studier med data på aktuelle miljøgiftsnivåer og blandinger av miljøgifter, i tillegg til lengre oppfølgingstid, er nødvendig for å kunne bekrefte disse funnene.

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Finansieringskilde: Regionalt samarbeidsorgan for utdanning, forskning og innovasjon (Samarbeidsorganet Helse Midt-Norge RHF)

Ovennevnte avhandling er funnet verdig til å forsvares offentlig

for graden PhD i medisin.

Disputas finner sted i Auditorium MTA, Medisinsk Teknisk Forskningssenter, NTNU, Trondheim

Torsdag 21. Juni 2018, kl. 1215.

Contents

Acknowledgements

This doctoral thesis is based on work carried out between May 2012 and February 2018 at the Department of Public Health and Nursing, Norwegian University of Science and Technology (NTNU), and had not been possible without the help and support from others.

The work was funded by the Liaison Committee Central Norway Regional Health Committee-NTNU. Norway's National research school in population-based epidemiology (EPINOR) provided funding for conference participation.

First, I want to thank all individuals in the Scandinavian SGA study, both study-participants and staff.

My sincere appreciation goes to my supervisory team, and especially my main supervisor Geir W. Jacobsen, for sharing his immense knowledge, expertise and enthusiasm for public health and perinatal epidemiology, and for his thorough peer-reviews on all the manuscripts and this thesis. Torbjørn Øien has been one of my co-supervisors, and I would like to thank him for invaluable feedback, careful reading and helpful criticism. I would like to express my gratitude to my co-supervisor Torkjel M. Sandanger for sharing his extensive knowledge and experience in the environmental research field, and for his hospitality and friendliness all the times I came to visit in Tromsø. Many thanks to my co-supervisors Jon Øyvind Odland, for his experience and knowledge in the obstetrical and environmental research field, and Margot van de Bor for her immense knowledge in paediatric and environmental medicine and research. Tricia L. Larose has been one of my co-supervisors the last couple of years, and her epidemiological knowledge, genuine encouragement, friendliness, prompt and thorough peer-reviews of all manuscripts have been key to land this thesis.

I also wish to express my gratitude to Charlotta Rylander, Therese Nøst, Vivian Berg and the rest of the staff at NILU for their great expertise, support and tremendous help with the POP analyses, and for their hospitality, friendliness and interesting discussions in Tromsø. I would also thank the staff at the Institute National de Santé Publique de Quebec for conducting the OC analyses. A special appreciation goes to Jennifer Hutcheon at Boston University for peer-reviewing my last manuscript and for her immense knowledge and enthusiasm regarding infant growth trajectories.

I am grateful for invaluable feedback and encouragement from colleagues located across all floors at the Department of Public Health and Nursing. A special gratitude to my colleagues at the 3rd floor, including my officemate through 5 years Bente Mjølstad, for their friendliness, support and interesting discussions during our weekly lunches. My new colleagues at the Department of Occupational Medicine at St. Olavs Hospital, including the Head of Department Siri Slåstad, deserve an appreciation for their support, friendliness and for keeping up with me during the final stage of writing this thesis.

I would also like to thank my family and friends for generous support and long endurance. A special appreciation to my parents for all help, support and encouragement. And, finally, many thanks to my husband Kim, and our children Andreas, Martin and Sara, for putting the PhD-process into perspective. Thank you for your unconditional love and support, and for reminding me of the most important things in my life.

List of papers

The thesis is based on the following papers:

Paper I: Lauritzen HB, Larose TL, Oien T, Odland JO, van de Bor M, Jacobsen GW, Sandanger TM. Factors Associated with Maternal Serum Levels of Perfluoroalkyl Substances and Organochlorines: A Descriptive Study of Parous Women in Norway and Sweden. PLoS One. 2016;11(11):e0166127.

Paper II: Lauritzen HB, Larose TL, Oien T, Sandanger TM, Odland JO, van de Bor M, Jacobsen GW. Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatric research. 2017;81(1-1):33-42.

Paper III: Lauritzen HB, Larose TL, Oien T, Sandanger TM, Odland JO, van de Bor M, Jacobsen GW. Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study. Environmental health : a global access science source. 2018;17(1):9.

Abbreviations and acronyms

AMAP – Arctic Monitoring and Assessment Programme

BMI – Body mass index

BPD – Biparietal diameter

CDC – Centre for Disease Control and Prevention

CIs – Confidence intervals

CG – Cockroft-Gault

CV – Coefficient of variation

CYP – Cytochrome P

DAG – Directed acyclic graph

DOHaD- The Developmental Origins of Health and Disease

EDCs – Endocrine disrupting chemicals

FGR – Foetal growth restriction

GC – Gas chromatography

GFR – Glomerular filtration rate

HCB – Hexachlorobenzene

HCHs – Hexachlorocyclohexanes

IUGR – Intrauterine growth restriction

LBW – Low birth weight

LOD – Limit of detection

MS – Mass spectrometry

NILU – Norwegian Institute for Air Research, Tromsø, Norway

NMDR – Non-monotonic dose-response

OCs – Organochlorines

PCBs – Polychlorinated biphenyls

PFASs – Perfluoroalkyl substances

PFOA – Perfluorooctanoic acid

PFOS – Perfluorooctane sulfonic acid

POPs – Persistent organic pollutants

POSF – Perfluorooctane sulfonyl fluoride

PPAR-γ – Peroxisome proliferator-activated receptor-γ

p,p´- DDE - 1,1- dichloro- 2,2- bis(p- chlorophenyl)ethylene

p,p´- DDT - 1,1,1- trichloro- 2,2- bis(p- chlorophenyl)ethane

QA- QC – Quality assurance- quality control

REK – The Regional Committee for Medical and Health Research Ethics

SGA – Small for gestational age

SGA study – Scandinavian Successive Small-for-Gestational Age Births Study, 1986-94

SRM – Standard reference material

t-nonachlor – trans-nonachlor

UPLC – Ultra-high pressure liquid chromatography

VIFs – Variance inflation factors

WHO – World Health Organization

Summary

Humans have been exposed to an increasing amount of persistent organic pollutants (POPs) during the 20th century. Perfluoroalkyl substances (PFASs) and organochlorines (OCs) are two important groups of POPs. Concentrations of PFASs increased extensively until the 1990s and decreased from around 2000, while OCs have decreased since the 1980s due to world-wide bans and restrictions on use. POPs have persistent properties, they bio-accumulate in the food chain, and diet is the main exposure route for humans. They are transferred from the mother to the foetus via the placenta during pregnancy and via breastmilk to infants after birth. These POPs are thought to act as "endocrine disrupting chemicals" (EDCs), and it has been hypothesized that prenatal exposure to EDCs could potentially lead to harmful developmental consequences in the offspring, including foetal growth restriction (FGR) and/or later childhood obesity, but the evidence is sparse and inconclusive.

This doctoral thesis uses data from the *Scandinavian Successive Small-for-Gestational Age (SGA) Births Study*. The SGA Births Study is a large, prospective, population-based multicentre study, conducted in Trondheim and Bergen (Norway) and Uppsala (Sweden) from 1986 to 1994. The women were followed from early pregnancy and screened at their study centre in gestational weeks 17, 25, 33, 37, and at delivery. After birth, a follow-up study examined selected children through their first year of life up to five years of age. Maternal serum samples, donated during the second trimester, were analysed for several PFASs and OCs.

Three peer-reviewed and published papers make up this doctoral thesis. Paper I reports maternal serum PFAS and OC concentrations and examines associated maternal factors. Overall, Swedish women had higher serum PFAS and polychlorinated biphenyl (PCB) 153 concentrations compared to Norwegian women. Previous breastfeeding duration, sampling

date, maternal age and time since last breastfeeding period influenced maternal PFAS and OC concentrations collected from 1986 to 1988. All maternal serum concentrations of PFASs and OCs were inversely associated with previous breastfeeding duration. Throughout the recruitment period maternal serum PFAS concentrations increased while OC concentrations declined. Maternal age and time since last breastfeeding period were positively associated with maternal serum concentrations of OCs and PFASs, respectively. Maternal pre-pregnancy BMI, smoking status, education level and alcohol consumption were also associated with some maternal serum PFAS and OC concentrations. A full description of the results can be found in the Results section and the appended published manuscripts.

In Paper II, maternal serum concentrations of perfluorooctanoic acid (PFOA), PCB 153 and HCB were associated with indices of impaired foetal growth (including higher odds for SGA birth), but only among Swedish mother-child pairs. Some indications of stronger associations among Swedish male offspring were found.

In Paper III, maternal serum concentrations of PFOS and PFOA were positively associated with offspring BMI and triceps skinfold z-scores and higher odds for offspring overweight/obesity at five-year follow-up. In country-stratified analyses, these associations only remained among Norwegian mother-pairs. Some evidence of non-linearity implied possible involvement of non-monotonic dose-response relationships.

This work highlights challenges in establishing causal effects of POPs on pre- and postnatal growth patterns due to the complexity of developmental processes, as well as the intricacy of correlated exposures, lack of monotonicity, and multiple possible exposures routes and concentrations of POPs between different regions. However, the results indicate a possible influence of background exposures to POPs on pre- and postnatal growth. Well-designed prospective studies with contemporary data on current environmental concentrations

and mixtures of POPs, as well as longer follow-up are needed to replicate and confirm these findings.

1. Introduction

1.1 Preamble

The overarching topic of this doctoral thesis is exposure to maternal persistent organic pollutants (POPs) and foetal growth, as well as later growth and childhood obesity. By using data from the Scandinavian SGA Births Study, I have examined maternal serum concentrations of two perfluoroalkyl substances (PFASs) and five organochlorines (OCs) in mothers from Norway and Sweden. I have further examined the associations between maternal serum and indices of offspring foetal growth, as well as later growth and obesity in offspring up to five years of age.

In most countries, the use of these chemicals is presently banned or restricted (1), but due to their persistent and bioaccumulating properties, adverse health outcomes related to background levels of exposure are still of great concern (2). Furthermore, even though a few PFASs were banned or included in the Stockholm Convention as restricted for use in the 21st century (1, 3), there are still countries, including China, that continue to produce PFASs (4). Despite a worldwide ban, some OCs are still being used. The pesticide and insecticide dichlorodiphenyltrichloroethane (DDT) is reported to still be in use for vector control in malaria infected countries, including India and some southern African states (5). Release of α-hexachlorohexane (α-HCH) and β-HCH is continued due to production of lindane (γ-HCH), which was permitted for use as a second line drug for control of head lice and scabies, or from environmental stockpiles (6).

POPs are transferred from the mother to the foetus via the placenta during pregnancy and via breastmilk to infants after birth (7-9). These POPs are thought to act as "endocrine disrupting chemicals" (EDCs) (10, 11), and it has been hypothesized that prenatal exposure to EDCs could potentially lead to harmful developmental consequences in the offspring,

including foetal growth restriction (FGR) and/or later obesity, but the evidence is sparse and inconclusive.

"Foetal programming" was proposed as a mechanism for which unfavourable foetal environment or adverse environmental exposures could result in chronic diseases in adulthood (12, 13). A recent report from the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) recognized EDCs as an emerging health challenge to vulnerable individuals in society, particularly foetuses and children, and further strengthening of the knowledge of EDCs and its potential adverse effects is needed (14).

1.2 Persistent organic pollutants (POPs)

POPs are ubiquitous, persistent and bio-accumulative chemicals that have been detected in maternal serum during pregnancy and in cord blood at delivery. Two important groups of POPs are perfluoroalkyl substances (PFASs) and organochlorines (OCs). Although the use of some of these substances is presently banned or restricted in most developed countries (1), adverse health outcomes related to background levels of exposures are still of utmost concern (2).

1.2.1 Perfluoroalkyl substances (PFASs)

PFASs include aliphatic substances that contain one or more carbon atoms where at least one hydrogen atom is replaced by fluoride (15). PFASs are used in a wide variety of commercial and industrial applications, like fire-fighting foam, metal plating and cleaning, as water and stain proofing agents, paper products and lubricants (16). Their chemical and thermal stability, as well as their hydrophobic and lipophobic nature, are major reasons for the extensive use in diverse applications (17). Production of PFASs started in the 1950s, and

increased extensively up to the early 1990s. Since then, the production of PFASs has remained constant until the major manufacturer, 3M, announced a phase-out of their perfluorooctane sulfonyl fluoride (POSF) based industry in 2000 (18). Perfluorooctane sulfonic acid (PFOS) was classified as a POP due to concerns over the bioaccumulation potential and its persistence in the environment, as well as the risk for toxicological effects on animals and humans (1). PFOS was banned in Norway in 2007 and by the European Union in 2011, and included in the Stockholm Convention that recommended restricted use in 2009 (1). In 2014, production, import and export of perfluorooctanoic acid (PFOA) and PFOA-containing products and textiles were banned in Norway (3). Other countries, like China, are still producing PFASs (4).

1.2.2 Organochlorines (OCs)

OCs are comprised of numerous substances, but only a few are considered in this thesis. Polychlorinated biphenyls (PCBs) are thermally stable industrial chemicals, produced since the 1930s for commercial uses in paints, plastic and electrical transformer fluids (19). PCBs comprise of 209 possible congeners, and were widely used during the 20th century. They were banned in several countries from the 1970s. Organochlorine pesticides covered in this thesis are chlordanes, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane *(p,p'*-DDT) and its metabolites, hexachlorohexanes (HCHs) and hexachlorobenzene (HCB). They have diverse chemical structures, but comparable toxic properties designed to control different pests and diseases. Global initiatives for reduction in the use of these compounds were initiated due to concerns for damaging effects of these chemicals on the environment and human health. However, DDT is still being used as a vector control agent in India and some southern African states (5), and production of lindane (γ-HCH) is permitted for use as a second line drug for control of head lice and scabies (6).

1.3 Human exposure to POPs

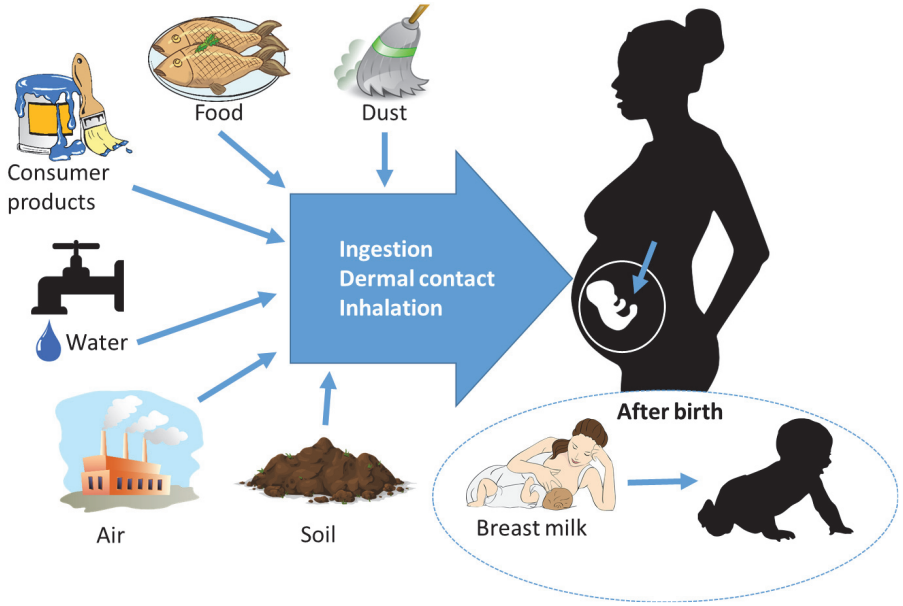

Figure 1 Illustration of the main exposure sources and intake routes of POPs. The figure is made by the author.

Industrial use and degradation of precursor compounds enhances the occurrence of POPs in the environment (16). As POPs have persistent properties, they bio-accumulate in the food chain, and diet is the main exposure route for humans (20-22), particularly in post-ban periods and in background exposed populations. POPs are also passed on through contaminated air, building materials, house dust, drinking water, soil, and consumer products containing POPs (21, 23) (Figure 1). POPs are transferred through the placenta to the foetus (See Section 1.5 for more details), and to infants through breastfeeding (7-9). Hence, the exposure pathways differ between foetuses, infants and children, as well as between females and males and across adult ages (23). Both the historic and current production and use of POP compounds probably contribute to the relative importance of different exposure pathways among persons with background exposure levels.

PFASs are both lipophilic and hydrophobic, they bind to proteins, and reside in the blood, liver and kidneys (24, 25). OCs, on the other hand, are lipophilic compounds, and

accumulate in fatty tissues (26). In humans, the metabolism and elimination of POPs are slow, and there is no known metabolism of PFOS (27). Enzymes in the liver (cytochrome P (CYP) enzymes) can modify compounds and make them more water-soluble, to assist in the excretion from the body (28). Some PCBs can induce their own metabolism by inducing CYP enzymes, and cigarette smoking may enhance elimination of dioxins and dioxin-like PCBs by such induction (29). Excretion of OCs occurs mainly through faeces and urine, whereas some PFASs are excreted through the kidneys (30). Among women, excretion of POPs also occurs through breastmilk and menstruation (30). The half-life of a compound corresponds to the time it takes for an initial concentration to be reduced by half. The chain length of the compound is important for its half-life. PFASs with shorter carbon-chains degrade more rapidly than those with longer chains (31). Similarly, lower chlorinated PCBs are more readily metabolized compared to higher chlorinated congeners (32). Consequently, estimated half-lives of PCBs range from 1-27.5 years (33), while serum HCB and DDT concentrations are halved in 6 and 7 years, respectively (34). Estimated half-lives of PFOS and PFOA are 4.6 and 3.4 years, respectively (35).

Biomonitoring programs, like the Arctic Monitoring Assessment Programme (AMAP) have demonstrated that POPs are ubiquitous compounds. Declining trends in use and emissions of OCs during the 1980s and 1990s have led to decreasing concentrations in air and biota in Northern Europe (36-38). As for PFASs, the concentrations in humans and biota have increased during the same period (39-41). After phase-out of the PFAS based industry, studies monitoring humans have shown a decrease of PFASs in human serum from around year 2000 (41-44).

1.4 Maternal factors associated with serum POP concentrations

Several epidemiological studies have reported demographic, pregnancy-related, dietary and lifestyle factors associated with POP concentrations in humans, mainly with a cross-sectional design. In studies among pregnant women, maternal age or birth year and maternal BMI have commonly been associated with maternal serum OC concentrations (28, 45), while similar associations have been inconsistent for maternal serum PFAS concentrations (46-48). Parity and/or previous breastfeeding duration have been negatively associated with maternal serum PFAS and OC concentrations in cross-sectional studies (46, 47, 49, 50), probably due to elimination of these compounds through the placenta and breast milk. Moreover, inter-pregnancy interval has been positively associated with serum PFAS concentrations in a cross-sectional study (46), whereas glomerular filtration rate (GFR) was negatively associated with maternal serum PFAS concentrations in a recent cross-sectional study from early pregnancy (48). In another cross-sectional study, maternal smoking was negatively associated with dioxin-like PCBs in breastmilk in a dose-dependent manner (29), perhaps due to induction of CYP enzymes. Maternal smoking has been inconsistently associated with other OCs or PFASs in serum among pregnant women (46, 51).

Assessments of dietary patterns have indicated that fish consumption is positively associated with serum OC (28, 52, 53) and PFAS (22, 46) concentrations. Differences in mean serum POP concentrations between countries might stem from dissimilar contamination patterns in fish from different areas. For example, one study from the early 1990s found that herring and white fish from the Baltic Sea had much higher PCB 153 concentrations than herring from the West Coast of Sweden (54).

It has also been hypothesized that the relative importance of associated factors (e.g., parity, breastfeeding duration and inter-pregnancy interval) may differ in periods before and after POPs were banned or restricted in use (20, 33, 55).

1.5 Foetal exposure to POPs

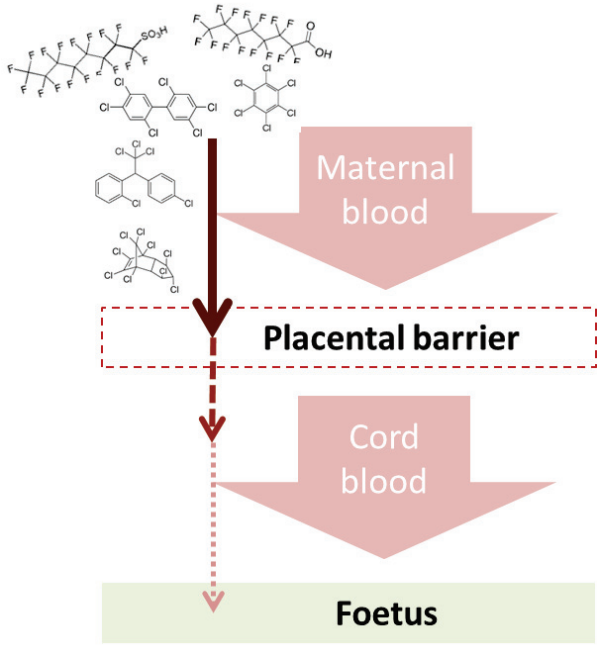

Figure 2 Schematic drawing of transfer of PFASs and OCs across the placenta barrier. The figure is made by the author.

Pregnant women are daily exposed to a wide range of different substances via lifestyle factors (e.g. smoking, personal care products, alcohol), maternal medication or environmental exposures. Many different compounds are transported across the placenta, and may therefore impact the foetal development. Foetal exposures to environmental and medical substances may influence the growth of foetus (e.g. cigarette smoke) or development of foetal organs (e.g. methylmercury and thalidomide).

The foetal circulating system connects with the maternal circulating system through the umbilical cord, and exchange of substances occur in a temporary organ called the placenta (Figure 2). The placenta plays a protective role as a barrier that allows transfer of nutrients and antibodies, filters out certain infectious agents and drugs, and metabolizes xenobiotics with enzyme expression (56). Exchange of nutrients, gases and waste products between the foetus and the mother occurs through simple diffusion, active transport, osmosis and vesicular

transport (56). This transport is enabled by many tiny villi and a thin membrane with trophoblast and epithelial cells in the placenta.

Although POPs have been reported to enter the foetal system (7-9), the detailed mechanism of the placental transfer of POPs is not clear. Studies using maternal to cord blood ratios to estimate the placental transfer efficiency of PFASs have shown that PFOA is transferred more efficiently than PFOS (57, 58). The carbon chain length probably influences the transfer efficiency in that shorter chained PFASs seem to transfer more easily across the placenta than longer chained PFASs. The length of the carbon chain is probably also influencing the hydrophobic capacity, so that the more hydrophobic PFASs (PFOS>PFOA) (59) are retained in maternal tissues and less transferred to the foetus.

Circulating OCs are transported by lipoproteins and albumin in blood vesicles due to their lipophilicity. Studies have identified receptors for lipoproteins in the placental membrane, and demonstrated that lipoproteins in the microvilli are decomposed into fatty acid and cholesterol (60). By diffusion and specific transport mechanisms, decomposed fatty acids and cholesterol can transfer into the foetal system. Hence, the assumed mechanism for transport of OCs across the placental barrier is related to the transport mechanism of lipoproteins, such as passive diffusion and uptake by lipoprotein receptors (61). In agreement with this, studies have shown that the PCB distribution between cord blood and placenta samples is dependent on the molecular size and lipophilicity, in that concentrations of low chlorinated congeners are higher in the cord blood (61).

1.7 Endocrine disruptive chemicals (EDCs)

The human endocrine system is responsible for controlling several processes, from embryonic development and organogenesis early on, to the control of tissue and organ

functions in adulthood (62). Endocrine glands produce hormones that are transported with the blood to exert effects on cells and tissues via complex signalling pathways. Over 50 different hormones and hormone-related molecules control normal body functions throughout life.

Several POPs, including OCs and PFASs, are classified as endocrine disrupting chemicals (EDCs) (63). An EDC is "an exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action" (10, 11). EDCs probably affect all hormonal systems, including those controlling the development and function of reproductive organs, as well as tissues and organs that regulate metabolism and satiety (64). Obesity, reduced fertility, learning difficulties, adult-onset diabetes and cardiovascular disease are only a few of the endpoints that have been associated with EDCs. Chemicals can disrupt hormone action either directly via one hormone-receptor protein complex or on a specific protein that controls hormone delivery to the receptor. Like hormones that act via binding to receptors, also EDCs are able to act at low concentrations (9). Several EDCs can work together and exert synergistic or antagonistic effects not seen when only an individual chemical is considered (65). Like hormones, EDCs can also act via non-monotonic or non-linear dose-response curves (66). Consequently, it is not always possible to extrapolate low-dose from the high-dose effects of EDCs.

There is significant concern for EDC exposures during developmental periods for foetuses and children during development, due to immature metabolic pathways and elevated weight-adjusted body burdens. Exposure during sensitive windows of susceptibility, e.g., prenatally, may be particularly important for adverse effects that can appear later in life (67).

1.6 Foetal programming

In 1977, the Norwegian doctor Anders Forsdahl first noticed that nutrition and living conditions during early life could have life-long consequences (68, 69). This was followed by Professor David Barker's (1938-2013) "foetal programming" hypothesis in 1986. He hypothesized that an unfavourable foetal environment or adverse environmental exposures could lead to chronic diseases later in life (12, 13, 70). The Developmental Origins of Health and Disease (DOHaD) hypothesis arose from Barker's foetal programming hypothesis (71).

The placenta and its many functions are pivotal for successful reproductive outcomes. Throughout pregnancy, the ambient environment may influence these functions, changing the genetic programming that is needed to sustain a healthy pregnancy and ensure appropriate foetal development. A cohort study of 2,414 children born around the time of the Dutch famine in World War, reported that exposure to famine during early stage of gestation was associated with glucose intolerance, coronary heart disease, increased stress responsiveness and more obesity later in life (72). The findings of the Dutch famine birth cohort study broadly support the foetal origins hypothesis, and suggest that chronic diseases originate in the womb through adaptations made by the foetus in response to undernutrition. Also, higher maternal pre-pregnancy body mass index (BMI) and higher gestational weight gain are associated with increased birth weight and increased BMI in young and adult age (73, 74). Epigenetic changes related to environmental contaminant exposure may result in altered developmental programming (75). Numerous important reproductive outcomes, including early pregnancy loss, FGR, congenital syndromes, preterm birth, and preeclampsia are related to epigenetic alterations.

Several maternal exposures have been shown to induce epigenetic change in the offspring (71). Maternal smoking during pregnancy has been found to influence offspring

DNA methylation in genes involved in fundamental developmental processes (76). One longitudinal study that followed 800 mother-child pairs found persistently perturbed patterns of methylation in offspring at 17-year follow-up after maternal smoking, and that the major contribution to altered methylation was attributed to a critical window of *in utero* exposure (77). This highlights the importance of considering exposures *in utero* in relation to later health and chronic diseases.

1.8 Foetal growth

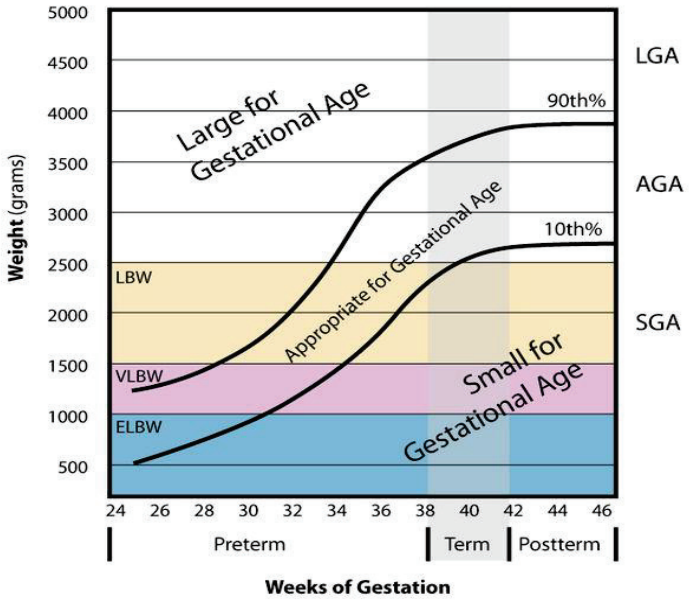

Figure 3 Fetal growth chart. AGA, appropriate-for-gestational-age; ELBW, extremely low birth wight; LBW, low birth weight; LGA, large-for-gestational-age; SGA, small-for-gestational-age; VLBW, very low birth weight. From Wikimedia Commons, the free media repository, with permission.

On a population level, birthweight is strongly related to infant and adult morbidity and mortality (78), and is routinely measured in clinical practice and recorded in medical registries. Hence, birthweight has become a key measure of exposure and landmark outcome in clinical and epidemiological studies. However, it is important to remember that birthweight

is a consequence of both different growth patterns throughout pregnancy and gestational age at birth.

Birthweight is considered the most important outcome characteristic for perinatal mortality. However, as birthweight is profoundly dependent on gestational age, it might be difficult to disentangle these two variables. Wilcox et al. separated *relative birthweights* for any given gestational age from gestational age at delivery, and found that gestational age could independently explain much of the perinatal mortality related to birthweight (79).

Earlier on, birthweight was dichotomized into low birth weight (LBW) (birth weight below 2,500 g) and normal birth weight (birth weight above 2,500 g), and for several years, premature delivery was the assumed reason for LBW children. However, not all small new-borns are premature, and not all premature new-borns are small. The scientific community now prefers to use "preterm" on a baby born early. Nonetheless, term new-borns who weigh less than 2,500 g still have an elevated risk of mortality and morbidity. The term intrauterine growth restriction (IUGR) or foetal growth restriction (FGR) emerged, and the most frequently used definition is "small-for-gestational-age" (SGA), the lowest 10 percentiles of birth weight for each gestational age. SGA is often adjusted for parity and sex as well (80) (Figure 3).

Every year, over 20 million LBW babies are born, which corresponds to 17% of all births in low and middle income (LMIC) countries. In high income countries (HIC), prematurity is the major cause of low birthweight, while foetal malnutrition and growth retardation are main causes for of birthweight in LMICs. Approximately 40% of all LBW babies in LMICs comes from India (81), wherein 75% are born to term with IUGR and are labelled as being SGA.

1.8.1 Risk factors for foetal growth restriction

The multifactorial aetiology of foetal growth restriction includes maternal, placental and foetal factors, but several aetiologies are not identified (82). Foetal factors include intrauterine infections, chromosomal abnormalities, congenital malformations and multiple gestation, and has been estimated to cause 15-20% of all FGR cases (82). Placental factors comprise abnormal development of the placenta, insufficient implantation or villi dysfunction (82). Maternal factors are categorized in maternal diseases, and demographic and environmental factors. In the former group, maternal chronic vascular disease (i.e. renal disease or hypertension, and particularly in combination with preeclampsia) is one of the most dominant causes of FGR (82). Risk factors in the latter group include a previous LBW infant, young or old age (below 17 or above 34 years), short stature and low socioeconomic status. Moreover, environmental factors like undernutrition and toxins (tobacco, alcohol, medications and illicit drugs) contribute to a harmful foetal environment and may cause FGR. In up to 40% of SGA infants the cause of IUGR cannot be determined, and unknown environmental factors could have a possible implication (81).

1.9 Childhood obesity

The prevalence of childhood overweight and obesity has increased dramatically over the last four decades. From 1990 to 2010, the estimated prevalence of childhood overweight and obesity increased from 4.2% to 6.7% (83). This trend is expected to continue and the World Health Organization (WHO) predicts that in 2020, a total of 60 million preschool children (9.1%) will be overweight or obese (83). Among US children aged 2-19 years, 32% are overweight (BMI>85th percentile) and 17% are considered obese (BMI >95th percentile) (84).

Childhood overweight and obesity is an important public health concern and is of special interest because of possible long-term associations with adult weight status and morbidity. Children who are overweight or obese are more likely to be obese as adults (85). Adult obesity is a risk factor for several life-threatening and chronic diseases such as diabetes, cardiovascular disease, musculoskeletal disorders, and some forms of cancer (86). Obesity prevention is an essential element of overall health and well-being.

Although the increasing prevalence of obesity is mostly attributed to changes in diet, sedentary lifestyle and genetic predisposition, they do not completely account for the obesity epidemic (87). The foetal environment is important for later growth and development. One theory is that the rise in ambient emissions of environmental chemicals has led to higher prenatal exposure to hazardous substances that may disrupt foetal development.

1.10 Prenatal exposure to POPs and foetal growth and birth outcome

1.10.1 Mechanisms

Endocrine-disrupting properties of PFASs and OCs may be involved in the biological mechanisms that affect human foetal growth, although this is still uncertain. The prenatal period is a vulnerable period for hormonal changes, and normal foetal development is, among others, highly dependent on thyroid and sex steroid hormones. During development, thyroid hormones are crucial for somatic growth and differentiation of tissues (88, 89), and prenatal oestrogens are important in the promotion of foetal growth (90). A recently published review regarding POPs and thyroid function concluded that both PFASs and OCs may disrupt thyroid hormones (91). Increasing levels of maternal serum PFAS and OC concentrations have been associated with lower levels of circulating thyroid hormones in background exposed populations (92, 93). Hypothesized actions of estrogenic and anti-estrogenic PCB congeners

are competitive binding to estrogenic receptors, disruption of enzymes or inhibition of the effects of endogenous oestrogens, and may consequently lead to inhibition of foetal growth (94). Moreover, several PFASs seem to possess the potential *in vitro* to interfere with the function of the oestrogen and/or the androgen (95). Cellular growth and epigenetic alterations that occur early in foetal development could be affected by prenatal exposure to POPs, and may have long lasting impacts on diverse health outcomes, including foetal growth (96). Some studies have shown that high prenatal exposure to PFASs is associated with DNA demethylation in neonates (97-99). A recent in vitro study reported that chronic exposure of foetal cells to low doses of PCBs causes permanent genomic and epigenetic instability, which could influence both prenatal and postnatal growth up to adulthood (100).

