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Prenatal exposure to persistent organic pollutants and physical, mental and motor development in young children

PhD dissertation

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Preface

The studies presented in this PhD thesis were performed between 2011 and 2014 at the Danish Ramazzini Centre, Department of Occupational Medicine, Aarhus University Hospital, which provided an excellent research environment.

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Birgit Bjerre Høyer
Aarhus, July 2014

The thesis is based on the following manuscripts:

I. Motor development following *in utero* exposure to organochlorines: a follow-up study of children aged 5–9 years in Greenland, Ukraine and Poland. [In review]

II. Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5–9 years – a prospective study. [In review]

III. Body mass index in young school-age children in relation to organochlorine compounds in early life: a prospective study. Int J Obes (Lond), 2014, apr;

IV. Anthropometry at 5 to 9 years of age in relation to prenatal exposure to perfluorinated alkyl substances. [In review]

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List of abbreviations

ADHD, attention deficit hyperactivity disorder

BMI, body mass index

BSID, Bayley Scales of Infant Development

CB-153, 2,2',4,4',5,5'-hexachlorobiphenyl

CDC, Centers for Disease Control and Prevention

CI, confidence interval

DCD, Developmental Coordination Disorder

DCDQ'07, Developmental Coordination Disorder Questionnaire 2007

DDE, Dichlorodiphenyldichloroethylene

DDT, Dichlorodiphenyltrichloroethane

DNA, deoxyribonucleic acids

INUENDO, Biopersistent organochlorines in diet and human fertility. EU funded project running 2002 to 2005

IOTF, International Obesity Task Force

MSCA, McCarthy Scales of Children's Abilities

NES, neurobehavioural evaluation system

OR, odds ratio

PCBs, polychlorinated biphenyls

PDI, Psychomotor Development Index

PFAS, perfluorinated alkyl substances

PFOA, perfluorooctanoate

PFOS, perfluorooctane sulfonate

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

SD, standard deviation

SDQ, Strength and Difficulties Questionnaire

T4, total thyroxine

WHO, World Health Organisation

WHtR, waist-to-height ratio

1. English summary

Prenatal exposure to persistent organic pollutants and physical, mental and motor development in young children

Backgrounds

Persistent organic pollutants (POPs) are widespread in the environment, have long half-lives in humans and are able to cross the placenta causing exposure to the fetus. Developmental programming states that adverse environmental exposures during critical periods of development may influence the risk of disease later in life. The POPs are believed to be endocrine disruptors, possibly with obesogenic and adverse neuro-behavioural properties.

Aims

The overall aim of the thesis was to investigate the association between prenatal exposure to POPs and child development. First, we examined the association between 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p'*-DDE) and infancy developmental milestones and motor development in school-age children. Secondly, we examined the association between *in utero* perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) exposure and motor and behavioural development at school-age. Thirdly, we examined the association between measured prenatal and estimated postnatal exposure to CB-153 and *p,p'*-DDE and body mass index and overweight at school-age. Finally, *in utero* exposure to PFOA and PFOS was investigated in relation to overweight and waist-to-height ratio.

Methods

A cohort of 1,441 pregnant women was established in 2002–2004 in Greenland, Warsaw (Poland) and Kharkiv (Ukraine). The women provided a blood sample and responded to a detailed questionnaire. In 2009–2012, when the children were between 5 and 9 years of age, 1,121 children were identified, and a follow-up examination of height and weight and an interview concerning motor milestones, motor development and behaviour were performed. Covariate data were collected at baseline and follow-up. The concentrations of CB-153, *p,p'*-DDE, PFOA and PFOS in the pregnant women were analysed, and postnatal levels of CB-153 and *p,p'*-DDE were estimated by use of a toxicokinetic model. Different multivariable regression models were used to evaluate the exposure-outcome associations.

Results

We observed no association between prenatal exposure to CB-153 and *p,p'*-DDE and infancy developmental milestones or motor development at school-age. PFOA and PFOS were not associated with motor development. In Greenland, elevated PFOA concentrations were associated with more hyperactive behaviour, and PFOS was associated with more behavioural problems.

No clear association was observed between prenatal and estimated postnatal exposure to CB-153 and *p,p'*-DDE and body mass index and overweight at school-age. Prenatal PFOA and PFOS exposures were not unequivocally associated with overweight. Elevated PFOS exposure was associated with elevated risk of having a waist-to-height ratio > 0.5, and similar tendencies were observed in relation to PFOA exposure.

Conclusions

PFOA and PFOS seem to have more adverse effects on neuro-behavioural and adiposity outcomes than CB-153 and *p,p'*-DDE. Both waist-to-height ratio and behaviour were associated with either or both studied perfluorinated alkyl substances, whereas no associations were observed between the studied organochlorines and any outcome.

2. Dansk resumé – Danish summary

Prænatal eksponering for persistente organiske forureningsstoffer og børns fysiske, mentale og motorisk udvikling

Baggrund

Persistente organiske forureningsstoffer (POPs) findes overalt i miljøet, har lange halveringstider i menneskeligt serum og kan passerer placenta, hvilket betyder at fostret eksponeres for forureningsstofferne. Ifølge hypotesen om føtal programmering kan udsættelse for ugunstigt intrauterint miljø i kritiske perioder af udviklingen påvirke risikoen for sygdom senere i livet. POPs er mistænkt for at forstyrre kroppens hormonelle balance muligvis med egenskaber, der har negativ indflydelse på vægt, motorik og adfærd.

Formål

Det overordnede formål med afhandlingen var at undersøge sammenhængen mellem prænatal eksponering for POPs og børns udvikling. Først undersøgte vi sammenhængen mellem 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) og 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p'*-DDE) og spædbørns udviklingsmæssige milepæle og den motoriske udvikling i skolealderen. Desuden undersøgte vi sammenhængen mellem prænatal eksponering for perfluorooctanoate (PFOA) og perfluorooctane sulfonate (PFOS) og motorisk og adfærdsmæssig udvikling i skolealderen. Herudover undersøgte vi sammenhængen mellem målt prænatal og estimeret postnatal eksponering for CB-153 og *p,p'*-DDE og body mass index og overvægt i skolealderen. Afslutningsvis undersøgte vi sammenhængen mellem prænatal eksponering for PFOA og PFOS og overvægt og talje-højde ratio.

Metoder

I 2002–2004 blev en graviditetskohorte etableret bestående af 1441 kvinder i Grønland, Warszawa (Polen) og Kharkiv (Ukraine). Kvinderne afgav en blodprøve og besvarede et detaljeret spørgeskema. I 2009–2012, da børnene var mellem 5 og 9 år gamle, blev 1121 børn identificeret og en opfølgende undersøgelse blev udført inklusiv mål af højde og vægt og interview vedrørende udviklingsmæssige milepæle, motorisk udvikling og adfærd. Kovarianter blev indsamlet ved graviditetsinterviewet og ved opfølgningsinterviewet. Graviditetsniveauerne af CB-153, *p,p'*-DDE, PFOA og PFOS blev

analyseret og postnatale niveauer af CB-153 og *p,p'*-DDE blev estimeret ved hjælp af en toksiko-kinetisk model. Sammenhænge mellem eksponeringer og udfald blev analyseret ved hjælp af relevante multiple regressionsmodeller.

Resultater

Vi fandt ingen sammenhæng mellem CB-153 og *p,p'*-DDE og spædbørns udviklingsmæssige milepæle og motorisk udvikling i skolealderen. Prænatal PFOA og PFOS eksponering var ikke associeret med motorisk udvikling. I Grønland var højere prænatal PFOA koncentrationer associeret med mere hyperaktiv adfærd, og prænatal PFOS eksponering var associeret med flere adfærdsmæssige problemer. Vi fandt ingen klar sammenhæng mellem prænatal og estimeret postnatal eksponering for CB-153 og *p,p'*-DDE og body mass index og overvægt i skolealderen. Prænatal eksponering for PFOA og PFOS var ikke entydigt associeret med overvægt. Højere prænatal eksponering for PFOS var associeret med forhøjet risiko for at have talje-højde ratio > 0,5, og lignende tendenser blev observeret i relation til prænatal PFOA eksponering.

Konklusion

Prænatal PFOA og PFOS eksponering lader til at have flere ugunstige effekter på adfærds-, motorik- og overvægtsmæssige udfald end CB-153 og *p,p'*-DDE. Både talje-højde ratio og adfærd var associeret med prænatal eksponering for de undersøgte perfluorerede stoffer, hvorimod der ikke blev observeret nogen sammenhænge mellem de undersøgte organokloriner og noget udfald.

3. Introduction

Approximately 5–6% of children suffer from the neuro-behavioural disorder developmental coordination disorder (DCD), which includes delays in achieving motor milestones in infancy among others [1-3]. Moreover, the worldwide prevalence of behavioural disorders such as attention deficit hyperactivity disorder (ADHD) is estimated to be between 5 and 10% among more than 100 prevalence studies from North America, South America, Europe, Oceania, Asia and Africa [4], and over the last decades, increasing numbers of children have been diagnosed with the disorder [5]. The prevalence of ADHD is highest in South America, North America and Europe, whereas the Asian prevalence is around 2%. Twin studies of behavioural disorders suggest a high level of heritability, but fetal environmental pollutant exposures are likely also to play a role [6].

At the same time, the prevalence of overweight, including obesity among children 6-17 years of age, is reported to be between 18–49% in various surveys around the world, depending on country of study and definition of overweight [7-9]. The major predictors of childhood overweight and obesity are unhealthy diet, sedentary lifestyle and low parental education [8,10], but overweight has a complex and multifactorial aetiology that also includes genetic, environmental, metabolic and behavioural factors [11,12].

During the past decades, an increased attention has been focused on the possible association between the persistent organic pollutants (POPs) and health outcomes in general [13-19], and *in utero* exposure to different POPs and various health-related outcomes, including neuro-behaviour and anthropometry, have also been investigated [20-27].

3.1 POP exposure

POPs like the organochlorine pollutants polychlorinated biphenyls (PCBs), organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and its main metabolite dichlorodiphenyldichloroethylene (DDE) and the perfluorinated alkyl substances (PFAS) perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are ubiquitous in the environment, have half-lives of several years in human serum, and they have bio-accumulative properties [28-32]. POPs are able to cross the placenta causing exposure to the fetus [33-36], and they have been found worldwide in serum

samples [29,36-39]. In addition, the organochlorines and PFOS are found in human milk [37,40-43].

Different PCB congeners are found to have estrogenic, anti-estrogenic and anti-androgenic effects [44] and several molecular mechanistic studies have found endocrine disrupting effects in various PCB congeners [45-47]. PCBs may interfere with cell proliferation and they have the potential to exert thyroid hormone disruption [48]. Moreover, PCBs down-regulate thyroid function in mice and rats [49] and decrease levels of free thyroxine (T4) in human cord and neonatal blood [50], which could possibly lead to an increase in adipocyte lipid accumulation [51]. *In vivo* and *in vitro* studies have reported PFOA and PFOS to have weak estrogenic effects [52,53] and a new in vitro study reported PFOA and PFOS to have potential to interfere with the estrogene receptor and the androgene receptor [14]. Prenatal PFOS exposure has, furthermore, been associated with depressed free thyroxine in rat pups [54], and a new *in vitro* study found PFOA and PFOS to possess endocrine disrupting potential by interfering with the thyroid hormone and the aryl hydrocarbon receptor [13]. These endocrine abilities may affect the developing brain and the metabolism and thereby affect offspring neuro-behavioural attributes [55] and anthropometry [56].

PCBs are man-made chemicals and consist of a group of 209 congeners. PCBs have been widely used in industrial production, e.g. in capacitors, sealants, plasticisers, fire retardants and hydraulic fluids, but they were banned in the late 1970s in the United States and the early 1980s in Europe because of their environmental persistence [57,58].

Also, the insecticide DDT and its main metabolite DDE are highly persistent in the environment, and although the agricultural use of DDT has been severely restricted through the regional United Nations Economic Commission for Europe and the Stockholm Convention [59], it is still used as disease vector control in some developing countries [60]. PCBs and DDE are lipophilic compounds which bio-accumulate in adipose tissue, and, thus, the main exposure is through food consumption and breast feeding [31,32]. The half-lives of different PCBs in children are estimated to be between 3 and 9 years [61], while the estimated half-life of DDE in humans is between 6 and 10 years [62].

PFOA and PFOS belong to a group of PFAS which have been used extensively in various consumer products such as textiles, leather, paper and food wrapping due to their water-, dirt- and oil-repellent properties [63]. A phase out of the production of PFOA and PFOS was initiated in 2000 by the major U.S. producers [64], and PFOS was added to an appendix of the Stockholm Convention list in 2009 [65]. However, as the estimated half-lives in humans are 3.8 and 5.4 years for PFOA and PFOS, respectively [28] and precursor substances are able to transform into PFOA and PFOS, we are still being exposed for instance through diet, packaged food, drinking water and dust [63]. However, the serum concentrations of the PFOA and PFOS as well as PCBs and DDE in humans are slowly declining in accordance with reduced production and the ratification of the Stockholm Convention [66-68].

3.2 Developmental programming

Studies from the late 1980s reporting associations between low birth weight and increased mortality of coronary heart disease [69,70] were the onset of the "fetal origins" hypothesis (later called "developmental programming" hypothesis) [71,72]. This hypothesis suggested that individuals exposed to an adverse *in utero* environment, often resulting in restricted growth, had a permanently changed structure and physiology, increasing the risk of diabetes and heart disease later in life [71,72]. At least three mechanisms are involved in the developmental programming: 1) epigenetic change, which involves permanent, environmentally induced changes in gene expression, either through changes in deoxyribonucleic (DNA) methylation or histone modifications; 2) changes in cell-cycle regulation; and 3) changes in cellular or tissue differentiation [73]. The hypothesis provided the rationale for examining *in utero* exposure to POPs and several health outcomes later in life.

3.3 Prenatal exposure to POPs and developmental milestones and developmental coordination disorder

Developmental motor milestones in infancy include crawling, standing with support and walking without support. According to the World Health Organisation (WHO), the average age at which these milestones are achieved is wide. The median (5^{th}–95^{th} percentile) age of crawling is 8.3 (6.1–11.3) months, while it is 7.4 (5.5–10.1) months for standing with support and 12.0 (9.4–15.3) months for walking without support [74]. Even though the age range of achieving the milestones is wide, marked delays in

achieving motor milestones may be related to DCD [75]. DCD is a neuro-developmental disorder characterised by being a chronic condition of motor impairment that interferes with the daily activities and academic achievements of the child [2,3]. Children suffering from DCD often have problems with fine or gross motor skills or both [3], and it is more commonly diagnosed in boys than in girls (2:1) [2]. In addition, it is more prevalent in children born with very low birth weight or very pre-term [3], and it often co-occurs alongside ADHD, specific language impairment and dyslexia [3]. The diagnosis according to the American Psychiatric Association is presented in Table 1.

Table 1. Diagnostic criteria for DCD according to the American Psychiatric Association [75].

DCD diagnostic criteria
A. Performance in daily activities that require motor coordination is substantially below that expected given the person's chronological age and measured intelligence. This may be manifested by marked delays in achieving motor milestones (e.g., walking, crawling, sitting), dropping things, "clumsiness," poor performance in sports, or poor handwriting.
B. The disturbance in Criterion A significantly interferes with academic achievement or activities of daily living.
C. The disturbance is not due to a general medical condition (e.g., cerebral palsy, hemiplegia, or muscular dystrophy) and does not meet the criteria for a Pervasive Developmental Disorder.
D. If mental retardation is present, the motor difficulties are in excess of those usually associated with it.

Abbreviations: DCD, developmental coordination disorder

Animal studies have found decreased muscular strength in relation to high prenatal exposure to tetrachlorobiphenyl [76]. Moreover, prenatal exposure to the PCB congener CB-180 has been associated with reduced motor activity in male rats, and prenatal exposure to another congener, CB-138, was associated with reduced motor activity in both genders [77].

Epidemiological studies on the association between prenatal exposure to PCBs and DDE and developmental milestones and motor development have reported inconsistent findings (Table 2). Briefly, high *in utero* exposure to PCBs was related to a significant decrease in motor abilities, indicated by a 7- to 8-point decrease of the Psychomotor Development Index (PDI) scores in Bayley Scales of Infant Development (BSID) (a screening tool measuring children's motor and mental abilities) compared with low exposure at 6, 12 and 24 months [78,79], which is in line with a recent study [80], whereas many other studies reported associations only at some but not all follow-up ages [81-85] or only among two separate congeners of a whole range of congeners [86]. By contrast, the Collaborative Perinatal Project from 12 hospitals in the United States found no association between prenatal exposure to PCBs and motor development in terms of PDI scores [87], which is consistent with several other studies [88-97].

Some studies reported associations between prenatal DDE exposure and motor development. *In utero* exposure to DDE has been associated with hyporeflexia at 1

month in a cohort of North American children [78] and a 4 points decrease PDI score for each doubling of DDE [96] and poor performance on the continuous performance test [89]. Moreover, a 2-point decrease in PDI scores for every 10-fold increase of DDE has been observed at 6 months of age, but not at 12 and 24 months [98]. In contrast, no associations were found between prenatal DDE and various motor developmental outcome measures [79-81,88,93,99-102].

To my knowledge, only one study has investigated the association between prenatal PFOA and PFOS exposure and motor development, and they observed an association between PFOS (but not PFOA) and gross motor function at 2 years of age [26].

Table 2. Follow-up studies of the association between prenatal exposure to PCBs and DDE and developmental milestones and motor development by cohort, enrolment year and age of the children.

Cohort Enrolment	Authors Publication year	Age at follow-up	*N*	Exposures	Outcome	Summary of results
The Collaborative Prenatal Project, USA 1959-65	Daniels et al. 2003 [87]	8 months	1,207	Prenatal **PCBs**. 3rd trimester maternal serum. Total-PCB = Sum of 11 congeners; PCB-28, 52, 74, 105, 118, 138, 153, 170, 180, 194 and 203 Lipid adjustment[a]. Median (95% CI) PCB: 2.7 (1.8, 3.7) ng/ml	BSID-PDI	No association between prenatal **PCBs** and PDI.
The North Carolina Breast Milk and Formula Project, USA 1978-82	Rogan et al. 1986 [78]	7-31 days	912	Prenatal **PCBs** and **DDE**. Maternal blood, cord blood, breast milk and placenta samples. No lipid adjustment. Levels: PCB:0–> 4 mg/L in milk fat DDE:0–> 6 mg/L in milk fat.	Brazelton neonatal behavioural assessment scales	Elevated prenatal **PCB** levels were associated with less muscle tone and activity. Elevated prenatal **DDE** exposure was associated with hyporeflexia.
	Gladen et al. 1988 [79]	6 and 12 months	858	Prenatal **PCBs** and **DDE**. Maternal blood, cord blood, breast milk and placenta samples. Levels: PCB:0–> 4 mg/L in milk fat DDE:0–> 6 mg/L in milk fat No lipid adjustment.	BSID-PDI	Elevated prenatal **PCB** exposure was associated with decreased PDI at 6 and 12 months. No association between prenatal **DDE** and PDI at 6 and 12 months.

Cohort Enrolment	Authors Publication year	Age at follow-up	*N*	Exposures	Outcome	Summary of results
	Rogan et al. 1991 [81]	18 and 24 months	676/670	Prenatal **PCBs** and **DDE**. Maternal blood, cord blood, breast milk and placenta samples. Levels: PCB:0–> 4 mg/L in milk fat DDE:0–> 6 mg/L in milk fat. No lipid adjustment.	BSID-PDI	Elevated prenatal **PCB** exposure was non-significantly associated with decreased PDI score at 18 months and significantly associated with decreased PDI at 24 months. No significant association between prenatal **DDE** and PDI at 18 or 24 months.
	Gladen et al. 1991 [88]	3-5 years	859	Prenatal **PCBs** and **DDE**. Maternal blood, cord blood, breast milk and placenta samples. Levels: PCB:0–> 4 mg/L in milk fat DDE:0–> 6 mg/L in milk fat. No lipid adjustment.	MSCA	No associations between prenatal exposure to **PCBs/DDE** and PDI.
Faroe Islands 1986-87	Grandjean et al. 2012 [89]	7 years	923	Pre- and postnatal **PCBs** and **DDE**. Cord **PCBs** geometric mean (interquartile range): 1.86 (1.16–3.16) µg/L Postnatal PCB at 7 years: 1.71 (1.06–2.64) µg/g lipid DDE level not presented. No lipid adjustment.	NES2 Finger Tapping; NES2 Hand-Eye Coordination average deviation; and NES2 Continuous Performance Test	No association between prenatal **PCBs** and motor function. Increasing prenatal **DDE** exposure was statistically significantly associated with a poorer continuous performance test but no other motor outcome.

Groningen/ Rotterdam, The Netherlands 1990-92	Huisman et al. 1995 [90]	18 months	418	Prenatal **PCBs**. Maternal median (5^{th}–95^{th} percentile) total-PCB: Breast-fed group: 2.2 (1.1–4.0) µg/L Formula-fed group: 1.9 (0.95–3.6) µg/L. No lipid adjustment.	Age-specific neurological examination of motor functioning: grasping, sitting, crawling, standing and walking in a normal, mildly abnormal or abnormal way.	No association between prenatal **PCBs** and neurological optimality score.
	Lanting et al. 1998 [91]	3.5 years	394	Prenatal **PCBs**. Maternal total-PCB (118, 138, 153, 180) Median (5^{th} –95^{th} percentile): 0.4 (0.2–0.9) µg/L. No lipid adjustment.	Neurological optimality score and fluency cluster score.	No association between prenatal **PCBs** and motor function.
Subsample of the Dutch PCB/Dioxin study, Rotterdam, The Netherlands 1990-92	Koopman-Esseboom et al. 1996 [82]	3, 7 and 18 months	207	Prenatal **PCBs.** (118, 138, 153 and 180) (PCB-plasma-sum) Maternal 3^{rd} trimester serum sample, cord blood and milk samples. Maternal PCB-plasma-sum: 2.2 (1.2–3.2) ng/g. Lipid adjustment [b.]	BSID-PDI	Each doubling of prenatal **PCBs** exposure decreased PDI by 3 points at 3 months. No association between prenatal **PCBs** and PDI at 7 and 18 months.
The Dutch PCB/Dioxin study, Groningen/ Rotterdam, The Netherlands 1990-92	Vreugdenhil et al. 2002 [83]	6.5 years	354	Prenatal **PCBs**. Total-PCB (118, 138, 153, 156, 170, 180 and 189) in maternal blood and cord blood. Median (range) total-PCBs Breast-fed group: 2.2 (0.7–7.4) µg/L Formula-fed group: 1.9 (0.6–5.1) µg/L No lipid adjustment.	MSCA	No association between prenatal **PCBs** and motor performance. Among formula-fed children with disadvantage backgrounds, prenatal **PCBs** exposure was associated with decreased motor scores.

Cohort Enrolment	Authors Publication year	Age at follow-up	*N*	Exposures	Outcome	Summary of results
Oswego, NY USA 1991-94	Stewart et al. 2003 [84]	38 months 54 months	212	Prenatal **PCBs**. Median (25^{th}–75^{th} percentile) total PCBs: 0.52 (0.17–1.11) ng/g wet weight. No lipid adjustment.	MSCA	The children exposed to the highest prenatal **PCBs** levels had a statistically significantly decreased motor score at 38 months compared to low exposed children. No association between prenatal **PCBs** and motor function at 54 months.
Düsseldorf, Germany 1993-95	Winneke et al. 1998 [92]	7 months	171	Prenatal **PCBs**. Mean cord total-PCBs (138, 153, 180) 0.55 ng/ml. No lipid adjustment.	BSID-PDI	No association between prenatal **PCBs** and PDI.
	Walkowiack et al. 2001 [85]	7, 18 and 30 months	116	Prenatal **PCBs**. Median (5^{th} –95^{th} percentile) cord PCB: 0.39 (0.11–0.83) ng/ml. Lipid adjustment [b.]	BSID-PDI	No association between prenatal **PCBs** and PDI at 7 months. Elevated prenatal **PCB** exposure was associated with decreased PDI at 18 and 30 months.
Nunavik, Northern Quebec, Canada 1993-96	Despres et al. 2005 [93]	5 years	110	Prenatal **PCBs** and **DDE**. Cord blood PCB-153 geometric mean: 99.6 ng/g lipid. *p,p'*-DDE geometric mean: 371.9 ng/g lipid. Lipid adjustment [b.]	Gross motor function by use of Huttenlocher Fine neuromotor performance By use of the Catsy's system: postural hand tremor, reaction time and postural sway.	No association between prenatal exposures to **PCBs** and **DDE** and gross or fine motor function.
Faroe Islands 1994-95	Coccini et al. 2009 [94]	7 years	105	Prenatal **PCBs**. Total maternal PCBs geometric mean (range) 1.12 (0.04–18.4) µg/g lipid. Lipid adjustment [b.]	NES2 Continuous Performance Test; Finger Tapping;, Santa Ana Peg Board.	No association between prenatal **PCBs** and motor outcomes.

The New York State Angler Cohort, USA 1996-99	Lynch et al. 2012 [95]	24 months	44	Prenatal **PCBs**. The median (range) total-PCBs in maternal serum: 991.7 (585.5–1538.3) ng/g lipid. Lipid adjustment [b.]	BSID-PDI	No association between prenatal **PCBs** and PDI.
Subsample of INMA cohort, Flix, Spain 1997-99	Ribas-Fito et al. 2003 [96]	13 months	92	Prenatal **PCB**-28, 52, 101, 118, 138, 153 and 180 and ***p,p'*-DDE** in cord blood. No exposure levels presented. No lipid information.	BSID-PDI	Prenatal **PCBs** were marginally associated with decreased PDI (p = 0.1). Prenatal **DDE** was associated with decreased PDI.
Subsample of INMA cohort, Gipuzkoa, Sabadell and Valencia, Spain 2003-08	Gascon et al. 2013 [80]	14 months	1,175	Prenatal **PCBs** and **DDE**. Maternal geometric mean (25^{th}–75^{th} percentile): PCB-153: 39.8 (28.2–60.7) ng/g lipid DDE: 132.5 (76.6–204.6) ng/g lipid. Lipid adjustment [b.]	BSID-PDI	Increasing prenatal **PCB-153** levels were associated with decreased PDI scores. No association between prenatal **DDE** and PDI.
Subsample of INMA cohort, Menorca, Spain 1997-98	Forns et al. 2012 [144]	4 years	355	Prenatal **PCBs**. Total-cord PCB (CB-118, 138, 153,180) Median (25–75 percentile): 0.71 (0.54–0.97) ng/ml. No lipid adjustment.	MSCA	No association between prenatal **PCBs** and motor function.
Subsample of INMA cohort, Ribera d'Ebre cohort 1997-99 & Menorca cohort Spain 1997-98	Ribas-Fito et al. 2006 [99]	4 years	475	Prenatal **DDE**. Cord serum median (25^{th}–75^{th} percentile) DDE: Ribera: 0.9 (0.5–1.7) ng/ml Menorca: 1.0 (0.6–19.5) ng/ml. No lipid adjustment.	MSCA	No association between prenatal **DDE** and motor function.