1.10.2 Results from epidemiological studies

Among PFASs, PFOA is one of the most abundant and studied compounds related to child health (101). Two systematic reviews concluded that there was "sufficient" human evidence that prenatal exposure to PFOA reduces foetal growth (102), whereas the evidence was less consistent for PFOS (102, 103). However, a recent review found no quantitative toxicological evidence to support this epidemiological association, as the effective animal extrapolated serum concentrations were 102-103 times higher than those in humans (104). Thus, the biological plausibility of a causal relationship is uncertain. Only a few studies have assessed associations between prenatal exposure to PFASs and SGA or LBW birth (49, 105-108), with conflicting results. One study found greater odds for SGA birth with high prenatal exposure to PFOS (105), whereas another study found that higher maternal PFOS concentrations were associated with an increased odds for LBW, but only among male offspring (108).

Several epidemiological studies have assessed prenatal exposure to OCs and foetal growth. One large meta-analysis of 12 European birth cohorts enrolled from 1990 through 2008 assessed the associations between prenatal exposure to PCBs and *p,p'*-DDE and birth weight, and concluded that low-level exposure to PCBs impairs foetal growth, but that exposure to *p,p'*-DDE does not (109). The associations between prenatal exposure to other OCs, like HCB, and foetal growth, are not consistent. Some authors have reported an inverse relationship between prenatal exposure to HCB and foetal growth, including length at birth (110, 111), length of gestation (111-113), birth weight (114), weight and length at birth among smoking mothers (115), length of male infants (116) and weight of female infants (117). Other studies have found no significant associations (118-123).

1.11 Prenatal exposure to POPs and childhood overweight and obesity

1.11.1 Mechanisms

It is proposed that development of obesity may be induced *in utero* by exposure to EDCs (63, 87). Evidence from animal and *in vitro* studies suggest that mechanisms include disturbance of lipid metabolism to promote storage of fat, changes to metabolic set points or alterations of hormonal control for appetite and satiety (63). Modifications of the epigenome that result in production of adipocytes at the cost of bone is another proposed mechanism (124). Exposure to *p,p'*-DDE has been associated with adipose dysfunction in experimental studies via increased adipocyte differentiation expression patterns of the peroxisome proliferator-activated receptor-γ (PPAR-γ), i.e., the main transcription factor that regulates the adipogenic process (125). In animal models, one study found that exposure to PFOA *in utero* led to weight gain in offspring mice (126).

1.11.2 Epidemiological findings

Epidemiological studies investigating associations between maternal serum POP concentrations during pregnancy and offspring postnatal growth and obesity are sparsely available. Regarding PFASs, longitudinal studies have reported both positive (127-134) and no associations (135-137) between maternal serum levels and offspring growth and obesity measures. Recent non-systematic reviews have reported moderate evidence regarding prenatal exposure to OCs and childhood obesity (101, 138). In more than 12 prospective studies, prenatal exposure to *p,p'*-DDE has been associated with accelerated weight gain in infancy, accelerated postnatal BMI trajectories and greater risk of childhood obesity (139-146), while less consistent findings were reported for associations between prenatal PCB and HCB exposure and childhood obesity (140, 142-147). The evidence was classified as "insufficient" due to inconsistencies in findings from studies for PCBs and HCB, and as "moderate" for *p,p'*-DDE (101).

Most previous studies relied on anthropometry measures, including BMI, rapid weight gain, weight-for-height ratio or waist circumference, while fewer studies utilized direct measures of adiposity, e.g. skinfold thickness or adipokines (128, 130, 142). Unlike BMI that indirectly measures obesity, skinfold measurements are direct measures of subcutaneous fat tissue and are highly correlated with body fat mass (148).

2. Aims

The research aims for Paper I-III included in this doctoral thesis were:

1) To determine the concentrations of POPs and their associated maternal factors, in a Scandinavian pregnant population with background exposure levels in the late 1980s

2) To investigate associations between maternal serum POP concentrations and indices of foetal growth (including SGA birth), and to explore potential effect modification by offspring sex and geography (country of residence)

3) To investigate associations between maternal serum POP concentrations and measures of childhood obesity at five-year follow-up, and explore effect modification by offspring sex and geography (country of residence)

3. Materials and Methods

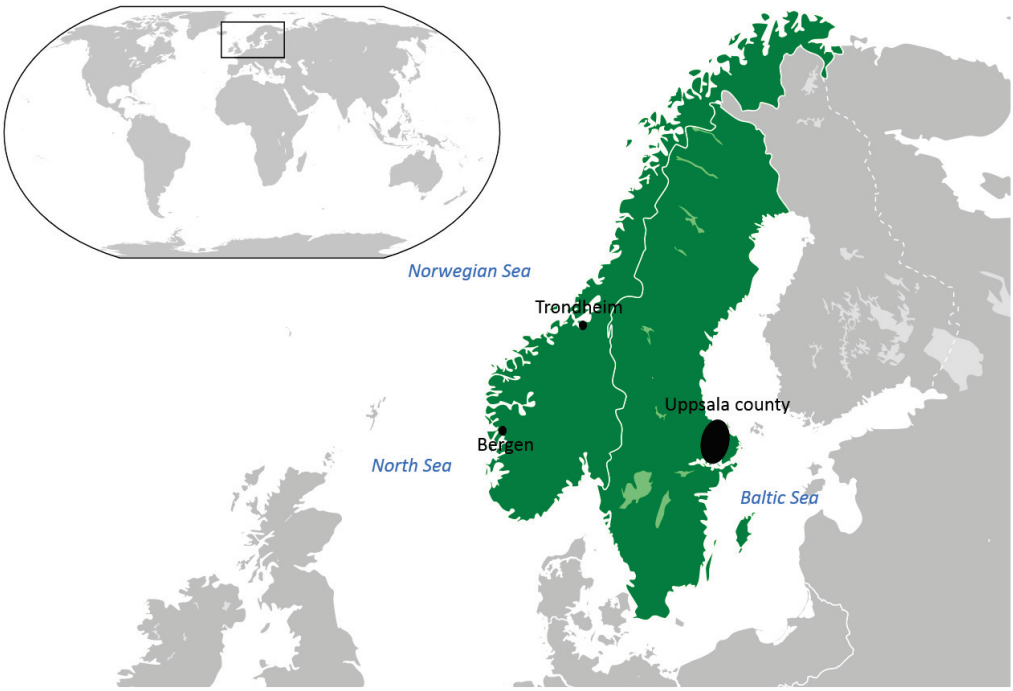

Figure 4 Map of the Scandinavian SGA-study area (reproduced and changed by permission from Wikimedia Commons)

3.1 Scandinavian SGA-study

All three papers were based on the *Scandinavian Successive Small-for-Gestational Age (SGA) Births Study.* This large, prospective, population-based multicentre study was conducted in Trondheim and Bergen (Norway) and Uppsala (Sweden), between January 1986 and March 1988 (Figure 4). It was organized and funded by The U.S. National Institute of Child Health and Human Development. The study aimed to investigate causes and consequences of foetal growth restriction in successive pregnancies (80).

Local general practitioners and obstetricians referred eligible participants who consented to participate at their nearest University Hospital in either Trondheim, Bergen

(Norway) or Uppsala (Sweden). The women were followed from early pregnancy and screened at their study centre in gestational weeks 17, 25, 33, 37, and at delivery. After birth, a follow-up study examined selected children through first and up to five years of age.

Due to the nature of the study, eligible participants were second and third time mothers of Caucasian origin who spoke one of the Scandinavian languages, had a singleton pregnancy, and were registered prior to gestational week 20 (Figure 5). In total, 6,354 women were recruited from which 5,722 made their first study visit. Three study groups were defined according to the protocol: (1) a 10% random sample was constructed to serve as a population reference (n=561), (2) a high-risk group (n=1,384), and (3) a low-risk group. The sealed envelope method was used to set up the random sample. To ensure a sufficiently large study sample of SGA infants, a high-risk group of women was constructed. Mothers with one of the following criteria were categorized as high-risk: (1) a previous low birth weight (LBW) child, (2) a previous perinatal death, (3) maternal low pre-pregnancy weight (<50 kg), (4) smoking cigarettes at conception, and (5) chronic maternal hypertension or renal disease. Due to the high prevalence (~ 30%) of smokers, just half of the women who reported smoking at the first study visit was randomly selected to the high-risk group. The low-risk group consisted of women without any risk factors and served as a "rest population". Women from the random sample and the high-risk group were invited to the above detailed follow-up throughout pregnancy and at birth.

The five-year follow-up was organized as a prospective study of growth and development during the first five years of life. All SGA children identified at birth were invited, as well as all non-SGA children from the 10% random sample (Figure 5). Hence, this part of the study can be characterized as a case-cohort study (149), in that cases (SGA children) and non-cases (non-SGA children) came from the same parent cohort, and were identified at time t_1(at birth), after baseline (prior to gestational week 20). Also, non-cases

were randomly selected from the parent cohort (10% random sample), and the cohort members had been assessed for risk factors prior to t_1.

3.2 Analysis cohort

This thesis and papers herein are based on secondary analyses of primary data collected from the Scandinavian SGA study. Figure 5 is a graphic representation of the analysis cohort used in Papers I-III.

The analysis cohort for *Paper I-III* is based on the Norwegian and Swedish mother-child pairs that were both followed through pregnancy and attended the five-year follow-up study (n=538). In total, 79% of the subjects (n=424) had sufficient serum volume from second trimester for analysis, and were considered for *Paper I and II*. Of these, 97% (n=412) had one or more anthropometric measurement registered at five-year follow-up, and were thus considered for *Paper III.*

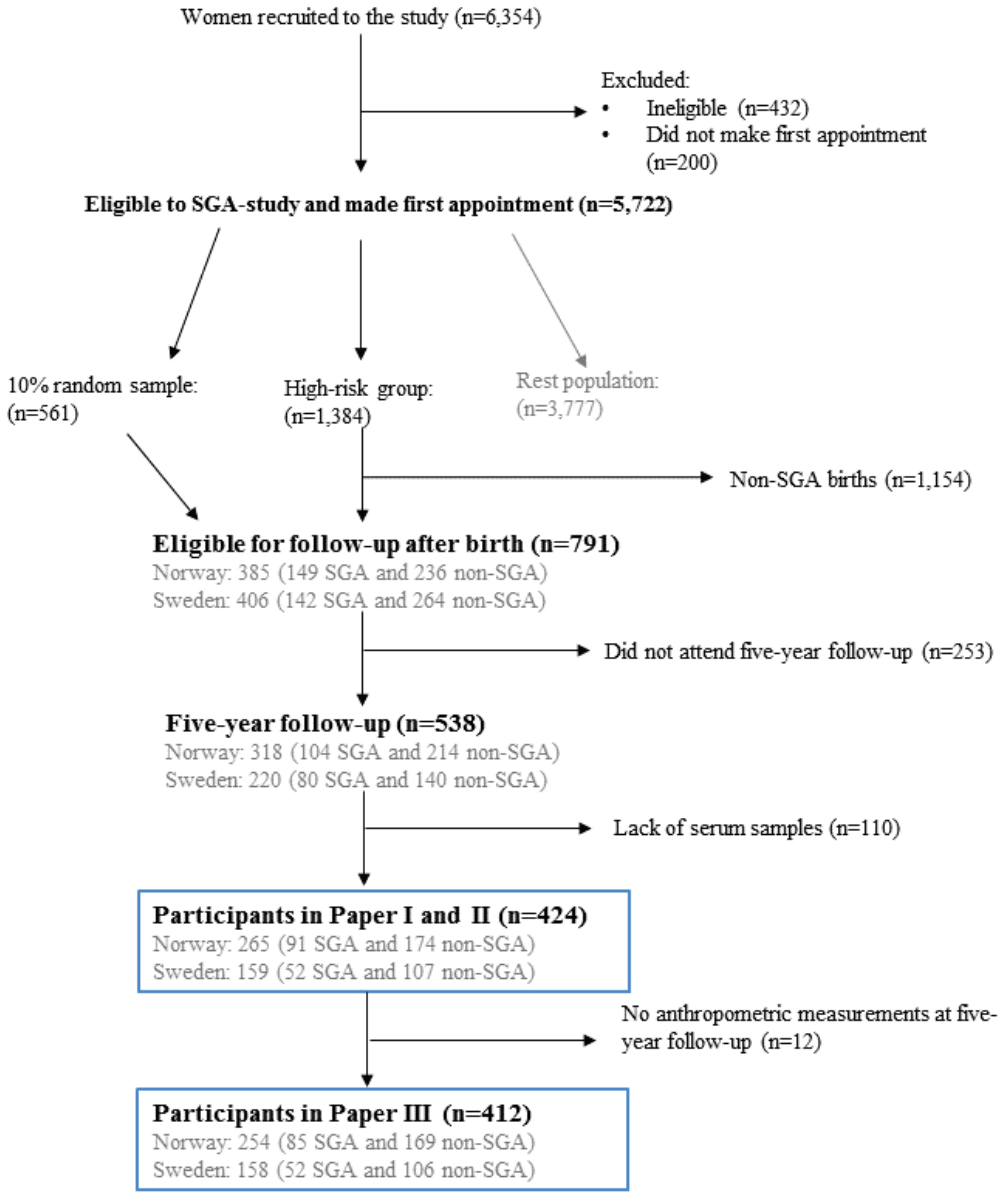

Figure 5 Flow chart illustrating the selection of participants in Paper I, II and III

3.3 Study variables

3.3.1 Serum POPs measurements

Maternal serum samples were collected during second trimester around gestational week 17 (range 13-20). They were stored at minus 80 °C until analysis. Wet weight concentrations of PFASs and lipid-adjusted serum concentrations of OCs were used in the analyses (150). Total lipid values were calculated based on measurements of triglycerides and cholesterol: total lipids = 1.33*triglycerides + 1.12*cholesterol + 1.48 (g/l). This formula showed good correlation with complete formulas including phospholipids (151).

3.3.1.1 Analyses of PFASs

Analyses were conducted for *Paper I, II and III* at the laboratories of Norwegian Institute for Air Research, Tromsø, Norway (NILU). In short, samples were extracted using sonication facilitated liquid-liquid extraction and activated ENVI-carb was used for clean-up according to Hanssen et al. (58). This method is based on the method developed by Powley (152). Samples were quantified and analysed by ultrahigh pressure liquid chromatography triple-quadrupole mass-spectrometry (UHPLC-MS/MS). PFASs measured were perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS).

The quantification was conducted with the LC Quan software, version 2.6.0 (Thermo Fisher Scientific Inc, Waltham, MA, USA) and the internal-standard addition method with isotope-labelled PFASs (58). Concentrations of PFASs in all samples were within the linear range of the instrument and the calibration curve. In the mass spectrometry analyses, a second mass transition served to confirm compound specificity for each compound. The quality of the analysis was verified by repetitive analysis of blank samples and reference samples (SRM 1957, NIS, Gaithersburg, MD, USA). Participation in the AMAP ring test program and results

from SRM material indicate a coefficient of variation (CV) of 15% for PFOA and 10% for PFOS. The linear PFOS isomers were chromatographically separated from the branched isomers and quantified separately. All PFOS results discussed are the sum of linear and branched isomers.

3.3.1.2 Analyses of OCs

OCs were analysed using the POPs Method E-446 (ISO 1 7025 accreditation) at the Institut National de Santé Publique du Quebec, Centre Toxicologie, Quebec. This laboratory is the organiser of the AMAP ring program. In short, 0.5–1 ml serum sample was extracted using hexane (2x6 ml), ethanol (2 ml) and saturated ammonium sulphate solution (2 ml). This method is a slight modification of the one described by Sandanger et al. (153), where the samples were cleaned up using 1 g of activated fluorisil on an automated Liquid handler system before GC-MS analysis described by Sandanger et al. (154). Results from the AMAP ring test program and SRM material indicate a CV of 5–10% for the OCs analysed. OCs measured were hexachlorobenzene (HCB), oxychlordane, PCB 52, PCB 101, PCB 118, PCB 153, PCB 156, PCB 170, PCB 180, *p,p'*-dichlorodiphenyldichloroehylene (*p,p'*-DDE), *p,p'*-dichlorophenyltrichloroethane (*p,p'*-DDT), *beta*-hexachlorohexane (*β*-HCH) and *trans*-nonachlor (*t*-NC). Only OCs detected in more than 99% of the samples were used in the regression analyses. PCBs were highly correlated; hence, we chose to report PCB 118, representing dioxin-like PCBs and PCB 153, representing non-dioxin like PCBs.

3.3.2 Maternal study variables

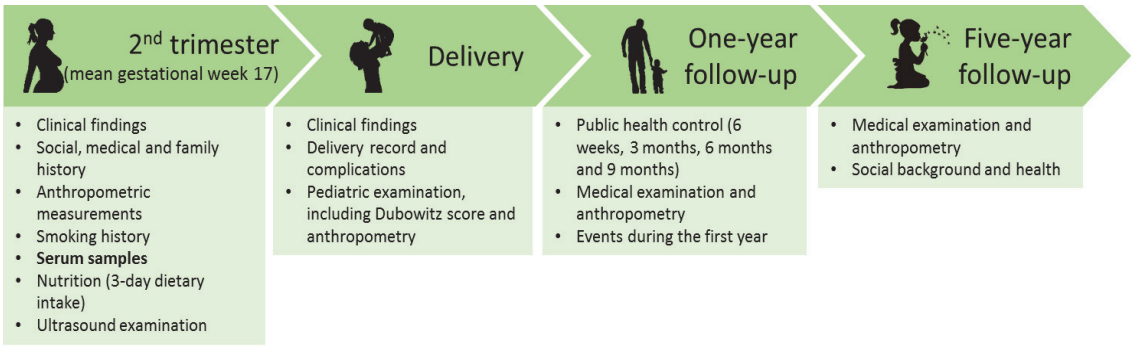

Figure 6 Flow chart for the events in the SGA Births study. Made by the author.

At study enrolment, the woman's social, medical and family history, diet and smoking habits were recorded (Figure 6). Maternal height and weight were measured by a clinician and serum samples were collected at the first study visit and stored according to standard procedures (80). We calculated maternal weight gain up to 17 weeks of gestation as the difference between self-reported pre-pregnancy weight and clinically recorded weight closest to gestational week 17 (done by the woman's own midwife or GP).

Serum sample date was based on the calendar date for enrolment, and was calculated as the number of days from January 1986 until the first visit for the individual woman. Lifestyle habits and pregnancy events were further recorded by either self-reported questionnaires or by study midwives in gestational week 25, 33 and 37.

3.3.2.1 Socio-demographic variables

Age at time of enrolment was included as continuous variables in *Paper I-III*. Maternal education was self-reported at first study visit (second trimester). In *Paper I*, the highest level of completed education was categorized in five ordinal categories (1: <9 years, 2: 9-11 years, 3: 12 years, 4: higher education, non-university level and 5: higher education,

university level. In *Paper II and III*, education was stratified into primary school (≥9 years), middle school (10-12 years) and high school (≥13 years).

3.3.2.2 Lifestyle variables

We classified smokers based on reported smoking habits at conception. As self-report is an inaccurate method of identifying smokers affected by the stigma associated with smoking during pregnancy (155, 156), we hypothesized that under-reporting might be less problematic at conception than later in pregnancy. In *Paper I*, women were dichotomized as either smokers or non-smokers based on self-reports on smoking at conception. In *Paper II and III* smoking habits were categorized in 0, 1-9 and ≥10 cigarettes a day. As a sensitivity analysis regarding the relationship between cigarette smoking and maternal serum concentrations of POPs, we used serum cotinine levels from second trimester in a randomly selected sub-sample of n=88 women. Alcohol consumption during pregnancy was reported in gestational week 33, and used as an ordinal variable with five categories (never, <once a month, once a month, 2-3 times a month and >once a week) in *Paper I*.

At gestational week 17-20, three days of dietary records were collected among the Norwegian women (157). Data was collected during the same three weekdays (Tuesday, Wednesday and Thursday). The amounts of food consumed were given in household measures, supplemented by food models presented as booklet with full scale drawings. Internal validity was tested against a food frequency questionnaire in a comparable group of non-pregnant Norwegian women. Maternal fish consumption was calculated as gram consumed of lean and fatty fish, shellfish and fish spread, and categorized as 0, 1-50 and >50 grams per day. We used fish consumption in sensitivity analyses in *Paper II and III*.

3.3.2.3 Pregnancy-related variables

Parity was categorized as 1 or 2, according to the number of children prior to this pregnancy. Previous breastfeeding duration was included as a continuous variable (in months), and was based on self-reported duration of both exclusive and partial breastfeeding of children born prior to study enrolment. Parity and previous breastfeeding duration was correlated (ρ=0.44), thus we only included one of these variables in the multivariate analyses. In *Paper II,* we used parity and in *Paper III*, we used previous breastfeeding duration. Inter-pregnancy interval refers to the number of months between the birth of the woman's latest child and the start of the current pregnancy, and was categorized in <1.5, 1.5-5 and >5 years, based on a known J-shaped association with adverse perinatal outcomes including restricted foetal growth (158). As a marker of pregnancy physiology, we measured serum creatinine in serum samples from second trimester in a sub-sample of n=88 women. Based on this, we calculated glomerular filtration rate (GFR) using the Cockroft-Gault (GFR-CG) formula: (140-age) x weight (g) x 1.04/serum creatinine (µmol/L).

3.3.3 Offspring study variables

At birth, the following offspring characteristics among others were recorded: sex, birth weight and length, head circumference and length of gestation. Gestational age (completed weeks) and expected date of delivery was estimated from ultrasound measurements of biparietal diameter (BPD) at 17 weeks of gestation. By design, SGA was defined as birth weight below 10^{th} percentile for a given gestational age, adjusted for offspring sex and parity.

After birth, medical history, nutrition, social and demographic factors, physical and neurological development were assessed regularly up to one year of age. At five-year follow-up, another thorough examination of the child was completed, including anthropometry

(Figure 6). Standing weight was recorded to the nearest 100 grams. Standing height was measured according to standard procedures and recorded to the nearest 0.1 cm (159). Age (in months) and sex-specific BMI z-scores, and BMI percentiles were based on the 2006 World Health Organization (WHO) child growth standards for children 5 years or younger (160), and the 2007 WHO growth standards for children and adolescents aged 5 to 19 years (161). Child BMI z-scores were analysed as a continuous outcome at 5 years, and in a category of overweight (BMI>85th percentile for age and sex) at 5 years, compared to BMI below the 85th percentile (162). Skinfold thickness was measured once using a Harpenden calliper (John Bull, British Indicators Ltd.) to the nearest 0.10 millimetre and 60 seconds after release of the grip to allow full tension placed on the compressed skinfold. Subscapular skinfold thickness was measured below the inferior angle of the left scapula, and triceps skinfold thickness was measured over the triceps in the middle of the left upper arm (159, 163). Age-and-sex-specific z-scores for triceps and subscapular skinfolds were calculated according to the Centre for Disease Control and Prevention (CDC) 2000 Growth Charts for children from 1.5 to 20 years of age (164).

3.4 Statistical analyses

All statistical analyses were conducted with SPSS statistic software version 22-24 (IBM SPSS Inc. Chicago, IL, USA).

In all papers in this thesis, normally distributed variables were presented as means, with 95% CI, standard deviation (sd) or range. Non-normally distributed variables were presented as medians, with corresponding 5th- to 95th-percentiles or range. Frequencies were presented as the number of observations and its proportion of total. The distribution of PFAS

and OC levels closely followed a log-normal distribution. Hence, serum PFAS and OC concentrations were transformed accordingly in all the analyses.

3.4.1 Paper I

This paper examined maternal second trimester serum concentrations of PFASs (PFOA and PFOS) and OCs (PCB 118, PCB 153, *p,p'*-DDE, *t*-NC, HCB and *β*-HCH), and examined maternal factors associated with the observed levels. We used multivariable linear regression to estimate associations between maternal factors and serum PFAS and OC levels, and reported adjusted estimates and 95% confidence intervals (CIs). Covariates included were chosen *a priori* based on previous literature and/or known toxicokinetic properties of the compounds. The multivariable models included serum sampling date, country of residence (Norway vs. Sweden), maternal age, education level, maternal height and pre-pregnancy body mass index (BMI), smoking status at conception, alcohol consumption during pregnancy, parity, previous breastfeeding duration and time since end of last breastfeeding period. We evaluated linear model assumptions using diagnostic plots of the residuals and checked the covariates for multicollinearity by variance inflation factors (VIFs). We calculated percent change in PFAS and OC levels for each independent variable by exponentiating regression coefficients, subtracting 1 and multiplying by 100.

In post hoc sub-analyses, we investigated potential dose-response relationships between cigarette smoking intensity (number of cigarettes smoked at conception and in early second trimester) and levels of PFOS, PCB 118 and *β*-HCH. As a sensitivity analysis, we used serum cotinine levels measured from samples collected at first study visit available from a randomly selected sub-sample of n = 88 women to further examine the association between

smoking and PFOS, PCB 118 and *β*-HCH levels. In the same n=88 serum samples, we examined if maternal PFAS levels were associated with glomerular filtration rate (GFR).

3.4.2 Paper II

Here, we examined the associations between maternal serum concentrations of PFASs (PFOA and PFOS) and OCs (PCB 153, *p,p'*-DDE, HCB, *t*-NC and *β*-HCH), and indices of foetal growth. We also studied possible interaction by country of residence and offspring sex based on knowledge from previous literature and findings of different serum concentration ranges between the Norwegian and Swedish mothers. Based on significant two-way interaction terms (POPs*country) on foetal growth indices as characterized by p-values <0.10, we performed the analyses separately on Norwegian (n=265) and Swedish (n=159) women. Within country-specific strata, we further considered possible effect modification by offspring sex. Group means were compared by independent t tests, medians by Wilcoxon-Mann-Whitney tests, and frequencies by chi-squared statistics. We used multivariable linear and logistic regression models to estimate the associations between PFASs and OCs and indices of foetal growth including birth weight and length, head circumference, gestational age at birth and SGA birth. Adjusted regression coefficients (β), adjusted odds ratios (aOR) and 95% CIs were reported. Possible confounding factors were identified by construction of a directed acyclic graph (DAG) (Figure 7), and then adjusted for in the analyses. Multivariable regression models included maternal age, height and pre-pregnancy BMI, maternal education level, smoking at conception, parity, inter-pregnancy interval and offspring sex. SGA birth was defined as birthweight below the 10^{th} percentile adjusted for gestational age, parity and offspring sex; hence, we did not adjust for parity or offspring sex in the models with SGA birth as outcome.

We did several sensitivity analyses to assess the robustness of our results. First, we evaluated associations between lifestyle variables (smoking status and fish consumption) and maternal serum PFAS concentrations, and additionally adjusted for fish consumption in the analyses of the Norwegian subgroup. Second, we checked for correlations between the studied POPs, and performed analyses with additional adjustment for the other POPs to assess which associations that remained statistically significant. Third, we analysed all models using wet weight values of OCs with adjustment for total lipids as an independent variable. Fourth, we checked if the estimates changed when we included gestational weight gain and alcohol consumption during pregnancy as covariates in the models. Finally, we tested the generalizability of our results by restricting the analyses to the random sample (i.e. the population reference). We conducted complete case analysis as missing data was less than 10% for included covariates.

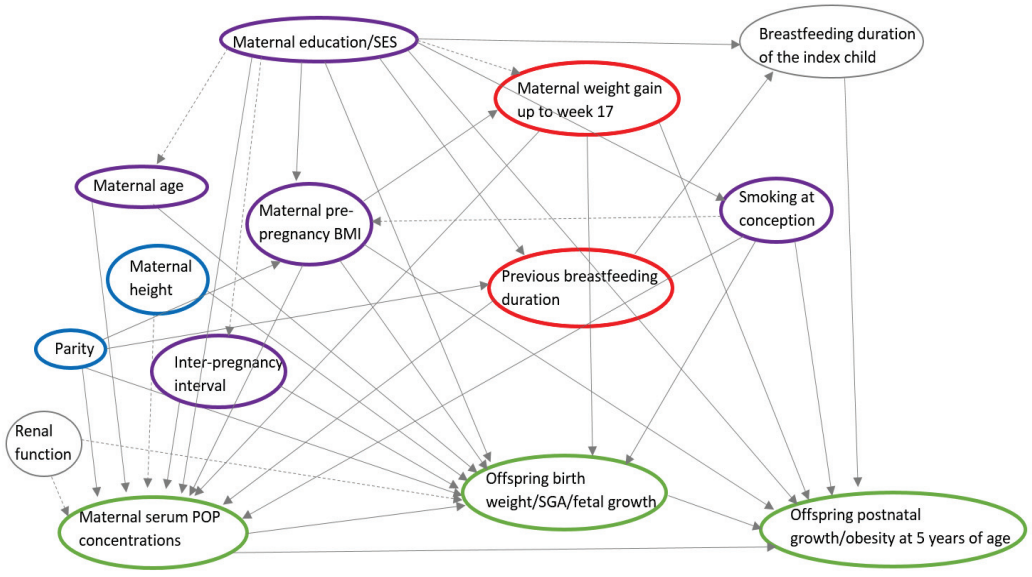

Figure 7 Directed acyclic graph (DAG) from *Paper II and III*. **Purple circles**: confounders included in both papers, **blue circles**: confounders included *in Paper II*, **red circles**: confounders included in *Paper III*, **green circles**: exposure, mediator and/or outcome in *Paper II and III*.

3.4.3 Paper III

In this paper, we used crude and adjusted linear regression models with 95% confidence intervals (CIs) to examine the association between ln-transformed maternal serum levels of two PFASs and five OCs and offspring sex-and-age-specific, first, BMI z-scores at five-year follow-up, and second, triceps and subscapular skinfolds at five-year follow-up. We used multivariable logistic regression to estimate adjusted odds ratios (aORs) and 95% CIs for the association between maternal serum POP concentrations and child overweight/obesity (BMI z-scores $\geq 85^{th}$ percentile for age and sex) at five-year follow-up. Possible confounding factors were identified by construction of a directed acyclic graph (DAG) (Figure 7), and then adjusted for in the analyses. The following variables were included in multivariable analyses as potential confounders: maternal age, pre-pregnancy BMI, education, smoking status at conception, previous breastfeeding duration, inter-pregnancy interval between the last two children, and maternal weight gain from conception up to gestational week 17. The pooled analyses were further adjusted by country. Prenatal growth was considered a mediator in the pathway between exposure to POPs and childhood overweight. As adjustment for a mediator may introduce bias if there are shared unmeasured causes of both SGA status and childhood overweight (165), we did not include prenatal growth or SGA status in the multivariate analyses. All models were tested for normality of residuals, heteroscedasticity, and multi-collinearity.

We examined linearity by scatter plots, assigning maternal serum POP concentrations to the horizontal axis, and measures of child adiposity to the vertical axis. Marginal relationship between maternal serum POP concentration and offspring BMI z-score at five-year follow-up was assessed by non-linear regression using 3-knot restricted cubic spline and 95% CI. We determined non-linear associations by examination of cubic spline graphs, and by the Wald test.

We had some missing data including 7.2% missing for maternal weight gain up to gestational week 17 and previous breastfeeding duration. Among children, we had 7.0% missing data on subscapular skinfold thickness and 6.1% missing data on triceps skinfold thickness. Overall, 80% of participants had complete data on all variables. Missing data were assumed missing at random. We used chained multiple imputation to generate and compare five complete data (166, 167). Complete case analyses widened the 95% CIs, but did not change the estimates substantially.

We evaluated possible effect modification by country and offspring sex based on *a priori* evidence from the literature (129, 168). We conducted several sensitivity analyses to assess the robustness of our results. First, we did stratum-weighted analyses to ensure generalizability of our reported estimates to the contemporary pregnant population as per the prevalence of i) SGA births, ii) maternal pre-pregnancy overweight, and iii) maternal smoking at conception. Such weighted analyses are recommended for analyses with case-control data or in other way unbalanced populations that may be subject to selection bias (169). Second, we additionally adjusted for maternal fish consumption during pregnancy among Norwegian participants. Finally, we considered a multi-pollutant model approach by mutually adjusting for maternal serum POPs that were found to be associated with offspring BMI.

3.5 Ethical considerations

All participants in the original Scandinavian SGA-study were informed about the study aims and objectives, and gave their written consent. Ethics Review Boards (ERB) within each study hospital approved the Scandinavian SGA study baseline protocol (1985). This doctoral thesis received ethics approval from The Regional Committee for Medical and Health Research Ethics (REK) in Mid Norway and utilized data and serum samples already

collected. All names and personal identifiers were removed from the data files. Nothing but anonymous case numbers were used to link questionnaire data and biological samples. Thus, the anonymity of all study participants was protected.

4. Main results

4.1 Maternal factors associated with serum PFAS and OC concentrations

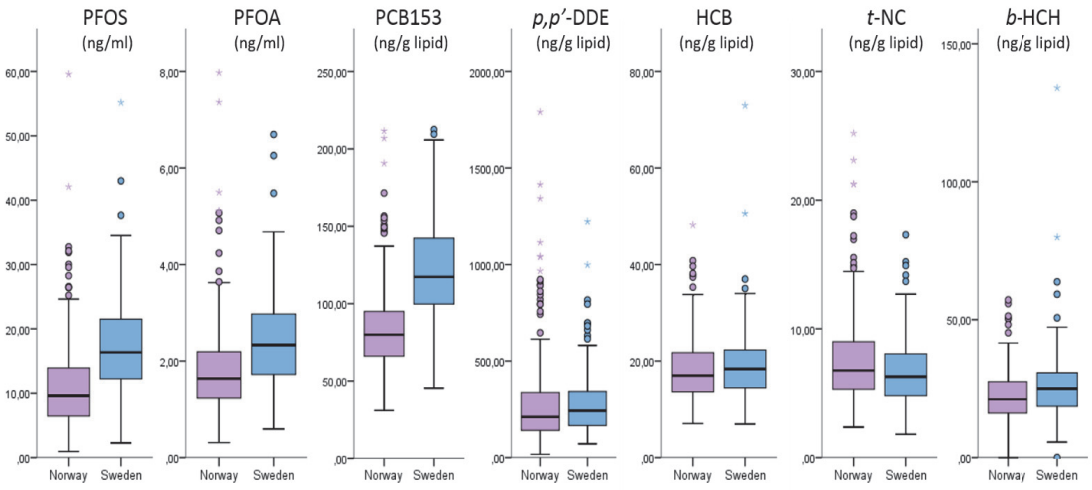

Figure 8 Maternal serum concentrations of PFASs (ng/ml) and OCs (ng/g lipid) by country of residence

Using data from the SGA Births Study, we assessed maternal serum concentrations of PFASs and OCs from second trimester and associated maternal demographic and pregnancy-related factors.