Cohort Enrolment	Authors Publication year	Age at follow-up	*N*	Exposures	Outcome	Summary of results
CHAMACOS cohort, California, USA (immigrants from Mexico), 1999-2000	Eskenazi et al. 2006 [98]	6, 12 and 24 months	330	Prenatal **DDE**. Mean maternal serum DDE: 1,437 ng/g lipid. Lipid adjustment [b.]	BSID-PDI	A 10-fold increase of prenatal ***p,p'*-DDE** was associated with a decrease of 2.14 PDI points at 6 months. No association between prenatal ***p,p'*-DDE** and PDI at 24 months.
Duisburg, Germany 2000-02	Wilhelm et al. 2008 [97]	12 and 24 months	111	Prenatal **PCBs**. Median (95th percentile) Total-PCBs (138, 153, 180) in milk: 181 (377) ng/g lipid. Lipid adjustment [b.]	BSID-PDI	No association between prenatal **PCBs** and PDI at 12 or 24 months.
Morelos, Mexico 2001-05	Bahena-Medina et al. 2011 [100]	1 month	265	Prenatal **DDE**. Maternal serum. Level: not reported. No lipid information.	BSID-PDI	No associations between prenatal **DDE** and PDI.
	Torres-Sanchez et al. 2007 [145]	1, 3, 6 and 12 months	244	Prenatal **DDE.** Mean maternal DDE concentration: 1st trimester: 6.4 ng/ml. No lipid adjustment.	BSID-PDI	High 1st trimester **DDE** was associated with decreased PDI during 1st year of life. No associations were observed between 2nd or 3rd trimester **DDE** and PDI.
	Torres-Sanchez et al. 2009 [101]	12, 18, 24 and 30 months	270	Prenatal **DDE.** Mean maternal DDE concentration: 1st trimester: 6.3 ng/ml. No lipid adjustment.	BSID-PDI	No association between prenatal **DDE** and PDI at any age.

	Torres-Sanchez et al. 2013 [102]	42, 48, 54 and 60 months (3.5-5 years)	203	Prenatal **DDE.** Median 1st trimester (10^{th}–90^{th} percentile) DDE 7.6 (1.8–23.1) ng/ml No lipid adjustment.	MSCA	No association between prenatal **DDE** and motor performance at any age.
Eastern Slovakia 2002-04	Park et al. 2010 [86]	16 months	760	Prenatal **PCBs**. PCB- 118, 138, 153, 156, 170, 180 and 189 in maternal blood and cord blood. Mean maternal PCBs: 0.59 ng/mg lipid Lipid adjustment [b.]	BSID-PDI	An increase of maternal **PCB-118** and **156** from 25^{th} to 75^{th} percentile was associated with a decrease of 1.4 PDI points. No association between maternal **PCB-**138, 153, 170 and 180 and PDI.

Abbreviations: BSID, Bayley Scales of Infant Development; CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; MSCA, McCarthy Scales of Children's Abilities; NES, neurobehavioural evaluation system; PCBs, Polychlorinated biphenyls; PDI, psychomotor development index

[a] Lipid adjustment by including total lipids as a covariate

[b] Lipid adjustment by calculation

3.4 Prenatal exposure to POPs and abnormal behaviour

Abnormal behaviour is often understood as undesirable behaviour deviating from the normal. In contrast to DCD, abnormal behaviour does not have as specific a definition. However, various aspects of child behavioural attributes are measurable by questionnaires (e.g. Strength and Difficulties Questionnaire (SDQ)) and include conduct, hyperactive/inattentive behaviour, peer relationship, emotional behaviour and pro-social behaviour [103]. The edge between normal and abnormal behaviour is arbitrary, but cut-offs have been suggested and validated in various populations [103].

Animal studies suggest associations between prenatal exposure to PFOA and PFOS and neuro-behavioural deficits [104-106]. Human observational studies on the relation between PFAS exposures and behaviour are sparse and results are inconsistent, but that may be due to different exposure levels and study designs. One cross-sectional study reported an association between PFOA and PFOS and ADHD [107], while another study observed an inverse J-shaped association between PFOA and ADHD across quartiles of exposure [108], indicating highest risk among those exposed to second quartile. Moreover, no association was observed between relatively high prenatal exposures to PFOA and PFOS and behaviour at 7 years of age among a sub-sample of the Danish National Birth Cohort [109].

3.5 Pre- and postnatal exposure to POPs and overweight

Overweight is abnormal or excessive fat accumulation, which presents a risk to health [110]. Body mass index (BMI) is the most commonly used measure for adult overweight and is calculated by weight (kg)/ (height (m))^2 [110]. The concept of overweight and the use of BMI and BMI cut-offs in children are controversial since children are growing, BMI changes substantially with age [111], and no universal cut-off is used [112]. Hence, three definitions of overweight and obesity are used in the epidemiological literature and include BMI cut-offs suggested by the WHO, the International Obesity Task Force (IOTF) and the Centers for Disease Control and Prevention (CDC). The WHO suggests cut-offs of > 1 standard deviation (SD) and > 2 SD for overweight and obesity, respectively, using the WHO reference population [113]. The IOTF suggests a cut-off for overweight > 91st percentile and for obesity > 98th percentile using the IOTF reference population [112]. The CDC suggests an

overweight cut-off of > 85th percentile and an obesity cut-off of > 95th percentile using the American 2000 CDC growth chart [114].

Due in part to the controversy regarding the use of BMI in children, increased attention has been directed towards other measures of adiposity. In addition, excess abdominal fat is believed to be of greater concern than a large lean mass because abdominal fat is associated with increased cardio-metabolic morbidity among children and adults [115,116]. Hence, measures such as skinfold thickness, waist circumference, waist-hip ratio and waist-to-height ratio (WHtR) are now being extensively used.

Few epidemiological studies have examined the relation between exposure to PCBs and DDE during pregnancy and BMI standardised by age and sex, and findings are inconsistent [27,117-120]. Two studies, from Spain and Belgium, have examined the association between prenatal exposure to PCBs and standardised BMI measures and reported that high prenatal PCB levels are associated with an increased risk of overweight and elevated BMI standard deviation scores [27,117].

Results of prenatal DDE exposure and BMI outcomes are more equivocal. In a Spanish cohort, 2nd prenatal DDE tertile (but not 3rd) was associated with increased relative risk (RR) of overweight compared to 1st tertile [27], and a Belgian study reported increasing prenatal DDE exposure associated with decreased BMI standard deviation scores; however, findings were statistically non-significant [117]. Moreover, a Spanish study found increased prenatal DDE exposure to be associated with elevated BMI at 14 months. But when stratified by maternal pre-pregnancy weight, the association was only evident in children of normal-weight mothers [118]. In addition, a 10-fold increase of prenatal *p,p'*-DDE was non-significantly associated with increased risk of obesity at 7 years in Mexican-American children in California [119]. By contrast, prenatal exposure to DDE was not associated with BMI standard deviation scores in a cohort of Mexican boys [120]. To my knowledge, only one study, from Spain, has examined postnatal exposure to these compounds in relation to BMI z-scores or related metrics, and it found no association [27].

In humans, prenatal exposure to PFOA was associated with obesity in adult females [25], and only one study has been performed in school-age children, finding no

association between *in utero* PFOA and PFOS exposure and overweight, BMI and waist circumference [121].

3.6 Conclusions leading to the present studies

Despite phase-out and limited production of POPs, we are still exposed to them worldwide. Motor developmental and behavioural problems have large consequences for the individual and are also related to high social, psychological and financial burdens [122], and moreover, obesity is highly prevalent and associated with an increased risk of co-morbidity. If prenatal exposure to POPs causes neuro-behavioural deficits and overweight, it will have a large impact on the public health if the emission of and exposure to these compounds is reduced. In addition, the equivocal conclusions in the existing literature justify further research into the association between prenatal exposure to POPs and subsequent neuro-behavioural and anthropometric outcomes.

4. Aims of the thesis

The aims of the thesis were to investigate the association between

- Prenatal exposure to 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p'*-DDE) and developmental milestones in infancy and motor development in school-age children
- Prenatal exposure to PFOA and PFOS and motor development and behaviour in school-age children
- Prenatal and estimated postnatal exposure to CB-153 and *p,p'*-DDE and childhood body mass index and overweight
- Prenatal exposure to PFOA and PFOS and childhood overweight and waist-to-height ratio

Table 3. Exposures and outcomes in the four studies

Study	Exposures	Outcomes
I	Prenatal PCBs and DDE	Developmental milestones
		Motor development
II	Prenatal PFOA and PFOS	Motor development
		Behaviour
III	Pre- and postnatal PCBs and	BMI z-scores[a]
	DDE	Overweight
IV	Prenatal PFOA and PFOS	Overweight
		Waist-to-height ratio > 0.5

[a] BMI z-scores = (observed value-mean value of reference population/standard deviation value of reference population

Table 3 presents the various exposures and the outcomes in the four studies. A more thorough introduction to the exposures and outcomes is presented in the method section.

5. Methods

In the following section, the methods used in the four studies included in the thesis will be introduced, briefly. Further descriptions of the methods are presented in the four papers (Appendices I-IV).

5.1 Study population at baseline

Between May 2002 and February 2004, 1,441 pregnant women were enrolled and provided a blood sample in the INUENDO (Biopersistent organochlorines in diet and human fertility) cohort from Greenland, Kharkiv (Ukraine) and Warsaw (Poland). Originally, the study was established to examine the associations between POPs and fertility, and these three populations were chosen because they represented very large POP exposure contrasts. At baseline, 2,478 women were eligible in Ukraine, 612 (25%) of whom participated. In Greenland, 665 women were eligible and 571 (86%) participated. In Poland, 690 women were eligible and 258 (37%) participated [16].

5.2 Data collection at baseline

The enrolled pregnant women participated in a detailed structured interview and provided a blood sample. One physician conducted the face-to-face interviews with participants in Greenland. In Poland, the interviews were performed face-to-face by 15 midwives, and 78 gynaecologists performed the interviews in Ukraine. Information concerning demographics, social factors, lifestyle, medical history as well as smoking and alcohol habits was obtained from the interview [16].

5.3 Study population at follow-up

The follow-up of the cohort was conducted between January 2010 and May 2012 when the children were between 5 and 9 years old. The mean age of the children was 8 years in Greenland and Poland and 7 years in Ukraine. A total of 1,116 singleton children were followed-up distributed between 531 mother-child pairs in Greenland, 493 in Ukraine, and 92 in Poland.

5.4 Data collection at follow-up

The follow-up interviews were primarily conducted face-to-face at the participants' residence or at the local hospitals in Greenland. One physician was the main interviewer in Greenland, assisted by local health workers. A telephone interview was performed when families lived in remote areas (n = 130) or participants had moved to Denmark (n = 34). In Poland, four interviewers conducted the interviews at the participant's residence or other local meeting points. In Ukraine, all interviews were conducted at eight paediatric polyclinics by a team of 59 paediatricians. Parents or guardians responded to questions concerning lifestyle, motor development, behaviour and other characteristics.

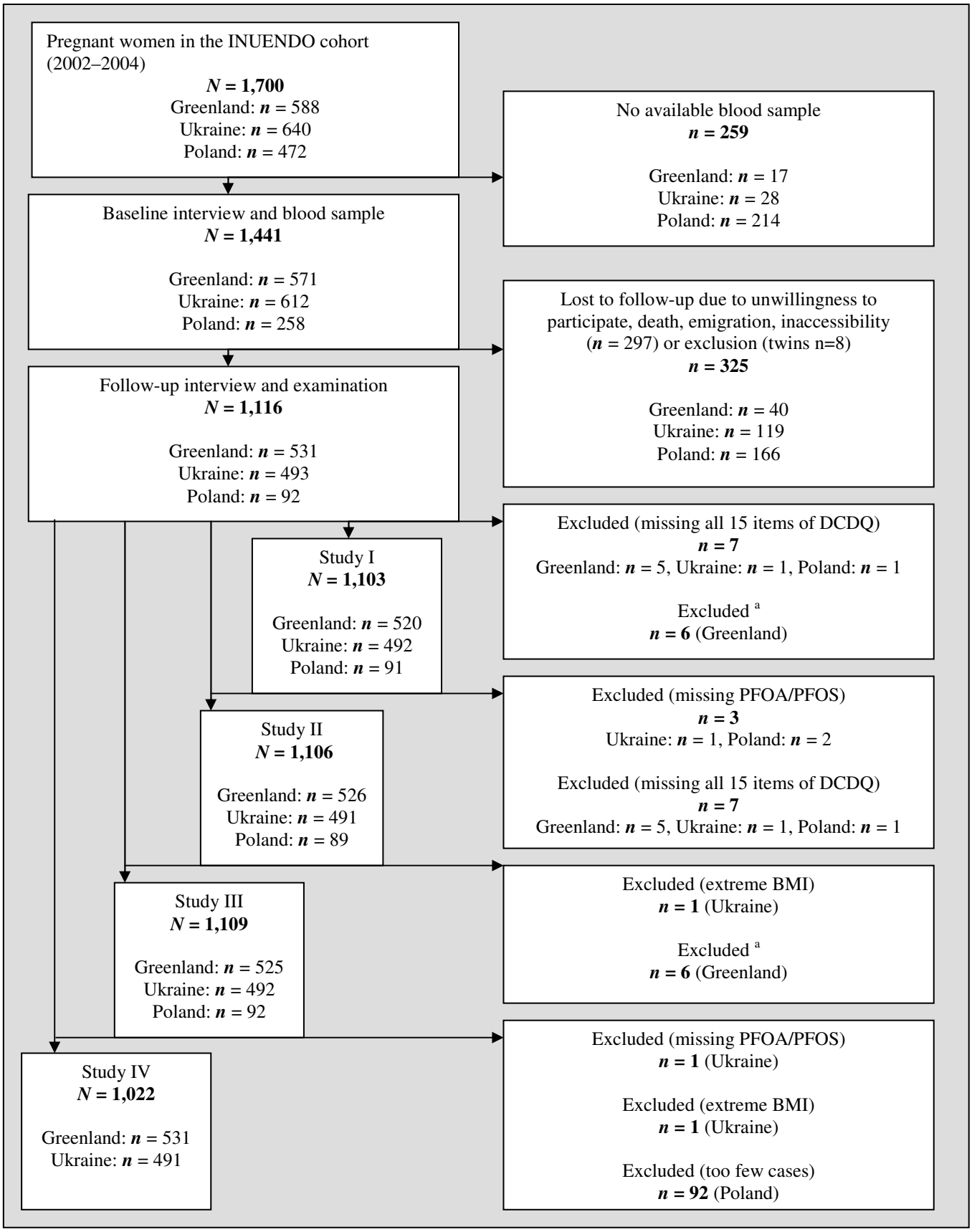

Figure 1. Flow chart of the study population.
[a] In studies I and III, one question was used to screen for participation at follow-up. In studies II and IV, more questions were used to screen for participation, meaning another six participants were included in studies II and IV.

5.5 Prenatal exposure assessment from pregnancy serum samples

Pregnancy samples from all three countries collected at any stage of pregnancy were analysed at the Department of Occupational and Environmental Medicine, Lund University, Sweden.

CB-153 and *p,p'*-DDE were analysed by gas chromatography-mass spectrometry following solid phase extraction as described elsewhere [38].

PFOA, PFOS and cotinine were analysed by liquid chromatography-tandem-mass spectrometry as described in detail elsewhere [39].

CB-153 was chosen as a biomarker for PCB exposure because studies have found this congener to be strongly correlated with total-PCB in plasma and serum (r = 0.9) [93,123]. Moreover, *p,p'*-DDE was chosen as a biomarker for DDT exposure because this metabolite occurs in high concentrations in serum [38]. Finally, PFOA and PFOS were chosen as biomarkers of the industrial PFAS as these two compounds are found in measurable levels in human serum [39,124,125].

5.6 Postnatal exposure assessment from toxicokinetic estimation

To estimate the postnatal cumulative contribution of CB-153 and *p,p'*-DDE for the first 12 months after birth, a toxicokinetic model developed by Verner et al. was used [126]. The model inputs were age of the mother at delivery, maternal pre-pregnancy weight, duration of exclusive breastfeeding, duration of partial breastfeeding, gestational age, child sex, birth weight, child's weight at follow-up, and up to two previously recorded weight measurements, maternal levels of CB-153 and *p,p'*-DDE during pregnancy (placental diffusion), gestational age at blood sampling and half-life of the compounds [126]. The toxicokinetic model was performed using acslX software (Aegis Technologies Group, Inc., Huntsville, AL, USA). We used this exposure metric since the contribution of persistent organic pollutants via breastfeeding is the most prevalent and important source of a child's postnatal exposure, and we had breastfeeding data up to the age of 12 months [126].

5.7 Developmental milestones and motor development

The parents retrospectively stated the age at which their child first crawled, stood-up with support and walked without support, using the definitions of child motor development milestones (crawling, standing-up and walking) from the WHO Multicentre Growth Reference Study [127]

To evaluate the motor development of early school-age children, we applied the country specific version of the Developmental Coordination Disorder Questionnaire 2007 (DCDQ'07). The DCDQ'07 is a parent report measure developed to assist in the identification of developmental coordination disorder in children aged between 5 and 15 years, using a 5-point Likert scale. It consists of 15 items grouped in three factors: 1) motor control, 2) fine motor and handwriting and 3) general coordination. The sum of the age-specific scores of the 15 items gives an indication of whether the child suffers from developmental coordination disorder [128]. The sum of the scores ranges from 15 to 75, low scores indicating motor problems.

5.8 Behaviour

Behaviour was assessed using the parent version of the standardised questionnaire SDQ comprising 25 items on five scales (emotional, conduct, hyperactivity, peer and pro-social behaviour) [129]. SDQ is a screening tool used to identify common mental disorders in children 4 to 16 years of age. The items were coded 0 "not true", 1 "somewhat true" or 2 "certainly true". Each scale had a summed score ranging from 0 to 10. A SDQ total score was calculated by summing four of the scales (emotional, conduct, hyperactivity and peer) giving a score range of 0 to 40. SDQ cut-offs were set according to standard (SDQ total: 0 to 13 = normal, 14 to 16 = borderline and 17 to 40 = abnormal) [103]. When outcomes were dichotomised, the cut-offs were normal/borderline versus abnormal. In all scales, except the pro-social subscale, a high score indicated problems.

5.9 Body mass index, overweight and waist-to-height ratio

The child's height was measured with the child standing barefoot against a wall, marking the top of the head and measuring the height to the nearest centimetre by use of ordinary measuring tape. The child's weight was measured to the nearest 0.1 kg by a weighing scale available at the family's home or at the clinics. All

measurements were performed by the interviewer except in those cases in which a telephone interview was used. Child BMI was calculated from weight (kg)/ (height (m))^2 . BMI was expressed as z-score representing the deviation in SD units from the mean of a standard normal distribution of BMI specific to sex and age (one month intervals). The standards were based on the WHO Growth Standards 2007, which are applicable regardless of ethnicity or country of origin [113]. Definition of overweight was based on the WHO recommendation of > 1 SD [113].
Waist circumference was measured by measuring tape across the abdomen corresponding to the umbilicus. WHtR was calculated from waist circumference (cm)/ height (cm). The cut-off of > 0.5 for WHtR was based on earlier child studies [115,130,131].

5.10 Missing data and multiple imputation

The number of missing values on any variable ranged from 0 to 55%. The variable with the highest percentage was in the DCDQ because one of the 15 items of the DCDQ was erroneously lacking in the Greenlandic version of the Questionnaire (but not in the Danish version used for some participants in Greenland), which meant that 55% of the DCDQs in Greenland were missing, because all items are needed to generate a total score. The median missing answers in the DCDQ items were 2%, and 85% of the Greenlandic population had answered at least 14 of the 15 items in the DCDQ. Other variables had a modest to moderate number of missing variables.

Because analysis of only complete cases (subjects with no missing variables) may lead to selection bias, we addressed the missing information problem using chained multiple imputation, allowing us to include participants with incomplete data in the statistical analyses [132]. Chained multiple imputation is a statistical method that creates several new complete datasets ($m > 1$) based on known subject characteristics in the complete dataset, incorporating the appropriate variability across the m datasets. The new m complete datasets are analysed, producing a single set of results accounting for the variability of the missing data [132]. Assuming the missing information to be missing at random (systematic differences between observed and missing values can be explained by differences in observed data), this approach will result in more precise and unbiased estimates [132,133].

Different models were made for the four studies, which are described further in the appended papers I–IV. In all studies, we generated 100 imputed datasets (m = 100) in a combined imputation that included the populations that were in the particular study. The predictors included all variables from the four models and other predictors believed to be related to the outcome of interest. To test the robustness of the final imputation models, different sensitivity analyses were performed, which included less and more predictors in the imputation model and generating less (m = 20) and more (m = 150) samples.

5.11 Statistical analysis

As described in the flowchart (Figure 1), the study sample varied between 1,022 and 1,109 mother-child pairs in the four papers. Spearman's rank correlation was used to assess the correlation between exposures in each of the four studies, and all multivariate models included the most important potential confounders among the available data, which were identified *a priori*. Participants were divided into tertiles of exposures. The crude relation between exposures and outcome was examined with lowest exposure tertile as the reference category. The adjusted relations of prenatal exposures on outcome were examined by means of different multivariable regression analyses with reference as above. When testing for trend, the three exposure strata were treated as continuous naturally logarithm transformed explanatory variables.

In all four studies, statistical analyses were performed in the statistical software STATA version 11.1–13.1 (StataCorp, College Station, TX, USA), and p-values < 0.05 were considered statistically significant.

6. Results

This section will summarise the main findings from the four papers. A more detailed description of the results is available in the four papers (Appendices I–IV).

6.1 In utero exposure to organochlorines and milestones and motor development

(Study I)

We saw no associations between the tertiles of CB-153 and *p,p'*-DDE and retrospective reports of the developmental milestones crawling, standing-up and

walking in infancy or the motor skills measured as developmental coordination disorder at young school-age.

We found the most distinct differences of mean age at crawling in Poland, with a difference of 1.5 (95% confidence interval (CI): -5.5, 2.5) months between low and medium CB-153 exposed groups and a difference of 1.8 (CI: -5.7, 2.1) months between medium and low *p,p'*-DDE exposed groups. Both estimates have wide confidence intervals and are not statistically significantly different from zero, and the results comparing the high exposure group with the low exposure groups are consistent with the rest of the results, indicating no difference according to exposure levels.

6.2 Prenatal exposure to perfluorinated alkyl substances and motor development and behaviour (Study II)

In the pooled analysis, the adjusted OR (95% CI) for hyperactivity was 3.1 (1.3, 7.2) comparing children prenatally exposed to high PFOA with those exposed to low PFOA. Comparing children prenatally exposed to high PFOS levels with those exposed to low PFOS levels gave a similar but statistically non-significant result (OR (95% CI) 1.7 (0.9, 3.2)). In Greenland, PFOS was associated with elevated SDQ total scores (β (95% CI) = 1.0 (0.1, 2.0)) and PFOA was associated with elevated hyperactivity sub-scale scores (β (95% CI) = 0.5 (0.1, 0.9)). Prenatal PFOS and PFOA exposures were not associated with motor difficulties.

6.3 Pre- and postnatal exposure to organochlorines and BMI and overweight (Study III)

No clear associations between pregnancy CB-153 and *p,p'*-DDE and child BMI were observed (the pooled differences in BMI z-score (95% CI) comparing 3rd tertile to 1st tertile were -0.07 (-0.32, 0.18) and -0.10 (-0.30, 0.10) kg/m^2, respectively). For postnatal CB-153 and *p,p'*-DDE and BMI, the overall differences in BMI z-score comparing 3rd tertile to 1st tertile were 0.12 (-0.15, 0.39) and -0.03 (-0.20, 0.27) kg/m^2, respectively. ORs of the association between maternal and estimated postnatal organochlorine concentrations and overweight were consistent with the results of the linear regression.

6.4 In utero exposure to perfluorinated alkyl substances and BMI and overweight

(Study IV)

For one log unit increase of pregnancy PFOA, the adjusted RR (95% CI) of offspring overweight was 1.11 (0.88, 1.38) in the pooled analysis of Greenlandic and Ukrainian children. Prenatal exposure to PFOS was not associated with an elevated risk of being overweight in country-specific or pooled analysis. The adjusted relative risk of having WHtR > 0.5 was increased for each log unit increase of prenatal PFOA (adjusted RR (95% CI) 1.30 (0.97, 1.74)) in the pooled analysis. For one log unit increase of *in utero* PFOS, the adjusted RR (95% CI) of having a WHtR > 0.5 was 1.38 (1.05, 1.82) in the pooled analysis of Greenlandic and Ukrainian children.

7. Discussion

In the following section, I will review the methodological problems in the four studies that may influence the conclusions of the studies.

7.1 Methodological considerations

7.1.1 Selection bias

Selection bias arises when the relation between exposure and outcome differ for those who participate in a study and those who do not. The way participants in a study are selected can bias either away from the null when results indicate an association even though there is none or towards the null when the results hide a true association [134].

The overall participation rate in the follow-up was 77%, distributed across 93% in Greenland, 36% in Poland and 81% in Ukraine, which makes the Polish results particularly prone to selection bias. However, the participants were unaware of the specific hypothesis investigated in the study and had no knowledge of their exposure levels, and thus, any selection bias would most likely be unrelated to exposure and outcome. Moreover, non-response analysis showed only a modest difference in relation to exposure level, and no difference on maternal educational level, maternal pregnancy smoking status, maternal age at baseline, maternal pre-pregnancy BMI

and paternal BMI between responders and non-responders, indicating no high risk of selection bias by loss to follow-up.

Missing data in an epidemiological study could introduce selection bias if those with missing data (not included in the analyses) differ from those with complete information (included in the analyses). To overcome the risk of introducing selection bias by only including those with complete data in the analysis (complete case analysis), we performed chained multiple imputation. In the analysis of the motor development data (studies I and II), 55% of the Greenlandic population had missing information on the outcome due to one missing item in the questionnaire. However, because 85% of the Greenlandic population had only one missing item, we believe the imputation model could predict the one item from the other 14 items of the questionnaire along with several other relevant predictors from the data set. Moreover, the general rate of missing data was not alarming, and we have no reason to believe that data were not missing at random. Hence, we believe imputation-based analysis is superior to complete case analysis.

7.1.2 Information bias

Information bias is caused by systematic errors in the classification of exposures, outcomes or covariates [134]. Information bias is often divided into differential and non-differential misclassification. Differential misclassification occurs when the misclassification of exposure is related to the outcome or vice versa and can result in bias in any direction. In non-differential misclassification, the misclassifications of the exposures and outcomes are unrelated to each other, which most often results in bias towards the null (no association) [134].

Misclassification of exposure variables

All of the prenatal exposure variables were measured bio-markers, and measurements were performed blinded to the outcome of interest, and were thus not prone to recall bias. Systematic measurement error, e.g. caused by incorrect calibration of equipment, cannot be ruled out but would most likely cause a non-differential misclassification of the exposures as they presumably would be unrelated to the outcome.

We had no measure of the children's postnatal organochlorine exposures and used a toxicokinetic model to estimate the accumulated concentrations during the first 12 months of life. The toxicokinetic model has proven robust in a validation study in similar settings, and a study suggests that the method is superior to the often used method of duration of breastfeeding multiplied by the prenatal exposure [126]. An exact measure of exposure of each child would, however, have been desirable because misclassification of the postnatal exposures cannot be completely ruled out.

Blood samples were collected throughout pregnancy, and as organochlorine and PFAS concentrations tend to decrease during pregnancy, there is a risk of exposure misclassification. However, we addressed this issue by adjusting for gestational age at blood sampling, although we recognise that this has not completely eliminated the possible misclassification.