Overall, Swedish women had on average 39%, 67% and 43% higher serum PFOA, PFOS and PCB 153 concentrations compared to Norwegian women, respectively (Figure 8). All serum OC and PFAS concentrations decreased by 1-3% per month of breastfeeding prior to this pregnancy. Serum PFAS concentrations increased by 3-5% per year since last breastfeeding period, while serum OC concentrations increased by 2-5% per year increase in maternal age. Maternal smoking at conception, pre-pregnancy BMI, education level and alcohol consumption was associated with serum concentrations of some OCs and PFASs.

In a random sub-sample (n=88), women with low glomerular filtration rate (GFR) had higher serum PFOS concentrations. We also found evidence of a consistently negative association between maternal smoking intensity (measured both as number of cigarettes and

serum cotinine levels) and maternal serum concentrations of PCB 118 in a dose-dependent manner.

4.2 Maternal serum POP concentrations and indices of foetal growth

The objective of this study was to examine the associations between prenatal exposure to EDCs and indices of foetal growth.

Based on evidence of effect modification by country of residence, we stratified the results in Norwegian and Swedish women. Among Swedish mothers, we found an increase in adjusted odds for an SGA birth per ln-unit increase in second trimester concentrations of PFOA (aOR=5.25 (95% CI: 1.68-16.4)), PCB 153 (aOR=5.59 (95% CI: 1.26-25.1)) and HCB (aOR=5.62 (95% CI: 1.26-25.1). In adjusted analyses, birth weight decreased by 359 g (95% CI: -596, -122) and 292 g (95%CI: -500, -84) per ln-unit increase in prenatal PFOA and PFOS levels among Swedish offspring.

The negative associations between PFOA and indices of foetal growth were stronger among Swedish boys compared to Swedish girls, although a limited sample size gave imprecise estimates.

Among Norwegian mother-child pairs, we observed no statistically significant associations between levels of PFASs or OCs and indices of foetal growth.

4.3 Maternal serum POP concentrations and offspring obesity

The objective of this study was to examine the associations between maternal serum PFAS and OC concentrations and offspring overweight or obesity at five-year follow-up.

Based on evidence of effect modification by country of residence, we presented both total and country-specific estimates. Overall, there were positive associations between increasing serum PFOS concentration and child BMI and triceps skinfold z-score. Adjusted OR for child overweight/obesity was 2.04 (95% CI: 1.11-3.74) per ln-unit increase in PFOS concentration. The data also suggested positive associations between increasing concentration of PFOA and child BMI z-score, triceps skinfold z-score and greater odds for child overweight/obesity.

These associations tended to be stronger among Norwegian mother-child pairs. In the latter case, adjusted odds for overweight/obesity was 2.96 (95% CI: 1.42-6.15) per ln-unit increase in PFOS concentration and 2.90 (95% CI: 1.10-7.63) per ln-unit increase in PFOA concentration. We found no evidence of effect modification by offspring sex.

No significant linear associations were observed among Swedish participants. However, based on a restricted 3-knot cubic spline model, we found evidence of non-linear associations between maternal serum PFOS and PCB 153 levels and offspring BMI z-scores among the Swedish mother-child pairs. This suggested potential non-linear or non-monotonic dose-response (NMDR) relationships between these POPs and measures of overweight/obesity at five-year follow-up.

5. Discussion

Our main findings from *Papers I-III*, a discussion of strength and limitations, and an appraisal of the findings, including comparisons to other studies, will be addressed in the following sections.

5.1 Summary of main results

Our key findings are hereby summarized:

- ✓ Variation in maternal PFAS and OC concentrations was explained by previous breastfeeding duration, serum sample date, time since last breastfeeding period, maternal age, pre-pregnancy BMI, smoking status, education level and alcohol consumption. Median serum PFOA, PFOS and PCB 153 concentrations were higher among Swedish compared to Norwegian women.
- ✓ Increasing maternal serum concentrations of PFOA, PCB 153 and HCB were associated with indices of impaired foetal growth (including higher odds for SGA birth), but only among Swedish mother-child pairs. There were also some indications of stronger associations among Swedish male offspring.
- ✓ Maternal serum concentrations of PFOS and PFOA were positively associated with offspring BMI and triceps skinfold z-scores and higher odds for overweight/obesity at five-year follow-up, but only in Norwegian mother-child pairs. In the Swedish part, we found some evidence of non-linearity that implied that non-monotonic dose-response (NMDR) relationships might be involved.

5.2 Methodological considerations

In an epidemiologic study, the overall goal is to obtain a valid and precise estimate of the effect of an exposure on the incidence of a disease in the source population under study (170). Accuracy implies that the value of the measured parameter is estimated with little error. Errors can be either random or systematic, and systematic errors are commonly referred to as biases. An estimate with little systematic error may be described as valid, and the validity of a study is usually separated into two components: internal validity (the extent to which a causal conclusion based on a study is acceptable) and external validity (the extent to which it is acceptable to generalize results to other contexts).

5.2.1 Precision (random error)

Random error refers to uncertainty due to chance findings, random variation and inaccurate measurements, and will be reduced with increased sample size (170). Lack of random error implies high precision of a study. Precision is often presented as a span of confidence around a point estimate, usually a 95% confidence interval (CI). Increased sample size will reduce variance and improve precision of the estimates. Consequently, large studies have increased precision and narrow CIs, while small studies have reduced precision and wide CIs. All three papers used 95% CIs to describe precision.

In *Papers I-III*, we had a relatively large sample of mother-child pairs with thorough evaluations through pregnancy, offspring examinations at birth and at several time points, up to five years of age. Due to recruitment of pregnancies with greater risk of SGA birth, we had a relatively substantial number of pregnancies with restricted foetal growth that resulted in SGA births. However, some sub-group analyses in Paper II and III had fewer within stratum

observations and wider CIs that indicated reduced precision. Hence, these estimates should be interpreted with caution.

5.2.2 Internal validity (systematic error)

Systematic error consists of diverse types of bias present in epidemiologic studies, and internal validity may be violated through three main categories of biases: selection bias, information bias and confounding. DAGs are graphical tools that illustrate the presumed causal associations between different variables, and may be useful to identify sources of systematic error (171).

5.2.2.1 Selection bias

Selection bias arises when the association between exposure and disease is different for those who participated and for all those who should have been eligible for study, including those who did not participate (170). The Scandinavian SGA Births Study was a prospective cohort study with pre-defined eligibility criteria for all study participants. Failure to include eligible subjects and loss to follow-up will be discussed in this section. Whether the eligibility criteria created a representative sample of the general population of pregnant women or not will be discussed in section “External validity”.

Only 200 (3%) of the 5,922 eligible women recruited to the study, failed to make a first appointment in the SGA Births Study. Approximately 50% of these failed to come to the first visit due to social constraints (e.g. “too time consuming”), whereas the rest failed due to unknown reasons (80).

In all three papers, the main reasons for exclusion were i) lack of follow-up throughout pregnancy, ii) lack of available stored serum samples from second trimester and iii) no attendance at five-year follow-up. By study design, a total of 791 mother-child pairs were closely followed up during pregnancy and were eligible for the five-year follow-up study. They included all participants in the 10% random sample, as well as SGA offspring from the High-risk group (Figure 5). Hence, any selection bias may arise from loss to follow up at five years (n=253), or unavailable serum samples (n=110).

Regarding loss to follow-up at five years, there was considerable difference in attendance rate between Norway (83%) and Sweden (54%). The main reasons for not attending was social inconvenience, lack of continued interest from the parents and/or the child, as well as moving to other areas during the period. Uppsala county is a much larger geographic area (6,989 km^2) compared to the city of Bergen (465 km^2) and Trondheim (341 km^2), and long travel distance was reported by the women to be the most important reason for the lower attendance rate in Sweden (80). If there are systematic reasons as to why participants were lost to follow-up, bias may have been introduced.

We compared maternal and new-born characteristics between the followed (n=424) and the non-followed (n=367) participants among the eligible mother-child pairs (n=791). Among Norwegian participants there were no notable differences between the two groups (data not shown). Among Swedish participants, the followed group (n=159) had fewer smokers (34% vs. 49%) and higher mean offspring birth weight (3,515g vs. 3,284g), but were otherwise like the non-followed group (n=247).

A great number of missing data in cohort studies may introduce bias as well as loss of statistical power, and missing data from survey non-response is another type of selection bias. As missing data was less than 10% for included covariates in Paper II, we conducted complete case analysis. In Paper III, 80% of participants had complete data on all variables,

while there were around 6-7% missing data each for these variables; maternal weight gain, previous breastfeeding duration, offspring subscapular and triceps skinfold thickness. To avoid loss of power we therefore used chained multiple imputation to generate and compare five complete data (166, 167). In a sensitivity analysis, we did complete case analysis that lead to widening of the 95% CIs, but it did not change the estimates substantially.

5.2.2.2 Information bias

Information bias arises if there is systematic error in the information collected about the study participants that results in an incorrect estimate of the association between exposure and outcome (170). Exposures and outcomes are often structured in categories, and *misclassification bias* occurs when the classification is wrong (170). In epidemiologic studies, there are two types of misclassification, namely *differential and non-differential misclassification*. If the probability of being misclassified differs across groups of study subject resulting in a different error rate in each comparable group, the misclassification is *differential.* In contrast, if all group subjects or categories of a variable have equal probability of being misclassified, the misclassification is *non-differential*. The effect estimate tends to attenuate in non-differential misclassification, while differential misclassification may bias the results in either direction. Differential misclassification is of special concern.

The potential association between an exposure and an outcome is dependent on the quality of measurements, when the variables are of measurable scale. To ensure precision and accuracy of serum POP concentrations, the analytical protocols included systematic quality assurance-quality control (QA-QC) procedures (Section “POPs analyses”). Also, as all analyses are done at the same time there is no risk of *differential* misclassification. Moreover, potential systematic errors in the measurements cannot alter the association, only the absolute

concentration when comparing to other studies. The serum samples chosen were from a narrow time frame (gestational week 17-20) to minimize potential bias related to changes in plasma volume and lipids during pregnancy (172). However, maternal serum levels of POPs were used as a proxy for prenatal exposure to POPs, and this may have caused bias. Still, assessment of POPs in serum is considered a good representation of circulating levels of POPs (173, 174), and maternal serum concentrations of POPs are highly correlated with offspring cord serum concentration of POPs due to placental transfer (175, 176). Hence, maternal serum levels of POPs are considered a good representation of prenatal exposure to these contaminants.

In *Paper III,* skinfold thickness z-score was used as a measure of subcutaneous fat, which is highly correlated with total amount of fat (148, 177). Skinfold measurements of the offspring may be prone to intra- and inter-observer errors (163), but it is unlikely that measurement precision was correlated with the maternal serum concentrations of POPs. In a sub-analysis in *Paper I*, associations between glomerular filtration rate and maternal serum PFAS concentrations were assessed. An indirect method was used to estimate glomerular filtration rate (GFR), based on a single-point measurement of serum creatinine, and this may introduce bias. GFR increases up to 60% in pregnancy, because renal function drastically changes during pregnancy with hyper filtration, systemic vasodilatation and plasma volume expansion (178). The gold standard is a direct method called inulin clearance (178). However, studies have shown that the difference between direct and indirect methods stayed the same with increasing GFR (178).

Some variables may have been misclassified due to self-report. Smoking status may be underreported, because of social stigma. However, measurements of cotinine levels in second trimester in a randomly selected sub-sample (n=88) were highly correlated with smoking status at conception, and was used as a sensitivity analyses in *Paper I*. Regarding previous

breastfeeding duration, recall bias is unlikely, as a study from this material showed that it was recalled quite accurately even 20 years after mothers gave birth (179). By using 3-day dietary intake as a measure of the overall fish consumption, it may be difficult to capture the "real" fish eaters, because seasonal or daily variations in dietary intake of fish may introduce non-differential misclassification.

5.2.2.3 Confounding

Confounding is an important concept in epidemiology, and means "mixing of effects" (170). There is confounding when we observe an association between an exposure and an outcome that really is due to an external factor (i.e. "confounder"). One example is the apparent association between birth order and prevalence of Down's syndrome, which is confounded by maternal age (170). Observational studies are highly prone to confounding because the compared exposure groups are not constructed at random. The confounder must be associated with both the exposure and the outcome, must not be along the causal pathway between the exposure and outcome, and must not be a consequence of the outcome (170). Identification of a confounder must be founded on expert and/or *a priori* knowledge, rather than statistical associations (170). There is a risk of either over-adjustment or missing important confounders if potential confounders are not critically evaluated (165).

The Scandinavian SGA Births Study consist of a large amount of data from each study participant collected throughout pregnancy, birth, infancy and childhood. This enabled us to control for a wide range of possible confounding factors in the regression models. Literature on maternal serum concentrations of POPs and potential health effects, like foetal growth restriction or obesity, is limited or inconsistent. In addition, there is great diversity between the studies regarding which variables they included as confounders. In *Paper I*, we lacked full

information on previous exclusive and partial breastfeeding duration. Therefore, the association between increased previous total breastfeeding duration and decreased POP concentrations may be subject to residual confounding. Nevertheless, after the *current* pregnancy, exclusive breastfeeding duration was highly correlated with total breastfeeding duration, and total breastfeeding duration was correlated in consecutive pregnancies (data not shown), making potential residual bias minimal.

In *Paper I*, we found a negative linear relationship between maternal GFR and maternal serum PFOS levels from a sub-sample (n=88). Because GFR describes the flow rate of the filtered fluid through the kidneys, and PFASs bind to albumin and reside in blood and kidneys, we postulated that higher GFR leads to more excretion of PFASs and thereby lower serum levels of PFASs. Consequently, GFR might be considered a potential confounder in epidemiologic studies of PFASs and foetal growth, because there have been indicated a possible association between low GFR and small size at birth (180). Unfortunately, we only had serum creatinine levels from the same sub-sample (n=88), and thus were unable to control for GFR in the main analyses. However, results from simulation models suggest that GFR drives only a portion of the association between PFASs and birth weight, but not all of it, and that its influence becomes more important with increasing gestational age (181).

Maternal weight gain during pregnancy, which may correspond both to lipid fat gain and/or plasma volume expansion, is considered a potential confounder in epidemiologic studies between POPs and foetal growth. However, studies suggest that GFR has less impact when using maternal serum from early pregnancy, compared to late in pregnancy or cord blood at birth (182). As increased alcohol consumption has been associated with higher levels of OCs (183) and has been related to adverse birth outcomes (184), it appears to be a potential confounder. However, sensitivity analyses revealed no change in estimates when we included maternal weight gain or alcohol consumption in the multivariate analyses in *Paper II*.

Finally, as persistent and bio-accumulative chemicals have comparable properties and thus, are highly correlated, we cannot dismiss residual confounding due to correlated unmeasured chemicals (e.g. lead, dioxins, mercury) in our analyses.

5.2.3 External validity (generalizability)

After considering the validity of the inferences as they relate to members of the source population, we now have to consider the validity of the inferences as they pertain to people outside the source population (170).

The population in the Scandinavian SGA Births Study is different from the general population in several aspects. First, as the initial intention was to examine the tendency to repeat an SGA offspring in consecutive pregnancies, only parous women were recruited. To increase the number of events of the main outcome of interest (SGA birth), the longitudinal study was enriched with women at higher risk of giving birth to an SGA child. Moreover, only pregnancies in Norway and Sweden were assessed, which may reduce the generalizability to other populations.

To ensure that the results in all three papers were not biased by the enrichment of SGA births, we 1) restricted the analyses to the 10% random sample (i.e. the population reference) and/or 2) performed stratum-weighted analyses with inverse probability weighting, where weights were the inverse probability of selection, according to the prevalence of SGA births, maternal smoking and maternal pre-pregnancy overweight (169), and the results did not change considerably. This further confirms the robustness of our results. However, the generalizability to other pregnant populations is dependent on variance in maternal serum concentrations of POPs and systematic lifestyle differences related to important exposure sources (e.g. fish vs. meat consumption, or region-specific contamination of drinking water).

5.3 Comparison with other studies and interpretation of findings

5.3.1 Serum concentrations of POPs

Overall, serum concentrations of POPs in the SGA Births Study population from the late 1980s can be considered low and reflect a background exposed population. When comparing our concentration ranges to other studies, it is important to consider that we only included second and third time mothers, and thus, expected overall lower median serum concentrations of POPs due to elimination through placenta and breast milk in earlier pregnancies. Human biomonitoring studies have reported an increase in serum PFAS concentrations from the 1960s until around 2000, and a subsequent decline thereafter (41, 43, 44). Consistent with this, maternal serum PFAS concentrations in our study (from 1986-1988) were lower compared to concentrations among women from Scandinavian countries from 1988 to early 2000s (43, 185-188). Moreover, we reported higher serum PFAS concentrations than comparable populations after late 2000s (47, 189). Serum OC concentrations were comparable to those reported in other pregnant populations when considering parity, fish intake and the year of sampling (185, 190-194). Also our finding of increase in serum PFAS concentrations and decrease in serum OC concentrations from start of enrolment (January 1986) to end of enrolment (March 1988) compare with these human biomonitoring studies and trends of historic production, restrictions and bans of use (195). In the coming years, POP exposures to humans are expected to decrease or level off, based on the knowledge of trends for emissions and concentrations in air and biota. However, there are large differences between continents where continued production of PFASs and continued use of some OCs contributes to high exposure in some populations (4-6) .

5.3.2 Geographical (country) differences in serum POP concentrations

Our findings of higher maternal serum concentrations of PCB 153, PFOS and PFOA may represent different exposure scenarios. Uppsala county in Sweden is situated close to the border of the Baltic Sea, whereas Bergen and Trondheim are situated along the west coast of Norway towards the North Sea (Figure 9). The Baltic Sea is a large brackish water body with an area of 415 266 km^2 and a volume of 21 721 km^3 (196). Its catchment area consists of nine countries that border the Baltic Sea (i.e. Sweden, Finland, Russia, Estonia, Latvia, Lithuania, Poland, Germany and Denmark), and it is only connected with the North Sea via a narrow and shallow opening that limits the water exchange and a water residence time between 20 and 30 years (196). This makes the Baltic Sea ecosystems vastly sensitive to human impacts and climate change, and may be considered a long-term reservoir for many POPs (197). The Baltic Sea ecosystem has since the 1950s been deteriorated by pollution from urban and industrial waste waters, air emissions and agricultural run-offs. Consequently, the concentrations of industrial by-products, such as PCBs and many agricultural pesticides have been generally higher in Baltic fish species than fish species from other sea areas (198). Studies have shown that consumption of fish from the Baltic Sea accounts for most of the exposure to POPs, due to the high concentrations of these compounds especially in fatty fish (199-201). Our findings of higher serum PCB 153 concentrations among the Swedish participants are also consistent with other cross-sectional studies (53, 202, 203). Regarding PFASs, there are no known PFAS manufacturing industries within the Baltic Sea catchment area, but there are industries that use PFAS formulations for various industrial applications (204). Dietary intake is also an important exposure source for PFASs (205), and particularly consumption of seafood in Scandinavian countries (206, 207). For instance, in a sub-analysis we found that Norwegian women with a fish consumption > 50 g/d in the second trimester had 20% and 19% higher PFOA and PFOS levels, respectively, compared to women who did

not eat fish (*Paper II*). Also, other exposure sources, like drinking water and household dust, may contribute to variability in serum concentrations between Norwegian and Swedish mothers, especially in periods, like ours, with continued use and emissions (205, 208).

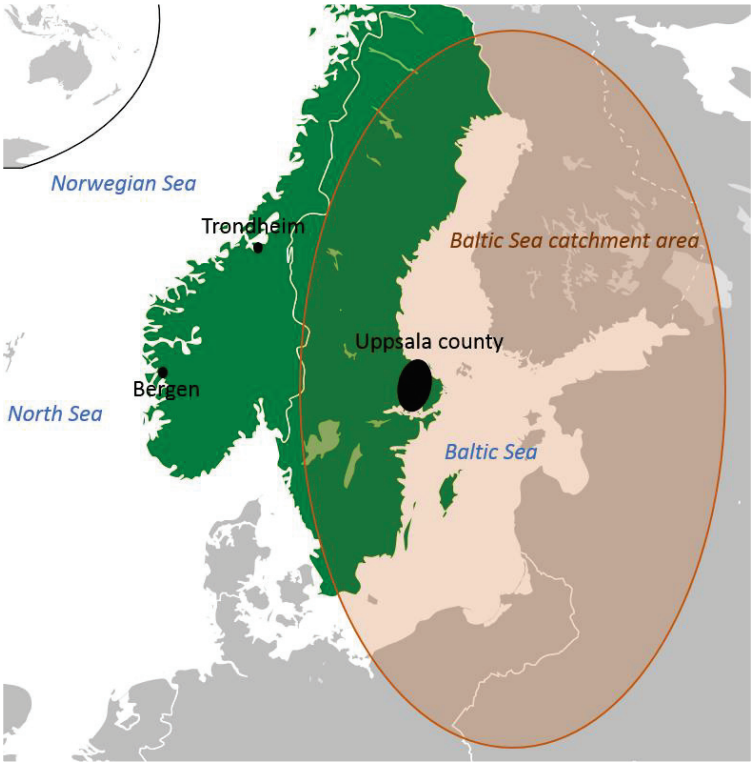

Figure 9 Baltic Sea catchment area. Reproduced and changed with permission from Wikimedia Commons.

5.3.3 Maternal factors associated with serum POP concentrations

Our strong associations between longer duration of previous breastfeeding and decrease in maternal serum concentrations of PFASs and OCs are consistent with other studies (46, 49, 50). This is explained by excretion of POPs in breastmilk which thereby lower maternal serum concentrations (209-211). This implies a variation in exposure to children who are breastfed (212), although a recent review has concluded that the benefits of breastfeeding probably outweigh the potential adverse effects (213). Elimination through

breast milk is assumed to be greater than the prenatal transfer through the placenta due to the placenta barrier (209). Our findings of null associations between parity and serum PFAS concentrations are consistent with this. The positive association between time since last breastfeeding period and maternal serum PFAS concentration is consistent with another Norwegian cross-sectional study (46), and possibly reflect bioaccumulation during periods without elimination through breastfeeding. Thus, this variable might confound associations in health effect studies as both short and long intervals between two back-to-back pregnancies are associated with adverse perinatal outcomes (158).

Like us, several other cross-sectional studies have reported positive associations between increasing maternal age and serum OC concentrations (53, 214). Longitudinal studies have shown that this association probably is caused by some cohort effect where older participants have lived during periods with higher exposure to OCs compared to younger participants (38).

High pre-pregnancy BMI was associated with low maternal serum PCB 153 and t-NC concentrations, which has been explained by dilution effects of OCs into adipose tissues (215). Still, other cross-sectional studies have observed both positive and negative associations (216). Possible explanations for diverse results include prolonged half-lives of OCs among obese people (217), increased serum concentrations after weight loss and decreased serum concentrations after weight gain (218, 219).

The inverse associations between maternal smoking status and maternal serum concentrations of PCB 118 support the hypothesis that cigarette smoking enhances the metabolism of PCB 118 through an induction of CYP-enzymes (29). This was further confirmed in dose-response sub-analyses with number of cigarettes and serum cotinine in second trimester. Maternal smoking was also negatively related to maternal serum PFOS concentrations, but the non-linear relationship between serum cotinine and serum PFOS

concentrations dispute a causal effect of cigarette smoking on elimination of PFOS. The relationship may rather be explained by differences in lifestyle patterns between smokers and non-smokers.

The inverse association between maternal GFR and maternal serum PFOS concentration in our previously mentioned sub-sample (n=88) is consistent with one previous study (48). It is hypothesized that higher GFR leads to a higher excretion rate and, thereby, a lower serum concentrations of PFOS. As one study reported an association between low GFR and low birth weight (180), GFR may be considered a potential confounder in health studies with foetal growth as outcome variable. Although some epidemiologic studies of associations between prenatal exposure to PFASs and birth weight have reported large attenuation with adjustment for maternal GFR (181), others have not revealed attenuation of the estimates (108, 220).

5.3.4 Maternal serum POP concentrations and indices of foetal growth

We reported associations between increasing maternal serum concentrations of PFOA, PFOS, PCB 153 and HCB and indices of impaired foetal growth (including SGA). However, in stratified analyses by country, we only found such associations among Swedish and not Norwegian participants. We also found some evidence for a stronger effect among Swedish male than female infants. Two systematic reviews (102, 103) reported "sufficient" human evidence of reduced foetal growth when exposed prenatally to PFOA. However, the evidence for PFOS was less consistent (103). One meta-analysis concluded that low-level exposure to PCBs impairs foetal growth (109), and some studies reported on inverse associations between prenatal exposure to HCB and birth weight (114). However, a systematic analysis of published epidemiological studies that assessed the relationship between prenatal exposure to

PCBs and birth weight found no association (221). Also, several studies assessing the relationship between prenatal exposure to HCB and foetal growth did not found any significant associations (118-123) or associations only in certain sub-groups like smoking mothers (115), only among male (116) or female (117) infants.

Our inverse associations between exposure to PFOA and foetal growth only among male infants may stem from different susceptibility to developmental disruptions between the sexes. It has long been known that foetal sex influence pregnancy outcomes. Thus, male sex is an independent risk factor for adverse pregnancy outcomes (222). Even an increased risk of maternal disease, such as gestational diabetes mellitus, is higher when the mother carries a male foetus (222). The placenta is one of the main drivers for adaptation of maternal metabolism to pregnancy, and may thus be involved in sex-dependent aspects of pregnancy disorders. Based on molecular and functional differences in placental tissues between the sexes, one may well hypothesize that male and female foetuses have different growth strategies (223). Male foetuses grow more rapidly potentially sacrificing placental growth for brain growth, thus leaving the foetus more vulnerable to undernourishment by the placenta (224). Sex-specific differences in DNA methylation profiles in the placenta are well studied in animal models, but human studies are limited (225). For these reasons, it seems fair to hypothesize that the associations between POPs and foetal growth is different depending on the offspring sex. However, any potential biological mechanism for the sex difference remains to be established. Moreover, our sex-specific findings differs from a Japanese study that found stronger associations between PFOS and birth weight among girls (226).

The different associations between exposures to POPs and foetal growth between the Norwegian and Swedish participants are consistent with a study covering the period from 1973 to 1991. The pregnant wives of fishermen from the East coast of Sweden were compared to those from the West coast. The authors found higher maternal serum

concentrations of PCB 153 and an increased number of infants with low birth weight (<3,000 g) among women from the East coast, whereas the opposite was the case for the West coast cohort (227, 228). The Norwegian Scientific Committee for Food Safety recently published a report that concluded on an overall beneficial effect of fish consumption on birth weight, as fatty acids and vitamins enhance foetal growth and outweigh potential negative effects from POPs (229). However, it seems important to stress that this report from 2015 was based on evidence from studies conducted when concentrations of POPs due to restrictions in use were much lower than our concentrations in 1986-88. The fact that high fish consumption is associated with high PFAS and OC concentrations and at the same time positively associated with foetal growth, may introduce negative confounding in epidemiological studies. This may lead to underestimation of the potential effects. We adjusted for fish consumption in the Norwegian cohort, but our estimates did not change (*Paper II*). However, when using a short time (3 days) dietary intake as a measure of the overall fish consumption, seasonal or daily variations in dietary intake of fish may introduce misclassification bias. Hence, we may not be able to capture the "real" fish eaters by this approach. In populations with overall lower fish intake in contrast to higher consumption of meat, negative confounding may be smaller, which make comparisons between populations difficult.

Heterogeneity in variables related to both maternal serum POP concentrations and foetal growth may give different results between our countries. Among Swedish women, smokers had overall 20% higher serum PFOA concentrations compared to non-smokers, whereas smokers in the Norwegian part had 19% lower serum PFOS concentrations than non-smokers. As smoking status is closely related to other socio-economic, dietary and lifestyle variables, we should expect the group of Swedish women with high PFAS concentrations to be vastly different from the group of Norwegian women with high PFAS concentrations.

We reported correlation coefficients as high as 0.73 between different POPs; thus, if a single compound is likely to cause a biological effect, relating this effect to a single compound may be difficult. There were moderate to high correlations within the PFASs (ρ=0.56-0.73) and within the OCs (ρ= 0.35-0.70), but only weak or no associations between the individual PFASs and OCs (ρ= -0.01-0.22) (Figure 10). The weak correlations between PFASs and OCs allowed for analyses with mutual adjustment for some other POPs. In single POP models, there was evidence of associations between PFOA, PCB 153 and HCB and SGA birth. However, after mutual adjustment, only the association between maternal serum PFOA concentration and SGA at birth remained statistically significant. This further supports that the association between PFOA and SGA birth is not explained by correlated exposures. However, high intercorrelations between HCB and PCB 153 (ρ=0.492-0.693) make it difficult to evaluate these individual effects on indices of foetal growth.

	Norway (N=265)							Sweden (N=159)						
	PCB 153	*p,p'*-DDE	HCB	*t*-NC	*β*-HCH	PFOA	PFOS	PCB 153	*p,p'*-DDE	HCB	*t*-NC	*β*-HCH	PFOA	PFOS
PCB 153		0.616	0.693	0.672	0.544	0.118	0.089		0.630	0.492	0.660	0.431	0.114	0.148
p,p'-DDE			0.522	0.361	0.473	0.217	0.067			0.351	0.456	0.374	0.042	-0.012
HCB				0.575	0.700	0.174	0.068				0.496	0.462	-0.013	0.078
t-NC					0.292	0.111	0.075					0.408	0.048	0.075
β-HCH						0.131	-0.021						0.157	0.159
PFOA							0.561							0.727
PFOS														

Figure 10 Correlation coefficients between maternal serum concentrations of PFASs and OCs by country of origin

5.3.5 Maternal serum POP concentrations and offspring obesity at five years of age

We have reported positive associations between maternal serum PFAS concentrations and child BMI and triceps skinfold z-scores, as well as child overweight/obesity at 5-year follow-up, with stronger associations among Norwegian participants (*Paper III*). This is consistent with a Danish study that found positive associations between PFOS and PFOA and waist-to-height ratio in 5-9-year old girls and boys (129). The maternal serum concentrations in that study (from gestational week 24±10) were comparable to the concentrations in ours (median PFOS: 10.8 ng/ml, PFOA: 1.3 ng/ml). However, inconsistent results are reported in studies with higher maternal serum PFAS concentrations. Positive associations between increasing maternal plasma PFAS concentrations and measures of child or adult obesity were found in three studies with median maternal plasma PFOS and PFOA concentrations in the range 19.6 - 24.8 ng/ml and 3.7 – 5.6 ng/ml, respectively (127, 128, 131). Inverse associations between maternal serum PFAS concentrations and infant weight and BMI during the first year of life were found in a Danish study with fairly high serum concentrations of PFASs (median PFOS: 33 ng/ml, PFOA: 5.2 ng/ml) (135). Yet another study with maternal serum PFOA concentrations higher than ours (median: 5.3 ng/ml), reported a non-linear dose-response relationship between maternal PFOA concentrations and BMI at 8 years of age (130).

We may hypothesize that these diverse associations depend on the range of observed PFAS concentrations in the study populations, and that high maternal serum PFAS concentrations may show positive and negative, as well as non-linear dose-response relationships with postnatal growth and child obesity. This is consistent with a recent review that reported possibilities for non-linear or non-monotonic dose-response relationships in epidemiologic studies with EDCs, and that the effects of high EDC concentrations poorly predict effects of EDCs at lower concentrations (66). Because EDCs may act like hormones, potential mechanisms for non-linear and non-monotonic dose-response relationships include

cytotoxicity, cell- and tissue-specific receptors and co-factors, receptor selectivity, receptor down-regulation and desensitization, receptor competition, and endocrine negative feedback loops (66). Moreover, disrupted postnatal growth patterns and childhood obesity are heterogenic conditions related to multiple exposures and conditions from conception and throughout childhood, which may complicate interpretation of results from different populations. Our differences between Norwegian participants (with lower maternal serum PFAS concentrations) and Swedish ones (with higher maternal serum PFAS concentrations) may stem from such mechanisms. A potential cytotoxic effect of high prenatal exposure to PFASs that results in a growth restricted offspring, is consistent with the reported higher odds for an SGA birth with increasing maternal serum PFAS concentrations among our Swedish participants (*Paper II*). Low birth weight is associated with an increased risk for disease in adulthood, such as type 2 diabetes and cardiovascular disease (13). Intrauterine growth restriction is often followed by rapid "catch-up" growth during the first two years of life, which again is associated with childhood and adult obesity (230). Consequently, both low and high birthweight may be associated with childhood obesity, and could distort the association between maternal serum PFAS and child obesity at 5-years. Also, a possible obesogenic effect may appear at later ages among growth restricted offspring. Moreover, non-linear or non-monotonic dose-response relationships have been proposed for relations between maternal serum PCB concentrations and offspring obesity (138). The authors reviewed nine prospective cohort studies and found that low maternal serum PCB concentrations (PCBs < 1,000 ng/g lipids) were associated with increased offspring BMI or body weight, while high concentrations (PCBs > 4,000 ng/g lipids) were associated with decreased offspring BMI or body weight (138). Although we have been unable to compare concentration ranges with our study that only reported PCB 153 concentrations, we found a non-monotonic dose-response

relationship between maternal serum PCB 153 concentrations and child overweight/obesity at 5-year follow-up among our Swedish participants.

In single POP models, we found evidence of associations between PFOS, PFOA and PCB 153 and BMI z-scores in Norwegian offspring at five years of age. As PFOA and PFOS are highly correlated (ρ=0.56-0.73) (Figure 10), it is difficult to relate this effect to a single compound. However, when we adjusted for PCB 153 in the models, both PFOA and PFOS remained significant in the models with BMI z-scores and childhood overweight as outcomes (Paper III). When we mutually adjusted for all three compounds (PFOA, PFOS and PCB 153), none remained statistically significant, although the association between PFOS and childhood overweight remained close to significance (Paper III). This suggest that PFOS may be the main driver in the associations.