Misclassification of outcome variables

We used parentally reported outcomes from questionnaires in study I (developmental milestones and motor skills) and study II (motor skills and behaviour). Parents were asked to retrospectively recall the age at which their child crawled, stood-up with support and walk without support, which potentially could lead to recall bias. However, as parents were unaware of the exposure levels, this recall bias would most likely be non-differential. The parents further assessed their child's current motor skills, and again the reporting was unrelated to the exposure levels, and any misclassification of the outcome would presumably be non-differential. Furthermore, developmental milestones and motor skills were used as continuous outcome measures, which are less prone to misclassification.

The median DCDQ score was lower in Ukraine than in Greenland and Poland. This may be due to cultural differences between study sites. The reliability and validity of DCDQ are acceptable [135], although previous studies have indicated that the original DCDQ may have poor sensitivity in a population sample compared to The Movement Assessment Battery for Children [136]. In addition, DCDQ'07 has been found to have low specificity when screening an adolescent population [137]. However, as we intended to examine whether there was a difference in motor score according to POP exposure levels, we do not believe this is of major concern for our study. In spite of a reported agreement of between 80 and 95% between DCDQ and expert opinion [135],

interpretation of the prevalence of DCD in the various countries is not recommended without an additional standardised motor test to confirm the screening result of DCDQ.

It is possible that the DCDQ is not sensitive enough towards moderate changes in motor skills as is expected in relation to POP exposure. The lack of an association in study I could be related to the possible insensitivity of the DCDQ and thus, the results of study I should be assessed in light of this limitation.

SDQ was used as a continuous and a categorical measure. In the analysis of the categorical SDQ, we chose to use the suggested cut-offs for behavioral problems as no country-specific cut-offs exist for Greenland, Ukraine and Poland. This enabled us to compare results between countries but may have resulted in misclassification of the SDQ outcome. SDQ as a screening tool has a high validity as well as reliability [138,139], and our sensitivity analysis, using top 10 percentile cut-offs in general, suggested consistent results, and we have no strong suspicion of misclassification.

In studies III and IV, where we used measured outcomes (BMI and WHtR), we used different scales for weighing children, which could introduce information bias. However, any misclassification would most likely not differ between exposure groups and would therefore be non-differential.

As these studies were performed in three different countries with a number of different observers, there is a risk of exposure misclassification of the outcome. Furthermore, 164 performed self-measurements of child weight as they were telephone-interviewed. As this possible misclassification is presumably unrelated to the exposure levels, the risk of differential misclassification is small. However, if overweight subjects have under-reported their true weight and underweight subjects have over-reported their true weight there is a risk of bias towards the null and any true association may have been attenuated.

Misclassification of confounding variables

All confounder data were collected blinded to the exposure levels and with no knowledge of the outcome of interest among participants. Most confounder variables were self-reported (e.g. maternal reports on age, education, parity, height, weight and

alcohol consumption) or parentally reported (e.g. duration of breastfeeding and child physical activity level). Any misclassification of the self-reported and parentally reported confounder variables is most probably non-differential.

Maternal smoking was based on self-reports in study I, whereas it was based on cotinine levels in studies II, III and IV. We cannot rule out the possibility of misclassification of smoking based on self-reports, but the women were unaware of the outcome at follow-up when they reported smoking status at the baseline interview, and moreover, they did not know the environmental exposure levels. Because the analysis of the cotinine levels was performed without knowledge of the environmental exposure levels and also without knowledge of the outcomes of interest and presumably, any misclassification of self-reported smoking or smoking based on cotinine levels would be non-differential.

The choice of a short term bio-marker (assessed late in pregnancy) of smoking exposure to indicate long term smoking during pregnancy can be debated. Presumably, women who have never smoked would probably not begin smoking during pregnancy and women, who were classified as smokers in late pregnancy, would probably have been smokers during the first trimester of pregnancy. Moreover, cotinine information was prospectively collected, without awareness of the outcome of interest, most likely resulting in non-differential misclassification of cotinine.

In study II, III and IV smokers were defined based on a cut-off of >10ng/ml serum cotinine, which could result in misclassification of the exposure, because passive smoking can increase serum cotinine. However, among passive smokers who had never smoked, mean serum cotinine concentration of 1.07 ng/ml (CI:0.87;1.26) have been reported [140] indicating that only very few if any passive smoke exposed women would be classified as smokers with the cut-off used in study II, III and IV.

Misclassification introduced by use of multiple imputation

The use of multiple imputation may cause information bias if the model does not impute the true answers. However, as we believe data were missing at random and as the imputation models were build on predictors of the outcomes, we have no such suspicion. Moreover, several sensitivity analyses, e.g. including more and less predictors in the imputation model, did not change the results.

7.1.3 Confounding

Confounding, which is a confusion of effects, is another concern in epidemiological studies. A variable that is an independent risk factor for the outcome and is associated with exposure and in addition is not an intermediate step in the causal path between the exposure and the outcome is a confounding variable. Confounding can lead to over- and underestimation of effects, and it can change the direction of the association under study, and should therefore be carefully dealt with in the data analysis [141].

It has been debated whether weight change during pregnancy may play a role in the levels of organochlorine compounds [134]. It is uncertain whether the same goes for the PFAS because PFAS tend to adhere to proteins with storage in the internal organs, whereas the organchlorines are lipophilic, and thus, adhere to fat. Unmeasured confounding by this variable is possible if indeed gestational weight gain is a confounder of the association of interest. However, a recent publication indicates that this is a minor concern [142].

The high correlation between CB-153 and *p,p'*-DDE made us choose not to perform mutual adjustment in the analyses in studies I and II, and thus possible confounding of the opposite organochlorines in the results cannot be ruled out.

The risk of residual confounding caused by too crude a categorisation of covariates or confounding by variables that we did not consider cannot be completely ruled out.

7.1.4 Effect measure modification

Effect measure modification is present when the effect of an exposure differs by another factor [143].

In studies I–IV, we tested for interactions between exposures and country. No statistically significant interactions were observed and, thus, in order to gain power, we pooled the total population in pooled analyses. However, individual results stratified by country were not always homogenous and hence, the country specific results may be more valid than pooled results.

Moreover, we tested for interaction between exposures and gender in studies II and IV. No statistically significant interactions were observed, except in the pooled analysis of Greenlandic and Ukrainian populations. Hence, the sex stratified results of the pooled results in study IV are more reliable than the pooled analysis.

7.1.5 Sample size and statistical issues

The Polish sample was small, which limited further stratification, and hence, the Polish results should be interpreted with care. The heterogenic population did not always permit pooling of populations (Greenland, Ukraine and Poland), and hence, we may not have detected a true associations (type II error). However, most analyses were performed both stratified by country and pooled.

7.2 Main findings in light of other studies

Elevated prenatal PCB exposure was associated with decreased motor skills in infancy and among school-age children in some studies [134]. Other studies have found associations between prenatal PCB exposure and motor development at some but not all follow-up ages, in sub-groups or in some but not all investigated congeners [78-80], whereas the majority found no association between PCBs and motor development in infancy and motor skills at school-age, which is consistent with our results [81-86]. Studies differ greatly with regard to exposure level, outcome measure, lipid adjustment, confounder adjustment and statistical analysis, which may explain the discrepancies among results.

We observed no association between prenatal exposure to DDE and developmental milestones in infancy or motor skills at school-age, which confirms earlier findings [87-97,144]. However, others reported associations between elevated prenatal exposure to DDE and poorer motor development in infancy and in school-age children [79-81,88,93,99-102]. Again, exposure levels vary considerably as well as the use of lipid adjustment or not. Furthermore, outcome measures and confounder adjustment also differ between studies, which may explain differences in results.

We observed no association between prenatal PFOA and motor development, which is in line with findings from the Danish National Birth Cohort [78,89,96,98,145]. In contrast, a small Taiwanese birth cohort study with exposure levels equivalent to exposure levels in this thesis observed associations between prenatal PFOS exposure

and gross motor functions at 2 years of age, whereas no association was found between prenatal PFOA exposure and motor development [109]. Direct comparison with this study is, however, hampered since their outcome measure was assessed by use of a questionnaire developed specifically for Chinese populations.

In the Danish National Birth Cohort, no associations were observed between prenatal PFOS and PFOA exposure and SDQ total or any of the SDQ sub-scales at 7 years, using top 10 percentile cut-offs [26], whereas we observed associations between PFOA and hyperactivity and statistically non-significant associations between PFOS and SDQ total. In our study, we used the cut-offs suggested by Youth in Mind [109], and furthermore, the children in our study were older, which may explain the discrepancies. In the C8 Health Project (which reports health outcomes in relation to PFOA contamination of drinking water from the Dupont factory in the United States) authors estimated *in utero* PFOA exposures by use of a pharmacokinetic model, and elevated PFOA levels were associated with fewer signs of ADHD [103]. In the aforementioned studies, different questionnaires were used; exposure levels differed extensively and in one study, exposure levels were estimated, which could account for the disparate results.

We observed no association between prenatal CB-153 exposure and BMI z-score. Our results are in line with results from the U.S. Collaborative Perinatal Study [146] and a Dutch cohort study [147]. In contrast, a recent study found associations between prenatal PCBs and increased BMI in girls [148] and another three studies reported elevated prenatal PCB exposure to be associated with elevated BMI SD scores or overweight in both boys and girls [22]. The difference in results could be caused by the duration of follow-up, as the children in this cohort were somewhat older, and potential associations earlier in life were not investigated. Moreover, exposure levels and confounder adjustment are very different among studies, and as children in the studies came from different parts of the world it is possible that ethnic differences occur in relation to susceptibility to prenatal exposure to POPs.

We observed no association between prenatal DDE and BMI z-scores, which is in line with results from a study within the U.S. Collaborative Perinatal Study [27,117,149] and two other studies with rather high prenatal DDE exposure levels [147]. By

contrast, a small Spanish study reported an association between medium prenatal DDE exposure and increased risk of overweight compared to low exposure [150,151].

One study observed a positive association between prenatal PFOA and overweight and central obesity (i.e. waist circumference) in young female adults [27], which we also found indications of among the Greenlandic girls in our study. Another study reported no association between prenatal PFOA and BMI z-scores and overweight at age 7 years [25].

We found no association between PFOS and overweight, which is consistent with prior studies [121]. In our study, PFOA and PFOS were associated with WHtR > 0.5, especially among girls, which is in contrast with findings in the Danish National Birth Cohort, where no associations were observed between prenatal PFOA and PFOS exposures and residuals of WHtR [25,121].

8. Conclusions

This thesis suggests that modest associations exist between prenatal exposure to POPs and child development. Prenatal exposure to PFOA and PFOS seem to have more adverse effects on neuro-behavioural and adiposity outcomes than CB-153 and *p,p'*-DDE. The following are the main conclusions of the four studies included in the thesis:

We found no association between *in utero* exposure to CB-153 and *p,p'*-DDE and parentally retrospectively assessed developmental milestones in infancy or parentally assessed motor development at 5 to 9 years.

Prenatal exposure to PFOA and PFOS may affect children's neuro-behavioural development modestly, specifically in terms of hyperactive behaviour. The associations were strongest in Greenland where exposure contrast is largest. No association was observed in relation to motor skills.

There was no overall association between pre- or postnatal exposure to CB-153 and *p,p'*-DDE and BMI and overweight at follow-up.

Prenatal PFOA and PFOS exposure may be associated with child WHtR > 0.5. Prenatal PFOA and PFOS exposure were not unequivocally associated with overweight.

9. Perspectives and future research

This thesis has contributed with new knowledge about the association between prenatal exposure to POPs and neuro-behavioural and anthropometric outcomes. Whether a decreased exposure to the POPs will be associated with a reduction in central obesity and inattentive behaviour is not yet known. As these compounds are possibly only a small part of the causes of central obesity and behavioural problems, further research in a broader perspective needs to be done.

The reduction and banning of the studied POPs has meant the development and production of new environmental pollutants, and the long-term effects of these exposures should be monitored in population-based studies. Moreover, follow-up could be performed also at older ages (i.e. post puberty) as some adverse effects may not be manifest until after puberty. In addition, more sensitive outcome measures and thorough physical examinations could be performed to obtain more precise results.

Little is known about the cellular mechanism involved in developmental programming concerning *in utero* exposure to POPs and later development, and future studies could disentangle this area further.
In this thesis, we only addressed one compound at a time. New methods enable researchers to study the effect and interaction of several compounds, which is useful because we are exposed to a cocktail of chemicals, and these chemicals may interact. Moreover, each individual person could be more or less prone to the effects of prenatal exposure to POPs, and gene-environment studies may possibly be able to disentangle the effects of *in utero* exposure to POPs in relation to genetic susceptibility.

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Appendix I

Paper I Motor development following *in utero* exposure to organochlorines: a follow-up study of children aged 5–9 years in Greenland, Ukraine and Poland. [In review]

Motor development following *in utero* exposure to organochlorines: a follow-up study of children aged 6-9 years in Greenland, Ukraine and Poland

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Abstract:

Background: Prior studies on the association between prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) and child motor development have found contradicting results. Using data collected in the INUENDO cohort in Kharkiv (Ukraine), Warsaw (Poland) and Greenland (N=1,103) between the years 2002 and 2012, we examined relations of prenatal exposure to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) and 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) on motor development and developmental milestones; crawling, standing-up and walking.

Methods: CB-153 and p,p'-DDE were measured in maternal blood in second or third trimester of pregnancy. Motor development was measured in terms of the parentally assessed screening tool Developmental Coordination Disorder Questionnaire 2007 and developmental milestones were assessed via retrospective parental reports of child age at the first time of crawling, standing-up and walking.

Results: We saw no associations between the tertiles of CB-153 and p,p'-DDE and retrospective reports of the developmental milestones crawling, standing-up and walking in infancy or the motor skills measured as developmental coordination disorder at young school age.

Conclusions: In utero exposure to CB-153 and p,p'-DDE was not associated with parentally retrospectively assessed developmental milestones in infancy or parentally assessed motor skills at young school age. The use of a more sensitive outcome measure may be warranted if subtle effects should be identified.

Keywords: Child motor development; developmental milestones; dichlorodiphenyldichloroethylene (DDE); organochlorines; polychlorinated biphenyls (PCBs); prenatal exposure.

Background

Polychlorinated biphenyls (PCBs) have been widely used in industrial production, e.g. in capacitors, sealants, plasticizers, fire retardants and hydraulic fluids, but they were banned in the late 1970s in the United States and the early 1980s in Europe because of their environmental persistence [1,2]). Also, the insecticide dichlorodiphenyltrichloroethane (DDT) and its main metabolite dichlorodiphenyldichloroethylene (DDE) are highly persistent in the environment, and although the agricultural use of DDT has been severely restricted through the regional United Nations Economic Commission for Europe and the Stockholm Convention [3], it is still used as disease vector control in some developing countries [4]. PCBs and DDE are lipophilic compounds which bio-accumulate in adipose tissue and, thus, the main exposure is through food consumption and breast feeding [5,6]. The compounds pass the placental barrier and the fetus is exposed to approximately the same concentration of DDE as the mother when calculated on lipid basis [7,8] . The level of PCBs in the fetus, however, seems to be somewhat lower than that of the mother although correlated [9]. PCBs and DDE can be detected in serum and breast milk samples from almost all women around the globe, causing the fetus and subsequent breast-fed baby to be exposed to the compounds during infancy [9-12].

A major contamination of rice oil by heated PCBs and degradation products in Taiwan in 1979 gave insight into the negative impact of high prenatal exposure on e.g. neurodevelopment [13,14]. Animal studies later found decreased muscular strength [15] after high prenatal exposure to tetrachlorobiphenyl and reduced motor activity [16] after prenatal exposure to PCBs. However, results from epidemiological cohort studies are inconsistent: Higher *in utero* exposure to PCBs (3000->4000 µg/L) was related to a significant decrease in motor abilities indicated by 7-8 points decrease of the Psychomotor Development Index scores (PDI) in Bayley Scales of Infant Development (BSID) (a screening tool measuring children's motor and mental abilities) compared with low exposure (0-900 µg/L) at six, 12 and 24 months in a birth cohort from North Carolina [17,18]. The Collaborative Perinatal Project from 12 hospitals in the United States found no association between background levels (0-16.50 µg/L of prenatal PCBs exposure and motor development in terms of PDI in a sample of prenatally PCB exposed children of 8 months of age [19]. A two-point decrease in PDI scores for

every 10-fold increase of DDE has been observed at six months of age, but not at 12 and 24 months [20], and further, no association was found between prenatal DDE and motor development according to McCarthy Scales of Children's Abilities (MSCA) in two Spanish birth cohorts [21]. In addition, no association was found between *in utero* DDE exposure and motor development using BSID or MSCA among infants of one month, toddlers of 30 months or children of 3.5-5 years [22-24]. However, *in utero* exposure to DDE has been associated with hyporeflexia at one month in a cohort of North American children [25] and 4 points lower PDI for each doubling of DDE [26]. Further, no inverse associations between prenatal DDE and motor skills were observed in later follow-ups of the large North Carolina cohort [17,18].

In this cohort, we follow three populations of 1,103 children from Arctic and Europe with a large exposure span, which enables us to examine the relation between different levels of organochlorines exposures and child motor development in various parts of the world. Thus, the aim of this follow-up study was to examine the relations between prenatal exposure to p,p'-DDE and CB-153 and developmental milestones in infancy measured retrospectively and motor development assessed by the questionnaire "Developmental Coordination Disorder Questionnaire" in six to nine year old children in Greenland, Ukraine and Poland.

Methods

Study population

Between May 2002 and February 2004, 1,441 pregnant women were enrolled and provided a blood sample in the INUENDO (Biopersistent organochlorines in diet and human fertility) cohort from Greenland, Kharkiv (Ukraine) and Warsaw (Poland). To be eligible at baseline, the women had to be born in the country of the study, be pregnant as well as at least 18 years of age. At baseline, 2,478 women were eligible in Ukraine of whom 612 (25 %) participated. In Greenland, 665 were eligible and 588 (88 %) participated. All Greenlandic women were Inuits. In Poland, 690 were eligible and 258 (37 %) participated. With few exceptions, the antenatal health programs covered all pregnant women in the localities.

At follow-up, 493 (81 %) singleton children with measured exposure information were accessible and willing to participate in Ukraine, 525 (89 %) in Greenland and 92 (36 %) in Poland. A total of 1,110 children were followed-up. Further details on distribution of the study population are provided elsewhere [27].

After recording of child birth anthropometrics shortly after birth, the first follow-up of the cohort was conducted between January 2010 and May 2012 when the mean age of the children was 8 years in Greenland and Poland and 7 years in Ukraine. The retrospective reports of the developmental milestones in infancy, the children's motor development at follow-up, and other characteristics were assessed by the parents (usually (95 %) the mother), through an interview-based questionnaire. Mother-child-pairs were eligible for the study if the woman had a live-born singleton baby who was still alive at follow-up.

Ethics statement

The study was approved by local ethical committees; Polish Bioethical Committee (approval no. 6/2002 of 3.07.2002), Ethical Committee for Human Research in Greenland (approval no. 2010-13) and the Commission on Ethics and Bioethics Kharkiv National Medical University in Ukraine (protocol number 7, October 7 2009) and all participating parents signed informed consent.

Exposure assessment

At baseline, the women were interviewed and had a blood sample drawn. In Greenland and Kharkiv, the blood samples were drawn when the women were on average 24 weeks pregnant and in Warsaw when the women were on average 33 weeks pregnant. Ten ml cubital vein blood samples were drawn into vacuum tubes for serum collection without additives (Becton Dickinson, Maylan, France). The sera were analysed for PCBs, measured as CB-153, and DDE, measured as p,p'-DDE, and used as bio-markers of the prenatal exposure to the compounds. All chemical analyses were performed at The Department of Occupational and Environmental Medicine in Lund, Sweden. The sera were analysed by gas chromatography-mass-spectrometry following solid phase extraction. All samples were

analysed twice at different days and the mean concentration of these two determinations was used. For an estimation of the imprecision in the method, the results of the analysed samples were divided into three equal sized groups, one with low levels, one with medium levels and one with the highest levels and the mean concentration for each group were calculated. Then the relative standard deviations were calculated from the duplicate determinations [28]. These were 18 % at 0.1 ng/mL (n=990), 10 % at 0.5 ng/mL (n=990) and 10 % at 2 ng/mL (n=990) for CB-153 and 11 % at 1 ng/mL (n=1058), 8 % at 3 ng/mL (n=1058) and 7 % at 8 ng/mL (n=1058) for p,p'-DDE. The detection limits were 0.05 ng/mL for CB-153 and 0.1 ng/mL for p,p'-DDE. For CB-153 there was 85 samples below the detection limit (LOD) and for p,p'-DDE there was 10 samples below LOD. If the concentration was less than LOD, the concentration was set to half the LOD based on fresh weight concentration. Sera were stored at –20˚C until analysis. CB-153 and p,p'-DDE levels were adjusted for serum concentrations of cholesterol and triglycerides which were determined by enzymatic methods. The inter-assay coefficients of variation for cholesterol and triglycerides were 1.5-2.0 %. Further details are described elsewhere [29]. The laboratory was blinded concerning the measured outcomes and the mothers' lifestyle etc during pregnancy.

Outcome assessment

To evaluate the motor development of the children in the early school age, we applied the country specific version of the Developmental Coordination Disorder Questionnaire 2007 (DCDQ'07). The DCDQ'07 is a parent report measure developed to assist in the identification of developmental coordination disorder in children between five and 15 years, using a five-point Likert scale. It consists of 15 items which are grouped in three factors: 1) motor control, 2) fine motor and handwriting and 3) general coordination. The sum of the age specific scores of the 15 items give an indication of whether the child suffers from a developmental coordination disorder [30]. The sum of the scores ranges from 15 to 75, lower scores indicating motor problems.

At follow-up, the parents retrospectively stated the age at which their child first crawled, stood-up with support and walked without support, using the definitions of child developmental milestones

(crawling, standing-up and walking) from the Multicentre Growth Reference Study by the World Health Organization [31]. Interviewers and parents were unaware of the level of prenatal exposure of the compounds.

Assessment of co-variates

Variables that might influence child motor development and developmental milestones and could be associated with the exposures were *a priori* identified in the literature, and data on these were harvested from questionnaires at baseline and follow-up. Baseline variables were: maternal pre-pregnancy smoking (yes/no), maternal pre-pregnancy alcohol intake (≤7, >7 servings of alcohol per week), maternal education (finished education before age 15 years, at 16-17 years, at or above 18 years), parity (1, 2-3, ≥4 child births), maternal age at the baseline interview (<30, 30-35, >35 years) and gestational age at blood drawing in weeks (continuous). Follow-up variables from immediately after birth were: sex of the child and gestational age (< 37, ≥ 37 gestational week) and current follow-up variables were: breastfeeding (<6, 6-12, > 12 months) and child age at interview in years (continuous).

Data collection

The interviews were primarily conducted face-to-face at the participants' residence or at the local hospitals in Greenland. A medical doctor was the main interviewer in Greenland, assisted by local health workers. A telephone interview was performed when families lived in remote areas (n=130) or participants had moved to Denmark (n=34). In Poland, four interviewers conducted the interviews at the participant's residence or other local meeting points. In Ukraine, all interviews were conducted at eight paediatric polyclinics by a team of 59 paediatricians. A proportion of the questionnaires were filled in by the parents without the face-to-face interview in Greenland. Also, one of the 15 items of the DCDQ was erroneously lacking in the Greenlandic version of the Questionnaire in Greenland (but not in the Danish version used for some participants in Greenland).

Statistical analyses

Missing information

A relatively large amount of the parents did not fill in all items in the questionnaire, leading to missing data on outcome and co-variates. If parents left one item unanswered, it was impossible to calculate a total DCDQ score. To overcome this and take into account that complete case analysis may cause biased estimates of the association under study [32], we performed chained multiple imputations on the dataset. This approach will result in more unbiased estimates if the data are missing at random (the missingness is based on other observed characteristics such as the observed outcome or covariates) [32].

Briefly, chained multiple imputation is a statistical method that creates several new complete datasets (m>1), based on known subject characteristics and other predictors in the complete dataset, incorporating the appropriate variability across the m datasets. The new m complete datasets are analysed, producing a single set of results accounting for the variability of the missing data [32].

We excluded subjects who had not filled in any of the 15 items in the DCDQ'07 from the chained multiple imputation model (n=7), leaving a final study population of 1,103 women and children. We made 100 imputed datasets (m=100) based on the following predictors: CB-153, p,p'-DDE, maternal education, maternal age at baseline interview, maternal pre-pregnancy smoking status, maternal pre-pregnancy alcohol consumption, parity, preterm birth, child sex, gestational age at blood drawing, child age at interview, the 15 items in the DCDQ'07, age at crawling, age at standing-up and age at walking. We performed different sensitivity analyses of the chained imputation models, including fewer and more predictors and generating less (m=20) and more (m=150) samples to check the robustness of the final imputation model. We present the results of the multiple imputation based analyses. Results of the complete-case analyses are available as supplementary material.

Data analyses

Study subjects were divided into tertiles of CB-153 and p,p'-DDE exposures. CB-153 and p,p'-DDE concentrations were adjusted for serum lipids and thus expressed as ng/g lipid. As a first step in the

data analysis, the correlation between CB-153 and p,p'-DDE was checked by use of Spearman's correlation. Secondly, the crude relation between CB-153 and p,p'-DDE and motor development and developmental milestones were examined with lowest exposure tertile as the reference category. Thirdly, the adjusted relations of prenatal exposure to CB-153 and p,p'-DDE on motor development were examined by means of multiple linear regression analyses with lowest exposure tertile as the reference category. When testing for trend, the three DDE/CB-153 exposure strata were treated as continuous naturally logarithm transformed explanatory variables. The relation between *in utero* exposure to the compounds and developmental milestones was studied, using multiple linear regression analyses, with referents as above. In a sub-analysis, we excluded children born before gestational week 37. *A priori* set variables that could affect child motor development, based on literature studies, were included in the regression model and kept in the model throughout the analyses. The possible interaction between exposures and country were tested by adding an interaction term to the regression models. All analyses were stratified by country as well as pooled (additionally adjusted for country).

A p-value of 0.05 or less was considered statistically significant. For all analyses the Stata statistical package was used (Version 12.1, StataCorp, College Station, Texas, USA).

Results

A total of 1,103 of the 1,441 baseline participants were included in the follow-up analyses (77 %). Although the pregnant women had to be at least 18 years of age to be included in the study, for unknown reasons, three women were 16 years old and 11 women were 17 years old in Ukraine, calculated based on the recorded date for filling in the questionnaire and date of birth.

In Greenland, Spearman's correlation between CB-153 and p,p'-DDE was 0.9, in Poland and Ukraine, it was 0.5. Mothers were slightly older in Poland than in the other countries and in Greenland, the majority of the women were multiparae in contrast to Ukraine and Poland where most women were primiparae. Most mothers smoked prior to pregnancy in Greenland while the majority of the pregnant women from Ukraine were non-smokers. Children were youngest in Ukraine, where the mean age

(SD) was 7 (1) years at follow-up. In Ukraine, median (10-90 percentile) developmental coordination disorder score (DCD) among the children <8 years of age was 47 (37-55) points and 47 [29-58] points among the children ≥8 years of age, while it was 62 (49-73) and 62 (29-58) points, respectively in Greenland and 64 (51-73) and 67 (58-74) points, respectively in Poland (Table 1).

As seen in Table 2, Greenlanders were most heavily exposed to CB-153, and Ukrainians were most heavily exposed to p,p'-DDE. The median (10-90 percentile) serum levels of CB-153 not adjusted for lipids were equivalent to 0.3 (0.1-1.7) μg/L in the total population and the ditto p,p'-DDE was 3.4 (1.0-8.7) μg/L (data not shown). The proportions of missing values are presented in Table 3. Missing values range from 0 to 59 %, mainly due to self-administration of a proportion of the Greenlandic questionnaires. The median missing answers in the DCDQ items were 2 %.

Crude results are presented as supplementary material (Additional file 1 and Additional file 2).

Medium CB-153 levels was positively associated with age at standing and walking in the total population, corresponding to approximately two weeks delay, compared to low exposed children. No associations were observed in the high exposed group, in analyses stratified by country or between DDE and either outcome.

In Table 4, results from the multiple imputation based analyses are presented, showing the adjusted mean differences of the developmental milestones crawling, standing-up and walking. We observed no associations between the exposures and the developmental milestones. Only very modest differences were seen between exposure groups in all three countries and in the pooled analysis. We found the most distinct differences of mean age at crawling in Poland, with a difference of 1.5 (95 % confidence interval (CI): -5.5, 2.5) months between low and medium CB-153 exposed groups and a difference of 1.8 (CI: -5.7, 2.1) months between medium and low p,p'-DDE exposed groups. Both estimates have wide confidence intervals and are not statistically significantly different from zero, and the results comparing the high exposure group with the low exposure groups are consistent with the rest of the results, indicating no difference according to exposure levels.

Table 5 shows results from the multiple imputation based analyses of CB-153 and p,p'-DDE and motor skills measured as developmental coordination disorder. No statistically significant differences were seen in any of the three countries or in the pooled analysis.

When restricting our analyses to subjects with complete information, we did not observe any associations between maternal serum CB-153 and p,p'-DDE levels and either of the child's developmental milestones in any of the three countries or in the pooled analysis (Additional file 3) except for mean age at walking that appeared to be slightly lower at higher serum p,p'-DDE levels in Greenland (p for trend, 0.04). In Poland, medium CB-153 exposed children had a lower mean age at standing compared to low exposed children; -1.0 (CI: -1.9, 0.1) months. In the complete-case analysis, results of adjusted mean differences between tertiles of CB-153 and p,p'-DDE exposures and parentally assessed motor skills at young school age are similar to the results of the multiple imputation based analyses (Additional file 4).

Using breastfeeding duration as the exposure in a sub-analysis, we found no relation to motor scores (data not shown). Excluding 37 preterm children in a sub-analysis gave roughly the same results except for a borderline statistically significant difference in Greenland. High DDE exposed children walked 0.7 months later than low exposed children (-0.7 (95 % CI:-1.4; 0.0)).

The four different sensitivity analyses performed on the imputation model did not change the direction or the magnitude of the estimates (data not shown). Also, stratification by maternal education gave no clear dose-response association according to maternal education. When testing for interaction between the exposures and country, no apparent signs of interaction were found.

Discussion

We investigated whether *in utero* exposure to p,p'-DDE and CB-153 was related to parentally retrospectively assessed delayed developmental milestones as age at crawling, standing-up and walking in infancy, and motor skills at 6-9 years of age as measured by DCDQ. We did not observe any adverse association of prenatal exposure to CB-153 and p,p'-DDE on developmental milestones or motor development.

Our results are consistent with some earlier studies with generally lower exposure levels [19,23,33]. Daniels et al. reported no association between PCBs and motor skills in terms of BSID among 8 months old infants [19], in line with a Canadian study among 5 year-old Inuits [33] and a Mexican study of DDE and motor skills in 30 months old children [23].

Studies with large exposure contrast between studies have found negative associations between *in utero* exposure to either or both of the compounds and parts of the motor development [17,20,25,26,34,35]. Some of the early studies on very high levels of maternal DDE and PCBs exposure and motor development in the offspring observed associations between PCBs and statistically significantly lower motor developmental scores according to BSID and between DDE and hyporeflexia according to Brazelton Neonatal Behavioral Assessment Scale in the same cohort [18,25] The discrepancies in results could be due to the higher exposure levels and their use of another outcome measure.

Among 92 toddlers, prenatal exposure to DDE has also been associated with lower PDI scores in Spain [26]. The age difference between the children of the cohorts and the use of different outcome measures could be the reason for the disparate results. A study found a 10-fold higher prenatal DDT exposure to be associated with a two-point lower psycho-motor development BSID score at six and 12 month old infants. High DDE-levels (geometric mean 1,437 ng/g lipid) were also associated to lower psycho-motor development scores at six months but not at 12 or 24 months, indicating DDT to be a psycho-motor toxic compound as well as DDE [20]. Mean DDE-levels in the study by Eskenazi et al. is more than twice the level of the highest mean level in our study, which may explain the dissimilarities in results.

Breastfeeding may potentially confound the results due to its association to postnatal exposure to PCBs and DDE. On the other hand, recent studies have found breastfeeding beneficial in relation to motor development, seemingly counterbalancing the possible negative consequences of DDE [20,26]. In our data, breastfeeding does not seem to be a risk or protective factor for motor development neither in general nor when stratified by prenatal exposure levels (data not shown). We did not intend to

evaluate the associations between postnatal PCB-153 or DDE exposure and motor development in the present study.

In this study, the blood sample was drawn in the second or third trimester of pregnancy as did many others [19,20,34,37]. As the half-life of both compounds of interest is several years in the human body it seems unlikely that large differences in concentration appear between trimesters of pregnancy. However, high levels of first trimester DDE has been associated with motor development in infants till the age of 12 months, whereas no association was seen between second and third trimester DDE and motor development [38]. This may be a chance finding since their study, to the best of our knowledge, is the only one reporting such results. Also, Longnecker et al. found the level of DDE to be highly correlated through the trimesters of pregnancy and they concluded that the timing of measurement was not important [39].

The three diverse populations of this study have enabled us to follow children in different settings with a large exposure span. The long follow-up of up till 9 years is important since it is of importance to disclose whether possible associations between prenatal organochlorine exposure and motor skills persist into school age. In addition, these associations have rarely been investigated in these populations.

This study has some limitations. Developmental milestones were assessed retrospectively, introducing the possibility of information bias. However, we have no reason to believe that differential misclassification by exposure levels has affected the estimates as parents were unaware of their exposure levels when answering the questionnaire. However, non-differential misclassification may have attenuated the true association.

We did have a considerable proportion of missing data in the dataset, in particular on the total DCDQ-score in Greenland. In the Greenlandic version of the questionnaire (as opposed to the Danish version) one item lacked due to an error, making a calculation of the total score impossible in the Greenlandic version before imputation. Thus, we performed chained multiple imputation on the dataset because of concern for selection bias caused by missing information. Analyses on a full dataset with no missing

items would have been preferable, however, when missing data does occur and data is missing at random, multiple imputation is superior to complete case analyses [36].

The median DCDQ-score was lower in Ukraine than in Greenland and Poland. This may be due to cultural differences among study sites. The reliability and validity of DCDQ is acceptable [40] although previous studies have indicated that the original DCDQ may have poor sensitivity in a population sample compared to the physical motor test The Movement Assessment Battery for Children (MABC) [41] and also, the later version, DCDQ'07, had low specificity when screening an adolescent population [42]. As we intended to examine whether there was a difference in motor score according to exposure levels of DDE and CB-153, we do not believe this is of major concern for our study. In spite of a reported agreement of between 80 and 95 % between DCDQ and expert opinion [40] interpretation of the prevalence of DCD in the various countries is not recommended without an additional standardized motor test to confirm the screening result of DCDQ.

Because of a relatively small study population in Poland and a substantial amount of covariates in the regression model, the results from Poland should be interpreted with care. In Poland, the participation rates at baseline and at follow-up were rather modest (37 % and 36 %, respectively) and thus, the Polish part of the cohort may not fully reflect the reference population. In Ukraine, the baseline participation rate was quite low (25 %) but very high at follow-up (81 %). The participation rates in Greenland were very high both at baseline (88 %) and at follow-up (89 %) suggesting a good representation of the reference population in Greenland. Unfortunately, we were not able to compare non-participating and participating women at baseline.

The relatively high correlation between CB-153 and DDE made us choose not to perform mutual adjustment in the analyses, and thus possible confounding of the opposite organochlorines in the results could not be eliminated.

Smoking and drinking alcohol prior to pregnancy were used as proxies for the habits during pregnancy as this information was unavailable. This may have lead to confounding of the estimates, as the women may have changed their behavior during pregnancy.

Omega-3 fatty acids may be positively related to motor development [43] and would co-occur with the exposures. Unfortunately we have no specific information on omega-3 fatty acid consumption, and thus, we can not rule out the possibility of omega-3 fatty acid counterbalancing the possible negative effects of the organochlorines.

The results of this follow-up study show consistently no indication of an adverse effect of prenatal exposure to CB-153 and p,p'-DDE on motor development in Arctic as well as in European countries representing countries with markedly different exposure levels. However, the lack of a relation between prenatal exposures to CB-153 and DDE and motor skills may be due to generally low exposure levels or a lack of variability in range of the exposures or both, compared to earlier studies. Also, developmental retrospectively recalled milestone may not be sensible enough to detect a difference according to exposures at these levels. The use of current motor skills (DCDQ) at school age, on the other hand are not prone to recall bias.

Conclusions

Prenatal exposure to CB-153 and p,p'-DDE were not associated with the parentally retrospectively assessed developmental milestones, age at crawling, standing and walking in infancy or parentally assessed motor skills in young school age children from Kharkiv (Ukraine), Warsaw (Poland) and Greenland, indicating no major effect of CB-153 or p,p'-DDE exposure on these outcomes. However, subtle effects can not be excluded and the use of a more sensitive outcome measure may be warranted.

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List of abbreviations

BSID, Bayley Scales of Infant Development

CB-153, 2,2',4,4',5,5'-hexachlorobiphenyl

CI, 95 % confidence interval

CLEAR, Climate change, environmental contaminants and reproductive health

DCD, Developmental coordination disorder score

DCDQ'07, Developmental Coordination Disorder Questionnaire 2007

DDE, Dichlorodiphenyldichloroethylene

DDT, Dichlorodiphenyltrichloroethane

Diff., adjusted mean difference

INUENDO, Biopersistent organochlorines in diet and human fertility

LOD, Detection limit

MABC, The Movement Assessment Battery for Children

MSCA, McCarthy Scales of Children's Abilities

PDI, Psychomotor Development Index scores

PCBs, polychlorinated biphenyls

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

Ref., reference group

SD, Standard deviation

Conflicts of interest

The authors declare that they have no competing interests.

Authors's contributions

JPB designed and initiated the INUENDO cohort. JPB and GT designed and initiated the CLEAR project. GT, HSP, LC, KG were responsible for collecting blood samples and interview data. BAGJ was responsible for the chemical analyses of the organochlorine biomarkers. JPB and GT coordinated

the execution of the INUENDO and the CLEAR projects. GT had main responsibility for creating the INUENDO and CLEAR databases. CHR-H contributed to the design, analyses and interpretation of data. BBH contributed to the design and data collection and were responsible for statistical analyses and writing the draft version of the manuscript. All authors revised the manuscript and approved the final version for publication.

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Table 1. Characteristics of mothers and their children (born 2002-2004), INUENDO cohort.

Characteristics	Greenland (n=520)	Ukraine (n=492)	Poland (n=91)
Mother			
Age at baseline interview (years), mean (SD)	27 (6)	25 (5)	29 (3)
Parity, child, n (%)			
1st	164 (33)	401 (82)	86 (95)
2nd/3rd	244 (49)	91 (18)	5 (5)
4th or more	88 (18)	0 (0)	0 (0.0)
Smoking before pregnancy, n (%)			
Yes	379 (87)	112 (31)	18 (47)
No	55 (13)	250 (69)	20 (53)
Alcohol before pregnancy, n (%)			
≤ 7 servings/week	460 (88)	492 (100)	86 (95)
> 7 servings/week	60 (12)	0 (0)	5 (5)
Educational level, Finished education at age (years), n (%)			
≤ 15	44 (10)	25 (6)	0 (0)
16-17	169 (36)	116 (27)	0 (0)
≥ 18	248 (54)	292 (67)	89 (100)
Child			
Sex, n (%)			
Male	280 (54)	260 (53)	54 (59)
Female	238 (46)	229 (47)	37 (41)
Age at follow-up (years), mean (SD)	8 (1)	7 (1)	8 (1)
Breastfeeding duration (months), n (%)			
0	17 (4)	42 (9)	2 (2)
< 6	118 (25)	164 (33)	19 (21)
6-12	124 (26)	177 (36)	37 (41)
> 12	211 (45)	107 (22)	33 (36)
Gestational age at birth (weeks), n (%)			
≥ 37	496 (95)	483 (98)	87 (96)
< 37	24 (5)	9 (2)	4 (4)
Exposure			
CB-153, ng/g lipid, Median (10-90 percentile)	107 (30-369)	27 (11-54)	11 (3-24)
p,p'-DDE ng/g lipid, Median (10-90 percentile)	300 (78-959)	639 (329-1303)	440 (160-718)
Outcome			
Crawling (months), Mean age (SD)	6.5 (2)	6.9 (2)	9.0 (8)
Standing-up (months), Mean age (SD)	8.8 (2)	8.9 (3)	9.8 (5)
Walking (months), mean age (SD)	12.2 (2)	11.2 (1)	12.6 (4)
DCDQ-score < 8 years (points) Median [10-90 percentile]	62 [49-73]	46 [37-55]	64 [51-73]
DCDQ-score ≥ 8 years (points) Median [10-90 percentile]	62 [51-75]	47 [29-58]	67 [58-74]

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; DCDQ, Developmental coordination Disoreder Questionnaire; p,p'-DDE; 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene; SD, standard deviation.

Table 2. Pregnancy tertiles of CB-153 and p,p'-DDE

Exposures ng/g lipids	**Tertile**	**Greenland n=520**	**Ukraine n=492**	**Poland n=91**	**All N=1,103**
CB-153	Low	2.6-74.9	2.4-20.4	2.5-7.3	2.4-26.8
	Medium	75.0-167.9	20.5-34.5	7.4-13.3	26.9-75.0
	High	168.0-2,223.7	34.6-533.4	13.4-74.8	75.1-2,223.7
p,p'-DDE	Low	5.3-208.9	147.0-488.3	88.3-302.8	5.3-330.5
	Medium	209.0-445.0	488.4-790.6	302.9-471.2	330.6-636.0
	High	445.1-3,122.0	790.7-4,835.7	471.3-1,750.1	636.1-4,835.7

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl p,p'-DDE; 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene.

Table 3. Number (%) of missing values according to country

Variables	Greenland (n= 520)	Ukraine (n= 492)	Poland (n=91)
	n (%)	n (%)	n (%)
Exposures			
Prenatal exposures	0 (0.0)	0 (0)	0 (0)
Motor skills			
DCDQ-score	306 (59)	11 (2)	3 (3)
Crawling	114 (22)	14 (3)	29 (32)
Standing	174 (34)	7 (1)	17 (19)
Walking	47 (9)	3 (1)	3 (3)
Maternal and child characteristics			
Maternal age at baseline	37 (7)	25 (5)	0 (0)
Parity	25 (5)	0 (0)	0 (0)
Smoking before pregnancy	0 (0)	0 (0)	0 (0)
Alcohol before pregnancy	0 (0)	0 (0)	0 (0)
Maternal educational level	59 (11)	59 (12)	2 (2)
Child sex	2 (0)	3 (1)	0 (0)
Breast feeding	50 (10)	3 (1)	0 (0)
Gestational age at birth	0 (0)	0 (0)	0 (0)
Child age at follow-up	52 (10)	5 (1)	0 (0)

Abbreviations: DCDQ, developmental coordination disorder questionnaire.

Table 4. Mean differences (months)[a] for developmental milestones in relation to maternal tertiles of CB-153 and p,p'-DDE

		Greenland, n=520			**Ukraine, n=492**			**Poland, n=91**			**All, n=1,103**		
		Diff. (95%CI)	Diff. (95%CI)	P[b]	Diff. (95%CI)	Diff. (95%CI)	P[b]	Diff. (95%CI)	Diff. (95%CI)	P[b]	Diff. (95%CI)	Diff. (95%CI)	P[b]
Exposures	Milestone	Medium	High		Medium	High		Medium	High		Medium	High	
CB-153	Crawl	-0.1 (-0.7, 0.4)	0.0 (-0.6, 0.6)	0.57	0.3 (-0.3, 0.8)	0.2 (-0.4, 0.8)	0.55	-1.5 (-5.5, 2.5)	-0.8 (-5.1, 3.4)	0.96	0.2 (-0.3, 0.7)	0.1 (-0.6, 0.8)	0.68
	Stand-up	-0.2 (-0.8, 0.4)	0.3 (-0.4, 0.9)	0.70	0.2 (-0.5, 0.9)	0.6 (-0.1, 1.3)	0.19	-0.7 (-2.7, 1.4)	1.0 (-1.4, 3.3)	0.36	0.5 (0.0, 1.0)	0.5 (-0.2, 1.1)	0.12
	Walk	-0.3 (-0.8, 0.2)	0.1 (-0.4, 0.6)	0.78	0.0 (-0.5, 0.6)	0.3 (-0.3, 0.8)	0.53	-0.1 (-1.9, 1.7)	0.6 (-1.5, 2.7)	0.25	0.4 (0.0-0.9)	0.3 (-0.2-0.9)	0.21
p,p'-DDE	Crawl	-0.1 (-0.7, 0.4)	-0.1 (-0.6, 0.5)	0.45	0.0 (-0.6, 0.5)	0.1 (-0.5, 0.7)	0.88	-1.8 (-5.7, 2.1)	-0.7 (-4.9, 3.5)	0.42	-0.1 (-0.6, 0.4)	-0.2 (-0.8, 0.3)	0.28
	Stand-up	-0.2 (-0.8, 0.3)	0.2 (-0.5, 0.8)	0.99	-0.2 (-0.9, 0.5)	-0.2 (-0.9, 0.6)	0.79	0.0 (-2.1, 2.0)	0.0 (-2.3, 2.3)	0.84	0.3 (-0.2, 0.8)	0.3 (-0.2, 0.8)	0.78
	Walk	-0.1 (-0.6, 0.4)	-0.1 (-0.6, 0.4)	0.51	-0.5 (-1.0, 0.1)	-0.2 (-0.7, 0.4)	0.65	0.6 (-1.2, 2.4)	0.6 (-1.5, 2.6)	0.45	0.3 (-0.2, 0.7)	0.1 (-0.3, 0.6)	0.83

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; CI, confidence interval; Diff., adjusted mean difference (months); p,p'-DDE , 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene. [a] Adjusted for: maternal pre-pregnancy smoking, maternal pre-pregnancy alcohol-intake, maternal education, parity, maternal age at baseline interview, breast-feeding, preterm birth, gestational age at blood-sampling, child sex and child age at interview. Low exposure is the reference group. [b] P-values are two-sided and express the continuous exposure in linear regression analyses. CB-153 and p,p'-DDE were natural logarithm transformed in test for trend. Imputation-based analyses.

Table 5. Mean differences (points)[a] for DCDQ-score in relation to pregnancy tertiles of CB-153 and p,p'-DDE

		Greenland, n=520			**Ukraine, n=492**			**Poland, n=91**			**All, n=1,103**		
		Diff. (95%CI)	Diff. (95%CI)	P[b]	Diff. (95%CI)	Diff. (95%CI)	P[b]	Diff. (95%CI)	Diff. (95%CI)	P[b]	Diff. (95%CI)	Diff. (95%CI)	P[b]
Exposures	Outcome	Medium	High		Medium	High		Medium	High		Medium	High	
CB-153	DCDQ	0.1 (-1.8, 2.0)	-0.6 (-2.6, 1.4)	0.60	-1.2 (-2.8, 0.4)	-0.5 (-2.2, 1.2)	0.58	-2.7 (-8.2, 2.8)	-0.9 (-6.7, 5.0)	0.70	0.2 (-1.1, 1.5)	0.5 (-2.2, 1.3)	0.78
p,p'-DDE	DCDQ	-0.4 (-2.3, 1.5)	0.3 (-1.7, 2.2)	0.86	1.1 (-0.5, 2.7)	0.6 (-1.0, 2.3)	0.35	-2.5 (-7.9, 2.9)	-2.0 (-7.8, 3.8)	0.45	-0.5 (-1.8, 0.8)	-0.5 (-1.9, 0.9)	0.80

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; CI, confidence interval; DCDQ, developmental coordination disorder questionnaire; Diff, adjusted mean difference (points); p,p'-DDE , 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene; Ref., reference group. [a] Adjusted for: maternal pre-pregnancy smoking, maternal pre-pregnancy alcohol-intake, maternal education, parity, maternal age at birth, breast-feeding, preterm birth, gestational age at blood-sampling, child sex and child age at interview. Low exposure is the reference group. [b] P-values are two-sided and express the p-value of the continuous exposure in linear regression analyses. CB-153 and p,p'-DDE were natural logarithm transformed in test for trend. Imputation-based analyses.

Additional file 1. Crude mean differences (months) for developmental milestones in relation to maternal tertiles of CB-153 and p,p'-DDE

		Greenland, n=520			Ukraine, n=492			Poland, n=91			All, n=1,103[a]		
Exposures	Milestone	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]
CB-153	Crawl	-0.1 (-0.6-0.5)	0.1 (-0.5-0.6)	0.76	0.3 (-0.3-0.8)	0.2 (-0.4-0.7)	0.65	-2.7 (-6.4-1.1)	-1.9 (-5.6-1.9)	0.49	0.2 (-0.3-0.7)	0.1 (-0.6-0.8)	0.66
	Stand-up	-0.1 (-0.7-0.4)	0.4 (-0.2-1.0)	0.31	0.1 (-0.5-0.8)	0.5 (-0.1-1.2)	0.23	-0.7 (-3.0-1.5)	1.1 (-1.2-3.4)	0.18	0.5 (0.0-1.0)	0.5 (-0.1-1.2)	0.07
	Walk	-0.3 (-0.8-0.2)	0.1 (-0.4-0.6)	0.67	0.0 (-0.6-0.5)	0.3 (-0.3-0.8)	0.51	-0.3 (-2.5-1.8)	0.7 (-1.5-2.8)	0.15	0.4 (0.0-0.8)	0.3 (-0.2-0.8)	0.22
p,p'-DDE	Crawl	-0.1 (-0.6-0.4)	0.0 (-0.6-0.5)	0.54	0.0 (-0.6-0.5)	0.0 (-0.5-0.6)	0.63	-2.2 (-5.9-1.5)	-2.0 (-5.8-1.8)	0.17	-0.1 (-0.6-0.4)	-0.3 (-0.8-0.3)	0.23
	Stand-up	-0.2 (-0.7-0.4)	0.3 (-0.3-0.9)	0.64	-0.2 (-0.9-0.4)	-0.2 (-0.8-0.5)	0.71	0.0 (-2.3-2.3)	0.9 (-1.4-3.2)	0.32	0.3 (-0.2-0.8)	0.4 (-0.2-0.9)	0.62
	Walk	0.0 (-0.5-0.5)	0.0 (-0.1-0.4)	0.61	-0.5 (-1.0-0.1)	-0.2 (-0.7-0.4)	0.66	0.6 (-1.5-2.7)	1.2 (-0.9-3.3)	0.19	0.2 (-0.2-0.6)	0.1 (-0.3-0.6)	0.87

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; CI, confidence interval; Diff., adjusted mean difference (months); p,p'-DDE , 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene. [a] Adjusted for country. [b] P-values are two-sided and express the continuous exposure in linear regression analyses. CB-153 and p,p'-DDE were natural logarithm transformed in test for trend. Imputation-based analyses.

Additional file 2. Crude mean differences (points) for DCDQ-score in relation to pregnancy tertiles of CB-153 and p,p'-DDE

		Greenland, n=520			Ukraine, n=492			Poland, n=91			All, n=1,103[a]		
Exposures	Outcome	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]
CB-153	DCDQ	-0.2 (-2.1-1.6)	-0.8 (-2.7-1.1)	0.91	-1.0 ()-2.6-0.6	-0.6 (-2.2-1.0)	0.40	-1.2 (-6.7-4.4)	-1.1 (-6.7-4.4)	0.47	0.1 (-1.2-1.4)	-0.7 (-2.4-1.0)	0.60
p,p'-DDE	DCDQ	-0.4 (-2.3-1.4)	0.0 (-1.9-1.8)	0.56	1.3 (-0.3-2.9)	0.8 (-0.8-2.4)	0.31	-0.7 (-6.2-4.8)	-1.6 (-7.1-3.9)	0.33	-0.3 (-1.6-1.0)	-0.5 (-1.8-0.9)	0.70

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; CI, confidence interval; DCDQ, developmental coordination disorder questionnaire; Diff, adjusted mean difference (points); p,p'-DDE , 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene; Ref., reference group. [a] Adjusted for country. Low exposure is the reference group. [b] P-values are two-sided and express the p-value of the continuous exposure in linear regression analyses. CB-153 and p,p'-DDE were natural logarithm transformed in test for trend. Imputation-based analyses.

Additional file 3. Mean differences (months)[a] for developmental milestones in relation to maternal tertiles of CB-153 and p,p'-DDE

			Greenland				**Ukraine**		
Exposures	Milestone	n	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	n	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]
CB-153	Crawl	275	0.1 (-0.4, 0.6)	0.2 (-0.3, 0.8)	0.77	366	0.0 (-0.3, 0.3)	0.2 (-0.1, 0.5)	0.21
	Stand-up	223	0.1 (-0.7, 0.9)	0.5 (-0.3, 1.3)	0.7	371	0.1 (-0.2, 0.4)	0.3 (0.0, 0.6)	0.10
	Walk	317	-0.5 (-1.1, 0.1)	-0.3 (-0.9, 0.4)	0.19	375	0.0 (-0.4, 0.4)	0.1 (-0.3, 0.4)	0.82
p,p'-DDE	Crawl	273	0.0 (-0.5, 0.4)	0.0 (-0.4, 0.5)	0.50	366	0.1 (-0.2, 0.4)	-0.2 (-0.5, 0.2)	0.34
	Stand-up	223	0.2 (-0.6, 1.0)	0.4 (-0.5, 1.2)	0.77	371	0.2 (-0.1, 0.5)	0.2 (-0.1, 0.5)	0.52
	Walk	299	-0.2 (-0.9, 0.4)	-0.6 (-1.3, 0.1)	0.04	374	-0.2 (-0.5, 0.2)	0.0 (-0.4, 0.4)	0.7
			Poland				**All**		
			Medium	High			Medium	High	
CB-153	Crawl	59	-0.5 (-2.1, 1.0)	0.5 (-1.1, 2.2)	0.21	698	0.1 (-0.2, 0.4)	0.3 (-0.2, 0.7)	0.51
	Stand-up	68	-1.0 (-1.9, 0.1)	0.1 (-0.9, 1.1)	0.99	658	0.1 (-0.2, 0.4)	0.4 (-0.2, 0.9)	0.20
	Walk	81	0.0 (-0.9, 1.0)	0.0 (-1.0, 1.0)	0.64	765	0.2 (-0.2, 0.5)	-0.1 (-0.6, 0.4)	0.44
p,p'-DDE	Crawl	56	-1.0 (-2.2, 0.3)	-1.0 (-2.2, 0.6)	0.21	690	0.1 (-0.2, 0.3)	-0.2 (-0.5, 0.1)	0.17
	Stand-up	68	0.3 (-0.6, 1.3)	-0.4 (-1.5, 0.6)	0.43	658	0.1 (-0.3, 0.5)	0.0 (-0.4, 0.4)	0.50
	Walk	80	0.3 (-0.7, 1.3)	-0.3 (-1.4, 0.7)	0.66	745	0.0 (-0.4, 0.4)	-0.1 (-0.5, 0.3)	0.09

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; CI, confidence interval; Diff, adjusted mean difference (months); p,p'-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene; Ref., reference group. [a] Adjusted for: maternal pre-pregnancy smoking, maternal pre-pregnancy alcohol-intake, maternal education, parity, maternal age at baseline interview, breast-feeding, preterm birth, gestational age at blood-sampling, child sex and child age at interview. Low exposure is reference group. [b] P-values are two-sided and express the continuous exposures in linear regression analyses. CB-153 and p,p'-DDE were natural logarithm transformed in test for trend. Complete case analyses.