6. Conclusion and future perspectives

Maternal serum concentrations of POPs were lower or comparable to other studies in the late 1980s, with higher concentrations among Swedish compared to Norwegian participants. This may suggest different exposure sources and patterns between the countries, and may stem from high pollution of fat fish in the Baltic Sea on the East Coast of Sweden. Variation in maternal POP concentrations is explained by demographic and pregnancy-related variables, where breastfeeding and child birth(s) are important elimination sources. Thus, foetuses and infants are exposed to POPs during sensitive developmental periods throughout pregnancy and after birth.

Prenatal exposure to PFOA, PCB 153 and HCB was associated with higher odds for SGA birth, but only among Swedish participants in the SGA study. There were indications of stronger associations among Swedish male offspring, but these results must be considered with caution. The observed country-specific estimates may stem from differences in concentrations, exposure patterns and sources, or maternal characteristics. This warrants further investigation.

Prenatal exposure to PFASs was positively associated with indices of childhood obesity at five-year follow-up, but the estimates were stronger among Norwegian participants compared to Swedish. Non-linear or non-monotonic relationships were observed between PFOS and PCB 153 and childhood obesity in the Swedish part. This may indicate hormone disrupting effects and/or different postnatal growth patterns among growth restricted infants, and highlight the importance of assessing non-linear or non-monotonic dose-response relationships from POP exposure. Prospective studies on the relationship between prenatal exposure to POPs and overweight/obesity among older children and adults are also needed.

The totality of human environmental exposures from conception and onwards could advance understanding of disease aetiology, and an increased focus should be on the "exposome". To further enhance the understanding of substance mixtures, future studies should strive to include numerous contaminants. Mechanisms for the proposed effects in this study are still mostly unknown and debated, and future studies are needed to elucidate these. Combination of basic research and population studies are needed to investigate effects with epigenetics, biomarkers and disease development.

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Papers I-III

Paper I

PLOS ONE

RESEARCH ARTICLE

Factors Associated with Maternal Serum Levels of Perfluoroalkyl Substances and Organochlorines: A Descriptive Study of Parous Women in Norway and Sweden

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OPEN ACCESS

Citation: Lauritzen HB, Larose TL, Øien T, Odland JØ, van de Bor M, Jacobsen GW, et al. (2016) Factors Associated with Maternal Serum Levels of Perfluoroalkyl Substances and Organochlorines: A Descriptive Study of Parous Women in Norway and Sweden. PLoS ONE 11(11): e0166127. doi:10.1371/journal.pone.0166127

Editor: Pal Bela Szecsi, Gentofte Hospital, DENMARK

Received: May 27, 2016

Accepted: October 24, 2016

Published: November 8, 2016

Data Availability Statement: Due to ethical restrictions related to protecting participant confidentiality, underlying data cannot be made publicly available. These data are available upon request from the principal investigator in the SGA-study, Geir W. Jacobsen, e-mail: geir.jacobsen@ntnu.no.

Funding: This work was funded by grants from the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology

Abstract

Introduction

Perfluoroalkyl substances (PFASs) and organochlorines (OCs) are ubiquitous and persistent in the environment and proposed endocrine disrupting chemicals (EDCs). They can be transferred across the placenta during pregnancy, and studies suggest that the prenatal period may be particularly sensitive for influences on fetal growth and development. Several studies have investigated socio-demographic and pregnancy related factors associated with maternal serum PFAS and OC levels, but few studies have been conducted in time periods with increasing emissions of PFASs and recent emissions of OCs.

Methods

Serum from 424 pregnant women participating in the NICHD Scandinavian Successive Small-for-gestational Age (SGA) births study was collected in 1986–1988, and analyses of two PFASs and six OCs were conducted. Associations between EDCs and geographic, time dependent, socio-demographic and pregnancy related variables were evaluated by using multivariable linear regression models.

Results

Previous breastfeeding duration, time since last breastfeeding period, sampling date and country of residence were important factors associated with serum levels of PFOS and PFOA. Smoking status and pre-pregnancy BMI were negatively associated with PFOS, and maternal height was borderline negatively associated with PFOS and PFOA. Glomerular filtration rate (GFR) was negatively associated with PFOS in a sub-sample. Maternal serum levels of OCs were positively associated with maternal age, and negatively

(NTNU). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

associated with previous breastfeeding duration and sampling date. Smoking had a consistently negative association with PCB 118 in a dose-dependent manner. Education level, pre-pregnancy BMI and alcohol consumption varied in importance according to the compound under study.

Conclusions

Several maternal factors, including potentially modifiable factors, markers of pregnancy physiology and factors also related to perinatal outcomes were associated with EDC levels. Results from this study are relevant to populations with still high PFAS and OC levels, i.e. developing countries. Moreover, we can use this knowledge about associated factors on emerging EDCs with similar properties.

Introduction

Perfluoroalkyl substances (PFASs) are synthetic chemicals that have been widely used in consumer products including lubricants, paper products and textiles since the 1950s [1]. Numerous PFASs are persistent substances, with approximate human half-lives of 2–5 years [2]. Organochlorines (OCs) comprise several substances, including polychlorinated biphenyls (PCBs) and pesticides. PCBs have been produced for commercial use in paints, plastics and electrical transformer fluids, whereas pesticides were used to control pests and disease [3]. PFASs are proteinophilic, primarily bound to albumin in serum and reside in blood, liver and kidneys [4], whereas OCs are lipid soluble and accumulate in fatty tissues [5]. PFASs and OCs can cross the placental barrier [6, 7], and *in utero* exposure have been associated with adverse effects of growth and development in both animal and epidemiological studies [8–10]. These environmental pollutants have been categorized as endocrine disruptive chemicals (EDCs) due to their disruption in the regulation of estrogen and thyroid hormones [11, 12]. In 2012, a report from the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) identified EDCs as an emerging health challenge to vulnerable individuals in society, particularly fetuses and children [13]. Maternal serum EDC levels are relevant biomarkers of fetal exposure.

There are large inter-individual differences in maternal serum levels of EDCs, but reasons behind the large variation have not been consistent. A better understanding of factors associated with serum levels of EDCs would improve the basis for advices with the purpose to lower the body burdens of young women during pregnancy. This knowledge may also provide information regarding exposure routes in addition to distribution and elimination of EDCs in the body.

Most previous studies on factors associated with PFAS and OC levels were conducted in post-ban periods when contaminant levels were declining in most Western countries. Important sources of exposures and associated maternal factors will change over time as emission history is changing. New knowledge about associated factors in historical periods of increasing PFAS emissions and recent OC emissions is important because biomonitoring studies have revealed minimal temporal declines and even increases in PFAS and OC levels among some populations, i.e. some developing countries [3, 14]. Moreover, we can use this knowledge about associated factors on new emerging EDCs with similar properties. Although epidemiologic studies have reported associations between maternal serum levels of PFASs and OCs and

indices of fetal growth [15, 16], potential confounders of these associations are not consistently reported and important to examine. This descriptive study of Scandinavian parous women examines factors associated with maternal serum levels of PFASs and OCs from years of continued emissions of PFASs and recent emissions of OCs (1986–1988) [17].

Materials and Methods

Ethics statement

All participants provided written informed consent for continued use of data and biomaterial, which was documented at the first study visit. The study, including the consent procedure, has been reviewed and approved by the Central Norway Regional Committee for Medical and Health Sciences Research Ethics (REK Midt 2010/1449-5).

Study area and population

Participants were from the NICHD Scandinavian Successive Small- for- Gestational Age (SGA) births study; a population based prospective multicenter study conducted in Trondheim and Bergen (Norway) and Uppsala (Sweden) (1986–1988) [17]. The SGA births study was designed to study the etiology and consequences of intrauterine growth restriction. Eligible participants included para 1 and para 2 women of Caucasian origin who spoke one of the Scandinavian languages and had a singleton pregnancy. In total, 5,722 women were eligible and made the first appointment, from which three groups were defined: a 10% random sample (n = 561); a group at high risk for SGA birth (n = 1,384), and a rest of population group (n = 3,777). Both the random sample and high risk groups were included for detailed follow-up. The high risk for SGA birth group was defined by one or more of the following risk factors: a) a prior low birth weight (LBW) child, b) maternal cigarette smoking at conception, c) low pre-pregnancy weight (<50 kg), d) a previous perinatal death, or 5) the presence of chronic maternal disease. Serum samples from n = 424 mothers were randomly selected for PFAS and OC analyses including a 2:1 ratio of non-SGA births to SGA births for future analysis (Fig 1).

Data on maternal characteristics

Data on socio-demographics, body mass index (BMI), lifestyle variables, and previous obstetric history were collected at first study visit. Socio-demographic variables included maternal age (continuous; years) and level of education (ordinal; 5 levels from low to high). Body weight and height were reported and pre-pregnancy BMI was calculated as weight in kilograms divided by height in meters squared (continuous; kg/m^2). Lifestyle variables included smoking status at conception (categorical; smokers or non-smokers), number of cigarettes smoked at conception and in 2^{nd} trimester, and alcohol consumption during pregnancy (ordinal; 5 levels from low to high). Smokers were women who at first study visit reported daily smoking at the time of conception. Obstetric variables included parity (binary; 1 or 2), previous breastfeeding duration (continuous; months) and time since end of last breastfeeding period (continuous; per year). We categorized serum collection by sampling date (continuous; for each 100 days since study inception) and gestational age at serum sampling (completed weeks).

Measurement of PFAS and OCs

Maternal serum samples were collected during the 2^{nd} trimester at approximately week 17 (Table 1), and stored at -80°C for later analysis. In total, a random selection of n = 424 serum samples were included for PFAS and OC analyses (Fig 1).

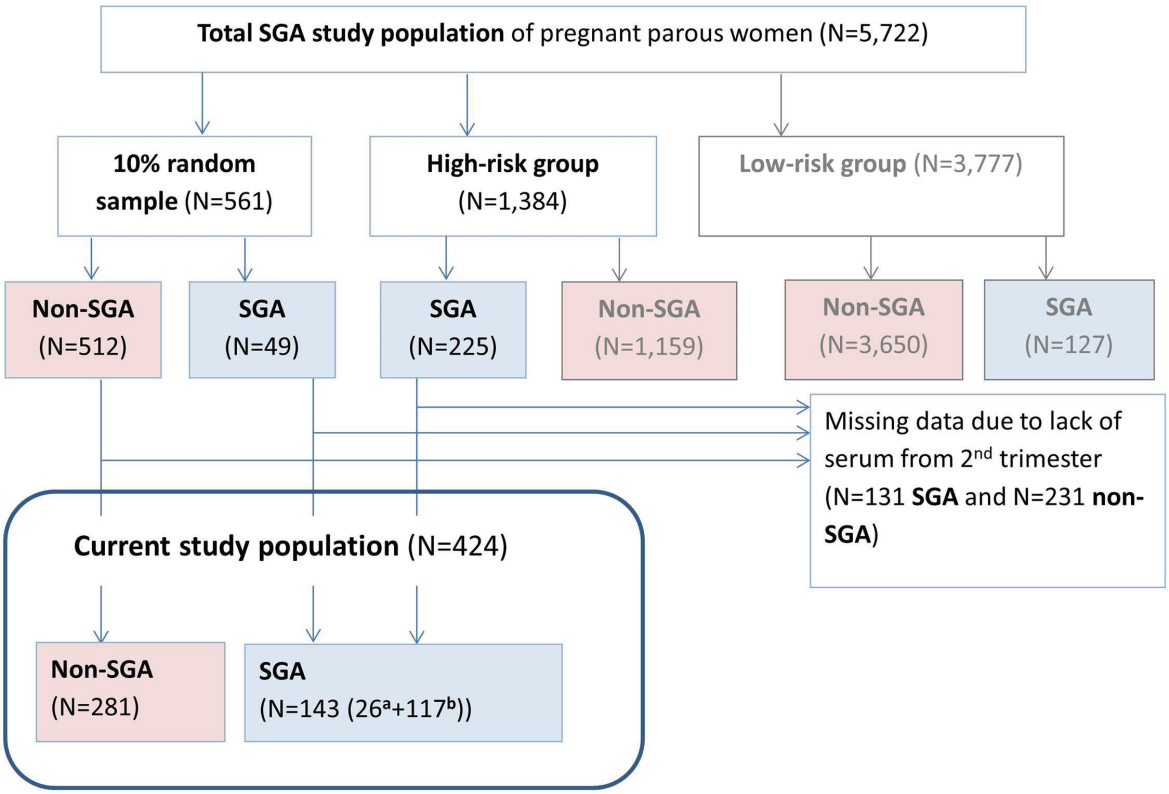

Fig 1. Flow chart of the participant selection for PFAS and OC analysis. [1]From 10% random sample.[2]From High-risk group.

doi:10.1371/journal.pone.0166127.g001

Chemical analyses of PFAS. Analyses were performed at the laboratories of Norwegian Institute for Air Research, Tromsø, Norway (NILU). PFASs measured were perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). Samples were analyzed using sonication-facilitated liquid-liquid extraction, activated ENVI-carb clean-up [18], quantified and analyzed by ultrahigh pressure liquid chromatography triple-quadrupole mass-spectrometry (UHPLC-MS/MS).

Sample preparation and extraction were performed as described by Hanssen et. al [19] except for minor volume changes.

The quantification was conducted with the LC Quan software, version 2.6.0 (Thermo Fisher Scientific Inc, Waltham, MA, USA). The internal-standard addition method with isotope-labeled PFASs was used to quantify the contaminants [19]. Concentrations of PFASs in all samples were within the linear range of the instrument and the calibration curve. In the mass spectrometry analyses, a second mass transition served to confirm compound specificity for each compound. The quality of the analysis was verified by repetitive analysis of blank samples and reference samples (SRM 1957, NIS, Gaithersburg, MD, USA). Participation in the AMAP ring test program and results from SRM material indicates a coefficient of variation (CV) of 15% for PFOA and 10% for PFOS. The linear PFOS isomers were chromatographically

Table 1. Maternal characteristics of the current sample of Scandinavian parous pregnant women in the SGA-study (N = 424).

		% or mean (range)
Gestational age[1] at serum sampling		17 (13–20)
Sampling date	1986	41.2%
	1987	49.8%
	1988	7.8%
Country of residence	Norway	62.5%
	Sweden	37.5%
Maternal age (years)		29.0 (18–41)
Maternal height (cm)		166 (150–182)
Maternal pre-pregnancy BMI (kg/m^2)		21.5 (16–33)
Education level	<9 years	16.5%
	9+1–2 years	28.3%
	9+3 years	21.2%
	Higher education, non-university level	24.8%
	Higher education, university level	8.0%
Parity	1	68.9%
	2	31.1%
Smoking at conception	No	54.5%
	Yes	45.5%
Number of cigarettes per day at conception		5.4 (0–25)
Smoking in early 2nd trimester	No	58.7%
	Yes	40.1%
Number of cigarettes per day at 2nd trimester		4.1 (0–25)
Alcohol consumption during pregnancy[2]	Never	47.4%
	< once a month	33.7%
	About once every month	9.2%
	2–3 times per month	4.7%
	$\geq$ once a week	1.9%
Previous breastfeeding duration (months)		7.4 (0–24)
Time since end of last breastfeeding period	<2 years	42.7%
	2–4 years	28.3%
	>4 years	21.9%

[1]Gestational age based on ultrasound measurements (completed weeks)

[2]Infomation collected in week 33 of pregnancy

doi:10.1371/journal.pone.0166127.t001

separated from the branched isomers and quantified separately. Unless otherwise specified, the PFOS results discussed are the sum of linear and branched isomers.

Chemical analyses of OCs. OCs were analyzed using the POPs method E-446 (ISO 1 7025 accreditation) at the Institut National de Santé Publique du Quebec, Centre Toxicologie, Quebec. This laboratory is the organizer of the AMAP ring program. OCs measured were hexachlorobenzene (HCB), oxychlordane, PCB 52, PCB 101, PCB 118, PCB 153, PCB 156, PCB 170, PCB 180, *p*,*p*'-dichlorodiphenyldichloroehylene (*p*,*p*'-DDE), *p*,*p*'-dichlorophenyltrichloroethane (*p*,*p*'-DDT), *beta*-hexachlorohexane (*β*-HCH) and *trans*-nonachlor (*t*-NC). Only OCs detected in more than 99% of the samples and used in the regression analyses were included in Table 1. Because of high correlation between PCBs we chose to use and report PCB 118,

representing dioxin-like PCBs and PCB 153, representing non-dioxin like PCBs. In short, 0.5–1 ml serum sample was extracted using hexane (2x6 ml), ethanol (2 ml) and saturated ammonium sulphate solution (2 ml). This method is a slight modification of the one described by Sandanger et al. [20], where the samples were cleaned up using 1 g of activated fluorisil on an automated Liquid handler system before GC-MS analysis described by Sandanger et al. [21]. Results from the AMAP ring test program and SRM material indicate a CV of 5–10% for the OCs analyzed.

Statistical analyses

The distribution of PFAS and OC levels closely followed a log-normal distribution. We therefore transformed data accordingly. We used wet weight concentrations of PFASs and lipid-adjusted serum concentrations of OCs [22]. As a sensitivity analysis we used wet weight values of OCs and adjusted for total lipids as an independent variable, but neither point estimates nor 95% confidence intervals (95% CIs) showed considerable change (data not shown).

Total lipid values were calculated based on measurements of triglycerides and cholesterol: total lipids = 1.33*triglycerides + 1.12*cholesterol + 1.48 (g/l) [22]. This formula showed good correlation with complete formulas including phospholipids [23].

We used multivariable linear regression to estimate associations between maternal characteristics and serum PFAS (PFOA and PFOS) and OC (PCB 118, PCB 153, *p,p*'-DDE, *t*-NC, HCB and β-HCH) levels, and reported adjusted estimates and 95% confidence intervals (CIs). Based on a priori knowledge, the following co-variates were included in adjusted analyses: serum sampling date, study site (Norway/Sweden), maternal age, education level, maternal height and pre-pregnancy body mass index (BMI), smoking status at conception, alcohol consumption during pregnancy, parity (1 vs. 2), previous breastfeeding duration and time since end of last breastfeeding period. We evaluated linear model assumptions using diagnostic plots of the residuals and checked the covariates for multicollinearity by variance inflation factors (VIFs). We calculated percent change in PFAS and OC levels for each independent variable by exponentiating regression coefficients, subtracting 1 and multiplying by 100.

In sub-analyses we investigated potential dose-response relationships between smoking intensity (number of cigarettes smoked at conception and in early 2nd trimester) and levels of PFOS, PCB 118 and β-HCH. As a sensitivity analysis we used serum cotinine levels measured from samples collected at first study visit available from n = 88 women to further examine the association between smoking and PFOS, PCB 118 and β-HCH levels.

As markers of pregnancy physiology we examined glomerular filtration rate (GFR) in n = 88 of the samples. We calculated GFR (mL/min per 1.73 m^2) using the Cockroft-Gault (GFR-CG) formula (GFR-CG = (140-age) x weight (g) x 1.04/serum creatinine (μmol/L)).

We performed statistical analyses using SPSS statistical software, version 22 (IBM SPSS Inc. Chicago, IL, USA).

Results

Maternal baseline characteristics

Maternal baseline characteristics are presented in Table 1. The gestational age at serum sampling varied from 13 to 20 weeks, with a mean gestation age of 17 weeks. A larger proportion of participants came from Norway (63%), while the majority of participants were pregnant with their second child (69%). Nearly half of all participants reported smoking at conception (46%), with a slight reduction by first visit in early 2nd trimester (40% smokers). In gestational week 33, more than half of all study participants reported at least some alcohol consumption (47% no alcohol).

Serum PFAS and OC levels

Serum levels of two PFASs (PFOA and PFOS) and six OCs are presented in Table 2. All PFASs and OCs reported were detected in 100% of the samples, with the exception of *β*-HCH levels which were detected in 99% of the samples. In wet weight values, PFOS was the dominating compound, followed by PFOA, *p,p'*-DDE, PCB 153, *β*-HCH, HCB, PCB 118 and *t*-NC.

Maternal factors associated with PFAS and OC levels

PFASs. In adjusted analyses, Swedish women had 39% higher PFOA levels and 67% higher PFOS levels compared to their Norwegian peers. Previous breastfeeding duration was negatively associated, while time since last breastfeeding period was positively associated with PFOA and PFOS. An increase in both PFOA and PFOS per 100 days from study enrollment was observed (2.8% and 5.0% respectively). Smokers had on average 21% lower levels of PFOS than non-smokers (Table 3), and in sensitivity analyses, we found a linear relationship between increasing number of cigarettes smoked at conception ($p = 0.015$) and in early 2nd trimester ($p = 0.006$) and decreasing PFOS levels (Table 4). However, this finding was not replicated in a sensitivity analyses of n = 88 women with available cotinine levels from 2nd trimester ($p = 0.113$) (Table 4). Adjusted for maternal height, pre-pregnancy BMI and gestational age at serum sampling, GFR was negatively associated with PFOS in the same sub-sample (n = 88) (Table 5). Pre-pregnancy BMI was negatively associated with PFOS levels in multivariable linear regression model (Table 3).

OCs. In adjusted analyses, Swedish women had, on average, 43% higher levels of PCB 153 and 9% lower levels of *t*-NC than Norwegian women. All OC levels, except PCB 118 and *t*-NC, decreased from January 1986 through March 1988. Previous breastfeeding duration was negatively associated and maternal age positively associated with all OCs (Table 3). Pre-pregnancy BMI was negatively associated with PCB 153 and *t*-NC. Women who smoked at conception had on average 26% lower levels of PCB 118 and 9% higher levels of *β*-HCH, compared to non-smokers. In sensitivity analyses, we found a clear dose-response relationship between increasing number of cigarettes smoked both at conception and ($p<0.001$) and in early 2nd trimester ($p<0.001$) and decreasing PCB 118 levels (Table 4). This finding was confirmed in a sub-sample analysis of the n = 88 women with available serum cotinine levels ($p = 0.004$). We also found some evidence of a positive linear relationship between cigarettes smoked at conception and in 2nd trimester and *β*-HCH levels, but this could not be confirmed with cotinine levels

Table 2. Maternal serum levels of PFASs (ng/ml) and OCs (ng/ml and ng/g lipids) in the SGA study (N = 424).

	Wet weight (ng/ml)	Lipid weight (ng/g lipid)	LOD[1]	%>LOD[2]
	Median (range)			
PFOA	1.82 (0.31–7.97)	-	0.03	100
PFOS	12.3 (0.95–59.6)	-	0.03	100
PCB 118	0.08 (0.03–0.27)	13.9 (5.38–86.2)	0.01	100
PCB 153	0.52 (0.17–1.40)	89.9 (31.0–212)	0.01	100
***p,p'*-DDE**	1.30 (0.10–11.0)	223 (16.7–1791)	0.09	100
HCB	0.10 (0.04–0.38)	17.7 (6.98–73.0)	0.04	100
***β*-HCH**	0.13 (<LOD-0.76)	22.1 (<LOD-134)	0.01	99
***t*-NC**	0.04 (0.01–0.14)	6.51 (1.82–25.2)	0.01	100

[1]LOD: Limit of detection (ng/ml)

[2]%>LOD: percentage of samples in which the analyte was detected

doi:10.1371/journal.pone.0166127.t002

Table 3. Adjusted[1] associations from multivariable linear regression models for sociodemographic and pregnancy-related variables and ln-transformed maternal PFAS (ng/ml) and OC levels (ng/g lipids) in serum collected in 2nd trimester (n = 424).

	Ln (PFAS (ng/ml))		Ln (OC (ng/g lipid))					
	PFOA	**PFOS**	**PCB118**	**PCB153**	***p,p'*-DDE**	**HCB**	***β*-HCH**	***t*-NC**
	% change (95% CI)	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**
Sample date (per 100 days)	**2.8 (0.7, 4.9)**	**5.0 (2.4, 7.7)**	-1.6 (-3.4, 0.2)	**-2.5 (-3.7, -1.3)**	**-7.2 (-9.7, -4.7)**	**-2.4 (-3.7, -1.0)**	**-4.4 (-5.9, -2.8)**	-0.5 (-2.2, 1.3)
Country of residence								
Norway	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
Sweden	**39 (27, 53)**	**67 (49, 88)**	8.0 (-0.7, 17)	**43 (35, 52)**	2.0 (-11, 16)	-2.5 (-8.7, 4.2)	7.4 (-0.5, 16)	**-9.4 (-16, -1.7)**
Maternal height (per 10 cm)	*-6.7 (-14, 0.3)*	*-8.3 (-16, 0.6)*	5.6 (-1.1, 13)	1.1 (-3.4, 5.9)	10 (-0.6, 22)	1.9 (-3.3, 7.3)	0.1 (-5.7, 6.2)	1.1 (-5.2, 7.7)
Maternal BMI (per kg/m^2)	-1.4 (-3.0, 0.3)	**-2.3 (-4.2, -0.2)**	-0.5 (-1.9, 1.0)	**-2.6 (-3.6, -1.6)**	-1.1 (-3.3, 1.1)	-0.3 (-1.4, 0.9)	1.0 (-0.3, 2.4)	**-2.0 (-3.4, -0.6)**
Maternal age	-0.1 (-1.5, 1.3)	-0.1 (-1.9, 1.7)	**2.5 (1.2, 3.8)**	**2.9 (2.0, 3.8)**	**5.5 (3.5, 7.6)**	**2.6 (1.6, 3.7)**	**3.2 (2.0, 4.4)**	**3.5 (2.3, 4.8)**
Smoking at conception								
No	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
Yes	-4.7 (-15, 5.0)	**-21 (-37, -7.4)**	**-26 (-38, -16)**	0.9 (-5.3, 6.6)	3.0 (-11, 15)	-4.4 (-12, 2.5)	**9.2 (1.9, 16)**	7.0 (-1.1, 14)
Alcohol consumption (5 groups from low to high)[3]	0.6 (-4.2, 5.5)	-0.4 (-6.2, 5.8)	**5.1 (0.8, 9.7)**	3.5 (0.4, 6.6)	3.3 (-3.2, 10)	3.2 (-0.2, 6.8)	2.5 (-1.3, 6.6)	**5.6 (1.3, 10)**
Education level (5 groups from low to high)[2]	2.0 (-2.4, 6.6)	3.0 (-2.5, 8.7)	2.3 (-1.6, 6.3)	1.6 (-1.1, 4.4)	**7.0 (0.9, 14)**	1.4 (-1.7, 4.6)	3.3 (-0.3, 7.0)	**4.8 (0.9, 8.8)**
Parity								
1	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
2	-3.1 (-13, 7.9)	-6.7 (-19, 6.8)	8.1 (-1.8, 19)	7.2 (0.3, 15)	7.6 (-7.1, 25)	2.1 (-5.4, 10)	1.2 (-7.2, 10)	0.3 (-8.6, 10)
Previous breastfeeding duration (per month)	**-1.3 (-2.3, -0.2)**	**-1.6 (-2.8, -0.3)**	**-2.3 (-3.2, -1.4)**	**-2.1 (-2.7, -1.4)**	**-3.5 (-4.9, -2.2)**	**-1.9 (-2.6, -1.2)**	**-2.7 (-3.6, -1.9)**	**-1.0 (-1.9, -0.1)**
Time since last breastfeeding period (per year)	**4.8 (2.9, 6.8)**	**2.8 (0.5, 5.2)**	-0.5 (-2.1, 1.1)	-0.4 (-1.5, 0.7)	-0.6 (-3.0, 1.9)	-0.2 (-1.5, 1.0)	0.2 (-1.2, 1.7)	-0.01 (-1.6, 1.6)

[1]Multivariable models were adjusted for all variables included in the table.

[2]Education level (ordinal): 1 = <9 years, 2 = 9–11 years, 3 = 12 years, 4 = higher education, non-university level, 5 = higher education, university level.

[3]Alcohol consumption during pregnancy (ordinal): 0 = never, 1 = <once a month, 2 = once a month, 3 = 2–3 times a month, 4 = >once a week.

doi:10.1371/journal.pone.0166127.t003

(p = 0.428). Increased length of education was significantly associated with increased *p,p'*-DDE, *t*-NC, and *β*-HCH levels, and alcohol consumption during pregnancy was positively associated with serum PCB153, *t*-NC and HCB levels (Table 3).

Discussion

In this descriptive study of n = 424 Scandinavian parous women, Swedish women had higher levels of PFOS, PFOA and PCB 153 levels compared to Norwegian ones. Our results indicate that several maternal factors, including potentially modifiable lifestyle factors, are independently associated with maternal serum EDC levels. We demonstrated that long previous breastfeeding duration was associated with lower PFAS and OC levels, and that long time since last breastfeeding period was associated with higher PFAS levels. Maternal smoking showed a consistently negative association with PCB 118 in a dose-dependent manner.

Table 4. Adjusted[1] associations from multivariable linear regression models between smoking intensity and ln-transformed maternal PFOS, PCB 118 and β-HCH levels in serum collected in 2nd trimester (n = 424).

	PFOS		PCB 118		*β*-HCH	
	% change (95% CI)	p-value	% change (95% CI)	p-value	% change (95% CI)	p-value
Smoking status at conception						
no smoking	*ref.*		*ref.*		*ref.*	
1–5 cig/day	-11 (-29, 11)	0.292	-14 (-23, -4.9)	0.004	1.1 (-12, 16)	0.876
6–10 cig/day	-9.1 (-21, 4.1)	0.166	-20 (-25, -14)	<0.001	13 (4.3, 23)	0.003
>10 cig/day	-15 (-26, -2.7)	0.019	-24 (-29, -19)	<0.001	13 (3.7, 23)	0.005
p for trend		*0.015*		*<0.001*		*0.001*
Smoking status at week 17						
no smoking	*ref.*		*ref.*		*ref.*	
1–5 cig/day	-7.7 (-23, 11)	0.384	-21 (-28, -15)	<0.001	1.1 (-10, 13)	0.845
6–10 cig/day	-17 (-28, -4.5)	0.010	-23 (-28, -18)	<0.001	15 (5.9, 26)	0.001
>10 cig/day	-13 (-26, 1.9)	0.084	-25 (-31, -20)	<0.001	6.9 (-2.9, 18)	0.173
p for trend		*0.006*		*<0.001*		*0.010*
Serum cotinine levels at 2nd trimester[2]	-2.0 (-4.5, 0.5)	0.113	-1.8 (-3.0, -0.6)	0.004	0.8 (-1.1, 2.7)	0.428

[1]Adjusted for maternal pre-pregnancy BMI, country of residence and maternal PCB 153-levels.

[2]Only analyzed for a subset of 88 women. Numbers shown are percent change in POPs levels for each 100-unit increase in serum cotinine (range 0–1856).

doi:10.1371/journal.pone.0166127.t004

Serum levels of PFASs and OCs; sampling date and geographic factors

Both maternal PFAS and OC levels found in our study were lower than levels among a group of Danish pregnant women sampled in 1988–1989 [24]. PCB levels were slightly lower than levels among women from the Netherlands (1990–1992) [25] and higher than levels among Swedish primiparous women enrolled in 1996–1999 [26]. Our study, conducted in 1986–1988, had only parous women that may have led to lower levels of PFASs and OCs due to elimination through the placenta and breast milk in earlier pregnancies.

The increase in PFAS levels over time (i.e. from January 1986 to March 1988) is consistent with other studies showing a rise in PFOA and PFOS levels from the 1960s to around 2000, and a subsequent decrease thereafter [27–29]. In contrast, almost all OC levels declined from January 1986, which is consistent with findings from longitudinal studies that observed a declining trend of most OCs after 1986 [30–32]. These changes compare with trends of historic production, restrictions, and use and bans on use of PFASs and OCs, leading to declining levels of these toxicants in air and biota [3].

Table 5. Adjusted associations from multivariable regression models between GFR, maternal height and pre-pregnancy BMI, and ln-transformed maternal PFOA and PFOS levels in serum collected in 2nd trimester from a subset of n = 88 mothers.

	PFOA		PFOS	
	Without GFR[2]	With GFR[3]	Without GFR	With GFR
	% change (95% CI)	% change (95% CI)	% change (95% CI)	% change (95% CI)
GFR (per 10 ml/min per 1.73 m2)[1]	-	2.6 (-7.6, 2.8)	-	**-8.6 (-15, -2.3)**
Maternal height (per 10 cm)	-12 (-24, 3.2)	-9.0 (-23, 7.3)	**-19 (-33, -0.7)**	-10 (-27, 10)
Maternal BMI (per kg/m^2)	0.2 (-3.2, 3.6)	1.6 (-2.9, 6.3)	2.1 (-2.3, 6.6)	**7.4 (1.5, 14)**

[1]Mutually adjusted estimates, in addition to adjustment for gestational age at serum sampling.

[2]Analyses performed without adjustment for GFR.

[3]Analyses performed with adjustment for GFR.

doi:10.1371/journal.pone.0166127.t005

Higher levels of PCBs among Swedish women seem reasonable and correspond to higher levels reported in fish from the Baltic Sea [33, 34]. Dietary intake is probably also the most important exposure source to PFASs [35], and particularly seafood in Scandinavian countries [36]. However, other sources of exposure such as drinking water and dust may contribute to variability in levels between the study sites, particularly in time periods with continued use and emissions [35, 37].

Despite declining serum levels of PFASs and OCs in most Western countries, biomonitoring programs still demonstrate high levels of both PFASs and OCs among certain populations and countries today [3]. So, this study may be of great importance to certain populations with still high environmental levels of PFASs and OCs, i.e. developing countries [14].

Maternal socio-demographic and pregnancy related factors associated with PFAS levels

Our results emphasize that information on previous breastfeeding duration is important in the evaluation of PFAS levels in women. The negative linear association between breastfeeding duration and PFAS levels is also consistent with other studies [38, 39]. Due to the placenta barrier, elimination through breast milk is thought to be greater than the prenatal transfer to the fetus [40]. Several studies from post-ban periods (after year 2000) have found parity as an important predictor of PFAS levels [41]. The null association between parity and PFAS levels in the current study is in line with a study from the pre-ban period (1978–2001) when parity was not identified as a predictor of PFOS levels [42]. This suggests that the relative importance of parity as a predictor differs between pre- and post-ban periods. We found the same null association between parity and PFAS levels when we excluded breastfeeding duration from the multivariable models (data not shown). We also included time since last breastfeeding period in the analyses because the importance of parity likely depends on how recent the previous pregnancy was.