Additional file 4. Mean differences (points)[a] for DCDQ-score in relation to tertiles of maternal CB-153 and p,p'-DDE

		Greenland				Ukraine		
Exposures	n	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]	n	Diff. (95%CI) Medium	Diff. (95%CI) High	P[b]
CB-153	152	1.2 (-2.1, 4.5)	0.1 (-3.6, 3.7)	0.31	369	-1.4 (-3.2, 0.5)	-0.3 (-2.1, 1.5)	0.78
p,p'-DDE	152	0.5 (-0.3, 3.9)	0.1 (-3.5, 3.6)	0.52	369	1.4 (-0.3, 3.2)	1.6 (-0.3, 3.4)	0.41
		Poland				**All**		
CB-153	81	-3.2 (-9.5, 3.1)	-3.1 (-9.6, 3.4)	0.46	594	-0.7 (-2.3, 0.9)	-0.3 (-3.1, 2.5)	0.84
p,p'-DDE	81	-2.4 (-8.5, 3.6)	-1.9 (-7.9, 4.2)	0.71	594	-0.5 (-2.3, 1.4)	0.1 (-1.9-2.1)	0.61

Abbreviations: CB-153, 2,2´,4,4´,5,5´-hexachlorobiphenyl; CI, confidence interval; DCDQ, developmental coordination disorder questionnaire; Diff., adjusted mean difference (points); p,p'-DDE;, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene. [a] Adjusted for: maternal pre-pregnancy smoking, maternal pre-pregnancy alcohol-intake, maternal education, parity, maternal age at birth, breast-feeding, preterm birth, gestational age at blood-sampling, child sex and child age at interview. Low exposure is reference group.
[b] P-values are two-sided and express continuous exposures in linear regression analyses. CB-153 and p,p'-DDE were natural logarithm transformed in test for trend. Complete-case analyses.

Appendix II

Paper II Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5–9 years – a prospective study. [In review]

Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5-9 years – a prospective study

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Abstract

Background: In animal studies, perfluorinated alkyl substances affect growth and neuro-behavioural outcomes. Human epidemiological studies are sparse.

Objectives: To investigate the association between pregnancy serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) and offspring behaviour and motor development at 5-9 years of age.

Methods: Maternal sera from the INUENDO cohort (2002-2004) comprising 1,106 mother-child pairs from Greenland, Kharkiv (Ukraine) and Warsaw (Poland) were analysed for PFOS and PFOA, using liquid-chromatography-tandem-mass-spectrometry. Exposures were grouped into country specific as well as pooled tertiles as well as being used as continuous variables for statistical analyses. Child motor development and behaviour at follow-up (2010-2012) were measured by the Developmental Coordination Disorder Questionnaire 2007 (DCDQ) and Strength and Difficulties Questionnaire (SDQ), respectively. Exposure-outcome associations were analysed by multiple logistic and linear regression analyses.

Results: In the pooled analysis, odds ratio (OR) (95% confidence interval (CI)) for hyperactivity was 3.1 (1.3, 7.2) comparing children prenatally exposed to high PFOA with those exposed to low PFOA. Comparing children prenatally exposed to high PFOS levels with those exposed to low PFOS levels showed similar but statistically non-significant result (OR (95 % CI) 1.7 (0.9, 3.2)). In Greenland, PFOS was associated with higher SDQ-total scores (β (95 % CI) =1.0 (0.1, 2.0)) and PFOA was associated with higher hyperactivity sub-scale scores (β (95 % CI) = 0.5 (0.1, 0.9)). Prenatal PFOS and PFOA exposures were not associated with motor difficulties.

Conclusions: Prenatal exposure to PFOS and PFOA may affect children's neuro-behavioural development modestly, specifically in terms of hyperactive behaviour. The associations were strongest in Greenland where exposure contrast is largest.

Keywords: Behaviour; child; child development; cohort study; motor development; Perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS); prenatal exposure, delayed effects

Abbreviations

ADHD: attention deficit hyperactivity disorder

CI; confidence interval

DCD; developmental coordination disorder

DCDQ; Developmental Coordination Disorder Questionnaire 2007

OR; odds ratio

PFAS; Perfluorinated alkyl substances

PFOA; perfluorooctanoate

PFOS; perfluorooctane sulfonate

SDQ; Strength and Difficulties Questionnaire

1. Introduction

Perfluorinated alkyl substances (PFAS) are man-made persistent chemicals, which have previously been widely used due to their water and oil-repellent properties. Biomonitoring studies have shown that they are found globally in a variety of living organisms, including humans (Butenhoff et al. 2006; Harada and Koizumi 2009; Roosens et al. 2010; Sundstrom et al. 2011). Exposure pathways are mainly through diet and drinking water (Halldorsson et al. 2008; Vestergren and Cousins 2009). Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are widespread in the environment, have long serum half-lives in humans (Olsen et al. 2007) and are known to cross the placenta (Midasch et al. 2007), which is worrying as fetal and early life is considered to be the vulnerable period of normal brain development (Rice and Barone 2000).

Some of the most prevalent neuro-developmental disorders are developmental coordination disorder (DCD) and attention deficit hyperactivity disorder (ADHD) and they may be related through common causal pathways (Gillberg 2003). The disorders are related to various predictors such as fetal alcohol exposure (Bhatara et al. 2006) and fetal smoking exposure (Pauly and Slotkin 2008) although newer studies report little evidence of a causal relation between prenatal smoking exposure and development of ADHD (Obel et al. 2011). Twin studies of ADHD suggest a high level of heritability, but fetal environmental pollutant exposures are also likely to play a role (de Cock et al. 2012).

Animal studies suggest associations between prenatal exposure to PFOS and PFOA and neuro-behavioural deficits (Fuentes et al. 2007a; Fuentes et al. 2007b; Johansson et al. 2008). Human observational studies on the relation between PFC exposures and behaviour are sparse and results are inconsistent, which may be due to different

exposure levels and study designs. One cross-sectional study reported a positive association between PFOS and PFOA and ADHD (Hoffman et al. 2010), while another study observed an inverse J-shaped association between PFOA and ADHD across quartiles of exposure (Stein and Savitz 2011), indicating highest risk among those exposed to medium exposure level. No association was observed between relatively high fetal PFOS and PFOA exposure and behaviour at 7 years of age (Fei and Olsen 2011), while another follow-up study with lower PFC exposures observed a relation between higher levels of PFOS (but not PFOA) and worse gross motor function in 2-year old children (Chen et al. 2013).

The present longitudinal study with a relatively large number of participants and relatively large exposure spans investigated the associations between pregnancy concentrations of PFOS and PFOA at background exposure levels and motor development and behaviour in 5-9-year old children in the INUENDO (Biopersistent organochlorines in diet and human fertility) birth cohort.

2. Methods and materials

2.1. Study population and data collection

During the period from May 2002 to February 2004, 1,441 pregnant women from Greenland, Warsaw (Poland) and Kharkiv (Ukraine) were enrolled in the INUENDO birth cohort from antenatal health care clinics and provided a blood sample at any stage of pregnancy. To be eligible for the study, the woman had to be born in the country of study, be pregnant and at least 18 years of age. At baseline, 2,478 women were eligible in Ukraine of whom 612 (24.7 %) participated, and non-participants were slightly younger than participants but were otherwise similar. In Greenland, 665 were eligible and 571 (85.9 %) participated, and participants and non-participants

were very similar. In Poland, 690 were eligible and 258 (37.4 %) participated. Unfortunately, we have no information available for the non-participants from Poland, limiting our ability to compare participants and non-participants. Further details on the baseline study population are available elsewhere (Toft et al. 2005). A follow-up was conducted from January 2010 to May 2012, when the children were between 5 and 9 years old. Parents or guardians responded to questions concerning lifestyle, motor development, behaviour and other characteristics in a face-to-face interview or by filling in a questionnaire themselves.

At follow-up, a total of 1,113 mother-child-pairs (singleton births) with measured exposure information participated of the 1,441 pregnant women enrolled at baseline. After exclusion of mother-child-pairs with missing information on all 15 DCDQ items (n=7) the study population consisted of 1,106 children, distributed between Greenland (n=526 (47.6 %)), Poland (n=89 (8.0 %)) and Ukraine (n=491(44.4 %)).

2.2. Determination of PFOS and PFOA and cotinine

Plasma concentrations of PFOA, PFOS and cotinine were analysed, using liquid chromatography-tandem-mass-spectrometry (LC/MS/MS) at The Department of Occupational and Environmental Medicine in Lund, Sweden. A detailed description of the method for PFOS and PFOA is presented elsewhere (Lindh et al. 2012). Cotinine was analysed by the same method. Briefly, aliquots of 100 µl serum were added 25 µl of a water:acetonitrile (50:50) solution containing labelled internal standards. Proteins were precipitated with acetonitrile and vigorously shaking for 30 minutes. The samples were then centrifuged and the supernatant analyzed using a LC (UFLCXR, SHIMADZU Corporation, Kyoto, Japan) connected to a hybrid triple quadrupole linear ion trap mass spectrometer (QTRAP 5500, AB Sciex, Foster City,

CA, USA). All samples were above limits of detection, which were 0.2 ng/ml and 0.04 ng/ml for PFOS and PFOA, respectively. Coefficient of variation of duplicate samples worked-up and analyzed on different days were 9 % for PFOS and 11 % for PFOA. The analyses of PFOA and PFOS are part of the Round Robin inter-comparison program (Professor Dr. Med. Hans Drexler, Institute and Out-patient Clinic for Occupational-, Social- and Environmental Medicine, University of Erlangen-Nuremberg, Germany) with results within the tolerance limits.

2.3. Assessment of child behaviour and motor abilities and covariate data

Behaviour was assessed using the parent version of the standardized questionnaire "The Strength and Difficulties Questionnaire" (SDQ), comprising 25 items on five scales (emotional, conduct, hyperactivity, peer and pro-social behaviour) (Goodman 1997). SDQ is a screening tool used to identify common mental disorders in children 4 to 16 years of age. The items were coded 0 "not true", 1 "somewhat true" or 2 "certainly true". Each scale had a summed score ranging from 0 to 10. A SDQ-total score was calculated by summing four of the scales (emotional, conduct, hyperactivity and peer) with a score range of 0 to 40. Cut-offs on the SDQ were set according to standard (SDQ-total:0 to 13= normal, 14 to16= borderline and 17 to 40= abnormal; Emotional symptoms: 0 to 5= normal, 6= borderline and 7 to 10= abnormal; Conduct problems: 0 to 3= normal, 4= borderline and 5 to 10= abnormal; Hyperactivity: 0 to 5= normal, 6= borderline and 7 to 10= abnormal; Peer problems: 0 to 3= normal, 4 to 5=borderline and 6 to 10= abnormal; Pro-social behaviour: 6 to 10= normal, 5=borderline and 1 to 4= abnormal) (Youth in Mind 2013). When outcomes were dichotomised, the cut-offs were normal/borderline versus abnormal. In all scales, except the pro-social subscale, a high score indicated problems.

Parents were asked to assess their child's behaviour during the past six months.
To evaluate the motor development, parents compared their child's motor abilities to that of his or her peers using "Developmental Coordination Disorder Questionnaire 2007" (DCDQ). DCDQ is a screening tool to help identify motor difficulties in children 5-15-years of age, comprising 15 items on a 5-point Likert scale with a total score range of 15 to 75 (Wilson et al. 2009). A low score indicates problems. Only continuous DCDQ scores were used in this study as scores in three populations were heterogeneous and the validity of DCDQ has not been examined specifically in the countries where the present study takes place.
Covariate data were collected from the pregnancy and follow-up questionnaires and included information about e.g. lifestyle, health and personal characteristics.

2.4. Statistical analysis

2.4.1. Missing information

The number of missing values on behaviour, motor development and covariates ranged from 0 to 55 %. One of the 15 items of the DCDQ was erroneously lacking in the Greenlandic version of the Questionnaire (but not in the Danish version used for some participants in Greenland), which caused 55% missing of the DCDQ in Greenland, as all items are needed to generate a total score. In the Greenlandic population, the median of missing answers of the DCDQ were 2%, and 85% had answered at least 14 of 15 items of the DCDQ. To increase power and overcome the risk of introducing selection bias by analysing only the complete case dataset, we used chained multiple imputation, allowing us to maintain participants with incomplete data (Sterne et al. 2009). Briefly, multiple different imputed datasets (m>1) are created, and a set of random plausible values replace each missing value, based on

known subject characteristics and other predictors in the complete dataset. This incorporates an appropriate variability across the *m* datasets. The new *m* complete datasets are analysed, producing a single set of results accounting for the variability of the missing data (Sterne et al. 2009).

We generated 100 imputed datasets in a combined imputation that included all three countries. The predictors were: PFOA, PFOS, the 15 items of the DCDQ, the 5 subscales of the SDQ, maternal cotinine level during pregnancy, maternal alcohol consumption before conception, maternal educational level, maternal age at pregnancy, birth weight, gestational age at birth, gestational age at blood sampling, parity, breastfeeding duration, child age at follow-up and child sex.

2.4.2. Data analysis

A non-response analysis was performed to check for inconsistencies between responders (n=1,113) and non-responders (n=328). Spearman's rank correlation was used to assess the correlation between maternal pregnancy levels of PFOS and PFOA. We used logistic regression models in the primary analyses of PFOS and PFOA levels and behavioural problems (abnormal SDQ-total and sub-scale scores) and linear regression in the investigation of PFOS and PFOA exposure and motor development. Exposures were *a priori* decided to be categorized into tertiles per country as well as pooled, using the lowest tertile as reference group. Moreover, trends in exposure-outcome associations were further explored, using continuous, natural logarithm transformed exposures in linear regression models. To increase power, the associations between exposures and behavioural outcomes were also examined using linear regression models with exposures categorized into tertiles as well as a continuous variable. All estimates were adjusted for the most important potential

confounders among the available data, which were identified *a priori* and included maternal cigarette smoking during pregnancy (serum cotinine ≤10/ >10 ng/ ml); maternal alcohol consumption at conception (0, <7, ≥7 drinks per week); maternal age at pregnancy (continuous); child sex and gestational age at blood-sampling (continuous) (Bhatara et al. 2006; Fergusson and Woodward 1999; Pauly and Slotkin 2008; Zahn-Waxler et al. 2008). In addition, we explored possible interactions between the exposures and sex and exposures and country, adding interaction terms to the model. In a sensitivity analysis of the SDQ, we used the top 10 percentile cut-off from this sample as a marker of behavioural problems instead of the standard cut-offs suggested by Youth in Mind (Youth in Mind 2013) as also used in another study (Fei and Olsen 2011). Furthermore, duration of breastfeeding was added as covariate to adjust for postnatal PFAS exposures. Finally, the robustness of the imputation model was examined creating fewer (*m*=20) and more (*m*=150) datasets and using less and more predictors. Complete case results are presented as supplementary material (Tables S1-S3).

All analyses were performed stratified by population as well as pooled (adjusted for population). A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using the Stata statistical package (version 12.1, StataCorp, College Station, Texas, USA).

2.5. Ethics

The study was approved by local ethical committees; Polish Bioethical Committee (approval no. 6/2002 of 3.07.2002), Ethical Committee for Human Research in Greenland (approval no. 2010-13) and the Commission on Ethics and Bioethics

Kharkiv National Medical University in Ukraine (protocol number 7, October 7 2009). All participating parents signed informed consent.

3. Results

The non-response analysis showed no differences between responders and non-responders at follow-up concerning exposure levels, maternal educational level, pregnancy smoking status and maternal age at baseline (data not shown). The median (10-90 percentile) age of the children at follow-up was 8 (7 to 9) years in Greenland compared to 7 (7 to 8) and 8 (7 to 8) years in Ukraine and Poland, respectively. Personal characteristics of the study population are presented in Table 1.

In comparison with women from Greenland, women from Poland were older, had lower body mass index (BMI), more often expected their first child, were non-smokers and better educated. Women from Ukraine were younger at recruitment, had lower BMI, were more often non-smokers and drank less alcohol compared to women from Greenland (Table 1).

The median serum concentrations of PFOS and PFOA are presented in Table 2 alongside the exposure tertiles across the three countries and pooled. Median PFOS exposure levels were highest in Greenland (median (10^{th}–90^{th} percentile) PFOS 20.3 ng/ml (12.0, 37.0)), which is approximate 4 times higher than the median PFOS exposure level in Ukraine. The median PFOA levels were highest in Poland (median (10^{th}-90^{th} percentile) 2.7 ng/ml (1.5, 4.3)).

The Spearman's correlation coefficient between maternal pregnancy levels of PFOS and PFOA was 0.5 in Greenland, 0.6 in Poland and 0.5 in Ukraine. The highest median (10^{th} to 90^{th} percentile) PFOS concentration was seen in Greenland (20 (12 to 36) ng/ml) and the highest median PFOA level was observed in Poland (3 (2 to 4) ng/ml). The level of missing data is presented in Table 3, showing highest level of missing data on the DCDQ in Greenland.

In Table 4, results of the linear regression analyses between PFOS and PFOA and motor skills (DCDQ) are presented. In Greenland and Poland, prenatal PFOS exposure was associated with an increased DCDQ score, whereas it was associated with a decreased score in Ukraine. Prenatal PFOA exposure was mainly associated with a decreased score although not consistently so. However, all associations between PFOS and PFOA and motor development were statistically non-significant. No interaction was observed between exposures and sex and exposures and country.

The associations between PFOA and PFOS and behavioural problems (continuous SDQ-total and hyperactivity) are presented in Table 5. In the pooled analyses, high PFOA and PFOS exposures compared with low exposures were statistically significantly associated with higher hyperactivity scores (more hyperactive behaviour) (β (95 % CI) = 0.5 (0.0, 1.0) and 0.5 (0.1, 0.9), respectively). Higher PFOS levels were statistically significantly associated with higher SDQ-total scores (more behavioural problems) in Greenland (β (95 % CI) 1.0 (0.1, 2.0)) (Table 5) and medium and high PFOS exposed children had an OR (95% CI) of 1.3 (0.3, 2.4) and 1.1 (0.1, 2.2), respectively compared to low exposed children (Table 6). No significant associations were observed in Poland and Ukraine. PFOA was significantly positively associated with hyperactive scores in Greenland (β (95 % CI)

= 0.5 (0.1, 0.9)) but not in Poland and Ukraine. PFOA was not associated with SDQ-total in any of the populations. There was no evidence of interaction between exposures and sex or between exposures and country.

Table 6 presents results of the adjusted logistic regression analyses of the associations between PFOS and PFOA and dichotomized (normal/borderline versus abnormal) SDQ-total and hyperactivity. In the pooled analysis, OR (95 % CI) for abnormal SDQ-total was 2.7 (1.2, 6.3) comparing high PFOA exposure to low PFOA exposure. The medium PFOA exposure was borderline statistically significantly associated with abnormal SDQ-total and the trend was increasing across PFOA levels. In the Polish data, only a few had behavioural problems and, thus, the analysis could not be performed. In Greenland, the adjusted OR for hyperactive behaviour was statistically significantly higher comparing medium and high PFOA exposures to low PFOA exposure, although the 95 % CIs were wide (OR (95 % CI)= 5.4 (1.1, 25.6) and 6.3 (1.3, 30.1), respectively). In Poland, all ORs of the association between prenatal PFOA and hyperactivity were above 1, but results did not reach statistical significance, and in Ukraine, no associations were observed. PFOS was not associated with abnormal SDQ-total in any of the analyses.

Few associations were found for the other SDQ sub-scales (emotional, conduct, peer and pro-social) (Supplemental Material, Table S4). The OR for abnormal pro-social behaviour was also significantly above 1 in Ukraine but not in Greenland, and the analysis could not be performed in Poland. No associations were observed between PFOS and any of the SDQ subscales or SDQ-total in any of the populations (Table 6).

Results from complete-case analyses were generally similar to the imputation-based analyses presented above except for a statistically significant finding in the

imputation-based analysis of the association between categorical PFOA (medium) and hyperactivity in Greenland which was not observed in the complete-case analysis and a statistically insignificant association observed in the imputation-based analysis of the association between continuous PFOS and behavioural problems in Greenland which was significant in the complete-case analysis (Supplemental Material, Tables S1-S3).

In the sensitivity analysis using top ten percentile cut-offs, results were generally similar. However, in Ukraine, OR for PFOS and SDQ-total became statistically significantly different from 1, whereas the OR of PFOA and hyperactivity was no longer statistically significant in the pooled analysis. Adding duration of breastfeeding as a covariate to the models did not change direction or significance of results.

Results of sensitivity analyses of smaller and larger imputation models showed similar results, indicating a robust imputation model.

4. Discussion

We observed a positive association between pregnancy levels of PFOA and child hyperactive behaviour, which was relatively consistent using both categorical and continuous measures of PFOA, and treating the outcome as either continuous hyperactivity scores, or dichotomized into hyperactivity problems versus none. In Greenland, PFOS was associated with behavioural problems (SDQ-total) (not in Poland and Ukraine), whereas PFOA was associated with hyperactivity. In the pooled analysis, odds of having hyperactive behaviour increased comparing medium and high PFOA to low. Only minor associations appeared in relation to other SDQ sub-scales and none to motor development, as measured using DCDQ. In the analysis of categorical exposures stratified by country, we used country specific tertiles as

exposure levels differed considerably between countries. This could in part explain the inconsistencies in our findings. Furthermore, PFAS levels and SDQ and DCDQ results did vary in the three countries included in this study which may account for the inconsistent findings. As stated earlier, the PFOS level was approximately 4 times higher in Greenland than in Ukraine, and DCDQ results were lower in Ukraine than in Poland and Greenland. However, as we found no evidence of interaction between exposures and country, we choose to present results pooled as well as stratified by country. The pooled results were presented in order to gain power, although not all the stratified results indicated consistency between the countries. Hence, the country specific results may be more reliable than the pooled results.

The mechanism related to the association between prenatal PFAS exposure and behavioural and motor problem is unclear. However, an association between prenatal PFOS exposure and thyroid hormone disruption has been reported (Lau et al. 2003), and since thyroid hormones are essential for fetal and early life neurologic development, a disruption by prenatal PFC exposures could possibly impair healthy neurodevelopment.

The results of epidemiological studies examining the neurodevelopmental effects of PFAS are few and inconclusive. Fei et al. reported no associations between high pregnancy levels of PFOA (median (inter-quartile-range): 5.4 (4.0-7.1 ng/ml)) and PFOS (34.4 (26.6-44.5 ng/ml)) and DCD (assessed by DCDQ'07) at 7 years among a sub-sample of 537 participants from the Danish National Birth Cohort (Fei and Olsen 2011), which is in accordance with our results. In the same study, no associations were observed between PFOS and PFOA and SDQ-total or any of the SDQ sub-scales, using top 10 percentile cut-offs (Fei and Olsen 2011), whereas we observed

associations between PFOA and hyperactivity in Greenland and in the pooled analysis of the three countries. Furthermore, we observed a statistically non-significant association between PFOS and SDQ-total in the pooled analysis, whereas the country specific results were more heterogeneous. In the present study, we used the cut-offs suggested by Youth in Mind (Youth in Mind 2013) and included 804 children older than 7 years, which may explain the difference in our results.

A small Taiwanese birth cohort study with exposure levels equivalent to exposure levels in this study observed associations between prenatal PFOS and gross motor functions at 2 years of age, whereas no association was reported between PFOA and any neuro-behavioural outcome (Chen et al. 2013). Direct comparison with this study is, however, hampered since their outcome measure was assessed by use of a questionnaire developed specifically for Chinese populations. Some inconsistencies were present in our results of prenatal PFAS exposure and motor development, and in general, prenatal PFAS exposure was associated with a decreased DCDQ score, indicating more motor problems. However, in Ukraine where the PFOS exposure level was much lower than in Greenland and Poland, prenatal PFOS was associated with higher motor scores. We did not observe any consistent and statistically significant associations which could be due to older age at follow-up or lack of sensitivity of the DCDQ, as the original version of the questionnaire has high sensitivity in populations with high prevalence of motor difficulties and lower in a background population (Schoemaker et al. 2006).

The C8 Health Project (which reports health outcomes in relation to PFOA contamination of drinking water from the Dupont factory in the United States) reported only minor associations between high levels of childhood PFOA measured at 2-8 years of age and a range of neuropsychological outcomes assessed at 6-12 years

among a sub-sample of 321 children (Stein et al. 2013a; Stein et al. 2013b), however, an interaction between PFOA and sex was reported in the analysis of ADHD-like behaviour, indicating high PFOA exposure to be protective among boys and adverse among girls (Stein et al. 2013a). When *in utero* PFOA exposures were estimated in a pharmacokinetic model, higher PFOA levels were associated with fewer signs of ADHD (using the clinical confidence index of Conners' continuous performance test-II) (Stein et al. 2013b). Different study designs and exposure and outcomes assessments were used in these studies and also, the offspring had different ages at examination, which may explain their findings. We saw no signs of interaction between PFOA and sex. However, different questionnaires were used and exposure levels differed extensively, which could account for the disparate results.

There is a risk of exposure misclassification since blood samples were collected throughout pregnancy in our study and PFAS tend to decrease during pregnancy (Fei et al. 2007). However, we addressed this issue by adjusting for gestational age at blood sampling, although we recognize that this may not have completely eliminated the risk of information bias.

The DCDQ is developed as a screening tool and should not be used for diagnostics (DCDQ - Developmental Co-ordination Disorder Questionniare (DCDQ) August, 2013). The questionnaire has not been validated in the three countries represented in this study, and thus there is a risk of misclassification of motor problems. However, we believe the questionnaire is suitable for detecting a difference in motor abilities according to PFOS and PFOA exposure levels within the three countries, and since we did not use the cut-offs for indication of motor difficulties, this should not be a large concern.

We chose to use the suggested cut-offs for behavioral problems as no country specific cut-offs exist for Greenland, Ukraine and Poland. This enabled us to compare results between countries but may have resulted in misclassification of the SDQ outcome. SDQ as a screening tool has a high validity as well as reliability (Goodman and Goodman 2009; Obel et al. 2004), and our sensitivity analysis, using top ten percentile cut-offs in general suggested consistent results.

Missing data can cause selection bias and to overcome this challenge, we performed several multiple imputation analyses, and results were consistent. A lack of demographic information concerning the Polish non-participants at baseline limited our ability to compare participants and non-participants and selection bias in the Polish results can not be ruled out. Further, the relatively low participation rate of 34 % at follow-up in Poland poses a risk of selection bias. However, the non-response analysis showed no evidence of difference between responders and non-responders, indicating low risk of selection bias by loss to follow-up.

It is difficult to estimate to what extent postnatal PFAS exposure may have influenced the results. In a sensitivity analysis, we adjusted for duration of breastfeeding, which did not materially change the results. However, residual confounding by postnatal PFAS exposure can not be completely ruled out.

The Polish results should be interpreted with caution, since the Polish sample is small. However, samples from Greenland and Ukraine and the pooled analysis are of considerable size and, thus, results should be robust.