PFASs have the potential to bio-accumulate, and we hypothesize that non-pregnant periods without breastfeeding are important accumulation periods for women of fertile age. The positive association between PFAS levels and time since last breastfeeding period in the current study is consistent with a Norwegian study of pregnant women sampled in 2003 that also showed a rise in PFAS levels during pregnancy intervals [38]. Long intervals between two subsequent pregnancies have been associated with adverse perinatal outcomes [43], thus, it is important to consider that time period as a potential confounder in child health effect studies.

The lower levels of PFOS among women who smoked at conception were confirmed in additional analyses where increasing number of cigarettes smoked at conception and in early 2^{nd} trimester were associated with decreasing levels of PFOS. However, the non-linear relationship between serum cotinine and PFOS levels corresponds to other studies that found inconsistent associations with smoking overall, and no associations with smoking intensity [38, 44]. This suggests that the relationship between smoking and PFOS levels may be explained by different lifestyle patterns (e.g. diet) among smokers and non-smokers.

Only one other study has examined associations between GFR and PFASs among pregnant women [45], and like that study, we found an inverse association between GFR and PFOS ($p = 0.002$) in a sub-sample ($n = 88$). GFR describes the flow rate of filtered fluid through the kidney, and we postulate that higher GFR leads to more excretion of PFASs and thereby lower serum levels of PFASs. Because studies have indicated a possible association between GFR and infant birth weight [46], GFR might be considered a potential confounder in epidemiologic studies of PFASs and fetal growth. In fact, a recent study that examined the impact of GFR on

associations between PFASs and birth weight, suggested that associations were largely attenuated due to confounding by GFR [47].

The weak negative association we observed between pre-pregnancy BMI and PFOS is in contrast to other studies that reported positive [7, 38, 45] or null associations [42, 48]. This finding, together with the negative association between maternal height and PFAS levels, is not completely understood, but may be due to proposed poorer plasma volume expansion, leading to higher PFAS levels, among smaller sized women [49]. BMI was not associated with maternal height in our study, but lack of adjustment for GFR might give biased results in the multivariate models. GFR is positively related to maternal height and weight. In a sensitivity analysis (n = 88), we estimated the associations between GFR, maternal height and BMI and PFAS levels. When we included GFR in the model, the negative association between maternal height and PFOS attenuated and the association between BMI and PFOS became positive (Table 5). This suggests that BMI and maternal height might be proxies for other variables, like GFR, and results in multivariate linear models should be interpreted with caution.

Maternal socio-demographic and pregnancy related factors associated with OC levels

The strong inverse associations between breastfeeding duration and all OC levels in the current study are consistent with previous studies [50]. This is explained by excretion of OCs in the breast milk, resulting in lower maternal serum levels of OCs and exposure to breastfeeding children [51]. A recent review concluded that the benefits of breastfeeding far outweigh the potential disadvantages, but argues to plea for further global source-directed methods to reduce human exposure to OCs [52].

The positive association between maternal age and OC levels has been found in several other cross-sectional studies [33, 53]. Age indicates whether the subject lived during periods with higher exposure to OC levels. Based on longitudinal studies it has been shown that this association may be considered a cohort effect related to historical emission patterns, rather than age-dependent metabolism or bioaccumulation [32].

The inverse relationships between pre-pregnancy BMI and serum levels of PCB153 and *t*-NC levels may be explained by the dilution effects of OCs into adipose tissues [54]. However, obesity can also prolong the half-lives of OCs whereby positive associations can be seen as well [55]. Conversely, weight loss may lead to increased serum levels due to reduced storage capacity in the adipose tissues. Similarly, weight gain may lead to decreased serum levels through dilution of OCs into adipose tissue [56, 57]. The inverse association between BMI and OC levels as found in our study, is supported by other cross-sectional studies that have observed varying associations with BMI depending on the OC under study [58].

The lower levels of PCB 118 among smokers at conception in adjusted analyses, were further confirmed in additional analyses with smoking intensity both by number of cigarettes smoked at conception and at early 2^{nd} trimester, and by serum cotinine in 2^{nd} trimester (n = 88). These findings support the hypothesis that smoking cigarettes can enhance the metabolism of PCB118, leading to reduced levels in serum. It has been speculated that cigarette smoke can enhance the elimination rates of dioxins and dioxin-like PCBs, due to an induction of CYP-enzymes [59]. Reasons for higher levels of β-HCH among smokers are unclear, but several studies have found positive associations between OCs and smoking. This association may be attributed to either the use of these pesticides on tobacco plants until they were banned in the 1970s, or alternatively that cotinine influences the expression of CYP-enzymes that metabolize organochlorines [60].

The positive associations between education level and serum OC levels might be explained by different lifestyle patterns. More educated women with higher socio-economic status may have different dietary patterns, such as increased consumption of fish. The linear relationship between increased alcohol consumption and serum PCB153, *t*-NC and *β*-HCH levels might be attributed to changes in fatty acid composition [61]. However, the overall alcohol consumption was quite small, and other lifestyle factors might also have contributed to the differences in levels.

Strengths and limitations

This study benefitted from a large sample size and detailed information about socio-demographic, lifestyle, pregnancy related factors, obstetric history and markers of pregnancy physiology, that have been considered in only a few epidemiological studies. The serum samples were taken in a narrow time frame in the early 2nd trimester (from week 13–20) to ensure comparability. To ensure that the results were not biased by the enrichment of mothers with SGA offspring, we did stratum-weighted sensitivity analyses where weights were the inverse probability of selection [62]. We compared the results from the un-weighted and the weighted analyses, and found that they were not substantially different (S1 Table). Women expecting their first child were not included in the study, and this may have led to some selection bias, especially when considering excretion of EDCs through previous breastfeeding. We did not distinguish between *exclusive* and *partial* breastfeeding duration, and the lack of information about the exclusive breastfeeding duration may have led to residual confounding and spurious associations between PFASs and OCs and factors associated with breastfeeding duration. However, after birth we collected information about both *exclusive* and *partial* breastfeeding duration after the *current* pregnancy, and found that both exclusive and total (exclusive + partial) breastfeeding were highly correlated (r = 0.6). We also found total breastfeeding duration to be correlated between pregnancies, suggesting that potential bias is limited. Moreover, breastfeeding duration was recalled quite accurately 20 years after mothers gave birth in this study [63]. We selected our cohort from an original SGA study with a high risk for SGA birth group that included a high proportion of smokers. Although this may limit generalizability to other studies with fewer smokers, we were able to thoroughly study the relation between smoking status, smoking intensity and serum cotinine levels and serum PFAS and OC levels in an otherwise homogeneous population. To estimate GFR, we used an indirect method, based on a single-point measurement of serum creatinine, as opposed to inulin clearance, which is the gold standard method [64]. Renal function drastically changes during pregnancy, because of hyper filtration, systemic vasodilatation and plasma volume expansion, resulting in up to 60% increase in GFR compared to values obtained before pregnancy [64]. Hence, indirect methods may be biased. However, studies have shown that with increasing GFR, the differences between direct and indirect methods stayed the same, and that bias was limited [64].

Conclusions

In summary, our study observed higher maternal serum PFOA, PFOS and PCB 153 levels among Swedish compared to Norwegian women. Several maternal factors including potentially modifiable lifestyle factors, markers of pregnancy physiology and important factors related to perinatal outcomes were associated with maternal serum EDC levels. Interestingly, we demonstrated that long time since last breastfeeding period was associated with higher PFAS levels and that maternal smoking showed a consistently negative association with PCB 118 in a dose-dependent matter. Results from this study are particularly relevant to populations with still

high PFAS and OC levels, for instance in some developing countries. Moreover, we can use this knowledge about associated factors on new emerging EDCs with similar properties.

Supporting Information

S1 Table. Adjusted associations from multivariable linear regression models for sociodemographic and pregnancy-related variables and ln-transformed maternal PFAS and OC levels in serum from 2nd trimester (n = 424): un-weighted and stratum-weighted analysis.
(DOCX)

Acknowledgments

We gratefully acknowledge the participating women in the SGA-study. We would like to thank people at NILU—Norwegian Institute of Air Research in Tromsø, Norway, for conducting the PFAS analyses, and Institut National de Santé Publique du Québec, Centre de Toxicologie in Quebec, for the OC analyses. Special thanks to Charlotta Rylander, Therese H. Nøst, and Vivian Berg, for valuable input and advice.

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S1 Table. Adjusted[1] associations from multivariable linear regression models for sociodemographic and pregnancy-related variables and ln-transformed maternal PFAS (ng/ml) and OC (ng/g lipids) levels in serum from 2nd trimester (n=424): un-weighted and stratum-weighted[2] analysis.

		Ln (PFAS (ng/ml))		Ln (OC (ng/g lipid))					
		PFOA % change (95% CI)	**PFOS** % change (95% CI)	**PCB118** % change (95% CI)	**PCB153** % change (95% CI)	***p,p'*-DDE** % change (95% CI)	**HCB** % change (95% CI)	***β*-HCH** % change (95% CI)	***t*-NC** % change (95% CI)
Sample date (per 100 days)	A	**2.8 (0.7, 4.9)**	**5.0 (2.4, 7.7)**	-1.6 (-3.4, 0.2)	**-2.5 (-3.7, -1.3)**	**-7.2 (-9.7, -4.7)**	**-2.4 (-3.7, -1.0)**	**-4.4 (-5.9, -2.8)**	-0.5 (-2.2, 1.3)
	B	**3.2 (1.0, 5.4)**	**5.1 (2.3, 7.9)**	-1.3 (-3.1, 0.6)	**-2.2 (-3.5, -0.9)**	**-6.4 (-9.0, -3.8)**	**-2.7 (-4.1, -1.3)**	**-4.7 (-6.3, -3.1)**	-0.5 (-2.3, 1.3)
Country of residence									
Norway		*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
Sweden	A	**39 (27, 53)**	**67 (49, 88)**	8.0 (-0.7, 17)	**43 (35, 52)**	2.0 (-11, 16)	-2.5 (-8.7, 4.2)	7.4 (-0.5, 16)	**-9.4 (-16, -1.7)**
	B	**34 (21, 47)**	**59 (41, 79)**	7.6 (-1.4, 17)	**41 (33, 50)**	0.6 (-11, 14)	-5.3 (-11, 1.1)	3.8 (-3.8, 12)	**-10 (-17, -2.6)**
Maternal height (per 10 cm)	A	*-6.7 (-14, 0.3)*	*-8.3 (-16, 0.6)*	5.6 (-1.1, 13)	1.1 (-3.4, 5.9)	10 (-0.6, 22)	1.9 (-3.3, 7.3)	0.1 (-5.7, 6.2)	1.1 (-5.2, 7.7)
	B	-4.3 (-11, 3.0)	-6.9 (-15, 2.1)	**7.6 (0.7, 15)**	2.8 (-1.8, 7.5)	**13 (2.3, 24)**	1.9 (-3.2, 7.1)	1.7 (-4.0, 7.7)	1.8 (-4.3, 8.4)
Maternal BMI (per kg/m^2)	A	-1.4 (-3.0, 0.3)	**-2.3 (-4.2, -0.2)**	-0.5 (-1.9, 1.0)	**-2.6 (-3.6, -1.6)**	-1.1 (-3.3, 1.1)	-0.3 (-1.4, 0.9)	1.0 (-0.3, 2.4)	**-2.0 (-3.4, -0.6)**
	B	**-2.1 (-3.7, -0.4)**	**-3.0 (-5.0, -1.0)**	-0.5 (-1.9, 1.1)	**-2.2 (-3.2, -1.2)**	-1.2 (-3.4, 1.0)	-0.02 (-1.2, 1.1)	1.6 (0.2, 2.9)	*-1.4 (-2.8, 0.01)*
Maternal age	A	-0.1 (-1.5, 1.3)	-0.1 (-1.9, 1.7)	**2.5 (1.2, 3.8)**	**2.9 (2.0, 3.8)**	**5.5 (3.5, 7.6)**	**2.6 (1.6, 3.7)**	**3.2 (2.0, 4.4)**	**3.5 (2.3, 4.8)**
	B	0.1 (-1.3, 1.6)	-0.2 (-2.0, 1.6)	**3.2 (1.9, 4.6)**	**3.2 (2.3, 4.1)**	**6.6 (4.5, 8.6)**	**2.9 (1.8, 3.9)**	**3.3 (2.1, 4.4)**	**3.8 (2.5, 5.1)**
Smoking at conception									
No		*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
Yes	A	-4.7 (-15, 5.0)	**-21 (-37, -7.4)**	**-26 (-38, -16)**	0.9 (-5.3, 6.6)	3.0 (-11, 15)	-4.4 (-12, 2.5)	**9.2 (1.9, 16)**	7.0 (-1.1, 14)
	B	-5.4 (-16, 4.5)	**-22 (-38, -7.6)**	**-24 (-35, -13)**	1.7 (-4.5, 7.5)	6.4 (-6.6, 18)	-5.2 (-13, 1.8)	*7.4 (-0.1, 14)*	**8.9 (0.9, 16)**
Alcohol consumption (5 groups from low to high)[4]	A	0.6 (-4.2, 5.5)	-0.4 (-6.2, 5.8)	**5.1 (0.8, 9.7)**	3.5 (0.4, 6.6)	3.3 (-3.2, 10)	3.2 (-0.2, 6.8)	2.5 (-1.3, 6.6)	5.6 (1.3, 10)
	B	-1.0 (-5.7, 3.8)	-1.7 (-7.4, 4.4)	**5.0 (0.5, 9.6)**	3.8 (0.8, 6.9)	4.3 (-2.2, 11)	3.0 (-0.4, 6.4)	2.6 (-1.2, 6.6)	5.8 (1.5, 10)
Education level (5 groups from low to high)[3]	A	2.0 (-2.4, 6.6)	3.0 (-2.5, 8.7)	2.3 (-1.6, 6.3)	1.6 (-1.1, 4.4)	**7.0 (0.9, 14)**	1.4 (-1.7, 4.6)	3.3 (-0.3, 7.0)	**4.8 (0.9, 8.8)**
	B	2.4 (-2.0, 7.0)	2.4 (-3.1, 8.2)	0.1 (-3.8, 4.2)	0.4 (-2.3, 3.2)	4.4 (-1.5, 11)	0.3 (-2.7, 3.3)	1.6 (-1.9, 5.2)	**3.8 (0.0, 7.8)**
Parity									
1		*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*	*ref.*
2	A	-3.1 (-13, 7.9)	-6.7 (-19, 6.8)	8.1 (-1.8, 19)	7.2 (0.3, 15)	7.6 (-7.1, 25)	2.1 (-5.4, 10)	1.2 (-7.2, 10)	0.3 (-8.6, 10)
	B	-0.0 (-10, 11)	-4.6 (-17, 9.1)	8.0 (-2.0, 19)	6.0 (-0.8, 13)	3.9 (-10, 20)	1.6 (-5.6, 9.4)	2.6 (-5.7, 12)	1.6 (-7.2, 11)
Previous breastfeeding duration (per month)	A	**-1.3 (-2.3, -0.2)**	**-1.6 (-2.8, -0.3)**	**-2.3 (-3.2, -1.4)**	**-2.1 (-2.7, -1.4)**	**-3.5 (-4.9, -2.2)**	**-1.9 (-2.6, -1.2)**	**-2.7 (-3.6, -1.9)**	**-1.0 (-1.9, -0.1)**
	B	**-1.7 (-2.7, -0.6)**	**-1.8 (-3.1, -0.5)**	**-2.5 (-3.4, -1.6)**	**-2.1 (-2.8, -1.5)**	**-3.5 (-4.8, -2.1)**	**-2.0 (-2.7, -1.3)**	**-2.6 (-3.4, -1.8)**	**-1.3 (-2.1, -0.4)**
Time since last breastfeeding period (per year)	A	**4.8 (2.9, 6.8)**	**2.8 (0.5, 5.2)**	-0.5 (-2.1, 1.1)	-0.4 (-1.5, 0.7)	-0.6 (-3.0, 1.9)	-0.2 (-1.5, 1.0)	0.2 (-1.2, 1.7)	-0.01 (-1.6, 1.6)
	B	**4.8 (2.9, 6.8)**	*2.4 (-0.02, 4.8)*	-1.1 (-2.7, 0.6)	-0.7 (-1.9, 0.4)	-1.0 (-3.4, 1.5)	-0.1 (-1.4, 1.2)	0.7 (-0.8, 2.2)	-0.2 (-1.8, 1.4)

[1]Multivariable models were adjusted for all variables included in the table.

[2]Stratum-weighted: weights are the inverse probability of selection.

[3]Education level (ordinal): 1=<9 years, 2=9-11 years, 3=12 years, 4=higher education, non-university level, 5=higher education, university level.

[4]Alcohol consumption during pregnancy (ordinal): 0=never, 1=<once a month, 2=once a month, 3=2-3 times a month, 4=>once a week.

A: Un-weighted analysis, B: Stratum-weighted analysis

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Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case–cohort study

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BACKGROUND: The associations between prenatal exposure to endocrine disruptive chemicals (EDCs) and fetal growth are inconsistent, and few studies have considered small-for-gestational-age (SGA) birth as an outcome. Our current study of Scandinavian parous women aimed to address these inconsistencies and gaps in the literature.

METHODS: This case–cohort study included 424 mother–child pairs who participated in a prospective, multi-center study of parous women in Norway (Trondheim and Bergen) and Sweden (Uppsala). We used linear and logistic regression with 95% confidence intervals (CIs) to analyze the associations between two perfluoroalkyl substances (PFASs) and five organochlorines (OCs) from early second trimester and indices of fetal growth.

RESULTS: Among Swedish women, prenatal exposure to perfluorooctanoate (PFOA), polychlorinated biphenyl (PCB) 153 and hexachlorobenzene (HCB) were associated with higher odds for SGA birth. We found stronger associations among Swedish male offspring. In the Norwegian cohort, we found no significant associations between EDC exposure and indices of fetal growth.

CONCLUSIONS: Some populations may be more vulnerable to EDCs, possibly due to differences in exposure levels, exposure sources and/or modifiable lifestyle factors. Male offspring may be more vulnerable to endocrine disruption.

Fetal growth restriction (FGR) is defined as a pathologic inhibition of intrauterine fetal growth and failure to achieve the fetus' growth potential. FGR is associated with perinatal mortality and morbidity. Small-for-gestational-age (SGA) is a proxy for FGR and defined as birth weight below the 10th percentile for gestational age, sex, and parity (1).

Perfluoroalkyl substances (PFASs) and organochlorines (OCs) are persistent, bio-accumulative chemicals that have been detected in maternal blood during pregnancy and in cord blood at delivery. PFASs and OCs may act as endocrine disrupting chemicals (EDCs), and *in utero* exposure to EDCs may have consequential developmental effects on the fetus (2). A United Nations Environment Program/World Health Organization (UNEP/WHO) report from 2012 identifies human exposure to EDCs as an emerging challenge (3). Increased knowledge is required to ensure better protection for the most vulnerable members of society. Pregnant mothers and children are particularly vulnerable to developmental exposures like EDCs, and fetal programming may increase susceptibility to diseases later in life (3).

The associations between EDCs and indices of fetal growth have been studied in different populations, although results are inconsistent (4,5). Diet is considered an important exposure route for PFASs and OCs even in periods with simultaneous direct exposure from production and emission of chemicals (6). In Scandinavian populations, consumption of seafood have been associated with high PFAS and OC serum levels (7,8). The Baltic Sea, which is situated on the East coast of Sweden, has been vastly contaminated with OCs (9), and a study of births in Sweden from 1973–1991 found associations between high maternal intake of fish from the Baltic Sea and restricted fetal growth (10). However, most other studies report positive associations between higher maternal fish intake and better fetal growth (11). Dietary intake of fish from different water sources may result in population-specific EDC exposures. Taken together, source-specific fish intake may confound associations between EDCs and fetal growth.

Most observational studies have been underpowered to detect significant associations between EDCs and SGA birth, or only included birth weight as an outcome. A most recent systematic review of prenatal serum levels of PFASs and human fetal growth published in 2015, recommended that future EDC exposure studies should focus on SGA birth as an outcome of interest (4).

In the current study, we used a case–cohort design to study the association between maternal serum levels of PFASs and OCs and indices of fetal growth including birth weight, birth

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Received 23 March 2016; accepted 8 August 2016; advance online publication 26 October 2016. doi:10.1038/pr.2016.187

length, head circumference, and SGA birth in Scandinavian women from Norway and Sweden. We also explored possible effect modification by country and offspring sex.

RESULTS

Maternal and Offspring Baseline Characteristics

Overall, Swedish women had, on average, higher prepregnancy BMI (22 vs. 21 kg/m^2) compared to Norwegian women. A greater proportion of Swedish women were nonsmokers at the time of conception compared to their Norwegian peers (67 vs. 47% nonsmokers) (**Table 1**). The total cohort of Swedish mothers compared to Norwegian mothers did not differ significantly in other baseline characteristics (maternal age, height, education, parity, and interpregnancy interval). Swedish offspring were on average longer at birth compared to Norwegian offspring, but did not differ significantly in other characteristics (birth weight, head circumference, and gestational age).

Swedish women who gave birth to SGA offspring (SGA mothers) were on average shorter, had lower prepregnancy BMI, fewer years of education, and lower parity compared to Swedish women with non-SGA births (non-SGA mothers). A higher proportion of Swedish SGA mothers reported smoking at conception compared to Swedish non-SGA mothers (60 vs. 21% smokers). We observed a similar pattern in baseline characteristics when Norwegian SGA and non-SGA mothers were compared. Norwegian SGA mothers were also significantly younger than Norwegian non-SGA mothers (28 vs. 29 y of age). Swedish SGA offspring had, on average, lower birth weight, shorter birth length and smaller head circumference compared to Swedish non-SGA offspring. Norwegian SGA offspring had in addition significantly lower gestational age compared to the Norwegian non-SGA offspring.

PFAS and OC Levels

Overall, Swedish mothers had significantly higher median serum levels of PFOA (2.33 vs. 1.62 ng/ml), perfluoroctane sulfonate (PFOS) (16.4 vs. 9.74 ng/ml), PCB 153 (117 vs. 80.1 ng/g lipid), and β-HCH (25.0 vs. 21.2 ng/g lipid) compared to the total Norwegian cohort (**Table 2**, **Figure 1**). Swedish mothers had significantly lower median serum levels of *t*-NC (6.28 vs. 6.74 ng/g lipid) compared to Norwegian mothers (**Table 2**, **Figure 1**).

Median levels of PFOA and PCB 153 were significantly higher among Swedish SGA mothers compared to Swedish non-SGA mothers (**Figure 1**). Differences in levels of other EDCs (PFOS, *p,p'*-DDE, HCB, β-HCH and *t*-NC) were not statistically significant when Swedish SGA mothers and Swedish non-SGA mothers were compared (**Figure 1**).

We observed no significant differences in PFAS or OC levels between Norwegian SGA mothers and Norwegian non-SGA mothers.

Overall, there were medium to high correlations within the PFASs (ρ = 0.56–0.73) and within the OCs (ρ = 0.29–0.70) (**Supplementary Figure S1** online). However, the PFASs were not highly correlated with the OCs (ρ = −0.01–0.22) (**Supplementary Figure S1** online). The same pattern was observed in both Norwegian and Swedish cohorts.

Associations Between PFAS and OC Levels and Indices of Fetal Growth

In the pooled analyses including data from both Norway and Sweden, we found no significant associations between EDCs and indices of fetal growth after adjustment for important covariates (**Supplementary Table S1** online).

Table 3 shows associations between serum levels of PFASs and OCs and indices of fetal growth stratified by country of residence. In adjusted linear models for the Swedish cohort, birth weight decreased significantly by −359 g (95% CI: −596, −122) and −292 g (95% CI: −500, −84) per ln-unit increase in PFOA and PFOS, respectively. Birth length was also negatively associated with increasing levels of PFOA and PFOS. For each ln-unit increase in HCB levels, a significant decrease in head circumference (β = −1.0 cm (95% CI: −1.7, −0.2)) was observed after adjustment.

In multivariate logistic regression models for the Swedish cohort, we observed a significant increase in adjusted odds for SGA birth per ln-unit increase in PFOA (aOR = 5.25 (95% CI: 1.68–16.4)), PCB 153 (aOR = 5.59 (95% CI: 1.05–29.9)) and HCB (aOR = 5.62 (95% CI: 1.26–25.1)). We also observed increased aOR for SGA birth per ln-unit increase in PFOS (aOR = 2.51 (95% CI: 0.93–6.77)), although nonsignificant. Among Swedish women, there were no significant associations between levels of PFASs or OCs and gestational age (**Table 3**). Among Norwegian women, we observed no statistically significant associations between PFASs or OCs and indices of fetal growth (**Table 3**).

In the Swedish cohort, the *P* value for interaction between PFOA and infant sex was 0.046 in multivariate linear regression models with birth weight as an outcome. No other interaction terms between EDCs and infant sex were statistically significant ($P < 0.10$) (data not shown). In Swedish male offspring, we observed a significant decrease in birth weight (β = −526 g; 95% CI: −828, −222) and birth length (β = −1.6 cm; 95% CI: −2.9, −0.4) for each ln-unit increase in PFOA (**Table 4** and **Figure 2**). We also observed a significant increase in adjusted odds for SGA birth (aOR = 6.55; 95% CI: 1.14–37.5) among male Swedish offspring (**Table 4** and **Figure 2**). No statistically significant associations between PFOA and indices of fetal growth were observed among Swedish female offspring. We found no significant interaction terms between PFASs or OCs and offspring sex in the Norwegian cohort (data not shown).

DISCUSSION

In this case–cohort study of 424 Scandinavian parous women, we observed increased odds of SGA birth for each ln-unit increase in PFOA, PCB 153 and HCB, among Swedish but not Norwegian mothers. Increasing levels of PFOA, PFOS and HCB were negatively associated with other indices of fetal growth including birth weight, birth length and head circumference among the Swedish mothers. The associations

Table 1. Maternal and offspring baseline characteristics by country and SGA status

	Norway (Trondheim: *N* = 137, Bergen: *N* = 128)			Sweden (Uppsala: *N* = 159)		
	Non-SGA (*N* = 174)	SGA (*N* = 91)	Total (*N* = 265)	Non-SGA (*N* = 107)	SGA (*N* = 52)	Total (*N* = 159)
	Mean (95% CI) or *n* (%)	Mean (95% CI) or *n* (%)	Mean (95% CI) or *n* (%)	Mean (95% CI) or *n* (%)	Mean (95% CI) or *n* (%)	Mean (95% CI) or *n* (%)
Maternal characteristics						
High-risk group	0 (0)	74 (81)*	74 (28)	0 (0)	43 (83)*	43 (27)
10% random sample	174 (100)	17 (19)*	191 (72)	107 (100)	9 (17)*	115 (73)
Maternal age	29.3 (28.6–29.9)	27.9 (27.0–28.7)*	28.8 (28.3–29.3)	29.5 (28.7–30.3)	28.9 (27.5–30.4)	29.3 (28.6–30.0)
Maternal height	167.0 (166.1–167.9)	164.3 (163.2–165.4)*	166.1 (165.3–166.8)	166.6 (165.5–167.7)	164.2 (162.7–165.7)*	165.8 (164.9–166.7)
Maternal prepregnancy BMI	21.6 (21.2–22.0)	20.2 (19.7–20.7)*	21.1 20.8-21.4)	22.6 (22.1–23.1)	20.9 (20.2–21.6)*	22.1 (21.6–22.5)*
Education (years)						
9 or less	20 (12)	19 (21)*	39 (15)	16 (15)	15 (29)*	31 (20)
10–12	93 (53)	54 (60)	147 (56)	46 (44)	17 (33)	63 (41)
13 or more	61 (35)	17 (19)*	77 (29)	42 (40)	19 (37)	60 (39)
Smoking at conception (number of cigarettes)						
0	103 (59)	20 (22)*	123 (47)	85 (79)	21 (40)*	106 (67)*
1–9	21 (12)	14 (16)	35 (13)	4 (3.7)	3 (5.8)	7 (4.4)
10 or more	50 (29)	56 (62)*	106 (40)	18 (17)	28 (54)*	46 (29)
Parity						
1	122 (70)	65 (71)	186 (71)	66 (62)	39 (75)*	104 (66)
2	52 (30)	26 (29)	78 (29)	41 (38)	13 (25)	54 (34)
Interpregnancy interval (months)						
18 or less	43 (25)	14 (15)	57 (22)	26 (24)	20 (39)*	46 (29)
19–60	98 (56)	56 (62)	154 (58)	58 (54)	21 (40)	79 (50)
61 or more	33 (19)	21 (23)	54 (20)	23 (22)	11 (21)	34 (21)
Offspring characteristics						
Gender						
Male	87 (50)	47 (52)*	133 (50)	52 (49)	29 (56)*	81 (51)
Female	87 (50)	44 (48)	131 (50)	55 (51)	23 (44)	77 (49)
Weight (g)	3661 (3590–3732)	2882 (2815–2949)*	3402 (3335–3469)	3790 (3680–3900)	2891 (2820–2963)*	3503 (3403–3601)
Length (cm)	50.8 (50.5–51.1)	48.3 (47.9–48.8)*	50.0 (49.8–50.3)	51.4 (50.9–51.9)	48.4 (48.0–48.8)*	50.5 (50.1–50.8)*
Head circumference (cm)	35.4 (35.2–35.5)	34.0 (33.7–34.2)*	34.9 (34.7–35.1)	35.5 (35.2–35.8)	33.7 (33.4–33.9)*	34.9 (34.7–35.1)
Gestational age	40.0 (39.8–40.2)	39.7 (39.4–40.0)	39.9 (39.8–40.1)	40.2 (40.0–40.6)	39.5 (39.1–39.9)*	40.1 (39.9–40.3)

*__Total__ group: Significant difference between the total group in Norway vs. the total group in Sweden ($P < 0.05$) using Student *t*-test for normally distributed variables and Mann-Whitney *U*-test for categorical variables. **SGA**-group: Significant difference between SGA vs. non-SGA group within country strata ($P < 0.05$) using Student *t*-test for normally distributed variables and Mann-Whitney *U*-test for categorical variables.
SGA, small for gestational age.

between PFOA and SGA birth, birth weight, birth height and head circumference were stronger among male offspring in the Swedish cohort. We found no statistically significant associations between EDCs and indices of fetal growth among Norwegian women in this study population.

Birth weight, adjusted for gestational age at birth, is often used as a proxy for fetal growth in observational studies. However, adjusting for gestational age, which acts like a mediator in the association between birth weight and health outcomes, may introduce bias (12). SGA birth is a better marker for fetal growth restriction because it identifies babies with low birth weight accounting for the gestational age at birth. Only four studies have assessed associations between PFASs and SGA birth (13–16) with conflicting results. One study found increased odds for SGA with higher PFOS (13), and none found statistically significant associations with

Table 2. Wet-weight levels of PFASs, and wet-weight and lipid-adjusted levels of OCs in serum by country from the study population in the SGA-study, $N = 424$

	Norway		Sweden			
	Median (range)	AM (SD)	Median (range)	AM (SD)	LOD	%>LOD
Wet-weight (ng/ml)						
PFOA	1.62 (0.31–7.97)	1.83 (1.00)	2.33 (0.60–6.70)	2.42 (1.00)	0.03	100
PFOS	9.74 (0.95–59.6)	11.3 (7.02)	16.4 (2.28–55.2)	17.3 (7.45)	0.03	100
PCB 153	0.46 (0.17–1.30)	0.49 (0.18)	0.68 (0.25–1.40)	0.69 (0.21)	0.01	100
p,p'-DDE	1.20 (0.10–11.0)	1.65 (1.43)	1.30 (0.39–9.20)	1.62 (1.13)	0.09	100
HCB	0.10 (0.04–0.33)	0.11 (0.04)	0.10 (0.04–0.38)	0.11 (0.04)	0.04	100
t-NC	0.04 (0.01–0.14)	0.04 (0.02)	0.04 (0.01–0.10)	0.04 (0.02)	0.01	100
β-HCH	0.12 (<LOD-0.39)	0.13 (0.06)	0.13 (<LOD-0.76)	0.15 (0.08)	0.01	99
Lipid-adjusted (ng/g lipids)						
PCB 153	80.1 (31.0–212)	84.4 (28.4)	117 (45.4–212)	121 (32.8)	–	–
p,p'-DDE	209 (16.7–1791)	285 (238)	244 (71.2–1223)	284 (175)	–	–
HCB	17.2 (7.1–48.2)	18.4 (6.27)	18.3 (6.98–73.0)	19.2 (7.56)	–	–
t-NC	6.74 (2.37–25.2)	7.55 (3.52)	6.28 (1.82–17.3)	6.79 (2.79)	–	–
β-HCH	21.2 (<LOD-57.2)	22.7 (9.07)	25.0 (<LOD-134)	26.5 (13.5)	–	–

AM, arithmetic mean; HCB, hexachlorobenzene; LOD, limit of detection; %>LOD, percentage of samples over limit of detection. Levels below LOD were set to LOD/√2; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluoroctane sulfonate.

PFOA. In contrast, we observed increased odds for SGA birth with increasing levels of PFOA in our Swedish study population. Our finding of increased odds for SGA birth with increasing levels of PCB 153 in the Swedish cohort has not previously been reported, but is consistent with a recent meta-analysis that established an association between PCB 153 and birth weight (5). The increased odds for SGA birth with increasing levels of HCB among our Swedish women has not been established in previous studies.