We measured PFAS levels at all periods during pregnancy as a measure of fetal exposure. However, it is not clear to what extent this reflects the vulnerable window of exposure. Others have reported high correlation between 1st and 2nd trimester

PFOA levels and because PFOA and PFOS have long half-lives, we believe blood-sampling throughout pregnancy is of minor concern.

5. Conclusions

Prenatal exposure to PFOS and PFOA may affect children's neuro-behavioural development modestly at the age of 5-9 years, specifically in terms of hyperactive behaviour. The associations were largest in Greenland, where the exposure contrast is largest. No association was observed in relation to motor skills. Standardized measures of behavioural- and motor problems were used and results were consistent across numerous sensitivity analyses.

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Table 1. Characteristics of mothers and their children according to country

Characteristics	Greenland (n=526)	Ukraine (n=491)	Poland (n=89)
Outcome			
DCDQ-score (points) [Median (10^{th}-90^{th} percentile)]	63 (51-75)	46 (37-55)	64 (52-73)
SDQ-score (points) [Median (10^{th}-90^{th} percentile)]	7 (2-14)	8 (4-14)	8 (2-15)
SDQ, n (%)			
Normal	455 (88)	424 (86)	75 (85)
Borderline	34 (6)	40 (8)	8 (9)
Abnormal	31 (6)	27 (6)	5 (6)
Maternal characteristics			
Maternal age at pregnancy, years [Median (10^{th}-90^{th} percentile)]	26 (20-37)	24 (20-32)	29 (26-34)
Maternal pre-pregnancy BMI, kg/m^2 [Median (10^{th}-90^{th} percentile)]	24 (20-30)	21 (18-26)	21(19-24)
Parity, no. (%)			
0	167 (33)	399 (81)	84 (94)
1 or 2	246 (49)	92 (19)	5 (6)
3 or more	89 (18)	0 (0)	0 (0)
Maternal smoking during pregnancy, no. (%)			
Yes (serum cotinine >10 ng/ml)	296 (56)	76 (15)	2 (2)
No (serum cotinine ≤10 ng/ml)	230 (44)	415 (85)	87 (98)
Maternal alcohol consumption at conception, no. (%)			
≤ 7 drinks/week	465 (88)	491 (100)	84 (94)
> 7 drinks/week	61 (12)	0 (0)	5 (6)
Maternal educational level, left school at age, no. (%)			
≤ 15 years	44 (9)	25 (5)	0 (0)
16–17 years	173 (37)	114 (23)	0 (0)
≥ 18 years	249 (54)	293 (68)	79 (100)
Child characteristics			
Sex, no. (%)			
Male	282 (54)	260 (53)	53 (60)
Female	241 (46)	228 (47)	36 (40)
Total breastfeeding duration, no. (%)			
0 months	17 (4)	43 (9)	2 (2)
< 6 months	120 (25)	163 (33)	19 (22)
6–12 months	124 (26)	175 (36)	35 (39)
> 12 months	214 (45)	107 (22)	33 (37)
Gestational age, no. (%)			
≥ 37 weeks	499 (95)	479 (98)	65 (94)
< 37 weeks	24 (5)	9 (2)	4 (6)
Gestational age at blood sample, weeks [median (10^{th}–90^{th} percentile)]	25 (13-37)	23 (9-40)	33 (27-37)
Birth weight, grams [median (10^{th}–90^{th} percentile)]	3,600 (2,840-4,370)	3,285 (2,800-3,800)	3,460 (2,880-4,000)
Age at follow-up, years [median (10^{th}–90^{th} percentile)	8 (7-9)	7 (7-8)	8 (7-8)

BMI, body mass index; DCDQ, developmental coordination disorder questionnaire; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; SDQ, strength and difficulties questionnaire. All percentages are based on complete-case data

Table 2. Range (ng/ml) of PFOS and PFOA plasma concentrations, by tertile, in pregnant women

Exposure	Tertile	Greenland (n=526)	Ukraine (n=491)	Poland (n=89)	All (n=1,106)
Pregnancy PFOS	Median (10^{th}-90^{th} percentile)	20.3 (12.0-37.0)	5.0 (2.9-8.2)	8.0 (5.3-12.7)	10.0 (3.6, 27,4)
	Low tertile	4.1-16.8	0.7-4.2	2.5-7.0	0.7-6.2
	Medium tertile	16.8-23.9	4.2-5.9	7.1-9.6	6.2-16.6
	High tertile	23.9-87.3	5.9-18.1	9.7-21.3	16.6-87.3
Pregnancy PFOA	Median (10^{th}-90^{th} percentile)	1.8 (1.0-3.1)	1.0 (0.5-1.7)	2.7 (1.5-4.3)	1.4 (0.7, 3.0)
	Low tertile	0.5-1.5	0.2-0.8	1.0-2.2	0.2-1.1
	Medium tertile	1.5-2.2	0.8-1.1	2.2-3.1	1.1-1.9
	High tertile	2.2-5.1	1.1-9.8	3.1-9.8	1.9-9.8

PFOA, perfluorooctanoate ; PFOS, perfluorooctane sulfonate.

Table 3. Number (%) of missing values according to country

Variables	Greenland (n=526) n (%)	Ukraine (n=491) n (%)	Poland (n=89) n (%)
Exposures			
Prenatal PFOA and PFOS levels	0 (0)	0 (0)	0 (0)
Outcomes			
Behaviour (SDQ)	5 (1)	0 (0)	1 (1)
Motor development (DCDQ)	289 (55)	6 (1)	3 (3)
Maternal and child			
Maternal age at baseline	38 (7)	24 (5)	0 (0)
Smoking during pregnancy	0 (0)	0 (0)	0 (0)
Alcohol before pregnancy[a]	0 (0)	0 (0)	0 (0)
Maternal educational level	60 (11)	59 (12)	10 (11)
Child sex	3 (1)	3 (1)	0 (0)
Child age at follow-up	53 (10)	0 (0)	0 (0)
Gestational age at blood sample	16 (3)	32 (7)	2 (2)

DCDQ, Developmental coordination disorder questionnaire; SDQ, Strength and difficulties questionnaire.
[a] Missing answer was coded as no drinks.

Table 4. Adjusted mean differences (points)[a] for offspring continuous DCDQ-score[b] at school age in relation to continuous and categorised (tertiles) pregnancy concentrations of PFOS and PFOA (ng/ml)

Exposure		Greenland (n=526) β (95 % CI)	Ukraine (n=491) β (95 % CI)	Poland (n=89) β (95 % CI)	All (n=1,106)[d] β (95 % CI)
PFOS	Low	reference	reference	reference	reference
	Medium	-1.2 (-3.0, 0.7)	1.4 (-0.2, 3.1)	-0.3 (-6.0, 5.3)	-0.4 (-1.9, 1.2)
	High	-0.7 (-2.6, 1.2)	0.6 (-1.1, 2.2)	-2.1 (-7.8, 3.5)	-1.7 (-3.8, 0.5)
	Continuous[c]	-0.2 (-1.9, 1.4)	0.5 (-1.0, 2.0)	-2.4 (-9.1, 4.3)	-0.1 (-1.2, 1.1)
PFOA	Low	reference	reference	reference	reference
	Medium	-1.2 (-3.0, 0.7)	-0.2 (-1.8, 1.4)	1.5 (-4.1, 7.1)	-0.6 (-1.9, 0.7)
	High	-0.1 (-2.0, 1.7)	-1.2 (-2.8, 0.5)	-3.7 (-9.3, 1.9)	-0.4 (-1.9, 1.1)
	Continuous[c]	0.8 (-0.8, 2.5)	-0.6 (-1.9, 0.8)	-2.7 (-8.3, 2.8)	-0.2 (-1.2, 0.9)

CI, confidence interval; DCDQ, developmental coordination disorder questionnaire; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.
[a] Adjusted for: maternal smoking during pregnancy, maternal alcohol consumption at conception, maternal age at pregnancy, gestational age at blood-sampling and child sex. [b] DCDQ score range from 15 to 75. [c] β= the change in score according to one natural-log unit increase in PFOA and PFOS. [e] Additionally adjusted for country.
[d]Additionnaly adjusted for country.

Table 5. Adjusted continuous mean differences (points)[a] for offspring SDQ-total and hyperactivity score at school age in relation to continuous and categorised (tertiles) pregnancy concentrations of PFOS and PFOA (ng/ml)

			Greenland (n=526) β (95 % CI)	Ukraine (n=491) β (95 % CI)	Poland (n=89) β (95 % CI)	All (n=1,106)[e] β (95 % CI)
SDQ[b]	**PFOS**	Low	reference	reference	reference	reference
		Medium	1.3 (0.3, 2.4)	-1.0 (-2.0, 0.0)	-0.1 (-2.7, 2.5)	-0.1 (-1.0, 0.8)
		High	1.1 (0.1, 2.2)	-1.0 (-2.2-0.0)	2.0 (-0.6, 4.6)	1.1 (-0.1, 2.3)
		Continuous[d]	1.0 (0.1, 2.0)	-1.0 (-1.6, 0.2)	2.6 (-0.5, 5.7)	0.3 (-0.3, 1.0)
	PFOA	Low	reference	reference	reference	reference
		Medium	0.7 (-0.4, 1.7)	-0.9 (-1.9, 0.1)	0.8 (-1.9, 3.4)	0.3 (-0.5, 1.0)
		High	0.5 (-0.6, 1.5)	-0.3 (-1.3, 0.7)	1.8 (-0.8, 4.5)	0.7 (-0.2, 1.5)
		Continuous[d]	0.3 (-0.6, 1.3)	-0.5 (-1.3, 0.4)	2.1 (-0.4, 4.7)	0.1 (-0.5, 0.7)
Hyperactivity[c]	**PFOS**	Low	reference	reference	reference	reference
		Medium	0.4 (-0.1, 0.8)	-0.2 (-0.6, 0.2)	0.9 (-0.5, 2.4)	0.1 (-0.3-0.5)
		High	0.3 (-0.2, 0.7)	-0.1 (-0.5, 0.3)	1.3 (-0.1, 2.8)	0.5 (0.0, 1.0)
		Continuous[d]	0.3 (-0.1, 0.7)	-0.1 (-0.4, 0.3)	1.5 (-0.3, 3.2)	0.2 (-0.1, 0.5)
	PFOA	Low	reference	reference	reference	reference
		Medium	0.5 (0.0, 1.0)	-0.3 (-0.7, 0.1)	1.1 (-0.4, 2.6)	0.1 (-0.3, 0.4)
		High	0.6 (0.1, 1.1)	-0.2 (-0.6, 0.2)	0.9 (-0.6, 2.4)	0.5 (0.1, 0.9)
		Continuous[d]	0.5 (0.1, 0.9)	-0.1 (-0.5, 0.2)	1.2 (-0.3, 2.6)	0.3 (0.0, 0.5)

CI, confidence interval; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; SDQ, strength and difficulties questionnaire.
[a] Adjusted for: maternal smoking during pregnancy, maternal alcohol consumption at conception, maternal age at pregnancy, gestational age at blood-sampling and child sex.
[b] Score range 0 to 40. [c] Score range 0 to 10. [d] β= the change in score according to one natural-log unit increase in PFOA and PFOS.
[e] Additionally adjusted for country.

Table 6. Adjusted OR (95 % CI)[a] for offspring abnormal SDQ-total and hyperactivity sub-scale according to continuous and categorised (tertiles) pregnancy concentrations of PFOS and PFOA (ng/ml)

Scale	Greenland Adjusted OR (95 % CI) (n=526)	Ukraine Adjusted OR (95 % CI) (n=491)	Poland Adjusted OR (95 % CI) (n=89)	All[e] Adjusted OR (95 % CI) (n=1,106)
SDQ-total[b]				
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.7 (0.7, 4.0)	0.6 (0.2, 1.6)	0.3 (0.0, 3.8)	1.1 (0.5, 2.5)
High	0.8 (0.3, 2.2)	0.6 (0.2, 1.5)	-	1.5 (0.5, 4.8)
Continuous[d]	0.9 (0.4, 2.1)	1.0 (0.4, 2.5)	-	1.1 (0.6, 2.0)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	2.7 (1.0, 7.5)	1.3 (0.5, 3.5)	-	2.0 (1.0, 4.2)
High	1.9 (0.7, 5.4)	1.5 (0.6, 4.2)	-	2.7 (1.2, 6.3)
Continuous[d]	2.1 (0.8, 5.2)	1.0 (0.4, 2.3)	3.1 (0.2, 56.2)	1.5 (0.9, 2.6)
Hyperactivity[c]				
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.1 (0.4, 3.4)	1.2 (0.4, 3.3)	2.3 (0.5, 10.6)	1.2 (0.5, 2.5)
High	1.3 (0.4, 3.9)	1.4 (0.5, 3.9)	2.2 (0.5, 10.0)	1.4 (0.4, 4.9)
Continuous[d]	1.9 (0.7, 4.8)	1.3 (0.5, 3.4)	2.0 (0.4, 10.9)	1.7 (0.9, 3.2)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	5.4 (1.1, 25.6)	0.8 (0.3, 2.3)	2.4 (0.5, 11.0)	0.8 (0.4, 2.0)
High	6.3 (1.3, 30.1)	0.9 (0.3, 2.4)	2.2 (0.5, 10.0)	3.1 (1.3, 7.2)
Continuous[d]	3.6 (1.2, 3.7)	0.8 (0.3, 1.9)	1.8 (0.5, 7.0)	1.6 (0.9, 2.8)

CI, confidence interval; OR, odds ratio; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; SDQ, strength and difficulties questionnaire.

[a]Adjusted for maternal smoking during pregnancy, maternal alcohol consumption at conception, child sex, maternal age at pregnancy and gestational age at blood sampling.

[b]SDQ cut-offs: normal and borderline (0 to 16) versus abnormal (16 to 40)

[c]Hyperactivity cut-offs: normal and borderline (0 to 6) versus (6 to 10)

[d]PFOA and PFOS were natural logarithm transformed.

[e] Additionally adjusted for country.

Supplemental Material, Table S1. Adjusted OR (95 % CI)[a] for offspring SDQ sub-scales. Imputation-based results.

Emotional subscale	Greenland, n=526 Adjusted OR (95 % CI)	Ukraine, n=491 Adjusted OR (95 % CI)	Poland, n=89 Adjusted OR (95 % CI)	Pooled[c], n=1,006 Adjusted OR (95 % CI)
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.1 (0.5, 2.4)	0.3 (0.1, 0.9)	0.3 (0.0, 3.1)	0.8 (0.4, 1.9)
High	1.0 (0.4, 2.2)	0.4 (0.1, 1.0)	1.5 (0.3, 8.4)	0.9 (0.3, 2.6)
Continuous[b]	1.1 (0.5, 2.2)	0.6 (0.2, 1.3)	2.2 (0.2, 24.8)	0.9 (0.5, 1.5)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.7 (0.8, 3.7)	0.6 (0.2, 1.8)	0.8 (0.1, 5.9)	1.4 (0.7, 2.7)
High	1.0 (0.4, 2.3)	1.2 (0.5, 3.1)	1.0 (0.2, 6.4)	1.2 (0.6, 2.7)
Continuous[b]	1.0 (0.5, 2.0)	0.9 (0.4, 2.0)	0.9 (0.1, 5.8)	0.9 (0.5, 1.5)
Conduct subscale				
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.7 (0.9, 3.3)	0.6 (0.3, 1.5)	1.4 (0.2, 9.9)	1.5 (0.8, 2.9)
High	0.8 (0.4, 1.7)	0.8 (0.3, 1.7)	1.5 (0.2, 10.1)	1.7 (0.7, 4.3)
Continuous[b]	1.0 (0.6, 2.0)	1.3 (0.6, 2.8)	3.0 (0.3, 33.4)	1.2 (0.8, 1.9)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.0 (0.5, 2.0)	0.8 (0.4, 2.0)	2.9 (0.5, 18.5)	1.3 (0.7, 2.3)
High	1.2 (0.6, 2.3)	1.6 (0.7, 3.6)	0.8 (0.1, 7.2)	1.8 (1.0, 3.5)
Continuous[b]	1.3 (0.7, 2.4)	1.1 (0.8, 1.9)	1.5 (0.2, 9.3)	1.2 (0.8, 1.9)
Peer subscale				
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.2 (0.7, 2.1)	0.6 (0.3, 1.2)	0.8 (0.1, 7.4)	1.0 (0.6, 1.7)
High	1.6 (0.9, 2.7)	0.7 (0.4, 1.4)	1.9 (0.3, 12.5)	1.5 (0.7, 3.0)
Continuous[b]	1.5 (0.9, 2.4)	0.8 (0.4, 1.3)	1.8 (0.2, 17.6)	1.2 (0.8, 1.7)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	1.9 (0.3, 2.3)	1.0 (0.5, 1.9)	1.9 (0.3, 13.7)	1.3 (0.8, 2.0)
High	1.1 (0.6, 1.8)	1.2 (0.6, 2.3)	1.2 (0.2, 9.1)	1.4 (0.9, 2.3)
Continuous[b]	1.1 (0.7, 1.7)	1.1 (0.6, 1.8)	2.0 (0.3, 12.1)	1.1 (0.8, 1.6)
Pro-social subscale				
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	0.8 (0.3, 2.2)	0.5 (0.2, 1.4)	-	1.7 (0.8, 3.5)
High	0.5 (0.2, 1.7)	1.1 (0.4, 2.7)	-	1.2 (0.4, 3.8)
Continuous[b]	0.8 (0.3, 2.1)	1.5 (0.6, 3.7)	0.3 (0.0, 4.6)	1.1 (0.6, 2.0)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	0.4 (0.1, 1.3)	1.7 (0.6, 5.1)	-	1.5 (0.8, 3.0)
High	0.4 (0.1, 1.3)	3.2 (1.1, 9.0)	-	0.7 (0.3, 1.7)
Continuous[b]	0.5 (0.2, 1.3)	2.0 (1.0, 4.0)	0.3 (0.0, 3.7)	1.1 (0.6, 2.0)

CI, confidence interval; OR, odds ratio; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; ref., reference group.

[a]Adjusted for maternal smoking during pregnancy, maternal alcohol consumption, maternal age at pregnancy, gestational age at blood-sampling and child sex. [b] PFOA and PFOS were natural logarithm transformed. [c] Additionnaly adjusted for country.

Supplemental Material, Table S2. Adjusted mean differences (points)[a] for offspring DCDQ-score at school age in relation to lowest tertiles of pregnancy PFOS and PFOA concentrations. Complete-case results.

		Greenland (n=217)	Ukraine (n=444)	Poland (n=84)	All (n=745)[d]
DCDQ		**Difference (95 % CI)**	**Difference (95 % CI)**	**Difference (95 % CI)**	**Difference (95 % CI)**
PFOS	Medium[b]	-0.2 (-2.9, 2.6)	1.1 (-0.6, 2.8)	-0.6 (-6.6, 5.5)	-0.8 (-2.4, 0.8)
	High[b]	1.9 (-1.1, 5.0)	0.0 (-1.8, 1.7)	-2.4 (-8.3, 3.6)	-0.8 (-3.6, 1.9)
	Continuous[c]	1.8 (-0.8, 4.5)	-0.1 (-1.6, 1.5)	-2.5 (-9.4, 4.4)	-0.1 (-1.2, 1.5)
PFOA	Medium[b]	-1.8 (-4.7, 1.1)	-0.7 (-2.4, 1.0)	1.4 (-4.6, 7.3)	-0.6 (-2.1, 0.9)
	High[b]	-0.8 (-3.7, 2.1)	-1.1 (-2.9, 0.6)	-4.1 (-10.0, 1.8)	-0.7 (-2.6, 1.1)
	Continuous[c]	0.2 (-2.3, 2.7)	-0.4 (-1.8, 1.0)	3.2 (-9.1, 2.7)	-0.6 (-1.9, 0.7)

CI, confidence interval; DCDQ, developmental coordination disorder questionnaire; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

[a] Adjusted for: maternal smoking during pregnancy, maternal alcohol consumption at conception, maternal age at pregnancy, gestational age at blood-sampling and child sex. [b] Low exposure was reference group. [c] PFOA and PFOS were natural logarithm transformed. [d] Additionally adjusted for country.

Supplemental Material, Table S3. Adjusted mean differences (points)[a] for offspring SDQ-score at school age in relation to lowest tertiles of pregnancy PFOS and PFOA concentrations. Complete-case results.

Scale	Exposure		Greenland (n=468) Difference (95 % CI)	Ukraine (n=450) Difference (95 % CI)	Poland (n=86) Difference (95 % CI)	All (n=1,004)[d] Difference (95 % CI)
SDQ	**PFOS**	Medium[b]	1.5 (0.4, 2.6)	-1.0 (-2.1, 0.0)	-0.2 (-2.9, 2.5)	-0.2 (-1.1, 0.8)
		High[b]	1.2 (0.1, 2.3)	-1.1 (-2.1, 0.0)	1.9 (-0.7, 4.6)	1.1 (-0.2, 2.4)
		Continuous[c]	1.1 (0.1, 2.1)	-0.7 (-1.7, 0.2)	2.6 (-0.6, 5.7)	0.4 (-0.3, 1.0)
Hyperactivity		Medium[b]	0.5 (0.1, 1.0)	-0.1 (-0.5, 0.3)	1.0 (-0.6, 2.5)	0.1 (-0.3, 0.5)
		High[b]	0.4 (-0.1, 0.8)	-0.1 (-0.5, 0.4)	1.4 (-0.2, 2.9)	0.5 (0.0, 1.1)
		Continuous[c]	0.3 (-0.1, 0.8)	0.0 (-0.4, 0.3)	1.5 (-0.3, 3.2)	0.2 (0.0, 0.5)
SDQ	**PFOA**	Medium[b]	0.3 (-0.8, 1.5)	-0.7 (-1.7, 0.4)	0.7 (-2.0, 3.4)	0.2 (-0.6, 1.0)
		High[b]	0.5 (-0.6, 1.6)	-0.2 (-1.3, 0.8)	1.8 (-0.9, 4.5)	0.5 (-0.4, 1.4)
		Continuous[c]	0.4 (-0.6, 1.5)	-0.4 (-1.2, 0.5)	2.1 (-0.6, 4.7)	0.1 (-0.5, 0.8)
Hyperactivity		Medium[b]	0.4 (-0.1, 0.9)	-0.3 (-0.7, 0.1)	1.1 (-0.4, 2.6)	0.0 (-0.3, 0.4)
		High[b]	0.5 (0.1, 1.0)	-0.2 (-0.6, 0.3)	0.9 (-0.7, 2.4)	0.5 (0.1, 0.9)
		Continuous[c]	0.4 (0.0, 0.9)	-0.1 (-0.5, 0.2)	1.2 (-0.3, 2.7)	0.2 (-0.1, 0.5)

CI, confidence interval; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; SDQ, strength and difficulties questionnaire.
[a] Adjusted for: maternal smoking during pregnancy, maternal alcohol consumption at conception, maternal age at pregnancy, gestational age at blood-sampling and child sex. [b] Low exposure was reference group. [c] PFOA and PFOS were natural logarithm transformed. [d] Additionally adjusted for country.

Supplemental Material, Table S4. Adjusted OR (95 % CI)[a] for offspring SDQ-total and hyperactivity sub-scale. Complete-case results.

Scale	Greenland Adjusted OR (95 % CI) (n=468)	Ukraine Adjusted OR (95 % CI) (n=450)	Poland Adjusted OR (95 % CI) (n=53)	Pooled[c] Adjusted OR (95 % CI) (n=1,004)
SDQ-total				
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	2.4 (0.9, 6.2)	0.6 (0.2, 1.6)	-	1.1 (0.5, 2.6)
High	1.1 (0.3, 3.4)	0.5 (0.2, 1.5)	-	1.7 (0.5, 6.0)
Continuous[b]	1.1 (0.5, 2.8)	1.0 (0.4, 2.6)	-	1.3 (0.7, 2.3)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	2.6 (0.9, 7.4)	1.6 (0.6, 4.6)	-	1.9 (0.9, 4.2)
High	2.1 (0.7, 6.1)	1.6 (0.6, 4.8)	-	2.7 (1.1, 6.5)
Continuous[b]	2.7 (1.0, 7.3)	1.0 (0.4, 2.4)	2.8 (0.1, 53.3)	1.7 (0.9, 3.0)
Hyperactivity	**(n=468)**	**(n=450)**	**(n=84)**	**(n=1,004)**
PFOS				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	2.8 (0.7, 10.9)	1.1 (0.4, 3.2)	2.4 (0.5, 11.4)	1.2 (0.6, 2.6)
High	2.5 (0.6, 10.3)	1.4 (0.5, 4.0)	2.3 (0.5, 10.4)	2.1 (0.6, 8.2)
Continuous[b]	2.3 (0.8, 6.2)	1.3 (0.5, 3.5)	2.0 (0.4, 10.7)	1.9 (1.0, 3.6)
PFOA				
Low	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)	1.0 (ref.)
Medium	4.2 (0.8, 21.5)	0.8 (0.3, 2.3)	2.5 (0.5, 11.4)	0.8 (0.3, 1.8)
High	6.8 (1.4, 34.0)	0.8 (0.3, 2.3)	2.3 (0.5, 10.5)	2.9 (1.2, 7.0)
Continuous[b]	5.6 (1.5, 20.6)	0.8 (0.3, 1.9)	1.8 (0.5, 7.3)	1.6 (0.9, 2.9)

CI, confidence interval; OR, odds ratio; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; SDQ, strength and difficulties questionnaire.

[a] Adjusted for maternal smoking during pregnancy, maternal alcohol consumption at conception, child sex, maternal age at pregnancy and gestational age at blood-sampling. [b] PFOA and PFOS were natural logarithm transformed. [c] Additionnaly adjusted for country.

Appendix III

Paper III Body mass index in young school-age children in relation to organochlorines compounds in early life: a prospective study. Int J Obes (Lond), 2014, apr;

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PEDIATRIC ORIGINAL ARTICLE

Body mass index in young school-age children in relation to organochlorine compounds in early life: a prospective study

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OBJECTIVE: To investigate the association between maternal pregnancy and estimated postnatal serum concentrations of the organochlorines 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p′*-DDE) and body mass index (BMI) z-scores in 5- to 9-year-old children.

METHODS: Maternal sera from the INUENDO birth cohort (2002–2004) comprising mother–child pairs ($N = 1109$) from Greenland, Warsaw (Poland), and Kharkiv (Ukraine) were analysed for CB-153 and *p,p′*-DDE, using gas chromatography-mass-spectrometry, and were grouped into tertiles for statistical analyses. A toxicokinetic model was used to estimate the first 12 months cumulative exposure to the compounds. Associations between these compounds and child age- and sex-specific BMI z-scores were calculated at follow-up (2010–2012), using multiple linear regression analysis.

RESULTS: No clear associations between pregnancy CB-153 and *p,p′*-DDE and child BMI were observed (the pooled differences in BMI z-score (95% confidence interval) comparing 3rd tertile to 1st tertile were − 0.07 (−0.32 to 0.18) and − 0.10 (−0.30 to 0.10) kg m^{-2}, respectively). For postnatal CB-153 and *p,p′*-DDE and BMI, the overall differences in BMI z-score comparing 3rd tertile to 1st tertile were 0.12 (−0.15 to 0.39) and − 0.03 (−0.20 to 0.27) kg m^{-2}, respectively.

CONCLUSIONS: This follow-up study of Greenlandic, Polish and Ukrainian populations showed no clear association between pregnancy and postnatal exposure *to p,p′*-DDE and CB-153 and BMI at the age of 5–9 years.