The possible biological mechanisms for the effects of PFASs and OCs on fetal growth are uncertain, but their endocrine-disruptive properties may be involved. Normal development is highly dependent on thyroid and sex steroid hormones, and the prenatal period is a critical period for hormonal changes. Thyroid hormones are important for somatic growth and are involved in differentiation and functions of target tissues during development (17). Prenatal estrogens are also important in promoting fetal growth (18). Both PFASs and OCs have been associated with lower levels of circulating thyroid hormones (19,20). Competitive binding to estrogenic receptors, disruption of enzymes or inhibition of the effects of endogenous estrogens are hypothesized actions of estrogenic and antiestrogenic PCB congeners, and may lead to inhibition of fetal growth (21). Moreover, human *in vitro* studies have found that PFASs interfere with the estrogen and androgen receptor (22). Since sex steroids might be disrupted by PFASs and OCs, there could be different effects in male and female fetuses which may explain our findings of stronger negative associations between PFOA and indices of restricted fetal growth among male but not female offspring in the Swedish cohort. Our sex specific finding differs from a Japanese study from 2009 that showed stronger negative associations between PFOS and birth weight among girls (n = 428 mother–child pairs) (23). The biological mechanisms for the sex difference in the toxicity for PFOA remains to be established, and it is possible that decreasing the sample size by stratifying our groups by sex reduced the precision of our results. Thus, further studies are warranted to elucidate any sex-related differences in fetal growth indices based on *in utero* exposure to PFASs.

The conflicting estimates on fetal growth between the Norwegian and Swedish cohort may be explained by differences in exposure levels and heterogeneity in exposure routes. Seafood and particularly fatty fish have been associated with high PFAS and OC levels in Scandinavian populations (7,8). In our study, Norwegian women with fish consumption > 50 g/d in the second trimester had 20 and 19% higher PFOA and PFOS levels, respectively, compared to women who did not eat fish (**Supplementary Table S2** online). However, other food items, like meat, may be more important in other populations with less fish intake (24). Moreover, we cannot discount the possibility of differences in dietary patterns between Scandinavian countries, which may partially account for our results. Since fish consumption is associated with both EDC levels and fetal growth, this may introduce bias in epidemiological studies. A recent report from the Norwegian Scientific Committee for Food Safety concluded that overall, there is a *beneficial* effect of fish consumption on birth weight, because fatty acids and vitamins important for enhancing fetal growth outweigh potential negative effects from EDCs (11). However, it is important to note that this evaluation was based on evidence from studies conducted when EDC levels were lower than the EDC levels found during our study period (1986–88). Our estimates in the Norwegian cohort did not substantially change when we adjusted for fish consumption (data not

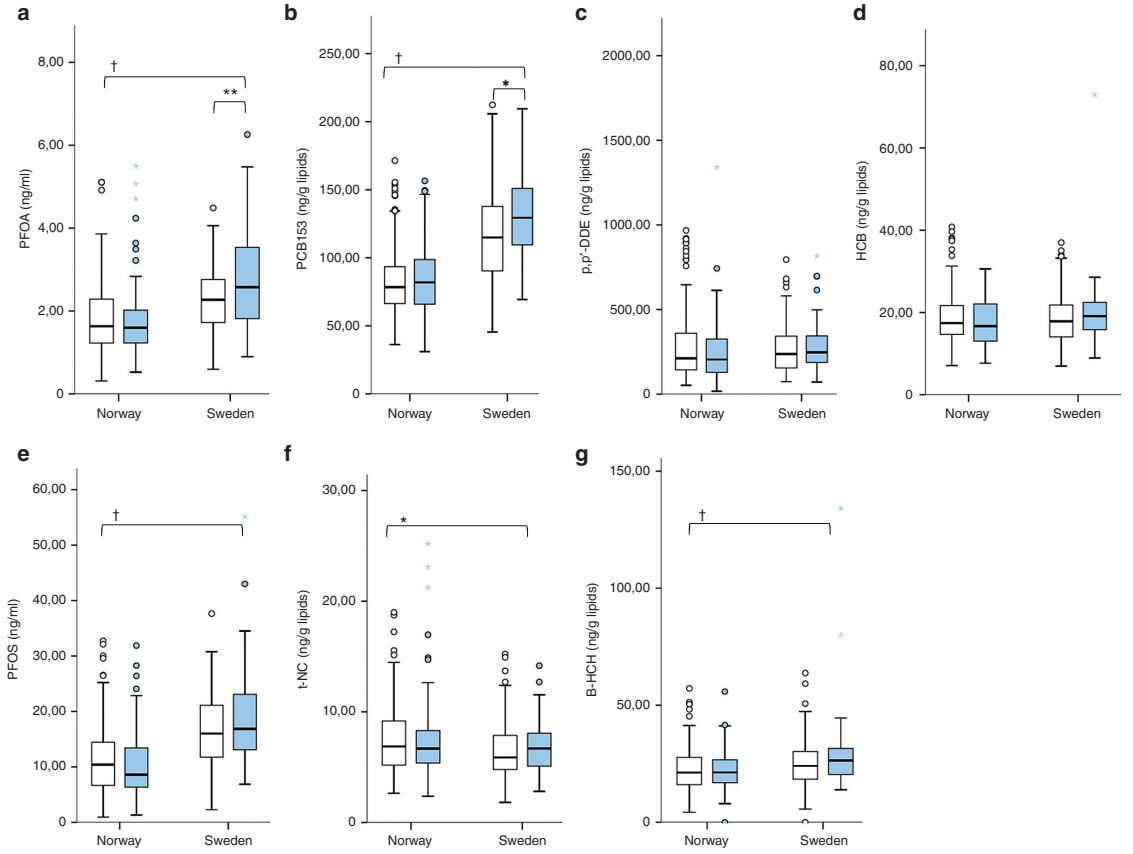

Figure 1. Levels of perfluoroalkyl substances (ng/ml) and organochlorines (ng/g lipids) analyzed in maternal serum in second trimester, stratified by country of residence and SGA status (**a**: PFOA, **b**: PCB 153, **c**: *p,p′*-DDE, **d**: HCB, **e**: PFOS, **f**: *t*-NC, **g**: *β*-HCH). White boxes: non-SGA mothers, light blue boxes: SGA-mothers. Boxes represent the 25th-75th percentiles, horizontal lines represents the median, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, respectively, and outliers are represented as data points. *$P \leq 0.05$, **$P \leq 0.01$, and †$P \leq 0.001$ for comparisons of levels between countries and between SGAs and non-SGAs within each country. HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; SGA, small for gestational age.

shown). Seasonal or daily variations in dietary intake of fish may introduce differential misclassification bias when using 3 d dietary intake as a measure of the overall consumption pattern, and we may not be able to capture the "real" fish eaters by this approach. We did not have information on fish consumption in the Swedish cohort.

In addition to different dietary patterns between populations and temporal trends of EDCs, there might be differences in contamination levels between fish sources. Studies have shown high OC contamination of the fish in the Baltic Sea on the East Coast of Sweden (9). Another study (1973–1991) that compared fishermen's pregnant wives from the East and West coast of Sweden, found that higher fish consumption from the East Coast resulted in higher maternal serum levels of PCB 153, which again resulted in increased risk of having an infant with low birth weight (25).

Smoking is often closely associated with socio-economic, dietary and lifestyle variables. In the Swedish cohort, smokers had 20% higher PFOA levels compared to nonsmokers (**Supplementary Table S2** online). Whereas, in the Norwegian cohort, smokers had 19% lower PFOS levels compared to nonsmokers. The fact that PFASs were higher among smokers in Sweden, but lower among smokers in Norway, speaks against a potential direct biologic effect of smoking on PFAS levels. In addition, we found no evidence of stronger associations between PFASs and fetal growth among the smokers compared to the non-smokers in Norway or Sweden, respectively. Taken together, this suggests that there might be differences in socio-economic status, lifestyle, and dietary variables between the women with high PFAS levels in Norway and Sweden, respectively, which will further confound the analyses. Problems with individual and local differences in EDC levels will be greater during time periods with continued use of contaminants, and possible contamination of the drinking water with PFASs in Sweden (26) may account

Table 3. Beta coefficients (β) and odds ratios (ORs) with 95% confidence intervals (95% CI) for associations between PFAS and organochlorines and indices of fetal growth (Norway: *N* = 265, Sweden: *N* = 159)

	Birth weight (g)[a]		Birth length (cm)[a]		Head circumference[a] (cm)		Gestational age[a] (weeks)		SGA[b]	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	OR (95% CI)	*P*
Norway										
PFOA	37 (−99, 174)	0.590	−0.1 (−0.7, 0.4)	0.656	0.2 (−0.2, 0.5)	0.354	−0.2 (−0.6, 0.2)	0.431	0.66 (0.33–1.33)	0.246
PFOS	74 (−31, 178)	0.167	−0.0 (−0.4, 0.4)	0.987	0.2 (−0.1, 0.4)	0.189	−0.01 (−0.3, 0.3)	0.952	0.71 (0.42–1.20)	0.201
PCB 153	100 (−104, 304)	0.334	−0.1 (−1.0, 0.7)	0.772	0.2 (−0.3, 0.7)	0.507	0.1 (−0.5, 0.7)	0.693	0.70 (0.25–1.98)	0.502
p,p'-DDE	46 (−47, 139)	0.327	0.2 (−0.2, 0.6)	0.306	0.1 (−0.2, 0.3)	0.531	−0.04 (−0.3, 0.2)	0.747	0.73 (0.46–1.15)	0.174
HCB	61 (−132, 255)	0.534	0.1 (−0.7, 0.9)	0.792	0.1 (−0.4, 0.6)	0.626	−0.1 (−0.6, 0.5)	0.756	0.52 (0.20–1.34)	0.176
B-HCH	−73 (−231, 86)	0.368	−0.5 (−1.1, 0.2)	0.152	−0.1 (−0.5, 0.3)	0.614	−0.1 (−0.6, 0.3)	0.592	0.76 (0.33–1.71)	0.500
t-NC	98 (−56, 253)	0.212	0.2 (−0.5, 0.8)	0.605	0.4 (−0.02, 0.8)	0.065	0.2 (−0.2, 0.7)	0.383	0.86 (0.40–1.84)	0.699
Sweden										
PFOA	−359 (−596, −122)	0.003	−1.3 (−2.3, −0.3)	0.010	−0.4 (−1.0, 0.1)	0.115	−0.3 (−0.9, 0.3)	0.318	5.25 (1.68–16.4)	0.004
PFOS	−292 (−500, −84)	0.006	−1.2 (−2.1, −0.3)	0.007	−0.4 (−0.9, 0.04)	0.073	−0.4 (−0.9, 0.2)	0.201	2.51 (0.93–6.77)	0.068
PCB 153	−11 (−374, 352)	0.953	0.1 (−1.4, 1.6)	0.891	−0.2 (−1.1, 0.6)	0.558	0.6 (−0.4, 1.6)	0.222	5.59 (1.05–29.9)	0.044
p,p'-DDE	38 (−147, 223)	0.688	0.3 (−0.4, 1.1)	0.400	0.2 (−0.2, 0.7)	0.273	0.4 (−0.04, 0.9)	0.072	1.70 (0.75–3.85)	0.200
HCB	−269 (−595, 57)	0.105	−1.0 (−2.4, 0.3)	0.134	−1.0 (−1.7, −0.2)	0.011	0.1 (−0.7, 1.0)	0.742	5.62 (1.26–25.1)	0.024
B-HCH	−161 (−446, 125)	0.268	−0.4 (−1.6, 0.8)	0.537	−0.4 (−1.1, 0.2)	0.209	0.1 (−0.7, 0.8)	0.852	3.20 (0.84–12.1)	0.087
t-NC	−92 (−379, 195)	0.529	−0.2 (−1.4, 1.0)	0.766	−0.4 (−1.1, 0.2)	0.213	0.3 (−0.5, 1.1)	0.452	2.04 (0.64–6.55)	0.229

[a]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m^2), education (<9, 9–12, >12 y), parity (1 or 2), smoking status at conception (0, 1–9, >10 cig/d), interpregnancy interval (<18, 19–60, >60 mo) and offspring sex (male/female). [b]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m^2), education (<9, 9–12, >12 y), smoking status at conception (0, 1–9, >10 cig/d), and interpregnancy interval (<18, 19–60, >60 mo).
HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

Table 4. Beta coefficients (β) and odds ratios (ORs) with 95% confidence intervals (95% CI) for associations between PFOA and indices of fetal growth in Sweden by offspring sex (*N* = 159)

		Birth weight (g)[a]		Birth length (cm)[a]		Head circumference[a] (cm)		Gestational age[a] (weeks)		SGA[b]	
		β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	OR (95% CI)	*P*
PFOA	*N*										
Boys	81	−526 (−828, −222)	0.001	−1.6 (−2.9, −0.4)	0.012	−0.6 (−1.3, 0.1)	0.103	−0.4 (−1.2, 0.5)	0.365	6.55 (1.14–37.45)	0.035
Girls	78	−156 (−541, 228)	0.419	−0.8 (−2.4, 0.8)	0.340	−0.1 (−1.0, 0.7)	0.728	−0.1 (−1.1, 0.9)	0.802	4.73 (0.79–28.3)	0.089

[a]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m^2), education (<9, 9–12, >12 y), parity (1 or 2), smoking status at conception (0, 1–9, >10 cig/d), inter-pregnancy interval (<18, 19–60, >60 mo), and offspring sex (male/female). [b]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m^2), education (<9, 9–12, >12 y), smoking status at conception (0, 1–9, >10 cig/d), and interpregnancy interval (<18, 19–60, >60 mo).
PFOA, perfluorooctanoate.

for some differences between populations. Although we did not find evidence of effect modification by smoking in our study, the high proportion of smokers in our study population may introduce bias and thereby underestimate true effects of the contaminants. In addition, due to the strong associations between smoking and fetal growth, the true effects of EDCs could be overshadowed and hard to detect, even in adjusted analyses.

Assessing potential causal effects of multiple EDCs on health outcomes are complicated because of the complex correlation of exposures. Associations found in some studies may largely reflect correlated EDC exposures instead of a specific EDC under study. In our study, we found moderate or high correlations within the PFASs (ρ = 0.56–0.73) and within the OCs (ρ = 0.35–0.70) (**Supplementary Figure S1** online), but only weak correlations between the PFASs and OCs (ρ = −0.01–0.22). The lack of correlation between PFASs and OCs made it possible to distinguish between the independent effects of PFASs and OCs. When assessing individual EDCs, we demonstrated positive associations between PFOA, PCB 153 and HCB and SGA birth (**Table 3**). However, the only association that remained statistically significant when including the other contaminants as covariates in final models was the association between PFOA and SGA birth (**Supplementary Table S3** online). This further confirms that the association between PFOA and SGA birth is not explained by correlated exposures. The association between HCB and SGA birth was also significant when we adjusted for PFOA. Based on these analyses, it seems like both PFOA and HCB are independently associated with SGA birth. Accounting for correlations within and between different EDCs strengthens our findings by distinguishing between contaminants

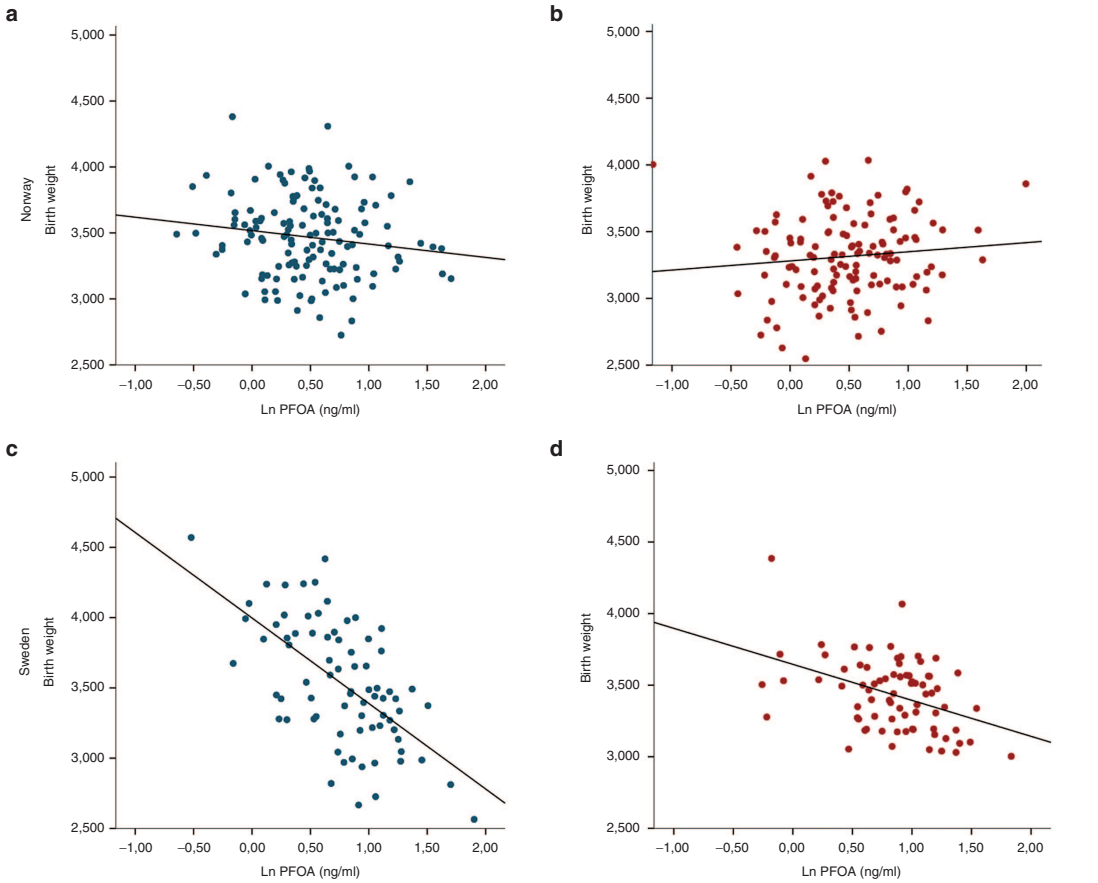

Figure 2. Scatter plot with ln (PFOA (ng/ml)) values on the X-axis and adjusted values of birth weight (g) on the Y-axis, stratified by country and offspring sex. Blue dots: boys, red dots: girls. (**a**) Norwegian boys, (**b**) Norwegian girls, (**c**) Swedish boys, (**d**) Swedish girls. PFOA, perfluorooctanoate.

that may have independent causal effects on restricted fetal growth.

This study has several strengths including a large, homogeneous population of mother–child pairs (n = 424). Ours is one of few studies to investigate a variety of PFAS and OC exposures. Since PFASs and OCs were not highly correlated, our results show an independent association between PFOA and fetal growth. The unique design of this case-cohort study accounts for a high proportion of SGA births as a marker for FGR, rather than birth weight alone. Therefore, this study has greater power to detect clinically relevant associations between EDCs and FGR using SGA birth as an outcome. We further tested the generalizability of our results by excluding the women from the high-risk group in all the linear and logistic models, and the associations in the Swedish cohort persisted (data not shown). The use of lipid-adjusted serum levels of OCs may be prone to bias in epidemiological studies (27). We therefore analyzed all linear and logistic models using wet weight values adjusted for total lipids as an independent variable with no considerable change in point estimates (data not shown). Maternal weight gain during pregnancy is suggested to be an important confounder in studies with lipophilic chemicals, but mainly in studies using serum from late pregnancy or cord blood (28). Since maternal weight gain also represents blood volume expansion, it may also be an important confounder in studies with PFASs, which largely are bound to albumin in the blood (4). Alcohol consumption may also be a confounder because it has been associated with higher levels of OCs (29). However, sensitivity analyses revealed that the estimates did not change when we included weight gain or alcohol consumption as covariates in the analyses (**Supplementary Tables S4 and S5** online).

This study also has some weaknesses. We have not corrected for multiple comparisons, which increases the chance of a rare event and enables false-positive results (i.e., Type I error). Measures of renal filtration have been proposed to be important confounders in studies with PFASs and fetal growth (30). Unfortunately, we only had glomerular filtration

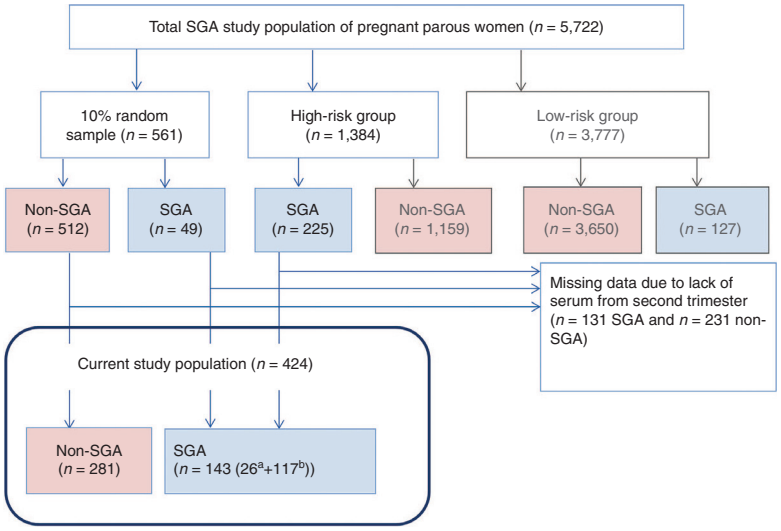

Figure 3. Flow chart of the study selection. [a]Number of participants from the 10% random sample. [b]Number of participants from the high-risk group.

rate (GFR) for 88 women, and could not include GFR as a covariate in adjusted models due to problems with missing data. Primiparous women were not eligible for study inclusion, which may contribute to some selection bias. However, excluding these women may largely reduce confounding due to parity because first time mothers have a higher risk of delivering small babies (31). At the same time, primiparous women may have higher serum EDC levels, because they lack previous excretion through placenta and breastmilk (2). Persistent and bio-accumulative chemicals with the same properties are highly correlated. Therefore, our point estimates may be subject to residual confounding due to some unmeasured chemicals (e.g., lead) in our analyses.

Conclusion

We found higher odds for SGA birth with increasing serum levels of PFOA, PCB 153 and HCB, but only in the Swedish cohort. Both PFOA and HCB were associated with SGA birth after accounting for correlation between EDCs. We observed stronger associations between increasing levels of PFOA and SGA birth in male offspring from the Swedish cohort. Associations between EDCs and indices of fetal growth in the Norwegian cohort were null. Our results suggest that some populations may be more vulnerable to EDCs, possibly due to differences in EDC levels, exposure sources and/or potentially modifiable lifestyle factors. The study also suggests that male offspring may be more vulnerable to endocrine disruption than female offspring.

METHODS

Ethics

The project is approved by the Central Norway Regional Committee for Medical and Health Science Research Ethics (REK Midt 2010/1449-5).

Study Population

Participants were from the US National Institute of Child Health and Human Development (NICHD) Scandinavian Successive Small-for-Gestational Age (SGA) births study; a population-based prospective multicenter study conducted in Trondheim and Bergen (Norway) and Uppsala (Sweden). Participant recruitment occurred over a 27-mo period (1986–1988). The SGA births study was designed to study the etiology and consequences of intrauterine growth restriction (32). Since the study also examined the tendency for individual women to repeat an SGA birth outcome in consecutive pregnancies, first time mothers were ineligible for study participation. Eligible participants were second and third time mothers of Caucasian origin who spoke one of the Scandinavian languages, had a singleton pregnancy, and were registered by the study center prior to the 20th gestational week. In total, we recruited 5,722 women, from which we defined three groups: a 10% random sample representative of the parous population at each study site (n = 561); a group at high risk for SGA birth (n = 1,384), and a low risk group (n = 3,777) (**Figure 3**). Both the random sample and high-risk group were included for detailed follow-up throughout pregnancy and at birth. The high risk group was defined by one or more of the following risk factors: (i) a prior SGA or low-birth-weight (LBW) child, (ii) maternal cigarette smoking at conception, (iii) low prepregnancy weight (<50 kg), (iiii) a previous perinatal death, or (iiiii) the presence of chronic maternal disease including essential hypertension or renal disease. In the current study, we used a case–cohort design (33) to estimate the associations between prenatal EDC exposure and indices of fetal growth. In total, 143 SGA births were selected as cases, and 281 non-SGA controls were selected from the 10% random sample group (total n = 424) (**Figure 3**). Selection was based on the availability of second trimester maternal serum samples.

Exposure Assessment

Serum samples were collected in second trimester (gestational week 17–20) in the SGA study (1986–1988) and serum was stored at −80 °C for later analysis. All 424 samples were included for PFAS and OC analyses. All chemicals presented were detected in 100% of the samples, except β-hexachlorohexane (β-HCH) that was detected in 99%. Limits of detection (LODs) are listed in **Table 2**, and values below LOD were replaced by LOD/$\sqrt{2}$.

Chemical Analyses of PFAS

Analyses were performed at the laboratories of Norwegian Institute for Air Research, Tromsø, Norway (NILU). All serum samples were

quantified for two target analytes; perfluorooctanoate (PFOA) and PFOS. They were analyzed using sonication-facilitated liquid-liquid extraction, activated ENVI-carb clean-up (34), quantified and analyzed by ultrahigh pressure liquid chromatography triple-quadruple mass-spectrometry (UHPLC-MS/MS). Sample preparation and extraction were performed as described by Hanssen *et al* (35) except for minor volume changes. The quantification was conducted with the LC Quan software, version 2.6.0 (Thermo Fisher Scientific, Waltham, MA). The internal-standard addition method with isotope-labeled PFASs was used to quantify the contaminants (35). Participation in the AMAP Ring Test (36) indicates that the uncertainties of the analysis are within ± 15–20% of the assigned values.

Chemical Analyses of OCs

OCs were analyzed at the Institut National de Santé Publique du Quebec, Centre Toxicologie, Quebec. This laboratory is the organizer of the AMAP Ring Test (36). OCs measured were hexachlorobenzene (HCB), oxychlordane, polychlorinated biphenyl (PCB) 52, 101, 118, 153, 156, 170, and 180, *p,p*'-dichlorodiphenyldichloroehylene (*p,p*'-DDE), *p,p*'-dichlorophenyltrichloroethane (*p,p*'-DDT), *β*-hexachlorohexane (*β*-HCH) and *trans*-nonachlor (*t*-NC). We chose to report PCB 153 representing total PCBs, and excluded *p,p*-DDT because of low detection limit (>50% of samples <LOD). In short, 0.5–1 ml serum sample was extracted using hexane (2 × 6 ml), ethanol (2 ml) and saturated ammonium sulphate solution (2 ml), a slight modification of Sandanger *et al.* (37).

Outcome Assessment

Indices of fetal growth including birth weight (continuous; grams (g)), birth length (continuous; centimeters (cm)) and head circumference (continuous; cm) were measured and recorded at birth. Gestational age (continuous; completed weeks) was determined by ultrasound scan at 17 wk of gestation. SGA birth was defined as birth weight below the 10th percentile adjusted for gestational age, parity and sex of child (32).

Covariates

Based on prior knowledge of PFAS and OC properties, and known risk factors for SGA birth, we included potential confounders from data collected at first study visit in gestational week 17. These involved maternal age (continuous; years), maternal height (continuous; cm), maternal prepregnancy BMI (continuous; kg/m^2), education level (categorical; 9 y or less, 10–12 y, or 13 y or more), smoking status at conception (categorical; 0, 1–9 or >10 cigarettes per day), parity (binary; 1 or 2) and interpregnancy interval (categorical; 18 mo or less, 19–60 mo, 61 mo or more). We categorized the interpregnancy interval based on a known J-shaped association to adverse perinatal outcomes including restricted fetal growth (38). Offspring sex (male/female) was registered at birth. Weight gain up to 17 wk was calculated based on recorded weight measurements done by midwives at the regular prenatal visits throughout pregnancy. Alcohol consumption was self-reported in gestational week 33. Dietary information from the Norwegian women consisted of a self-reported food frequency questionnaire over a 3-d period in week 17 and 33.

Statistical Analyses

PFAS and OC levels were logarithmically (ln) transformed to obtain normal distribution. We used wet weight concentrations of PFAS and lipid-adjusted serum concentrations of OCs (39). Total lipid values were calculated based on measurements of triglycerides and cholesterol:

Total lipids = 1.33*triglycerides + 1.12*cholesterol + 1.48 (g/l) (39). This formula showed good correlation with complete formulas including phospholipids (40).

In a complete case analysis we used uni- and multivariate linear regression with 95% confidence intervals (CIs) to estimate the adjusted associations between natural log-transformed (ln) serum levels of seven individual EDCs (*PFASs:* PFOA, PFOS; *OCs:* PCB153, *p,p*'-DDE, HCB, *t*-NC, and *β*-HCH) and indices of fetal growth including birth weight, birth length, head circumference and gestational age at birth. We evaluated linear model assumptions using diagnostic plots of the residuals. In all linear regression analyses we adjusted for the same covariates (maternal age, maternal height, prepregnancy BMI, maternal education level, smoking at conception, parity, interpregnancy interval, and offspring sex). We conducted uni- and multivariate logistic regression to estimate the crude and adjusted odds ratios (ORs and aORs) for SGA birth per unit increase in natural log-transformed (ln) serum levels of each PFAS and OC. In all the logistic regression analyses we adjusted for the same covariates (maternal age, maternal height, prepregnancy BMI, maternal education level, smoking at conception, and interpregnancy interval). SGA birth was defined as birthweight below the 10th percentile adjusted for gestational age, parity and offspring sex; hence, we did not adjust for parity or offspring sex in the models with SGA birth as an outcome. Based on the evidence of effect modification by geography, we stratified our results by country of residence. In multivariate logistic models with SGA as outcome variable, we found significant *P* values for interaction between the different EDCs and country of residence (PFOA $P = 0.043$; PCB 153 $P = 0.073$; HCB $P = 0.008$). In multivariate linear regression models with birth weight as an outcome, *P* values for the interaction term were 0.004, 0.001, and 0.036 for PFOA, PFOS, and HCB, respectively. Within country-specific strata, we further considered possible effect modification by offspring sex, because proposed endocrine disrupting properties might be sex-specific. Missing data was less than 10% for included covariates. All statistical analyses were conducted with SPSS statistical software, version 22 (IBM SPSS, Chicago, IL).

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/pr

ACKNOWLEDGMENTS

We gratefully acknowledge the participating women in the SGA-study. We would like to thank people at NILU – Norwegian Institute of Air Research in Tromsø, Norway, for conducting the PFAS analyses, and Institut National de Santé Publique du Québec, Centre de Toxicologie in Quebec, for the OC analyses. Special thanks to Charlotta Rylander, Therese H. Nøst, and Vivian Berg, for valuable input and advice.

STATEMENT OF FINANCIAL SUPPORT

This work was funded by grants from the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology.

Disclosure: The authors have nothing to disclose.

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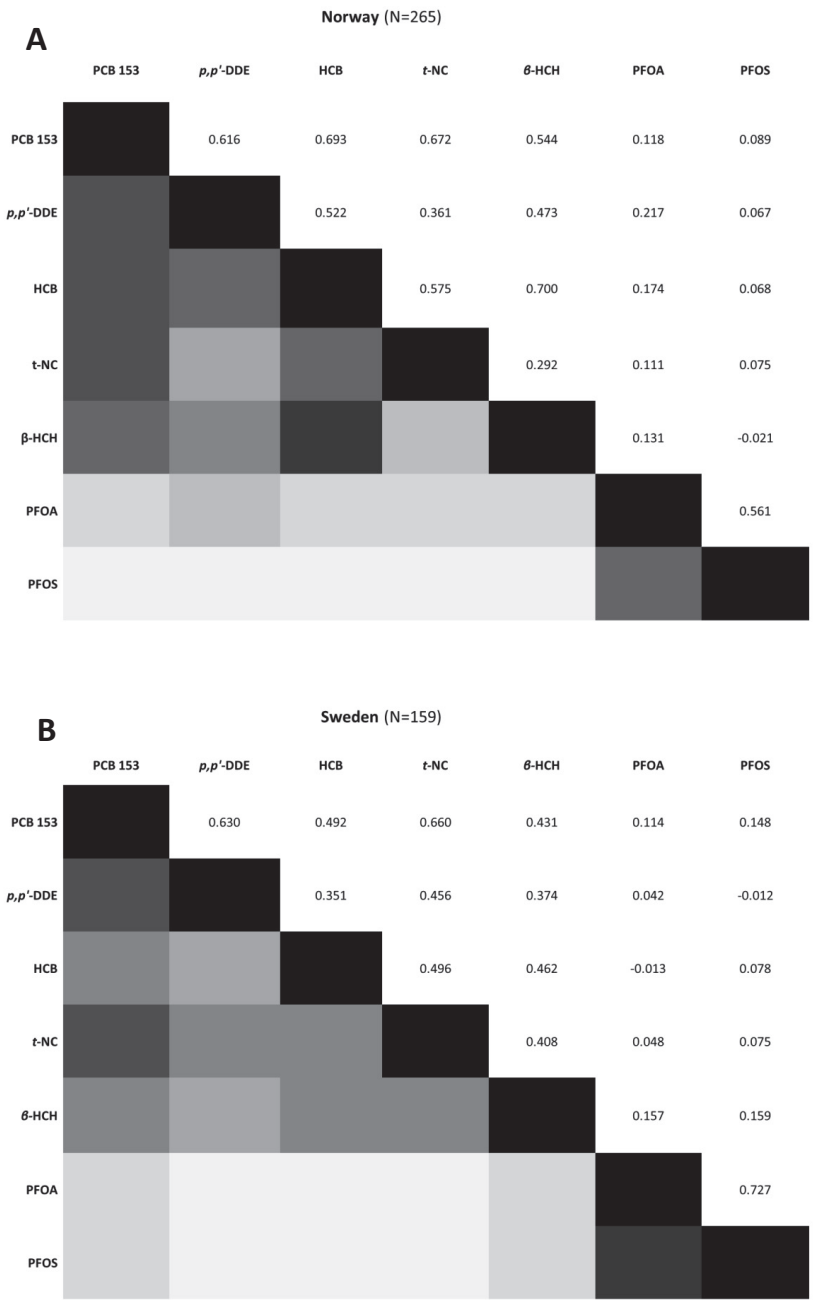
Supplemental Figure S1. Pearson's corelation coefficients based on levels of 5 OCs and 2 PFASs measured in 424 serum samples. The heat map depicts the correlation (darker shades of grey indicate stronger correlation). A: Norway. B: Sweden.



Supplemental Table S1: Beta coefficients (β) and odds ratios (ORs) with 95% confidence intervals (95% CI) for associations between PFAS and OCs and indices of fetal growth (N=424)

	Birth weight (g)[a]		Birth length (cm)[a]		Head circumference[a] (cm)		Gestational age[a] (weeks)		SGA[b]	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	OR (95% CI)	p
PFOA	-81.7 (-202, 39.2)	0.185	-0.49 (-0.99, 0.02)	0.060	-0.02 (-0.32,0.27)	0.878	-0.20 (-0.34, 0.14)	0.255	1.21 (0.69-2.11)	0.514
PFOS	-15.1 (-111, 80.7)	0.757	-0.30 (-0.70, 0.10)	0.139	0.04 (-0.19, 0.27)	0.748	-0.07 (-0.34, 0.20)	0.601	0.95 (0.62-1.48)	0.833
PCB 153	68.4 (-112, 248)	0.456	-0.05 (-0.80, 0.70)	0.902	0.03 (-0.40, 0.47)	0.888	0.27 (-0.23, 0.77)	0.289	1.16 (0.50-2.66)	0.731
***p,p'*-DDE**	48.7 (-35.9, 133)	0.258	0.26 (-0.10, 0.60)	0.156	0.11 (-0.09, 0.32)	0.278	0.10 (-0.14, 0.34)	0.417	0.84 (0.58-1.23)	0.370
HCB	-48.1 (-217, 121)	0.575	-0.23 (-0.93, 0.47)	0.518	-0.23 (-0.64, 0.18)	0.270	0.02 (-0.45, 0.50)	0.920	1.10 (0.52-2.34)	0.808
***t*-NC**	44.6 (-93.7, 183)	0.527	0.08 (-0.50, 0.66)	0.789	0.17 (-0.17, 0.50)	0.328	0.26 (-0.12, 0.65)	0.183	1.15 (0.62-2.15)	0.654
***β*-HCH**	-103 (-242, 37.4)	0.150	-0.46 (-1.05, 0.13)	0.125	-0.16 (-0.50, 0.18)	0.353	-0.03 (-0.43, 0.36)	0.872	1.27 (0.66-2.45)	0.468

[a] Adjusted for maternal age (years), height (cm), pre-pregnancy BMI (kg/m^2), education (<9, 9-12, >12 years), parity (1 or 2), smoking status at conception (0, 1-9, >10 cig/day), inter-pregnancy interval (<18, 19-60, >60 months) and offspring sex (male/female).

[b] Adjusted for maternal age (years), height (cm), pre-pregnancy BMI (kg/m^2), education (<9, 9-12, >12 years), smoking status at conception (0, 1-9, >10 cig/day) and inter-pregnancy interval (<18, 19-60, >60 months).

Supplemental Table S2: Associations between lifestyle variables and ln-transformed maternal PFAS (ng/ml) levels in serum collected in 2nd trimester by country of residence

	Norway					Sweden				
		PFOA		PFOS			PFOA		PFOS	
	N	% change (95% CI)	p	% change (95% CI)	p	N	% change (95% CI)	p	% change (95% CI)	p
Smoking status at conception										
Non-smoking	123	*ref.*		*ref.*		106	*ref.*		*ref.*	
Smoking	142	-4.4 (-16, -7.0)	0.448	-19 (-33, -4.5)	0.010	53	20 (6.2, 34)	0.005	4.1 (-12, 20)	0.602
Fish intake week 17[a]										
0 g	116	*ref.*		*ref.*		-	-	-	-	-
10-50 g	90	13 (-0.4, 25)	0.057	8.0 (-7.9, 24)	0.318	-	-	-	-	-
>50 g	59	20 (0.5, 35)	0.009	19 (0.7, 37)	0.042	-	-	-	-	-

[a] gram consumed of lean and fatty fish, shellfish and fish spread in week 17.

Supplemental Table S3. Beta coefficients (β) and odds ratios (ORs) with 95% confidence intervals (95% CI) for associations between PFOA, HCB and PCB 153 and indices of fetal growth in Sweden (N=159)

	Birth weight (g)[a]		Birth length (cm)[a]		Head circumference[a] (cm)		Gestational age[a] (weeks)		SGA[b]	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p	OR (95% CI)	p
PFOA										
Model A	-359 (-596, -122)	0.003	-1.3 (-2.3, -0.3)	0.010	-0.4 (-1.0, 0.1)	0.115	-0.3 (-0.9, 0.3)	0.318	5.25 (1.68-16.4)	0.004
Model B[c]	-346 (-582, -109)	0.005	-1.2 (-2.2, -0.3)	0.014	-0.4 (-0.9, 0.2)	0.158	-0.3 (-1.0, 0.3)	0.306	5.18 (1.59-16.9)	0.006
Model C[d]	-349 (-584, -113)	0.004	-1.3 (-2.2, -0.3)	0.013	-0.4 (-0.9, 0.1)	0.148	-0.3 (-1.0, 0.3)	0.294	5.05 (1.54-16.5)	0.007
HCB										
Model A	-269 (-595, 57)	0.105	-1.0 (-2.4, 0.3)	0.134	-1.0 (-1.7, -0.2)	0.011	0.1 (-0.7, 1.0)	0.742	5.62 (1.26-25.1)	0.024
Model B[e]	-233 (-551, 86)	0.152	-0.9 (-2.2, 0.4)	0.188	-0.9 (-1.6, -0.2)	0.015	0.2 (-0.7, 1.1)	0.684	5.08 (1.08-23.9)	0.039
Model C[f]	-486 (-930, -43)	0.032	-2.1 (-3.9, -0.2)	0.031	-1.5 (-2.5, -0.5)	0.003	-0.4 (-1.7, 0.8)	0.489	3.93 (0.47-32.9)	0.206
PCB 153										
Model A	-11 (-374, 352)	0.953	0.1 (-1.4, 1.6)	0.891	-0.2 (-1.1, 0.6)	0.558	0.6 (-0.4, 1.6)	0.222	5.59 (1.05-29.9)	0.044
Model B[g]	24.4 (-330, 378)	0.892	0.2 (-1.2, 1.7)	0.756	-0.2 (-1.0, 0.6)	0.627	0.6 (-0.3, 1.6)	0.197	4.45 (0.78-25.3)	0.093
Model C[h]	400 (-86, 889)	0.109	1.8 (-0.2, 3.9)	0.081	1.0 (-0.1, 2.1)	0.084	1.0 (-0.4, 2.3)	0.161	1.53 (0.14-17.3)	0.731

[a] Adjusted for maternal age (years), height (cm), pre-pregnancy BMI (kg/m^2), education (<9, 9-12, >12 years), parity (1 or 2), smoking status at conception (0, 1-9, >10 cig/day), inter-pregnancy interval (<18, 19-60, >60 months) and offspring sex (male/female).

[b] Adjusted for maternal age (years), height (cm), pre-pregnancy BMI (kg/m^2), education (<9, 9-12, >12 years), smoking status at conception (0, 1-9, >10 cig/day) and inter-pregnancy interval (<18, 19-60, >60 months).

[c] Additionally adjusted for HCB

[d] Additionally adjusted for HCB and PCB 153

[e] Additionally adjusted for PFOA

[f] Additionally adjusted for PFOA and PCB 153

[g] Additionally adjusted for PFOA

[h] Additionally adjusted for PFOA and HCB

Supplemental Table S4: Odds ratios (ORs) and 95% confidence intervals (95% CIs) for associations between PCB 153 and PFOA and SGA birth, obtained by adjusting for co-variates including *maternal weight gain up to 17 weeks*

		Norway	**Sweden**
		OR (95% CI)	*OR (95% CI)*
PCB 153	Model 1[a]	0.51 (0.17-1.53)	5.56 (1.04-29.6)
	Model 2[b]	0.49 (0.16-1.49)	5.52 (1.03-29.5)
PFOA	Model 1[a]	0.78 (0.38-1.61)	5.21 (1.67-16.2)
	Model 2[b]	0.79 (0.38-1.63)	5.13 (1.63-16.2)

[a] Adjusted for maternal age, height, BMI, education level, inter-pregnancy interval and smoking status

[b] Additionally adjusted for weight gain up to 17 weeks

Supplemental Table S5: Odds ratios (ORs) and 95% confidence intervals (95% CIs) for associations between PCB 153 and PFOA and SGA birth, obtained by adjusting for co-variates including *alcohol consumption during pregnancy*

		Norway *OR (95% CI)*	**Sweden** *OR (95% CI)*
PCB 153	Model 1[a]	0.70 (0.25-1.99)	4.72 (0.86-26.0)
	Model 2[b]	0.71 (0.25-2.02)	4.72 (0.86-26.0)
PFOA	Model 1[a]	0.62 (0.30-1.28)	5.63 (1.78-17.8)
	Model 2[b]	0.62 (0.30-1.29)	5.63 (1.78-17.8)

[a] Adjusted for maternal age, height, BMI, education level, inter-pregnancy interval and smoking status

[b] Additionally adjusted for alcohol consumption during pregnancy

Paper III

Lauritzen et al. Environmental Health (2018) 17:9
DOI 10.1186/s12940-017-0338-x

Environmental Health

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Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study

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Abstract

Background: Prenatal exposure to persistent organic pollutants (POPs), may influence offspring weight gain. More prospective epidemiological studies are needed to compliment the growing body of evidence from animal studies.

Methods: Serum from 412 pregnant Norwegian and Swedish women participating in a Scandinavian prospective cohort study were collected in 1986–88, and analyses of two perfluoroalkyl substances (PFASs) and five organochlorines (OCs) were conducted. We used linear and logistic regression models with 95% confidence intervals (CIs) to evaluate the associations between maternal serum POP concentrations at 17–20 weeks of gestation and child overweight/obesity (body mass index (BMI) ≥ 85th percentile) at 5-year follow-up. Results were further stratified by country after testing for effect modification. We also assessed potential non-monotonic dose-response (NMDR) relationships.

Results: In adjusted linear models, we observed increased BMI-for-age-and-sex z-score (β = 0.18, 95% CI: 0.01–0.35), and increased triceps skinfold z-score (β = 0.15, 95% CI: 0.02–0.27) in children at 5-year follow-up per ln-unit increase in maternal serum perfluorooctane sulfonate (PFOS) concentrations. We observed increased odds for child overweight/obesity (BMI ≥ 85th percentile) for each ln-unit increase in maternal serum PFOS levels (adjusted OR: 2.04, 95% CI: 1.11–3.74), with stronger odds among Norwegian children (OR: 2.96, 95% CI: 1.42–6.15). We found similar associations between maternal serum perfluorooctanoate (PFOA) concentrations and child overweight/obesity. We found indications of NMDR relationships between PFOS and polychlorinated biphenyl (PCB) 153 and child overweight/obesity among Swedish children.

Conclusion: We found positive associations between maternal serum PFAS concentrations and child overweight/obesity at 5-year follow-up, particularly among Norwegian participants. We observed some evidence for NMDR relationships among Swedish participants.

Keywords: Perfluoroalkyl substances, Organochlorines, Childhood obesity, Non-monotonic dose-response relationship, Pregnancy, Endocrine disrupting chemicals, Skinfolds

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Background

The prevalence of childhood overweight and obesity (BMI ≥ 85th percentile) has increased dramatically over the past decades [1]. From 1990 to 2010, the global estimated prevalence of overweight and obesity among preschool children increased from 4.2% to 6.7% [1]. This trend is expected to continue, and the World Health Organization (WHO) predicts that 60 million preschool children worldwide (9.1%) will be overweight or obese by 2020 [1]. Childhood obesity is a risk factor for several chronic diseases later in life including diabetes, cardiovascular disease, musculoskeletal disorders, and some forms of cancer [2]. Dietary influences, a sedentary lifestyle, as well as possible gene-environment interactions are important determinants of the increasing obesity trends, but they do not completely account for the obesity epidemic [3]. An increasing body of evidence suggests that in utero exposure to endocrine disrupting chemicals (EDCs) may contribute to obesity development in children and adults [3, 4]. Animal and in vitro studies suggest that EDCs may cause obesity through interference with lipid metabolism to promote fat storage, by altering the metabolic set points, or modifying hormonal control of appetite and satiety [4]. Obesity may be programmed in the intrauterine period, and fetal exposure to certain EDCs may modify the epigenome of stem cells to preferentially produce more adipocytes at the cost of bone [5].

Several persistent organic pollutants (POPs), including perfluoroalkyl substances (PFASs) and organochlorines (OCs), are classified as EDCs [4]. PFASs and OCs are ubiquitous, persistent and bio-accumulative chemicals that have been detected in maternal serum throughout pregnancy and in cord blood at delivery. Although the use of some POPs is presently banned or restricted in many countries [6], adverse health outcomes related to background levels of POP exposures are still a major public health concern [7].

Compared to animal studies, prospective epidemiological studies investigating the association between maternal serum POP concentrations during pregnancy and offspring postnatal obesity are less extensive [8, 9]. For PFAS exposures, longitudinal studies have reported both positive [10–15] and no associations [16, 17]. For OCs, prenatal exposure to *p,p'*-dichlorodiphenyldichloroethane (*p,p'*-DDE) has been associated with increased body mass index (BMI) in infancy and childhood [8, 9], but less consistent findings are reported for associations with prenatal polychlorinated biphenyl (PCB) and hexachlorobenzene (HCB) [8, 9]. Most previous studies used anthropometric indices, such as BMI, as proxies for offspring body composition [18]. However, children with the same amount of body fat can have quite different BMI values. For this reason, skinfold thickness may be a more informative measure of body fat mass in children [19].

The current study includes 412 mother-child pairs from a Scandinavian prospective cohort study with participants from Norway and Sweden. We aimed to evaluate the associations between maternal serum POP concentrations in early pregnancy and offspring anthropometry, including child overweight/obesity at 5-year follow-up.

Methods

Study participants

This current study uses data from the U.S. National Institute of Child Health and Human Development (NICHD) Scandinavian Successive Small-for-Gestational Age births study (The SGA Study) [20]. The SGA Study is a large multi-center prospective cohort study conducted in Trondheim and Bergen (Norway) and Uppsala (Sweden) from 1986 to 1988. The SGA Study was designed to study longitudinal fetal growth, as well as perinatal and postnatal outcomes among mother and child [20]. In brief, all pregnant women (< 20 weeks gestation) in the study catchment areas who were expecting their 2nd or 3rd child were eligible for study inclusion and made the first appointment (n = 5722) (Fig. 1). Women with elevated risk for an SGA birth were intentionally oversampled. Risk factors for SGA birth included a previous low birth weight child, previous perinatal death, low maternal prepregnancy weight (< 50 kg), maternal smoking at conception and/or chronic maternal hypertension or renal disease. All high-risk pregnancies resulting in an SGA birth (birth weight below 10th percentile adjusted for sex and parity), and a 10% random sample of the study population were invited for follow-up when children were five years of age (n = 791). Of these, 534 (68%) attended the 5-year evaluation. In the current study, 412 mother-child pairs (137 SGA births and 275 non-SGA births) were included in the analyses (Fig.1).

Exposure assessment of maternal serum POP concentrations

According to study protocol (1986–88), maternal serum samples were collected in the 2nd trimester (gestational week 17–20) and stored at minus 80 °C for later analysis. Analyses of maternal serum PFAS and OC concentrations were performed.

PFAS analyses

The PFAS analyses were performed at the laboratories of Norwegian Institute for Air Research, Tromsø, Norway (NILU). Maternal serum samples were quantified for two target analytes including perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). Detailed information about the sample preparation, extraction method, analytical method, reagents and instrumentation is previously reported [21, 22]. Maternal serum PFAS concentrations were determined using sonication-facilitated liquid-liquid

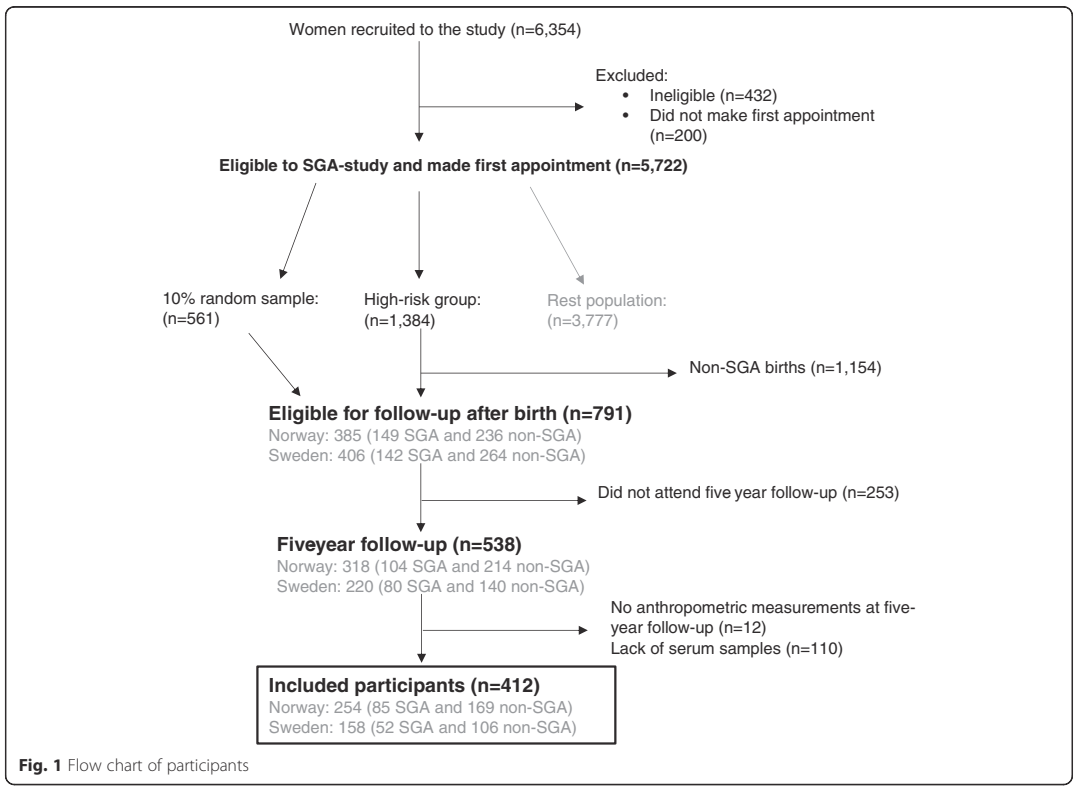

Fig. 1 Flow chart of participants

extraction, activated ENVI-carb clean-up [23], and analyzed by ultrahigh pressure liquid chromatography triple-quadruple mass-spectrometry (UHPLC-MS/MS). Participation in the AMAP Ring Test [24] ensures that the uncertainties of the analysis are within ±15–20% of the assigned values.

OC analyses

Maternal serum OC concentrations were analysed at the Institut National de Santé Publique du Quebec, Centre Toxicologie, Quebec. Several OCs were measured, including hexachlorobenzene (HCB), oxychlordane, polychlorinated biphenyl (PCB) 52, 101, 118, 153, 156, 170, and 180, *p,p'*-dichlorodiphenyldichloroehylene (*p,p'*-DDE), *p,p'*-dichlorophenyltrichloroethane (*p,p'*-DDT), *β*-hexachlorohexane (*β*-HCH) and *trans*-nonachlor (*t*-NC). In short, 0.5–1 ml serum sample was extracted using hexane (2 × 6 ml), ethanol (2 ml) and saturated ammonium sulphate solution (2 ml). This method is a slight modification of the one described by Sandanger et al. [25], where the samples were cleaned up using 1 g of activated fluorisil on an automated Liquid handler system before GC-MS analysis [26]. Uncertainties of the analyses are within ±15–20% of the assigned values, which are confirmed by participation in the AMAP Ring Test [24]. Lipids were enzymatically determined and the summed lipid amounts were calculated based on triglycerides and cholesterol measurements using the following formula:

Total lipids = 1.33*triglycerides +1.12*cholesterol +1.48 (g/l) [27]. This formula showed good correlation with complete formulas including phospholipids [28].

We report PCB 153 as a proxy for total PCBs, and excluded *p,p*-DDT because of low limit of detection (LOD) (> 50% of samples < LOD). LODs are listed in Table 2. Values below LOD were replaced by LOD/√2.

Outcome assessment of child overweight/obesity

Child weight was recorded at 5-year follow-up in the clinic by trained professionals using a standard procedure. Standing weight was recorded to the nearest 100 g. Standing height was measured according to standard procedures and recorded to the nearest 0.1 cm [29]. BMI was calculated from weight in kilograms (kg) divided by height in meters squared (kg/m^2). We used offspring age (in months), offspring sex and offspring BMI to calculate age-and-sex-specific BMI z-scores. BMI

percentiles were based on the 2006 WHO child growth standards for children 5 years or younger [30], and the 2007 WHO growth standards for children and adolescents aged 5 to 19 years [18]. We assessed child age-and-sex-specific BMI z-scores as a continuous outcome at 5-year follow-up. We also analyzed child overweight/obesity at 5-year follow-up categorically (BMI ≥ 85th percentile for age and sex compared to BMI below the 85th percentile) [31]. Skinfold thickness was measured once using a Harpenden caliper (John Bull, British Indicators Ltd.) to the nearest 0.10 mm and 60 s after release of the grip to allow full tension placed on the compressed skinfold. Subscapular skinfold thickness was measured below the inferior angle of the left scapula, and triceps skinfold thickness was measured over the triceps in the middle of the left upper arm [29, 32]. We calculated age-and-sex-specific z-scores for triceps and subscapular skinfolds according to the Center for Disease Control and Prevention (CDC) 2000 Growth Charts for children from 1.5 to 20 years of age [33].

Covariates

Information on maternal age, height, pre-pregnancy weight, education, smoking habits, previous breastfeeding duration and inter-pregnancy interval were collected via in-person interviews and self-report questionnaires during the original study period as per SGA Study protocol. Maternal pre-pregnancy BMI was calculated based on self-reported height and weight at first study visit. We calculated maternal weight gain up to 17 weeks of gestation as the difference between self-reported pre-pregnancy weight and clinically recorded weight closest to gestational week 17 (done by the woman's own midwife or GP). Based on a known J-shaped association with adverse perinatal outcomes including restricted fetal growth [34], we categorized the inter-pregnancy interval as <18 months, 19–60 months and > 60 months since their last birth.

Statistical analyses

Maternal serum PFAS and OC concentrations were logarithmically (ln) transformed to obtain normal distribution. We used wet weight maternal serum PFAS concentrations, and lipid-adjusted maternal serum OC concentrations [27].

We used multivariable linear regression with 95% confidence intervals (CIs) to examine the association between ln-transformed maternal serum concentrations of seven separate POPs (*PFASs:* PFOA, PFOS; *OCs:* PCB153, *p,p'*-DDE, HCB, *t*-NC and *β*-HCH) and offspring i) sex-and-age-specific z-scores for BMI at 5-year follow-up, and ii) sex-and-age-specific triceps and subscapular skinfolds at 5-year follow-up. We used multivariable logistic regression to estimate adjusted odds ratios (ORs) and 95% CIs for the association between maternal serum POP concentrations and child overweight/obesity (BMI z-scores ≥ 85th percentile for age and sex) at 5-year follow-up. We constructed a directed acyclic graph (DAG) to assess and select potential confounders (Additional file 1: Fig. S1). Prenatal growth was considered a mediator in the pathway between exposure to POPs and childhood overweight, due to positive associations between increasing prenatal levels and POPs and SGA birth in our study sample [35]. As adjustment for a mediator may introduce collider bias if there are shared unmeasured causes of both the mediator (SGA status) and the outcome (childhood overweight) [36], we did not include prenatal growth or SGA status in the multivariate analyses. The following variables were included in multivariable analyses as potential confounders: maternal age (continuous; years), maternal pre-pregnancy body mass index (BMI) (continuous: kg/m^2), maternal education (categorical: < 9 years, 10–12 years, or ≥ 13 years), maternal smoking status at conception (categorical: 0, 1–9 or ≥ 10 cigarettes per day), previous breastfeeding duration (continuous: months), inter-pregnancy interval between the last two children (categorical: ≤ 18 months, 19–60 months, ≥ 61 months), and maternal weight gain from conception up to gestational week 17 (continuous: kilograms). The pooled analyses were further adjusted by country (Norway or Sweden). All models were tested for normality of residuals, heteroscedasticity, and multi-collinearity.

We examined linearity by scatter plots, assigning maternal serum POP concentrations to the horizontal axis, and measures of child adiposity to the vertical axis. Marginal relationships between maternal serum POP concentrations and offspring BMI z-scores at 5-year follow-up were assessed by non-linear regression using 3-knot restricted cubic splines and 95% CIs. We determined non-linear associations by examination of cubic spline graphs, and by the Wald test.

We had some missing data including 7.2% missing for both maternal weight gain up to gestational week 17 and previous breastfeeding duration. Among children, we had 7.0% missing data on subscapular skinfold thickness and 6.1% missing data on triceps skinfold thickness. Overall, 80% of participants had complete data on all variables. Missing data were assumed missing at random. We used chained multiple imputation [37, 38] to generate and compare five complete data sets. Complete case analyses widened the 95% CIs, but did not change the estimates substantially.

We evaluated possible effect modification by country and offspring sex based on a priori evidence from the literature [12, 35]. We conducted several sensitivity analyses to assess the robustness of our results. First, we did stratum-weighted analyses to ensure generalizability of our reported estimates to the contemporary pregnant population according to the prevalence of i) SGA births,

ii) maternal pre-pregnancy overweight, and iii) maternal smoking at conception (See Additional file 1: Supplementary description S1 for details). Such weighted analyses are recommended for analyses with case-control data or in other way unbalanced populations that may be subject to selection bias [39]. Second, we additionally adjusted for maternal fish consumption during pregnancy among Norwegian participants (See Additional file 1: Supplementary description S2 for details). Finally, we considered a multi-pollutant model approach by mutually adjusting for maternal serum POPs that were found to be associated with offspring BMI.

All statistical analyses were conducted with SPSS statistical software, version 22 (IBM SPSS Inc. Chicago, IL) and Stata IC/13.1.

Results

Overall, mean maternal age at study start was 29 years, with 69% of women expecting their second child, and 31% expecting their third (Table 1). Mean duration of previous breastfeeding was 7 months. On average, mothers gained 3.2 kg from conception up to gestational week 17. Overall, 10% of mothers were underweight (BMI < 18.5 kg/m^2) at conception, and 9% were overweight or obese (BMI ≥ 25 kg/m^2), with some variation between countries. A higher proportion of Norwegian mothers were underweight (BMI < 18.5 kg/m^2) at conception compared to their Swedish peers (12% vs. 7%). A total of 53% of Norwegian mothers reported smoking at conception, compared to 33% of the Swedish mothers.

Children at 5-year follow-up were evenly distributed by sex (51% boys and 49% girls), wherein 1/3 were categorized as SGA births (reflecting the oversampling of SGA births for follow-up). Norwegian children had slightly lower birth weight (3401 vs. 3515 g), and were breastfed longer (6.8 vs. 6.0 months) than Swedish children. Norwegian children were also younger at 5-year follow-up (Norway: 61 months, Sweden: 65 months). A total of 55 children (12%) were considered overweight or obese at 5-year follow-up (Norway: 14%, Sweden: 10%). In our study population, Norwegian children had higher sex-and-age-adjusted z-scores of subscapular skinfold thickness (Norway: 0.18; Sweden: −0.32), and triceps skinfold thicknesses (Norway: 0.32; Sweden: −0.07) compared to Swedish children (Table 1).

Norwegian mothers had substantially lower median serum PFOA concentration (1.64 vs. 2.33 ng/ml), PFOS concentration (9.62 vs. 16.3 ng/ml), PCB 153 concentration (79.9 vs. 117 ng/g lipid) and β-HCH concentration (21.2 vs. 25.0 ng/g lipid) compared to Swedish mothers (Table 2). Norwegian mothers had higher median serum *t*-NC concentration (6.77 vs. 6.28 ng/g lipid) compared to Swedish mothers. Median maternal serum HCB concentrations (17.0 vs. 18.4 ng/g lipid) and *p,p'*-DDE concentrations (211 vs. 244 ng/g lipid) did not differ between countries (Table 2).

Adjusted linear and logistic associations between maternal serum concentrations of PFASs and OCs, and measures of child adiposity at 5-year follow-up are shown in Table 3. These results are stratified by country of residence based on some indication of effect modification by country ($p_{interaction} = 0.039$) between maternal serum PFOS concentrations and offspring BMI z-scores as well as child overweight/obesity at 5-year follow-up ($p_{interaction} = 0.098$). In the total cohort, adjusted BMI-for-age-and-sex z-score increased by 0.18 (95% CI: 0.01–0.35) and adjusted triceps skinfold z-score increased by 0.15 (95% CI: 0.02–0.27) for each ln-unit increase in maternal serum PFOS concentrations. For each ln-unit increase in maternal serum PFOS concentration, the adjusted OR for child overweight/obesity was 2.04 (95% CI: 1.11–3.74). The data also suggests positive associations between maternal serum PFOA concentrations and child BMI z-scores, triceps skinfolds z-scores and child overweight/obesity at 5-year follow-up (Table 3).

Among Norwegian children, we observed increased BMI-for-age-and-sex z-scores for each ln-unit increase in maternal serum PFOS concentration (β:0.30 (95% CI: 0.08, 0.51), and each ln-unit increase in maternal serum PFOA concentration (β:0.32 (95% CI: 0.05, 0.60) (Table 3). Norwegian children also showed increased triceps skinfold z-scores per ln-unit increase in maternal serum PFOS concentration (β:0.20, 95% CI: 0.06, 0.35) and maternal serum PFOA concentration (β: 0.24, 95% CI: 0.05, 0.42). BMI z-scores increased by 0.45 (95% CI: 0.03, 0.87) for each ln-unit increase in maternal serum PCB 153 concentration in the Norwegian part. No associations were observed among Swedish participants.

In adjusted logistic regression, we observed no overall association between maternal serum POP concentrations and child adiposity or overweight/obesity in the pooled analyses. Among Norwegian children, we observed increased odds for child overweight/obesity at 5-year follow-up for each ln-unit increase in maternal serum PFOS concentration ($OR_{adjusted}$: 2.96, 95% CI: 1.42–6.15) and maternal serum PFOA concentration ($OR_{adjusted}$: 2.90, 95% CI: 1.10–7.63).

To examine potential NMDR relationship between maternal serum POP concentrations and child overweight/obesity outcomes, we used a restricted 3-knot cubic spline model. Among Swedish participants, we observed some evidence of a NMDR relationship between maternal serum PFOS concentration and offspring BMI z-scores at 5-year follow-up ($p = 0.09$ for non-linearity, Fig. 2). We found also some indications of a NMDR relationship between maternal serum PCB 153 concentrations and offspring BMI z-scores at 5-year

Table 1 Maternal and child characteristics by country of residence ($N = 412$)

Maternal characteristics	Total ($n = 412$)	Norway ($n = 254$)	Sweden ($n = 158$)	p^a
Maternal age (mean years (sd))	29.0(4.3)	28.8(4.3)	29.2(4.4)	0.364
missing n(%)	3(0.7)	1(0.4)	2(1.3)	
Maternal pre-pregnancy BMI ($kg/m^{2)}$ n(%)				0.031
Underweight (BMI < 18.5)	42(10)	31(12)	11(7)	
Normal weight (BMI 18.5–25.0)	330(81)	205(81)	125(80)	
Overweight (BMI > 25.0)	38(9.3)	17(7)	21(13)	
missing n(%)	2(0.4)	1(0.4)	1(0.6)	
Maternal education (years) n(%)				0.011
less than 9	67(17)	36(14)	31(20)	
9–11	205(50)	142(56)	63(41)	
12 or more	135(33)	75(30)	60(39)	
missing n(%)	5(1.2)	1(0.4)	4(2.5)	
Maternal smoking at conception (number of cigarettes) n(%)				< 0.001
0	225(55)	120(47)	105(67)	
1–9	42(10)	35(14)	7(4)	
10 or more	145(35)	99(39)	46(29)	
Parity n(%)				0.245
1	285(69)	181(71)	104(66)	
2	127(31)	73(29)	54(34)	
Inter-pregnancy interval (years) n(%)				0.187
less than 1.5	102(25)	56(22)	46(29)	
1.5–5	225(55)	147(58)	78(49)	
5 or more	85(21)	51(20)	34(22)	
Previous breastfeeding duration (mean months (sd))	7.3(5.1)	7.5(5.2)	7.1(5.0)	0.546
missing n(%)	30(7.2)	13(5.1)	17(10.8)	
Maternal weight gain up to 17 weeks of gestation (mean kilograms (sd))	3.2(2.6)	3.1(2.6)	3.3(2.6)	0.601
missing n(%)	30(7.2)	28(11.0)	2(1.3)	
Child characteristics at birth				
Sex n (%)				0.987
Boys	211(51)	130(51)	81(51)	
Girls	201(49)	124(49)	77(49)	
Birth weight (mean grams (sd))	3445(592)	3401 (568)	3515 (625)	0.056
SGA status n(%)				0.908
non-SGA	275(67)	169(67)	106(67)	
SGA	137(33)	85(33)	52(33)	
Breastfeeding duration of the index child (mean months (sd))	6.5(3.6)	6.7(3.7)	6.0(3.2)	0.030
missing n(%)	16(3.9)	8(3.1)	8(5.0)	
Child characteristics at 5 year follow-up				
Exact age at five-year follow-up (mean years (sd)	5.2(0.2)	5.1(0.2)	5.4(0.1)	< 0.001
missing n(%)	1(0.2)	0(0)	1(0.6)	
Weight				0.708
Underweight (< 5th percentile)	12(3)	7(3)	5(3)	
Normal weight (5th–85th percentile.)	346(84)	211(83)	135(87)	

Table 1 Maternal and child characteristics by country of residence (*N* = 412) *(Continued)*

Maternal characteristics	Total (*n* = 412)	Norway (*n* = 254)	Sweden (*n* = 158)	*p*[a]
Overweight (> 85th percentile)	30(7)	21(8)	9(6)	
Obese (> 95th percentile)	22(5)	15(6)	7(4)	
missing n(%)	2(0.4)	0(0)	2(1.3)	
Subscapular skinfolds (mean mm (sd))	5.6(1.7)	6.0 (1.9)	5.1 (1.2)	< 0.001
Triceps skinfolds (mean mm (sd))	10.0(2.2)	10.4 (2.2)	9.2 (1.9)	< 0.001
Subscapular skinfold z-score	−0.01(0.8)	0.2(0.7)	−0.3(0.8)	< 0.001
missing n(%)	29(7.0)	16(6.3)	13(8.2)	
Triceps skinfold z-score	0.2(0.7)	0.3(0.6)	−0.1(0.7)	< 0.001
missing n(%)	25(6.1)	15(5.9)	10(6.3)	
BMI-for-sex-and-age z-score	0.1(1.0)	0.2(1.0)	0.1(0.9)	0.499
missing n(%)	2(0.4)	0(0)	2(1.3)	

Sd standard deviation, *BMI* body mass index, *SGA* small for gestational age
[a]*p*-values for comparison between the countries by using independent t-tests for continuous variables and χ^2 tests for categorical variables

follow-up ($p = 0.02$ for non-linearity, Fig. 3) in the Swedish part of the study.