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Keywords: body mass index; child; cohort study; dichlorodiphenyl dichloroethylene (DDE); polychlorinated biphenyls (PCBs); prenatal exposure delayed effects

INTRODUCTION

The man-made chemicals dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCBs) are ubiquitous in the environment, although the use of them has been banned (PCBs) or restricted (DDT). PCBs and DDT (and thereby the degradation product 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE)) bioaccumulate within the food chain and are measurable in human serum and milk around the world.[1–4] The compounds pass the placenta, causing exposure of the fetus to the chemicals.[1,5] This is of major concern, as exposure during prenatal development and early life may be related to a wide range of health outcomes in children, such as perturbation of the endocrine system, attention deficit hyperactivity disorder-like behaviour, decreased motor skills and cognitive impairments.[6–9]

Prenatal PCBs have been found to downregulate thyroid function in mice and rats[10] and to be associated with lower levels of free and total thyroxine (T4) in human cord and neonatal blood,[11] which could possibly lead to an increase in adipocyte lipid accumulation.[12] An upregulation of adipocytes in newborn mice has been reported in relation to prenatal exposure to the oestrogen diethylstilbestrol,[13] and as o,*p′*-DDT is known to have weak oestrogenic properties, mechanisms could be similar in *p,p′*-DDE. Also, the relation between other environmental compounds with supposed oestrogenic or antiandrogenic properties, such as bisphenol A and phthalates, and overweight has been discussed.[14,15] Few studies have examined the relation between pregnancy PCB and DDE exposures and body mass index (BMI) standardized by age and sex, and findings are inconsistent.[16–20]

To our knowledge, only one study examined postnatal exposure to these compounds in relation to BMI z-scores or related metrics and found no association.[16] Measurement of the child's postnatal exposure is rarely performed, and therefore a toxicokinetic model, estimating the postnatal levels of persistent organic pollutants was developed by Verner *et al*.[21] Using this model, we were able to estimate the child's serum level of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (*p,p′*-DDE) and thereby take the postnatal exposure of the compounds into account. Thus, the aim of this study was to investigate the associations between maternal pregnancy CB-153 and *p,p′*-DDE serum concentrations and the estimated accumulated concentrations of the compounds during the first 12 months of life in the offspring, and age- and sex-standardized BMI z-scores

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when the children reached young school-age. We hypothesized that higher exposure to CB-153 and *p,p′*-DDE *in utero* and early life would be associated with higher BMI.

MATERIALS AND METHODS

Study population

A total of 1441 pregnant women from Greenland, Warsaw (Poland) and Kharkiv (Ukraine) were enroled from antenatal health-care clinics and provided a blood sample in the INUENDO birth cohort between May 2002 and February 2004. At baseline, 567 (85%) of 665 eligible women participated in Greenland; 258 (37%) of 690 participated in Poland and 612 (25%) of 2478 eligible women participated in the study in Ukraine. Further details on the baseline study population are described elsewhere.[22] The follow-up of the children in the cohort was conducted between January 2010 and May 2012, when the children were between 5 and 9 years old. Parents were interviewed using an interview-based questionnaire with regard to child physical development and lifestyle. All participating parents signed informed consent, and the local ethical committees approved the study.

Data collection

The follow-up interviews were primarily conducted face-to-face at the participants' residence or at the local hospitals. A medical doctor, assisted by local health workers, was the main interviewer in Greenland. In Poland, interviews were conducted at local meeting points or at the participant's residence by a team of four interviewers. In Ukraine, all interviews were conducted by a team of 59 paediatricians at eight paediatric polyclinics. Telephone interviews were performed when families lived in remote areas of Greenland ($n=130$) or had moved to Denmark ($n=34$). Also, in Greenland, a proportion of the questionnaires was filled in by the parents without an interview.

Assessment of prenatal CB-153 and *p,p′*-DDE exposure

At baseline, the pregnant women had a blood sample drawn in order to identify the fetal exposures through placental diffusion. In Greenland, the blood samples were drawn when the women were (median (10–90 percentile)) in gestational week 24 (13–37); in Poland, in gestational week 33 (27–37); and in Ukraine, in gestational week 23 (9–40). Ten millilitre cubital vein blood was drawn into vacuum tubes for serum collection without additives (Becton Dickinson, Meylan, France). All centres used the same ethanol-rinsed sampling device obtained from Lund University, Sweden. Sera were stored at −80 °C until analyses, and all chemical analyses were performed at the Department of Occupational and Environmental Medicine at Lund University Hospital. The sera were analysed for CB-153 and *p,p′*-DDE and used as bio-markers of the prenatal exposure to PCBs and DDT/DDE, respectively. The sera were analysed by gas chromatography-mass-spectrometry following solid phase extraction.[23] Samples were analysed twice on different days and the mean concentration of these two determinations was used. The detection limits were 0.05 ng ml^{-1} for CB-153 and 0.1 ng ml^{-1} for *p,p′*-DDE. For CB-153, 85 samples were below the detection limit (LOD) and for *p,p′*-DDE, 10 samples were below the LOD. When concentrations were below the LOD, they were set to half the LOD based on fresh weight concentrations for all subsequent analyses. In-house prepared quality control samples were included in all analysed series. The relative standard deviations (s.d.) were calculated from samples analysed in duplicate at different days. These were used to estimate the imprecision. For this, the results of the analysed sera were divided into three groups: low, medium and high level, and the s.d. and the mean concentration for each group were calculated.[24] These were 18% in the range 0.05–0.8 ng ml^{-1} ($n=990$), 10% in the range 0.2–0.8 ng ml^{-1} ($n=990$) and 10% in the range 0.7–19 ng ml^{-1} ($n=990$) for CB-153 and 11% in the range 0.1–2 ng ml^{-1} ($n=1\,058$), 8% in the range 2–5 ng ml^{-1} ($n=1\,058$) and 7% in the range 3–37 ng ml^{-1} ($n=1\,058$) for *p,p′*-DDE. CB-153 and *p,p′*-DDE levels were adjusted for serum concentrations of triglycerides and cholesterol, which were determined by enzymatic methods and were expressed as ng g^{-1} lipids. The inter-assay coefficients of variation for cholesterol and triglycerides were 1.5–2.0%. Further details are described elsewhere.[23]

Estimation of postnatal CB-153 and *p,p′*-DDE exposure

To estimate the postnatal cumulative contribution of the compounds for the first 12 months after birth, a toxicokinetic model developed by Verner *et al.* was used.[21] The model was validated in a Canadian Inuit birth cohort and a Slovakian birth cohort, leading us to assume validity in our populations. The model inputs were: age of the mother at delivery, maternal pre-pregnancy weight, duration of exclusive breast feeding, duration of partial breastfeeding, gestational age, child sex, birth weight, child's weight at follow-up and age at measurement, and up to two previous recorded weight measurements, maternal levels of CB-153 and *p,p′*-DDE during pregnancy (placental diffusion), gestational age at blood sampling and half-life of the compounds.[21] The estimate of the postnatal exposure used in the regression models was the area under the curve, which is equivalent to cumulative accumulated CB-153 and *p,p′*-DDE levels during the first 12 months after birth. We used this exposure metric, as the contribution of persistent organic pollutants via breastfeeding is the most prevalent and important source of a child's postnatal exposure and we had breastfeeding data up until the age of 12 months.[21] As the toxicokinetic model used total and exclusive duration of breastfeeding as predictors, only children with this information were included in these analyses ($n=1047$). The toxicokinetic model was performed using acslX software (Aegis Technologies Group, Inc., Huntsville, AL, USA).

Assessment of anthropometric measures

The child's height was measured with the child standing barefoot against a wall, marking the top of the head and measuring the height to the nearest centimetre by use of ordinary measure tape. The child's weight was measured to the nearest 0.1 kg by a weighing scale available at the family's home or at the clinics. All measurements were performed by the interviewer except for those who were telephone-interviewed. Child BMI was calculated from weight (in kilograms)/ height × height (in metres). BMI was expressed as z-score representing the deviation in s.d. units from the mean of a standard normal distribution of BMI specific to age (1 month intervals) and sex. Positive z-values are above the 50 percentile and negative z-values are below the 50 percentile. The standards were based on the World Health Organization (WHO) Growth Standards 2007, which are applicable regardless of ethnicity or country of origin,[25] as population-specific growth data were not available for all populations.

Covariates

Variables that according to the literature might influence child growth were obtained from questionnaires. These variables were: maternal pre-pregnancy BMI (continuous), paternal BMI (continuous), maternal smoking (serum cotinine in pregnancy $\leqslant 10/>10$ ng ml^{-1}), maternal pre-pregnancy alcohol intake (<7, $\geqslant 7$ drinks per week), maternal educational level (left school at or before the age of 15 years, at the age of 16–17 years, at or above the age of 18 years), parity (1, 2–3, 4+ child births), maternal age at baseline (continuous), total breastfeeding duration (0, <6, 6–12, >12 months), child physical activity level (<2.75 h a day, $\geqslant 2.75$ h per day) and child diet (predominantly healthy versus predominately unhealthy; derived as sugary drinks or deserts <6–7 versus $\geqslant 6$–7 times a week).[26–32] Gestational age at blood sampling in weeks (continuous) was included as it was correlated with the exposure levels.

Statistical analyses

Missing information. The number of missing values on height, weight and covariates ranged from 0 to 27%. As complete case analysis may lead to selection bias, we addressed the missing information problem, using chained multiple imputation allowing us to include participants with incomplete data in the statistical analyses.[33] Assuming the missing information to be missing at random (systematic differences between observed and missing values can be explained by differences in observed data), this approach will result in more precise and unbiased estimates.[33,34]

Briefly, multiple different imputed data sets ($m>1$) are created, and each missing value is replaced with a set of random plausible values based on known subject characteristics and other predictors in the complete data set, incorporating the appropriate variability across the m data sets. The new m complete data sets are analysed, producing a single set of results accounting for the variability of the missing data.[33]

In a combined imputation across country, we generated 100 imputed data sets based on the following predictors: CB-153, *p,p′*-DDE, maternal height, maternal pre-pregnancy weight, paternal height, paternal weight, maternal educational level, maternal age at baseline, maternal smoking status during pregnancy, maternal pre-pregnancy alcohol consumption, parity, duration of breastfeeding, preterm birth, child sex, gestational age at blood drawing, child physical activity level, child diet, child age at

interview, birth weight, birth length, z-score of weight at follow-up and z-score of height at follow-up.

Data analysis

Study subjects were divided into population-specific and total population tertiles of exposure. A non-response analysis was performed to check for inconsistencies between responders and non-responders. Spearman's rank correlation was used to assess the correlation between maternal pregnancy levels of CB-153 and *p,p'*-DDE as well as the correlation between maternal pregnancy levels and postnatal levels of the two compounds. Crude associations between the exposures and age-standardized z-scores of BMI at follow-up were examined with lowest exposure tertile as the reference category, using univariate linear regression. Adjusted associations of pre- and postnatal exposure to CB-153 and *p,p'*-DDE on BMI z-score were examined, using multiple linear regression with lowest exposure tertile as reference category. We chose *a priori* to adjust for covariates, which were previously demonstrated to be associated with child growth and the exposures, as described above. In addition, odds ratios for the associations between organochlorines and overweight were calculated, using logistic regression. Overweight (defined as >+1 s.d. from the mean BMI of the WHO Growth Standards) was compared with normal weight (> −2 s.d. to ⩽1 s.d. from the mean of the WHO Growth Reference). Additional analyses were performed to check the robustness of the results: (a) stratified by sex, (b) birth weight and preterm birth. These were included in the model but were not included in the main analysis, as they may well be mediators of the relation between prenatal and postnatal exposure to CB-153 and *p,p'*-DDE and growth, (c) excluding maternal BMI from the model, as this also may be a mediator of the associations under study, (d) prenatal and postnatal exposures kept in the same model, (e) only adjusting for maternal age and maternal smoking and, finally, (f) sensitivity analyses using different chained imputation models and by creating a varying number of data sets (20 and 150) were performed to check the robustness of the imputation model.

All analyses were performed stratified by population as well as pooled (adjusted for population). A *P*-value less than 0.05 was considered statistically significant. The Stata statistical package (version 12.1, StataCorp, College Station, TX, USA) was used for all analyses.

RESULTS

At baseline, 1441 pregnant women participated in an interview and donated a blood sample. A total of 1117 (78%) women and their children were followed up when the median age of the children was 8 years in Greenland and Poland and 7 years in Ukraine. Twins ($n=8$) were excluded, leaving a total study population of 1109 at follow-up. The study population was distributed across Greenland (525 (47.3% of the study population)), Poland (92 (8.3%)) and Ukraine (492 children (44.4%)). The participation rates at follow-up were 89% in Greenland, 36% in Poland and 80% in Ukraine. The Spearman's correlation coefficient between maternal pregnancy levels of CB-153 and *p,p'*-DDE was 0.92 in Greenland, 0.47 in Poland and 0.46 in Ukraine. In Greenland, the Spearman's correlation coefficient between maternal pregnancy levels and estimated postnatal levels was 0.81 and 0.82 for CB-153 and *p,p'*-DDE, respectively. In Poland, the equivalents were 0.78 and 0.68, and in Ukraine, the correlations were 0.56 and 0.49, respectively. The non-response analysis showed only modest differences between responders and non-responders at follow-up concerning exposure levels and no difference in maternal pre-pregnancy BMI and paternal BMI (Supplementary Table 1). Characteristics of the study population are presented in Table 1. The median BMI (10–90 percentile) of the Greenlandic children was 17 kg m^{-2} (15–21) compared with 15 (13–19) and 16 (14–18) in Poland and Ukraine, respectively. In Greenland, 27% of the children were overweight or obese according to WHO standards, whereas the equivalents in Poland and Ukraine were 19% and 18%, respectively. In comparison with women from Greenland, women from Ukraine were younger, more often primiparous, less often smokers, more often breastfed for >12 months, had a lower pre-pregnancy BMI and their children were younger at follow-up. Women from Poland were older at enrolment, had lower pre-pregnancy BMI, were more often primiparous, had higher educational level and were less often smokers in comparison with the Greenlandic population. In Greenland, maternal median (10–90 percentile) serum CB-153 was 107 (30–369) ng g^{-1} lipids, which was considerably higher than in Poland and Ukraine. Maternal *p,p'*-DDE concentrations were highest in Ukraine, with maternal median (10–90 percentile) serum *p,p'*-DDE at 639 (329–1303) ng g^{-1} lipids. The estimated postnatal CB-153 exposure was highest in Greenland, whereas estimated postnatal *p,p'*-DDE exposure was highest in Ukraine, closely followed by Poland. The difference in prenatal and estimated postnatal exposure profiles are further presented in Table 2, describing the exposure tertiles stratified by country. The pattern of missing data in relation to country is presented in Table 3.

Table 4 presents crude and adjusted results of child BMI z-score in relation to prenatal exposure to the compounds based on the imputation-based analyses. Overall, there was no crude or adjusted dose–response relation between prenatal CB-153 and *p,p'*-DDE exposure and BMI z-scores. In Ukraine, crude and adjusted associations of maternal *p,p'*-DDE concentrations in the third tertile (>791 ng g^{-1} lipids) compared with the first tertile (<488 ng g^{-1} lipids) were inversely associated with child BMI z-scores (adjusted $\beta=-0.30$ (95% confidence interval (CI): −0.55 to −0.04)). Results from the complete case analyses were similar (Supplementary Table 2).

The results on the imputation-based associations between estimated first 12 months postnatal exposures and child BMI z-scores are presented in Table 5. Neither crude nor adjusted analyses indicated associations apart from a statistically significant association in Ukraine, where adjusted medium *p,p'*-DDE concentrations compared with low *p,p'*-DDE concentrations were associated with lower BMI z-scores (adjusted $\beta=-0.35$ (95% CI: −0.67 to −0.03)). Results from the complete case analyses were similar (Supplementary Table 3). ORs of the association between maternal and estimated postnatal organochlorine concentrations and overweight were consistent with the results of the linear regression (data not shown).

Several sensitivity analyses showed generally similar results as seen in Tables 4 and 5 (data not shown): (a) no difference was observed when the results were stratified by sex, (b) including preterm birth and birth weight in the model lead to similar results, (c) removing maternal BMI from the model had no impact on results, (d) having both prenatal and postnatal exposure in the model gave similar results, except for a nonsignificant result for the medium exposed group of postnatal *p,p'*-DDE in Ukraine and a statistically significant *P* for trend in Poland on postnatal CB-153 and DDE and BMI z-score (Supplementary Table 4), (e) analysing smaller models with only maternal age and smoking as covariates made the Ukrainian result on high prenatal *p,p'*-DDE and BMI z-score significant ($\beta=-0.35$ (95% CI: −0.60 to −0.10)) but did not change the results otherwise (Supplementary Table 5) and finally, (f) the four sensitivity analyses on the imputation model proved the imputation model robust, as the results did not materially change.

DISCUSSION

Overall, this study suggests no strong association between prenatal and early-life *p,p'*-DDE and CB-153 exposures and BMI z-scores at 5–9 years of age.

Our hypothesis was that higher early-life exposure *to* DDE and PCBs would be associated with higher BMI. However, the results did not support this. Our results are in line with a recent study within the US Collaborative Perinatal Study, which reported no associations between high levels of prenatal *p,p'*-DDE or total-PCB exposures and overweight or obesity at 7 years.[35]

Table 1. Characteristics of mothers, fathers and their children, INUENDO cohort (born 2002–2004)

Characteristics	*Greenland* (n = *525*)	*Poland* (n = *92*)	*Ukraine* (n = *492*)
Maternal and paternal characteristics			
Maternal age at pregnancy, years (median (10–90 percentile))	26 (20 to 36)	29 (26 to 33)	24 (19 to 32)
Maternal pre-pregnancy BMI, kg m^{-2} (median (10–90 percentile))	24 (20 to 30)	21 (19 to 24)	21 (18 to 26)
Paternal BMI, kg m^{-2} (median (10–90 percentile))	26 (21 to 31)	25 (22 to 30)	24 (20 to 28)
Parity, no. (%)			
1	166 (33)	87 (95)	401 (82)
2 or 3	247 (49)	5 (5)	91 (18)
4 or more	88 (18)	0 (0)	0 (0)
Maternal smoking during pregnancy, no. (%)			
Yes (serum cotinine >10 ng ml^{-1})	295 (56)	2 (2)	75 (15)
No (serum cotinine ⩽10 ng ml^{-1})	230 (44)	88 (98)	412 (85)
Maternal alcohol consumption when trying to conceive, no. (%)			
⩽7 Drinks per week	466 (89)	87 (95)	495 (100)
>7 Drinks per week	60 (11)	5 (5)	0 (0)
Maternal educational level, left school at the age (years), no. (%)			
⩽15	44 (9)	0 (0)	25 (6)
16–17	169 (37)	0 (0)	115 (26)
⩾18	251 (54)	82 (100)	293 (68)
Child characteristic			
Sex, no. (%)			
Male	284 (54)	54 (59)	263 (53)
Female	239 (45)	38 (41)	229 (47)
Total breastfeeding duration (months), no. (%)			
0	18 (4)	2 (2)	42 (9)
<6	120 (25)	19 (21)	164 (33)
6–12	124 (26)	37 (40)	175 (36)
>12	213 (45)	34 (37)	108 (22)
Gestational age (weeks), no. (%)			
⩾37	498 (95)	68 (94)	480 (98)
<37	25 (5)	4 (6)	9 (2)
Gestational age at blood sample, weeks (median (10–90 percentile))	25 (13 to 37)	33 (27 to 37)	23 (9 to 40)
Birth weight, g (median (10–90 percentile))	3593 (2835 to 4350)	3445 (2880 to 4000)	3290 (2800 to 3800)
Age at follow-up, years (median (10–90 percentile))	8 (7 to 9)	8 (7 to 8)	7 (7 to 8)
Exposures			
Maternal CB-153, ng g^{-1} lipids (median (10–90 percentile))	107 (30 to 369)	11 (3 to 24)	27 (8 to 54)
Maternal *p,p′*-DDE, ng g^{-1} lipids (median (10–90 percentile))	300 (75 to 954)	385 (160 to 718)	639 (329 to 1303)
Estimated postnatal CB-153, ng g^{-1} lipids (median (10–90 percentile))	2 647 (558 to 9618)	297 (83 to 742)	516 (104 to 1419)
Estimated postnatal *p,p′*-DDE,ng g^{-1} lipids (median (10–90 percentile))	7 075 (1282 to 23 133)	11 627 (4017 to 26 435)	12 535 (2914 to 34 724)
Anthropometric measures at follow-up			
Weight, kg (median (10–90 percentile))	29 (24 to 38)	26 (21 to 35)	24 (20 to 30)
Height, cm (median (10–90 percentile))	131 (122 to 138)	128 (122 to 138)	124 (117 to 131)
BMI, kg m^{-2} (median (10–90 percentile))	17 (15 to 21)	15 (13 to 19)	16 (14 to 18)
BMI z-scores, kg m^{-2} (median (10–90 percentile))	0.7 (−0.4 to 2.2)	−0.1 (−1.6 to 1.9)	0.2 (−1.1 to 1.5)
Overweight (+1 s.d.), no. (%)	93 (18)	12 (13)	66 (13)
Obese (+2 s.d.), no (%)	46 (9)	5 (5)	28 (6)
Underweight (< −2 s.d.), no. (%)	0 (0)	4 (4)	13 (3)

Abbreviations: BMI, body mass index; CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; *p,p′*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene.

We observed no association between prenatal CB-153 exposure and BMI z-score, whereas a study reported higher BMI s.d. scores in relation to cord blood total-PCBs at 1–3 years.[17] The difference in results could be caused by duration of follow-up, as the children in this cohort are somewhat older and potential associations earlier in life have not been investigated. Also, the observed association could be due to congeners other than CB-153. Another well-performed but rather small study found an association between high prenatal total-PCB exposures and risk of overweight in girls at 6.5 years compared with low exposures.[16] This was also observed in a cohort study of 5-year olds from the 1960s with high PCB exposure levels,[36] but these results are inconsistent with our study results. Also, in contrast to our study, a small Spanish study reported an association between medium prenatal DDE exposure and increased risk of overweight compared with low exposure,[16] whereas another study with rather high prenatal DDE exposure levels observed no independent association between prenatal DDE exposure and BMI or risk of overweight.[37] Our null finding for postnatal exposure to CB-153 supports a previous null finding in a small but well-performed Dutch cohort study examining weight and height as outcomes.[38] However, one small informative study has reported increased risk of overweight in relation to postnatal PCB and DDE levels.[16] Results of all the above discussed follow-up studies have reached inconsistent conclusions, which could be due to different exposure levels, measured congeners, choice of covariates in the model and age at the follow-up.

Table 2. Description of the prenatal and estimated postnatal CB-153 and *p,p'*-DDE exposure tertiles of children, INUENDO cohort (born 2002–2004)

Exposure [a]	*Tertile*	*Greenland*	*Poland*	*Ukraine*
Maternal CB-153	Low	2.6–73.0 ($n=175$)	2.5–7.3 ($n=31$)	2.4–20.4 ($n=165$)
	Medium	73.1–167.1 ($n=175$)	7.4–13.3 ($n=31$)	20.5–34.5 ($n=164$)
	High	167.2–2223.4 ($n=175$)	13.4–74.8 ($n=30$)	34.6–533.4 ($n=163$)
Maternal *p,p'*-DDE	Low	5.3–205.2 ($n=175$)	88.1–302.8 ($n=31$)	147.1–487.8 ($n=165$)
	Medium	205.3–439.3 ($n=175$)	302.9–471.2 ($n=31$)	487.9–790.4 ($n=164$)
	High	439.4–3122.0 ($n=175$)	471.3–1750.1 ($n=30$)	790.5–4835.6 ($n=163$)
Est. postnatal CB-153[b]	Low	21.6–527.0 ($n=164$)	7.5–143.4 ($n=24$)	11.1–312.4 ($n=162$)
	Medium	527.1–1627.7 ($n=164$)	143.5–432.3 ($n=24$)	312.5–732.6 ($n=162$)
	High	1627.8–72 266.5 ($n=163$)	432.4–1225.2 ($n=23$)	732.7–9108.2 ($n=161$)
Est. postnatal *p,p'*-DDE[b]	Low	169.2–4147.2 ($n=164$)	929.1–7653.3 ($n=24$)	429.5–8078.2 ($n=162$)
	Medium	4147.3–11 845.0 ($n=164$)	7653.4–15 130.7 ($n=24$)	8078.3–18 005.5 ($n=162$)
	High	11 845.1–111 728.9 ($n=163$)	15 130.8–51 498.9 ($n=23$)	18 005.6–165 654.9 ($n=161$)

Abbreviations: CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; Est., estimated; *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene. [a]Exposure range of CB-153 and *p,p'*-DDE is measured in ng g^{-1} lipids. [b]The estimated postnatal exposures are cumulative exposures to 12 months.

Table 3. Number (%) of missing values according to country, INUENDO cohort (born 2002–2004)

Variables	*Greenland* (n = *525*), n *(%)*	*Poland* (n = *92*), n *(%)*	*Ukraine* (n = *492*), n *(%)*
Exposures			
Prenatal exposures[a]	0 (0)	0 (0)	0 (0)
Estimated postnatal exposures[b]	0 (0)	0 (0)	0 (0)
Anthropometric measures			
Weight at follow-up	92 (18)	3 (3)	0 (0)
Height at follow-up	45 (9)	2 (2)	0 (0)
BMI at follow-up	97 (19)	3 (3)	0 (0)
BMI z-score	141 (27)	3 (3)	8 (2)
Maternal, paternal and child characteristics			
Maternal age at baseline	38 (7)	0 (0)	25 (5)
Pre-pregnancy BMI	2 (0)	2 (2)	8 (2)
Paternal BMI	132 (25)	1 (1)	16 (3)
Parity	24 (5)	0 (0)	0 (0)
Smoking during pregnancy	0 (0)	2 (2)	5 (1)
Alcohol before pregnancy[c]	0 (0)	0 (0)	0 (0)
Maternal educational level	62 (12)	10 (11)	59 (12)
Child sex	3 (1)	0 (0)	3 (1)
Total breastfeeding information	53 (10)	0 (0)	3 (1)
Gestational age at birth	0 (0)	0 (0)	0 (0)
Child age at follow-up	54 (10)	0 (0)	5 (1)
Gestational age at blood sample	16 (3)	3 (3)	32 (7)
Birth weight	6 (1)	20 (22)	1 (1)

Abbreviation: BMI; body mass index. [a]$N=1109$. [b]$N=1047$. [c]Missing answer was coded as no drinks.

The results of this study should be interpreted in light of some study limitations. In the main analyses, we chose to use the WHO Growth Standards for BMI z-scores as internally derived standardized BMI z-scores might have caused unreliable coefficients because of the small sample in Poland. As the WHO Growth Standards are generally representative across ethnicities,[39] we deemed this approach preferable, and sensitivity analyses proved the results to be robust.