We tested the generalizability of our results in a stratum-weighted analysis that accounted for the original SGA study design that included a higher proportion of SGA births, a lower prevalence of maternal overweight, and a higher prevalence of smoking mothers at conception, compared to the general pregnant population. Our stratum-weighted analysis did not substantially change our reported results (Additional file 1: Table S1). Adjustment for maternal fish intake among the Norwegian women also did not change the estimates (data not shown). Mutual adjustment between maternal serum PFOS and PCB 153 concentrations, and maternal serum PFOA and PCB 153 concentrations did not change the current estimates. However, adding both maternal serum PFOS and PFOA concentrations into the same model resulted in some attenuation of the estimates probably due to high correlation between the PFASs (Additional file 1: Table S2).

Discussion

In this prospective cohort study of 412 mother-child pairs from Norway and Sweden, we observed

Table 2 Wet-weight levels of maternal serum PFASs, and wet-weight and lipid-adjusted levels of maternal serum OCs, by country (*n* = 412)

	Norway (*n* = 254)		Sweden (*n* = 158)		*p*[a]	LOD	% > LOD
	Median	5th–95th perc.	Median	5th–95th perc.			
Wet-weight (ng/ml)							
PFOA	1.64	0.82–3.54	2.33	0.95–4.28	< 0.001	0.03	100
PFOS	9.62	3.78–24.6	16.3	7.17–30.5	< 0.001	0.03	100
PCB 153	0.46	0.26–0.83	0.68	0.37–1.10	< 0.001	0.01	100
p,p'-DDE	1.30	0.44–4.70	1.30	0.57–4.00	0.174	0.09	100
HCB	0.10	0.05–0.19	0.11	0.06–0.18	0.522	0.04	100
t-NC	0.04	0.02–0.09	0.04	0.02–0.08	0.020	0.01	100
β-HCH	0.12	0.06–0.25	0.13	0.07–0.28	0.007	0.01	99
Lipid-adjusted (ng/g lipids)							
PCB 153	79.9	46.8–146	117	69.4–179	< 0.001	–	–
p,p'-DDE	211	78.0–844	244	101–662	0.101	–	–
HCB	17.0	10.7–30.1	18.4	10.3–30.9	0.261	–	–
t-NC	6.77	3.62–14.5	6.28	3.25–12.4	0.034	–	–
β-HCH	21.2	10.7–39.5	25.0	12.6–44.6	< 0.001	–	–

PFAS perfluoroalkyl substance, *OC* organochlorine, *IQR* inter-quartile range, *SD* standard deviation, *LOD* limit of detection, *% > LOD* percentage of serum samples above LOD, *PFOA* perfluorooctanoate, *PFOS* perfluorooctane sulfonate, *PCB* polychlorinated biphenyl, *p,p'-DDE p,p'*-dichlorodiphenyldichloroethane, HCB
[a]*p*-values for comparison between the countries using Mann-Whitney U test

Table 3 Adjusted associations between ln-units of maternal serum POPs and BMI-for-age-and-sex z-scores, subscapular and triceps skinfold z-scores (*β*s and 95% CIs) and child obesity/overweight (OR and 95% CI) at 5 years of age, overall and by country

Maternal serum POPs	Child outcomes at five-year follow-up ($n = 412$)			
	All participants ($n = 412$)[a]	Norway ($n = 254$)[b]	Sweden ($n = 158$)[b]	
	BMI-for-age-and-sex z-score			
	β (95% CI)			p_{int} [c]
PFOS	0.18 (0.01, 0.35)	0.30 (0.08, 0.51)	−0.11 (−0.41, 0.19)	0.039
PFOA	0.18 (−0.03, 0.39)	0.32 (0.05, 0.60)	−0.07 (−0.41, 0.27)	0.129
PCB 153	0.30 (−0.03, 0.63)	0.45 (0.03, 0.87)	0.11 (−0.43, 0.65)	0.509
p,p'-DDE	0.03 (−0.12, 0.18)	0.04 (−0.15, 0.23)	0.02 (−0.24, 0.29)	0.932
HCB	0.16 (−0.13, 0.45)	0.36 (−0.03, 0.75)	−0.11 (−0.56, 0.34)	0.154
b-HCH	0.17 (−0.08, 0.43)	0.17 (−0.16, 0.51)	0.14 (−0.26, 0.55)	0.823
t-NC	0.11 (−0.13, 0.35)	0.23 (−0.07, 0.54)	−0.12 (−0.53, 0.29)	0.380
	Triceps skinfold z-score			
	β (95% CI)			
PFOS	0.15 (0.02, 0.27)	0.20 (0.06, 0.35)	−0.02 (−0.27, 0.22)	0.255
PFOA	0.14 (−0.02, 0.29)	0.24 (0.05, 0.42)	−0.05 (−0.33, 0.23)	0.223
PCB 153	−0.08 (−0.31, 0.16)	0.02 (−0.27, 0.31)	−0.33 (−0.77, 0.11)	0.638
p,p'-DDE	−0.04 (−0.15, 0.07)	0.004 (−0.13, 0.14)	−0.12 (−0.33, 0.10)	0.905
HCB	0.02 (−0.20, 0.24)	0.07 (−0.20, 0.35)	−0.11 (−0.48, 0.26)	0.914
b-HCH	0.10 (−0.08, 0.29)	0.10 (−0.13, 0.32)	0.07 (−0.27, 0.41)	0.271
t-NC	−0.03 (−0.20, 0.15)	0.05 (−0.16, 0.25)	−0.32 (−0.64, 0.02)	0.497
	Subscapular skinfold z-score			
	β (95% CI)			
PFOS	0.07 (−0.07, 0.20)	0.12 (−0.04, 0.29)	−0.07 (−0.35, 0.22)	0.304
PFOA	−0.03 (−0.20, 0.15)	0.04 (−0.18, 0.25)	−0.11 (−0.42, 0.21)	0.470
PCB 153	−0.05 (−0.32, 0.23)	0.07 (−0.26, 0.41)	−0.30 (−0.80, 0.20)	0.432
p,p'-DDE	−0.07 (−0.19, 0.06)	−0.04 (−0.18, 0.11)	−0.10 (−0.34, 0.14)	0.917
HCB	−0.08 (−0.33, 0.17)	−0.05 (−0.36, 0.27)	−0.21 (−0.62, 0.20)	0.977
b-HCH	0.14 (−0.07, 0.35)	0.10 (−0.16, 0.36)	0.17 (−0.21, 0.55)	0.528
t-NC	−0.13 (−0.34, 0.07)	−0.08 (−0.32, 0.16)	−0.34 (−0.71, 0.04)	0.698
	Overweight (≥ 85th percentile)			
	OR (95% CI)			
N overweight/total	52/412 (13%)	36/254 (14%)	16/158 (10%)	
PFOS	2.04 (1.11–3.74)	2.96 (1.42–6.15)	0.71 (0.21–2.45)	0.098
PFOA	1.61 (0.75–3.46)	2.90 (1.10–7.63)	0.50 (0.12–2.06)	0.119
PCB 153	1.37 (0.42–4.49)	2.13 (0.49–9.26)	0.60 (0.06–6.03)	0.944
p,p'-DDE	0.88 (0.52–1.49)	1.11 (0.58–2.14)	0.61 (0.20–1.83)	0.792
HCB	1.51 (0.55–4.13)	2.30 (0.62–8.50)	0.60 (0.10–3.62)	0.806
b-HCH	2.14 (0.86–5.32)	2.17 (0.67–6.99)	1.56 (0.29–8.39)	0.529
t-NC	1.44 (0.63–3.31)	1.69 (0.62–4.62)	0.78 (0.15–1.19)	0.745

[a]Adjusted for maternal age, education, smoking at conception, pre-pregnancy BMI, weight gain at 17 weeks, interpregnancy interval, previous breastfeeding duration and country of residence

[b]Adjusted for maternal age, education, smoking at conception, pre-pregnancy BMI, weight gain at 17 weeks, interpregnancy interval and previous breastfeeding duration

[c]*p*-value for interaction between exposure and country

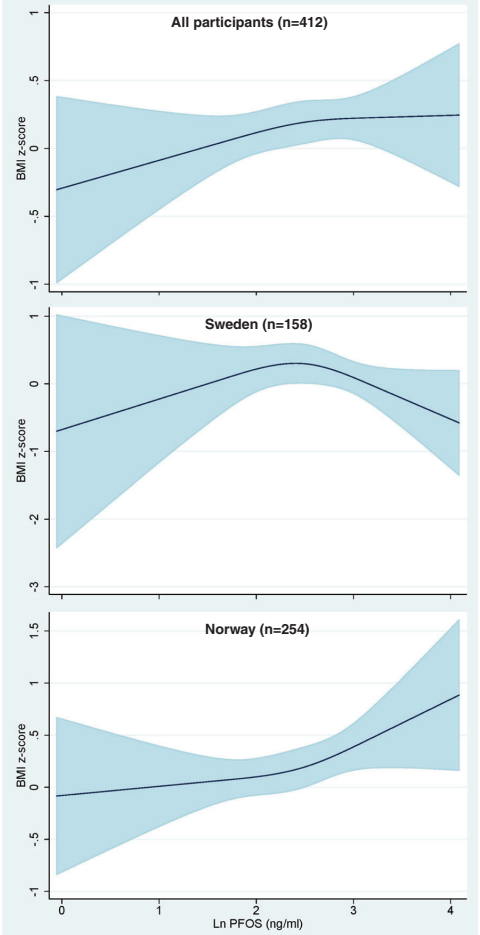

Fig. 2 Adjusted restricted cubic spline with 3 knots for maternal serum PFOS concentrations and offspring BMI-for-age-and-sex z-score at 5-year follow-up, overall and stratified by country. All models are adjusted adjusted for maternal age, pre-pregnancy body mass index, education, smoking status during pregnancy, inter-pregnancy interval, previous breastfeeding duration and maternal weight gain up to 17 weeks of gestation. The overall model is additionally adjusted for country of origin. The solid line represents the mean BMI z-score and the shaded area represents the 95% confidence interval

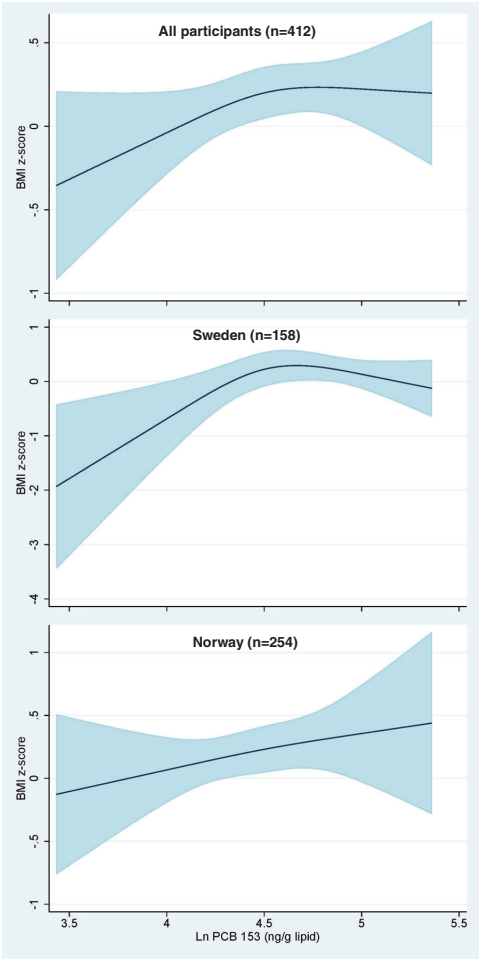

Fig. 3 Adjusted restricted cubic spline with 3 knots for maternal serum PCB 153 concentrations and offspring BMI-for-age-and-sex z-score at 5–year follow-up, overall and stratified by country. All models are adjusted for maternal age, pre-pregnancy body mass index, education, smoking status during pregnancy, inter-pregnancy interval, previous breastfeeding duration and maternal weight gain up to 17 weeks of gestation. The overall model are additionally adjusted for country of origin. The solid line represents the mean BMI z-score and the shaded area represents the 95% confidence interval

positive associations between maternal serum PFAS concentrations and child BMI and triceps skinfold z-scores, as well as child overweight/obesity at 5-year follow-up, particularly among Norwegian women. We also found evidence of NMDR relationships between maternal serum PFOS and PCB 153 concentrations and offspring BMI z-scores among Swedish participants.

Evidence of prenatal exposure to PFASs and child postnatal growth and obesity is limited and the results have been inconsistent. A Danish study with maternal plasma PFAS concentrations like ours (median PFOS: 10.8 ng/ml, PFOA: 1.3 ng/ml), found positive associations between PFOS and PFOA concentrations and waist-to-height ratio in 5–9-year-old girls and boys [12]. However, studies with higher maternal PFAS concentrations have been more

inconsistent. Three studies with higher maternal plasma PFAS concentrations than our study (median PFOS: 19.6–24.8 ng/ml, PFOA: 3.7–5.6 ng/ml) showed positive associations between increasing PFAS concentrations and measures of child obesity (10, 11, 14). However, another Danish study with even higher maternal plasma PFAS concentrations (median PFOS: 33 ng/ml, PFOA: 5.2 ng/ml) found no or inverse associations between increasing PFAS concentrations and BMI or waist circumference among 7-year-old children [16]. Another study with higher maternal serum PFOA concentrations (median: 5.3 ng/ml) than ours, observed a NMDR relationship between increasing PFOA concentrations and BMI at 8 years of age [13]. From this, we may speculate that low maternal serum or plasma PFAS concentrations show positive associations with child obesity, while higher maternal serum or plasma PFAS concentrations show positive, negative, or NMDR relationships with child obesity, depending on the range of PFAS concentrations measured in the study population. This is in agreement with a recent review suggesting that NMRD relationships may be observed with EDCs, and that the effects of high doses EDCs cannot predict the effects of EDCs at lower doses [40]. Possible mechanisms for these NMDR relationships include cytotoxicity, cell- and tissue-specific receptors and cofactors, receptor selectivity, receptor down-regulation and desensitization, receptor competition and endocrine negative feedback loops [40]. This is consistent with our different findings between Norwegian participants (with lower maternal serum PFAS concentrations) compared to Swedish participants (with higher maternal serum PFAS concentrations). These results support a potential cytotoxic effect of high levels of PFASs in utero that can result in growth restricted offspring, which is consistent with the positive associations we found between maternal PFAS concentrations and SGA birth among the Swedish participants in our study [35]. Consequently, this effect may distort the association between maternal serum PFAS concentrations and child obesity at 5-year follow-up. However, a possible obesogenic effect may appear later in the latter development of growth restricted offspring. NMDR relationships have also been proposed for associations between maternal serum PCB concentrations and offspring growth and obesity [41]. A recent review categorized $n = 9$ prospective cohort studies according to the level of maternal serum PCB concentrations whereby the authors suggested that low maternal serum PCB concentration (PCBs < 1000 ng/g lipids) was associated with increased offspring BMI or body weight, while high maternal serum PCB concentration (PCBs > 4000 ng/g lipids) was associated with decreased offspring BMI or body weight [41]. Taken together with our finding of a NMDR relationship between maternal serum PCB 153 concentrations and child overweight/obesity at 5-year follow-up among Swedish only participants, there is some indication that low maternal serum exposure concentrations may lead to offspring obesity, while higher concentrations of PCBs may exert cytotoxic effects on the fetus, resulting in poor fetal growth and development. However, it is difficult to compare concentration ranges in our study with results from this review, as the review considered total maternal serum PCB concentrations [41].

The current study has several strengths including the relatively substantial number of mother-child pairs ($n = 412$). We measured maternal serum PFAS and OC concentrations early in pregnancy, and evaluated mothers and children throughout pregnancy, infancy and into early childhood using detailed clinically based outcome assessments. The use of standardized anthropometric measurements may reduce possible misclassification and enhance the statistical precision of our estimates. To our knowledge, only one previous study has assessed the relationship between maternal serum prenatal PFAS concentrations and offspring triceps and subscapular skinfold thickness [11]. Studies that measure only BMI are limited by the fact that BMI is not a direct measure of fat distribution. As such, children with the same BMI may differ considerably in total amount of body fat [42]. Skinfold thickness, as applied in our study, is used as a measure of subcutaneous fat, which has been reported to be highly correlated with total amount body fat [19, 43]. We were also able to examine and/or adjust for several important prenatal and postnatal factors. Our study is one of few studies to investigate a variety of maternal serum PFAS and OC exposures.

The current study also has some limitations. Although we included a range of covariates in multivariate models, we cannot rule out possible residual confounding related to socio-economic and lifestyle differences between high-consumers of seafood in Norway compared to Sweden. However, adjustment for maternal fish intake among Norwegian participants did not change the results. The contamination pattern and POP concentrations in seafood from the Baltic Sea on the East Coast of Sweden is different compared to the seafood consumed in Norway [22]. Thus, the possible nutrient/contaminant interaction might be different in the two countries. We acknowledge that SGA births were overrepresented in our study cohort compared to the general pregnant population, which may introduce selection bias or problems with generalizability. Also, the high prevalence of maternal smoking and the low prevalence of maternal overweight in our study compared to recent pregnant populations [44], might distort the estimates. However, results from stratum-weighted analyses showed no change in reported results. Still, results from this study may not be generalizable to primiparous women, as only parous women were eligible for study inclusion.

Although skinfold measurements in children are more correlated with body fat mass, they are prone to intra- and inter-observer errors [32]. We had no information about the inter-observer reliability, but we assume that measurement precision was un-correlated with maternal serum EDC concentrations. Persistent and bioaccumulative chemicals with comparable properties are highly correlated. Therefore, our point estimates may be subject to residual confounding due to some unmeasured chemicals (e.g. lead) in our analyses. Finally, we must interpret our country-specific results with caution due to small numbers.

Conclusion

Our study shows that increasing maternal serum PFAS concentrations were associated with increasing child BMI and triceps skinfold z-scores, in addition to child overweight/obesity at 5-year follow-up, but this association may differ geographically and by maternal serum PFAS concentration. Our results also highlight the importance of assessing NMDR relationships for POP exposures. More prospective studies on the association between maternal serum POP concentrations and overweight/obesity among older children and adults are needed.

Additional file

Additional file 1: Supplementary figures, tables and information. (DOCX 323 kb)

Abbreviations

AMAP: Arctic Monitoring and Assessment Programme; BMI: body mass index; CI: confidence interval; DAG: directed acyclic graph; EDC: endocrine disrupting chemical; HCB: hexachlorobenzene; LOD: limit of detection; NICHD: National Institute of Child Health and Human Development; NILU: Norwegian Institute for Air Research; NMDR: non-monotonic dose-response; OC: organochlorine; OR: odds ratio; *p,p'*-DDE: *p,p'*-dichlorodiphenyldichloroethane; PCB: polychlorinated biphenyl; PFAS: perfluoroalkyl substance; PFOA: perfluorooctanoate; PFOS: perfluorooctane sulfonate; POP: persistent organic pollutant; SGA: small for gestational age; *t*-NC: trans-nonachlor; UHPLC-MS/MS: ultra-high pressure liquid chromatography triple-quadrupole mass-spectometry; WHO: World Health Organization.; *β*-HCH: beta-hexachlorohexane

Acknowledgements

We gratefully acknowledge the participating women in the SGA-study. We wish to present our sincere thanks to Associate Professor Jennifer Hutcheon at Department of Obstetrics & Gynaecology, Faculty of Medicine, University of British Columbia, for her thorough and valuable peer-review of this manuscript. We would like to thank people at NILU – Norwegian Institute of Air Research in Tromsø, Norway, for conducting the PFAS analyses, and Institut National de Santé Publique du Québec, Centre de Toxicologie in Quebec, for the OC analyses. Special thanks to Charlotta Rylander, Therese H. Nøst and Vivian Berg, for valuable input and advice.

Funding

This work was funded by grants from the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

Due to ethical restrictions related to protecting participant confidentiality, underlying data cannot be made publicly available. These data are available upon request from the principal investigator in the SGA-study, Geir W. Jacobsen, e-mail: geir.jacobsen@ntnu.no

Authors' contributions

GWJ were responsible for the conception and design of the SGA-study, and secured funding for the present study. HBL and GWJ designed the study and had full access to the data. HBL carried out the statistical analysis and wrote the initial draft of the manuscript.
All authors (i) provided substantial contributions to the conception/design of the work, acquisition, analysis or interpretation of the data, (ii) revised the manuscript critically, and (iii) approved the final version for submission. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants provided written informed consent for continued use of data and biomaterial, which was documented at the first study visit. The study, including the consent procedure, has been reviewed and approved by the Central Norway Regional Committee for Medical and Health Sciences Research Ethics (REK Midt 2010/1449–5).

Consent for publication

N/A.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Received: 14 July 2017 Accepted: 3 November 2017
Published online: 18 January 2018

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Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study

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Supplementary Figure S1. Directed acyclic graph (DAG) of the relationships between maternal serum POP levels and postnatal growth and obesity.

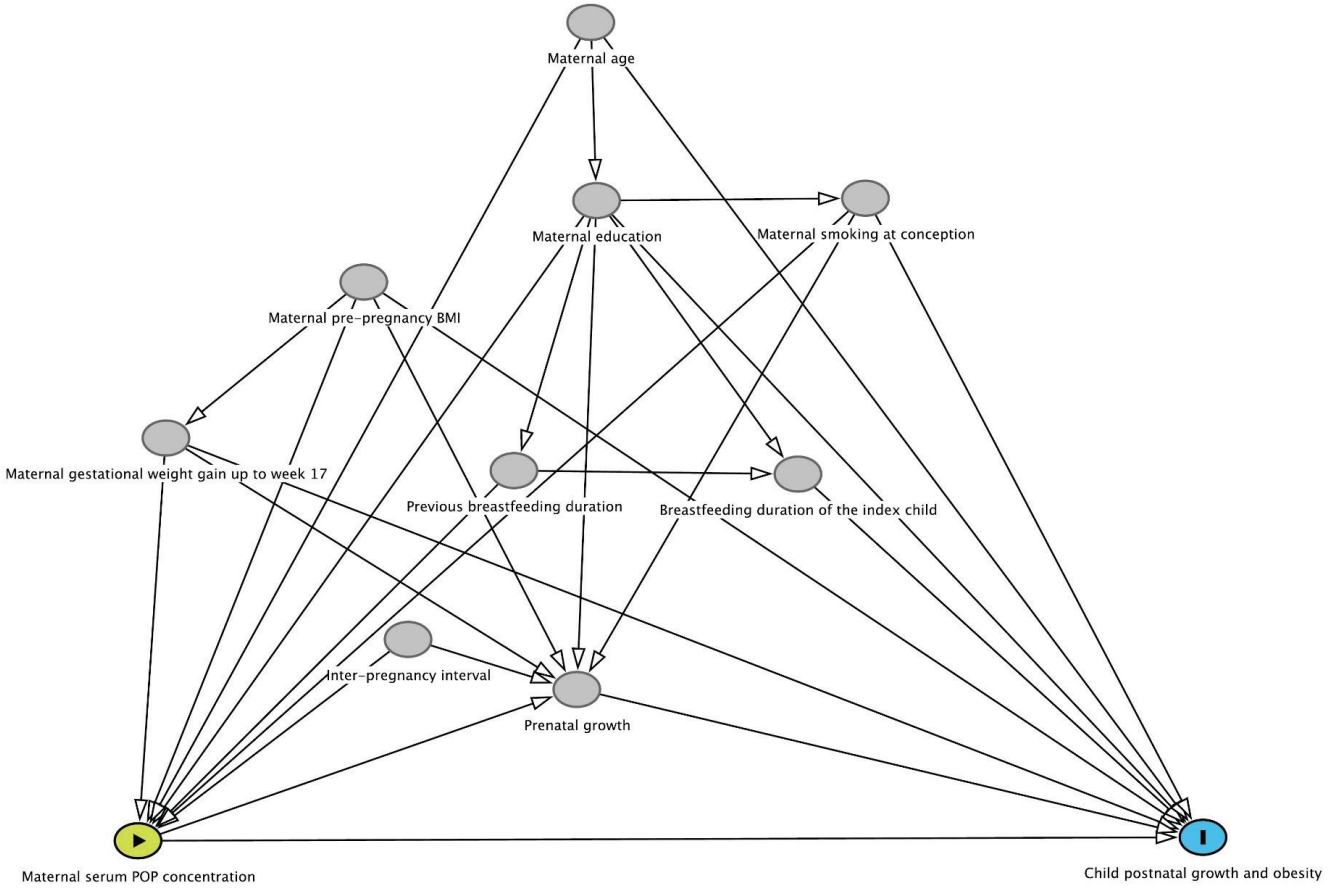
Supplementary description S1. Methods for weighting variables in sensitivity analyses.

Supplementary description S2. Methods for assessing fish consumption at gestational week 17-20.

Supplementary Table S1. Adjusted associations between ln-units of PFASs and BMI-for-age-and-sex z-scores, triceps skinfold z-scores (βs and 95% CIs) and overweight (OR and 95% CI) at 5 years of age, *un-weighted and weighted* according to prevalence of SGA offspring (10%), maternal overweight (30%) and smoking during pregnancy (3%), including all participants (n=412) and the Norwegian part (n=254).

Supplementary Table S2. Adjusted associations between ln-units of POPs and BMI-for-age-and-sex z-scores (β and 95% CI) and overweight (OR and 95% CI) at 5 years of age, with mutual adjustment for PFOS, PFOA and PCB 153, all participants (n=412) and Norwegian part (n=254).

Supplementary Figure S1. Directed acyclic graph (DAG) of the relationships between maternal serum POP levels and postnatal growth and obesity



Supplementary description S1. Methods for weighting variables in sensitivity analyses

Our cohort consisted of 33% SGA children in contrast to 10% in a general population, and to ensure generalizability, we did stratum-weighted sensitivity analyses where weights were the inverse probability of selection [1]. This means that the under-represented group get a weight larger than 1, and those in the over-represented group get a weight smaller than 1. We computed the weights by this formula: *percentage in general population divided by percentage in study sample*. Hence, SGA children were weighted 0.30 (=10% divided by 33%) and non-SGA children were weighted 1.35 (=90% divided by 67%) in these analyses.

We also performed weighted sensitivity analyses based on smoking prevalence and maternal overweight prevalence, which differed in our cohort compared to the general pregnant population today. In the former analyses, we weighted smokers 0.27 (=12% divided by 45%) and non-smokers 1.60 (=88% divided by 55%), and in the latter, we weighted mothers with overweight 3.26 (=31% divided by 9.5%), normal weight 0.83 (=66% divided by 80%) and underweight 0.29 (=3% divided by 10.5%). Prevalence of smoking during pregnancy and pre-pregnancy overweight was based on a prospective population-based pregnancy cohort study conducted in Norway from 1999-2008 [2].

Supplementary description S2. Methods for assessing fish consumption at gestational week 17-20.

At gestational week 17-20, three days of dietary records were collected among the Norwegian women [3]. Data were collected during the same three weekdays (Tuesday, Wednesday and Thursday). The amounts of food consumed were given in household measures, supplemented by food models presented as booklet with full scale drawings. Internal validity was tested against a food frequency questionnaire in a comparable group of non-pregnant Norwegian

women. Maternal fish consumption was calculated as gram consumed of lean and fatty fish, shellfish and fish spread, and categorized as 0, 1-50 and >50 grams per day.

Supplementary Table S1. Adjusted associations between ln-units of PFASs and BMI-for-age-and-sex z-scores, triceps skinfold z-scores (βs and 95% CIs) and overweight (OR and 95% CI) at 5 years of age, *un-weighted and weighted* according to prevalence of SGA offspring (10%), maternal overweight (30%) and smoking during pregnancy (12%), including all participants (n=412) and the Norwegian part (n=254)

Maternal serum levels	All participants (n=412)			Norway (n=254)		
	BMI z-score[1]	Triceps skinfold z-score[1]	Overweight (≥85th percentile)[1]	BMI z-score[2]	Triceps skinfold z-score[2]	Overweight (≥85th percentile)[2]
	β (95% CI)	β (95% CI)	OR (95% CI)	β (95% CI)	β (95% CI)	OR (95% CI)
PFOS						
A	**0.18 (0.01, 0.35)**	**0.15 (0.02, 0.27)**	**2.04 (1.11-3.74)**	**0.30 (0.08, 0.51)**	**0.20 (0.06, 0.35)**	**2.96 (1.42-6.15)**
B	**0.19 (0.02, 0.35)**	**0.19 (0.07, 0.31)**	**2.04 (1.13-3.67)**	**0.29 (0.08, 0.49)**	**0.25 (0.11, 0.39)**	**3.00 (1.47-6.13)**
C	**0.27 (0.10, 0.45)**	**0.19 (0.06, 0.32)**	**1.98 (1.12-3.51)**	**0.43 (0.20, 0.65)**	**0.25 (0.10, 0.41)**	**2.95 (1.48-5.87)**
D	0.15 (-0.01, 0.32)	**0.20 (0.07, 0.32)**	**3.05 (1.49-6.25)**	**0.24 (0.02, 0.46)**	**0.20 (0.04, 0.35)**	**6.87 (2.50-18.9)**
PFOA						
A	0.18 (-0.03, 0.39)	0.14 (-0.02, 0.29)	1.61 (0.75-3.46)	**0.32 (0.05, 0.60)**	**0.24 (0.05, 0.42)**	**2.90 (1.10-7.63)**
B	0.14 (-0.08, 0.35)	**0.18 (0.02, 0.33)**	1.40 (0.66-3.00)	0.21 (-0.06, 0.48)	**0.27 (0.08, 0.46)**	2.32 (0.90-5.95)
C	**0.22 (0.001, 0.44)**	**0.19 (0.02, 0.35)**	1.81 (0.84-3.89)	**0.33 (0.03, 0.63)**	**0.28 (0.08, 0.48)**	**3.03 (1.14-8.11)**
D	0.16 (-0.05, 0.37)	**0.19 (0.03, 0.35)**	2.10 (0.91-4.86)	**0.29 (0.01, 0.57)**	**0.24 (0.04, 0.44)**	**4.52 (1.42-14.3)**

[1]Adjusted for maternal age, education, smoking at conception, pre-pregnancy BMI, maternal weight gain at 17 weeks of gestation, interpregnancy interval, previous breastfeeding duration and country of residence

[2]Adjusted for maternal age, education, smoking at conception, pre-pregnancy BMI, maternal weight gain at 17 weeks of gestation, interpregnancy interval and previous breastfeeding duration

A: Un-weighted analysis

B: Weighted analysis; SGA offspring = 10%

C: Weighted analysis; maternal overweight = 30%

D: Weighted analysis; smoking during pregnancy = 12%

Supplementary Table S2. Adjusted associations between ln-units of POPs and BMI-for-age-and-sex z-scores (β and 95% CI) and overweight (OR and 95% CI) at 5 years of age, with mutual adjustment for PFOS, PFOA and PCB 153, all participants (n=412) and Norwegian part (n=254)

Maternal serum POPs	All participants (n=412)[1]	Norway (n=254)[2]
	BMI-for-age-and-sex z-score	
	β (95% CI)	
PFOS		
Adjusted	**0.18 (0.01, 0.35)**	**0.30 (0.08, 0.51)**
+PCB 153	0.17 (-0.01, 0.34)	**0.28 (0.07, 0.49)**
+PCB 153 and PFOA	0.12 (-0.11, 0.35)	0.22 (-0.07, 0.50)
PFOA		
Adjusted	0.18 (-0.03, 0.39)	**0.32 (0.05, 0.60)**
+PCB 153	0.16 (-0.05, 0.18)	**0.29 (0.02, 0.57)**
+PCB 153 and PFOS	0.06 (-0.23, 0.35)	0.10 (-0.27, 0.47)
PCB 153		
Adjusted	0.30 (-0.03, 0.63)	**0.45 (0.03, 0.87)**
+PFOS	0.30 (-0.03, 0.63)	*0.41 (-0.01, 0.83)*
+PFOS and PFOA	0.29 (-0.04, 0.62)	*0.42 (-0.002, 0.83)*
	Overweight (≥85th percentile)	
	β (95% CI)	
PFOS		
Adjusted	**2.04 (1.11-3.74)**	**2.96 (1.42-6.15)**
+PCB 153	1.72 (0.97-3.06)	**2.90 (1.39-6.08)**
+PCB 153 and PFOA	1.97 (0.88-4.44)	2.64 (0.97-7.20)
PFOA		
Adjusted	1.61 (0.75-3.46)	**2.90 (1.10-7.63)**
+PCB 153	1.44 (0.68-3.05)	**2.77 (1.04-7.39)**
+PCB 153 and PFOS	0.79 (0.28-2.23)	1.21 (0.33-4.47)
PCB 153		
Adjusted	1.37 (0.42-4.49)	2.13 (0.49-9.26)
+PFOS	0.67 (0.25-1.83)	1.75 (0.38-8.07)
+PFOS and PFOA	0.78 (0.27-2.23)	1.75 (0.34-8.99)

[1]Adjusted for maternal age, education, smoking at conception, pre-pregnancy BMI, weight gain at 17 weeks, inter-pregnancy interval, previous breastfeeding duration and country of residence.

[2]Adjusted for maternal age, education, smoking at conception, pre-pregnancy BMI, weight gain at 17 weeks, inter-pregnancy interval and previous breastfeeding duration.

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