We used different scales for weighing children at the follow-up examination. However, any misclassification most likely did not differ between exposure groups and therefore was non-differential. Further, we had no measure of the children's postnatal organochlorines exposure and used a toxicokinetic model to estimate the accumulated concentrations during the first 12 months of life. The toxicokinetic model has proven robust in a validation study in similar settings, and a study suggests that the method is superior to the often used method of duration of breastfeeding multiplied by the prenatal exposure.[21] As exclusive breastfeeding has been reported as having the largest relative influence on estimates of postnatal exposure,[21] we chose the first 12 months of life, where exclusive breastfeeding is present, as the postnatal period. An exact measure of exposure of each child would, however, have been desirable. Blood samples were collected throughout pregnancy and as organochlorine concentrations tend to decrease during pregnancy, there is a risk of exposure misclassification. However, we addressed this issue by adjusting for gestational age at blood sampling, although we recognize that this has not completely eliminated misclassification. Also, analysis of only complete cases in a follow-up study with missing data could cause selection bias and to overcome this challenge, we performed multiple imputation analyses. The considerable loss to follow-up in Poland (follow-up participation rate 36%) could introduce selection bias. However, non-response analysis showed only modest difference according to exposure

Table 4. The association between pregnancy CB-153 and *p,p'*-DDE concentrations and differences in child BMI z-scores at 5–9 years, INUENDO cohort (born 2002–2004)

Z-scores BMI	n	*Crude model*[a]		P *trend*[c]	*Adjusted model*[b]		P *trend*[c]
		Medium β *(95% CI) kg m*$^{-2}$	*High* β *(95% CI) kg m*$^{-2}$		*Medium* β *(95% CI) kg m*$^{-2}$	*High* β *(95% CI) kg m*$^{-2}$	
Greenland							
CB-153	525	−0.09 (−0.35 to 0.17)	−0.10 (−0.36 to 0.16)	0.79	−0.13 (−0.38 to 0.13)	−0.08 (−0.35 to 0.18)	0.93
p,p'-DDE	525	−0.08 (−0.34 to 0.18)	0.02 (−0.25 to 0.28)	0.75	−0.12 (−0.37 to 0.14)	−0.06 (−0.33 to 0.22)	0.60
Poland							
CB-153	92	−0.14 (−0.79 to 0.51)	−0.21 (−0.85 to 0.43)	0.46	0.00 (−0.67 to 0.66)	−0.15 (−0.85 to 0.56)	0.61
p,p'-DDE	92	0.23 (−0.41 to 0.88)	0.14 (−0.51 to 0.79)	0.49	0.35 (−0.33 to 1.03)	0.12 (−0.56 to 0.80)	0.40
Ukraine							
CB-153	492	0.07 (−0.17 to 0.31)	−0.02 (−0.26 to 0.22)	0.88	0.14 (−0.11 to 0.39)	0.06 (−0.20 to 0.32)	0.45
p,p'-DDE	492	−0.11 (−0.35 to 0.13)	−0.38 (−0.62 to −0.14)	0.01	−0.09 (−0.34 to 0.15)	−0.30 (−0.55 to −0.04)	0.11
Pooled							
CB-153	1109	−0.06 (−0.24 to 0.12)	−0.11 (−0.36 to 0.14)	0.87	−0.02 (−0.20 to 0.16)	−0.07 (−0.32 to 0.18)	0.73
p,p'-DDE	1109	0.06 (−0.13 to 0.25)	−0.12 (−0.32 to 0.08)	0.27	0.03 (−0.16 to 0.21)	−0.10 (−0.30 to 0.10)	0.41

Abbreviations: BMI, body mass index; CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CI, confidence interval; *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene. *β* is the difference in BMI z-score, compared with the lowest tertile of exposure. [a]The pooled estimates are adjusted for country. [b]Adjusted for maternal tobacco smoking during pregnancy, maternal age at baseline, maternal pre-pregnancy BMI, paternal BMI, maternal alcohol intake before pregnancy, parity, maternal education, duration of breastfeeding, gestational age at blood sampling, child physical activity level and child diet. [c]Exposures are natural logarithm transformed in test for trend using continuous linear regression analysis

Table 5. The association between estimated postnatal CB-153 and *p,p'*-DDE concentrations during the first 12 months of life[d] and differences in child BMI z-scores at 5–9 years, INUENDO cohort (born 2002–2004)

Z-scores BMI	n	*Crude model*[a]		P *trend*[c]	*Adjusted model*[a,b]		P *trend*[c]
		Medium β *(95% CI) kg m*$^{-2}$	*High* β *(95% CI) kg m*$^{-2}$		*Medium* β *(95% CI) kg m*$^{-2}$	*High* β *(95% CI) kg m*$^{-2}$	
Greenland							
CB-153	491	−0.02 (−0.30 to 0.26)	0.03 (−0.25 to 0.31)	0.78	0.01 (−0.27 to 0.29)	0.00 (−0.32 to 0.31)	0.82
p,p'-DDE	491	0.03 (−0.25 to 0.31)	0.03 (−0.25 to 0.31)	0.90	0.03 (−0.26 to 0.31)	−0.05 (−0.36 to 0.26)	0.57
Poland							
CB-153	71	−0.50 (−1.25 to 0.26)	0.20 (−0.56 to 0.97)	0.89	−0.41 (−1.35 to 0.52)	0.04 (−0.94 to 1.03)	0.71
p,p'-DDE	71	0.60 (−0.15 to 0.06)	−0.04 (−0.29 to 0.20)	0.20	0.47 (−0.43 to 1.38)	0.31 (−0.66 to 1.28)	0.53
Ukraine							
CB-153	485	−0.04 (−0.28 to 0.21)	0.21 (−0.04 to 0.45)	0.28	−0.04 (−0.32 to 0.25)	0.29 (−0.04 to 0.63)	0.57
p,p'-DDE	485	−0.19 (−0.43 to 0.06)	−0.04 (−0.29 to 0.20)	0.90	−0.35 (−0.67 to −0.03)	−0.24 (−0.61 to 0.13)	0.10
Pooled							
CB-153	1047	0.09 (−0.09 to 0.27)	0.13 (−0.10 to 0.37)	0.65	0.07 (−0.13 to 0.28)	0.12 (−0.15 to 0.39)	0.89
p,p'-DDE	1047	0.00 (−0.19 to 0.18)	0.09 (−0.10 to 0.27)	0.99	−0.05 (−0.26 to 0.15)	0.03 (−0.20 to 0.27)	0.34

Abbreviations: BMI, body mass index; CB-153, 2,2′,4,4′,5,5′-hexachlorobiphenyl; CI, confidence interval; *p,p'*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene. *β* is the difference in BMI z-score, compared with the lowest tertile of exposure. [a]The pooled estimates are adjusted for country. [b]Adjusted for maternal tobacco smoking during pregnancy, maternal age at baseline, maternal pre-pregnancy BMI, paternal BMI, maternal alcohol intake before pregnancy, parity, maternal education, duration of breastfeeding, gestational age at blood sampling, child physical activity level and child diet. [c]Exposures are natural logarithm transformed in test for trend using continuous linear regression analysis. [d]Estimated in a toxicokinetic model. Please see text for specifications.

levels, and no difference on maternal pre-pregnacy BMI and paternal BMI between responders and non-responders indicating no high risk of selection bias by the loss to follow-up.

Exposure levels vary according to population. Still, the results are somewhat consistent across population apart from the inverse association observed in Ukraine, which could be spurious. The lack of associations, however, may be a result of generally low exposure levels as both exposures are receding.

This study has some important strengths. First, the follow-up was continued for up till 9 years, enabling analyses of associations for a considerable period of time. Second, the sample size in Greenland and Ukraine made it possible to model the associations, including adjustment for a number of covariates, with substantial power in these two countries and in the pooled analyses. Third, the varying exposure levels in the three countries enhanced our chance to observe an association, if any. Finally, we used a standardized measure of BMI, which facilitates comparison across studies with populations of different ages. Our results were generally robust, as several sensitivity analyses showed similar results with few exceptions.

In conclusion, in this prospective birth cohort study of Greenlandic, Polish and Ukrainian populations, we found no overall association between pre- or postnatal exposure *to p,p'*-DDE and CB-153 and BMI at the age of 5–9 years.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Table S1. Characteristics of participants and those lost to follow-up.

	Greenland		Poland		Ukraine	
	Lost to follow-up n=42	Followed n=525	Lost to follow-up n=166	Followed n=92	Lost to follow-up n=120	Followed n=492
CB-153, ng g^{-1} lipids [Median (10-90 percentile)]	126 (39 to 309)	107 (30 to 369)	11 (3 to 21)	11 (3 to 24)	28 (11 to 68)	27 (11 to 54)
p,p′-DDE, ng g^{-1} lipids [Median (10-90 percentile)]	374 (108 to 663)	299 (75 to 954)	354 (179 to 785)	385 (160 to 718)	791 (355 to 1639)	639 (329 to 1303)
Maternal pre-pregnancy BMI [Mean (SD)]	24 (5)	24 (5)	22 (4)	22 (3)	22 (3)	22 (3)
Paternal BMI [Mean (SD)]	26 (3)	26 (4)	26 (3)	26 (3)	24 (3)	24 (3)

Abbreviations: CB-153, 2,2',4,4',5,5'-hexachlorobiphenyl; *p,p′*-DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene

Supplementary Table S2. The association between *p,p′*-DDE and CB-153 concentrations during pregnancy and differences in child body mass index z-scores at 5 to 9 years, INUENDO cohort (born 2002-2004). Complete-case analysis

		Crude model [1]				Adjusted model [2]		
Z-score BMI	n	Medium β (95 % CI) kg m^{-2}	High β (95 % CI) kg m^{-2}	p trend [3]	n	Medium β (95 % CI) kg m^{-2}	High β (95 % CI) kg m^{-2}	p trend [3]
Greenland								
CB-153	384	-0.13 (-0.39 to 0.14)	-0.06 (-0.32 to 0.20)	0.86	175	-0.05 (-0.44 to 0.35)	-0.09 (-0.52 to 0.33)	0.92
p,p′-DDE	384	-0.11 (-0.38 to 0.15)	0.04 (-0.22 to 0.31)	0.86	175	0.08 (-0.31 to 0.48)	-0.09 (-0.52 to 0.34)	0.39
Poland								
CB-153	89	-0.18 (-0.83 to 0.48)	-0.21 (-0.85 to 0.44)	0.44	73	0.01 (-0.65 to 0.67)	-0.01 (-0.69 to 0.67)	0.81
p,p′-DDE	89	0.23 (-0.42 to 0.88)	0.13 (-0.52 to 0.80)	0.50	73	0.38 (-0.29 to 1.04)	0.18 (-0.48 to 0.84)	0.59
Ukraine								
CB-153	484	0.07 (-0.17 to 0.31)	-0.02 (-0.26 to 0.22)	0.85	371	0.07 (-0.21 to 0.35)	-0.08 (-0.37 to 0.21)	0.81
p,p′-DDE	484	-0.12 (-0.36 to 0.12)	-0.39 (-0.63 to -0.15)	0.01	371	-0.08 (-0.36 to 0.19)	-0.34 (-0.62 to -0.06)	0.06
Pooled								
CB-153	957	-0.06 (-0.24 to 0.12)	-0.11 (-0.35 to 0.14)	0.63	619	-0.10 (-0.31 to 0.12)	-0.02 (-0.36 to 0.32)	0.66
p,p′-DDE	957	0.09 (-0.10 to 0.28)	-0.11 (-0.31 to 0.08)	0.24	619	0.13 (-0.11 to 0.37)	-0.11 (-0.37 to 0.14)	0.17

[1] The pooled estimates are adjusted for country

[2] Adjusted for maternal tobacco smoking during pregnancy, maternal age at baseline, maternal pre-pregnancy BMI, paternal BMI, maternal alcohol intake before pregnancy, parity, maternal education, duration of breastfeeding, gestational age at blood sampling, child activity level and child diet

[3] Exposures are natural logarithm transformed in test for trend using continuous linear regression analysis

β is the difference in BMI z-score, compared to the lowest tertile of exposure

Abbreviations: BMI, body mass index; CB-153, polychlorinated biphenyl congener 153; CI, confidence interval; *p,p′*-DDE, dichlorodiphenyl dichloroethylene

Supplementary Table S3. The association between estimated postnatal *p,p'*-DDE and CB-153 concentrations during the first 12 months of life[1] and differences in child body mass index z-score at 5 to 9 years, INUENDO cohort (born 2002-2004). Complete-case analysis

		Crude model [2]			Adusted model [2, 3]			
		Medium	High			Medium	High	
Z-score BMI	n	β (95 % CI) kg m^{-2}	β (95 % CI) kg m^{-2}	p trend [4]	n	β (95 % CI) kg m^{-2}	β (95 % CI) kg m^{-2}	p trend [4]
Greenland								
CB-153	364	-0.01 (-0.28 to 0.26)	0.02 (-0.26 to 0.29)		175	0.23 (-0.20 to 0.66)	0.06 (-0.44 to 0.56)	0.96
p,p'-DDE	364	0.02 (-0.25 to 0.29)	0.01 (-0.26 to 0.29)	0.58	175	0.26 (-0.19 to 0.70)	0.01 (-0.48 to 0.49)	0.48
Poland								
CB-153	69	-0.52 (-1.28 to 0.25)	0.21 (-0.56 to 0.98)	0.90	55	-0.13 (-0.93 to 0.89)	0.33 (-0.71 to 1.36)	0.84
p,p'-DDE	69	0.61 (-0.15 to 1.38)	0.57 (-0.20 to 1.34)	0.20	55	-0.55 (-0.92 to 0.81)	0.40 (-0.51 to 1.31)	0.75
Ukraine								
CB-153	478	-0.05 (-0.29 to 0.20)	0.20 (-0.04 to 0.45)	0.29	367	-0.23 (-0.55 to 0.08)	0.21 (-0.16 to 0.59)	0.95
p,p'-DDE	478	-0.19 (-0.43 to 0.06)	-0.04 (-0.29 to 0.20)	0.90	367	-0.52 (-0.87 to -0.17)	-0.40 (-0.81 to 0.01)	0.12
Pooled								
CB-153	911	0.08 (-0.10 to 0.26)	0.14 (-0.10 to 0.38)	0.75	597	0.00 (-0.25 to 0.24)	0.16 (-0.19 to 0.52)	0.71
p,p'-DDE	911	-0.02 (-0.20 to 0.16)	0.09 (-0.09 to 0.27)	0.75	597	-0.06 (-0.32 to 0.20)	-0.01 (-0.31 to 0.29)	0.19

[1] Estimated in a physiologically based toxicokinetic model. Please see text for specifications

[2] The pooled estimates are adjusted for country

[3] Adjusted for maternal tobacco smoke during pregnancy, maternal age at baseline, maternal pre-pregnancy BMI, paternal BMI, maternal alcohol intake before pregnancy, parity, maternal education, duration of breastfeeding, gestational age at blood sampling, child activity level and child diet

[4] Exposures are natural logarithm transformed in test for trend using continuous linear regression analysis

Abbreviations: BMI, body mass index; CB-153, polychlorinated biphenyl congener 153; CI, confidence interval; *p,p'*-DDE, dichlorodiphenyl dichloroethylene

β is the difference in BMI z-score, compared to the lowest tertile of exposure

Abbreviations: BMI, body mass index; CB-153, polychlorinated biphenyl congener 153; CI, confidence interval; p,p'-DDE, dichlorodiphenyl dichloroethylene

Supplementary Table S4. The association between estimated postnatal *p,p'*-DDE and CB-153 concentrations during the first 12 months of life[1] and differences in child

		Adjusted model [2]		
		Medium	High	
Z-scores BMI	n	β (95 % CI) kg m^{-2}	β (95 % CI) kg m^{-2}	p trend [3]
Greenland				
CB-153 [5]	491	0.12 (-0.23 to 0.47)	0.17 (-0.33 to 0.66)	0.8
p,p'-DDE [6]	491	0.08 (-0.29 to 0.44)	0.02 (-0.52 to 0.48)	0.8
Poland				
CB-153 [5]	71	-0.36 (-1.65 to 0.93)	0.12 (-1.64 to 1.89)	0.047
p,p'-DDE [6]	71	0.44 (-0.73 to 1.96)	0.29 (-1.38 to 1.95)	0.047
Ukraine				
CB-153 [5]	485	0.00 (-0.32 to 0.33)	0.40 (-0.05 to 0.84)	0.8
p,p'-DDE [6]	485	-0.16 (-0.52 to 0.19)	0.13 (-0.36 to 0.62)	0.5
Pooled [4]				
CB-153 [5]	1 047	0.14 (-0.08 to 0.38)	0.29 (-0.06 to 0.65)	0.7
p,p'-DDE [6]	1 047	0.02 (-0.21 to 0.25)	0.22 (-0.10 to 0.54)	0.6

body mass index z-scores at 5 to 9 years (adjusted for prenatal exposures), INUENDO cohort (born 2002-2004).

[1] Estimated in a toxicokinetic model. Please see text for specifications

[2] Adjusted for maternal tobacco smoking during pregnancy, maternal age at baseline, maternal pre-pregnancy BMI, paternal BMI, maternal alcohol intake before pregnancy, parity, maternal education, duration of breastfeeding, gestational age at blood sampling, child activity level and child diet

[3] Exposures are natural logarithm transformed in test for trend using continuous linear regression analysis

[4] The pooled estimates are additionally adjusted for country

[5] Additionally adjusted for prenatal CB-153 exposure

[6] Additionally adjusted for prenatal *p,p'*-DDE exposure

β is the difference in BMI z-score, compared to the lowest tertile of exposure

Abbreviations: BMI, body mass index; CB-153, polychlorinated biphenyl congener 153; CI, confidence interval; *p,p'*-DDE, dichlorodiphenyl dichloroethylene

Supplementary Table S5. The association between *p,p'*-DDE and CB-153 concentrations during pregnancy and differences in child body mass index z-scores at 5 to 9 years, INUENDO cohort (born 2002-2004).

		Simple adjusted model [1]		
		Medium	High	
Z-scores BMI	n	β (95 % CI) kg m^{-2}	β (95 % CI) kg m^{-2}	p trend [2]
Greenland				
CB-153	525	-0.10 (-0.36 to 0.16)	-0.13 (-0.40 to 0.14)	0.6
p,p'-DDE	525	-0.08 (-0.35 to 0.18)	0.01 (-0.28 to 0.27)	0.6
Poland				
CB-153	92	-0.17 (-0.82 to 0.48)	-0.36 (-1.03 to 0.31)	0.2
p,p'-DDE	92	0.28 (-0.37 to 0.93)	0.12 (-0.53 to 0.77)	0.6
Ukraine				
CB-153	492	0.10 (-0.14 to 0.35)	0.05 (-0.20 to 0.30)	0.7
p,p'-DDE	492	-0.09 (-0.13 to 0.25)	-0.35 (-0.60 to -0.10)	0.03
Pooled [4]				
CB-153	1 109	-0.05 (-0.24 to 0.13)	-0.11 (-0.36 to 0.14)	0.6
p,p'-DDE	1 109	0.06 (-0.13 to 0.25)	-0.12 (-0.32 to 0.08)	0.3

[1] Adjusted for maternal tobacco smoking during pregnancy and maternal age at baseline

[2] Exposures are natural logarithm transformed in test for trend using continuous linear regression analysis

[3] The pooled estimates are additionally adjusted for country

β is the difference in BMI z-score, compared to the lowest tertile of exposure

Abbreviations: BMI, body mass index; CB-153, polychlorinated biphenyl congener 153; CI, confidence interval; *p,p'*-DDE, dichlorodiphenyl dichloroethylene

Appendix IV

Paper IV Anthropometry at 5 to 9 years of age in relation to prenatal exposure to perfluorinated alkyl substances. [In review]

Anthropometry at 5 to 9 years of age in relation to prenatal exposure to perfluorinated alkyl substances

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Short running title

Prenatal exposure to PFAS and child overweight

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Conflict of interest statement

The authors declare they have no actual or potential competing financial interests.

Abstract

Background: In animal studies, perfluorinated alkyl substances are suggested to induce weight gain. Human epidemiological studies investigating these associations are sparse.

Objective: To examine pregnancy serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) and risk of offspring overweight (> 1 standard deviation) and waist-to-height ratio (WHtR) > 0.5 at five to nine years of age.

Methods: Sera from 1,022 pregnant women enrolled in the INUENDO cohort (2002–2004) from Greenland and Kharkiv (Ukraine) were analysed for PFOA and PFOS using liquid chromatography-tandem-mass-spectrometry. Relative risks (RR) of being overweight and having WHtR > 0.5 in relation to continuous and categorised (tertiles) PFOA and PFOS were calculated at follow-up (2010–2012) using generalised linear models.

Results: For each log-unit increase of pregnancy PFOA, the adjusted RR (95 % confidence interval (CI)) of offspring overweight was 1.11 (0.88–1.38) in the pooled analysis of Greenlandic and Ukrainian children. Prenatal exposure to PFOS was not associated with a higher risk of being overweight in country-specific or pooled analysis. The adjusted RR (95% CI) of having WHtR > 0.5 for each log-unit increase of prenatal exposure to PFOA was 1.30 (0.97–1.74) in the pooled analysis. For one log-unit increase of prenatal exposure to PFOS, the adjusted RR (95% CI) of having a WHtR > 0.5 was 1.38 (1.05–1.82) in the pooled analysis.

Conclusions: The results indicate that prenatal PFOA and PFOS exposure may be associated with child waist-to-height ratio > 0.5. Prenatal PFOA and PFOS exposure were not unequivocally associated with overweight.

Keywords: Body mass index; child; cohort study, perfluorooctane sulfonate (PFOS); perfluorooctanoate (PFOA); prenatal exposure delayed effects.

Introduction

Perfluorinated alkyl substances (PFAS) have been used extensively in various consumer products such as textiles, leather, paper and food wrapping due to their water, dirt and oil repellent properties (Fromme et al. 2009). A phase out of the production of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) was initiated in 2000 by the major U.S. producers (U.S. Environmental Protection Agency 2010). However, as both compounds have half-lives of several years in humans (Olsen et al. 2007) and precursor substances are able to transform into PFOA and PFOS as reviewed by Fromme et al., we are still being exposed for instance through diet, packaged food, drinking water and dust (Fromme et al. 2009).

The PFAS bind to albumin in blood (D'eon et al. 2010), and they are able to cross placenta (Midasch et al. 2007). *In vivo* and *in vitro* studies suggest PFOA and PFOS to be endocrine disruptors (Du et al. 2013a; Du et al. 2013b), and an animal study reported prenatal exposure to PFOA to be associated with overweight and elevated serum leptin and insulin in young adult female mice (Hines et al. 2009). In humans, *in utero* exposure to PFOA has been associated with lower birth weight (Fei et al. 2007; Fei et al. 2008) and smaller size at birth (Apelberg et al. 2007; Chen et al. 2012). Also, an inverse association between prenatal exposure to PFOA and PFOS and weight and BMI has been reported among infant boys (Andersen et al. 2010), and in a recent study, prenatal PFOA exposure was associated with obesity in adult females (Halldorsson et al. 2012).

To our knowledge, only one study has been performed in school-age children, finding no association between *in utero* PFOA and PFOS exposure and overweight, BMI and waist circumference (Andersen et al. 2013). Thus, the aim of this study was to investigate the association between prenatal exposure to PFOA and PFOS and subsequent anthropometry in the offspring at 5 to 9 years in European and Arctic birth cohorts.

Methods

Study population and data collection

A total of 1,183 pregnant women from Greenland (n = 571) and Kharkiv (Ukraine) (n = 612) were enrolled in the birth cohorts throughout pregnancy from antenatal health care clinics. The women provided a blood sample during the period from May 2002 to February 2004. To be eligible for the study, the woman had to be born in the country of study, be pregnant and at least 18 years of age. Further details on the baseline study population are available elsewhere (Toft et al. 2005). A follow-up was conducted from January 2010 to May 2012 when the children were between 5 and 9 years old (Hoyer et al. 2014). Parents or guardians responded to questions concerning lifestyle and other characteristics in a face-to-face interview or by filling in a questionnaire themselves.

A total of 1,023 mother-child-pairs (singleton births) had available blood samples and were followed up. One Ukrainian child was excluded due to an extreme BMI value, leaving a study sample of 1, 022 children. The study population was distributed between Greenland (n = 531 (52%)) and Ukraine (n = 491(48%)).

The study was approved by local ethical committees; Ethical Committee for Human Research in Greenland (approval no. 2010-13) and the Commission on Ethics and Bioethics Kharkiv National Medical University in Ukraine (protocol number 7, October 7 2009). All participating parents signed informed consent.

Determination of PFOA and PFOS

Plasma concentrations of PFOA and PFOS were analysed at The Department of Occupational and Environmental Medicine in Lund, Sweden using liquid chromatography-tandem-mass-spectrometry (LC/MS/MS). A detailed description of the method is presented elsewhere

(Lindh et al. 2012). Briefly, aliquots of 100 µl serum were added 25 µl of a water:acetonitrile (50:50) solution containing labelled internal standards. Proteins were precipitated with acetonitrile and vigorously shaking for 30 minutes. The samples were then centrifuged and the supernatant analyzed using a LC (UFLCXR, SHIMADZU Corporation, Kyoto, Japan) connected to a hybrid triple quadrupole linear ion trap mass spectrometer (QTRAP 5500, AB Sciex, Foster City, CA, USA). Limits of detection (LOD) were 0.04 ng/ml and 0.2 ng/ml for PFOA and PFOS, respectively, and all samples were above LOD. Coefficient of variation of duplicate samples worked-up and analyzed on different days were 11 % and 9 % for PFOA and PFOS, respectively.

Anthropometric measures

The child's weight was measured to the nearest 0.1 kg by a weighing scale available at the family's home or at the clinics. The child's height was measured with the child standing barefoot against a wall, marking the top of the head and measuring the height to the nearest centimetre by use of ordinary measuring tape. All measurements were performed by the interviewer except for those who were telephone-interviewed in which case parents performed the measurements. Child BMI was calculated from weight (kg)/ height^2 (m). BMI was expressed as a specific for age and sex z-score using the World Health Organization (WHO) Growth Standards 2007 (de Onis et al. 2007) to facilitate comparison of results with those shown in similar studies. Children with a BMI specific-for-age-an-sex z-score >1 SD were classified as overweight (de Onis et al. 2007).

Waist circumference was measured by measuring tape across abdomen corresponding to the umbilicus. Waist-to-height ratio (WHtR) was calculated from waist circumference (cm)/ height (cm). A cut-off of WHtR > 0.5 indicated high risk WHtR and was based on earlier studies on children (Goulding et al. 2010; Maffeis et al. 2008; Mokha et al. 2010) since a

WHtR > 0.5 has been associated with increased cardiometabolic morbidity in children (Mokha et al. 2010) and in adults (de Koning et al. 2007).

Statistical analysis

A non-response analysis was performed to check for inconsistencies between responders and non-responders. Spearman's rank correlation was used to assess the correlation between pregnancy levels of PFOA and PFOS. We used generalized additive models (GAM) to assess the shape of the relationship between pregnancy levels of PFOA and PFOS and BMI z-score, overweight, WHtR and WHtR > 0.5. As the generalised additive models (GAMs) not always showed linear association (p between 0.04 and 0.47), analyses were performed using both continuous exposures and using country specific tertiles. Generalized linear models with robust variance estimation were used to estimate the relative risk (RR) of overweight and WHtR > 0.5 (Zou 2004). Multivariate linear regression models were used to assess the association between exposures and continuous BMI z-scores and WHtR. Pooled analyses were performed on continuous exposures in Greenlandic and Ukrainian children as no sign of heterogeneity was evident (exposures*country interaction, $p > 0.05$).

Potential confounders of the associations between prenatal PFAS exposures and the anthropometric outcomes were identified in literature: maternal age at birth, parity, pre-pregnancy BMI, maternal smoking during pregnancy, maternal alcohol consumption when trying to conceive, maternal education, duration of total breastfeeding, child age at follow-up, child sex, gestational age at blood sampling, birth weight, preterm birth, child sugary intake, child fruit and vegetable intake and child physical activity level. Directed acyclic graphs (DAGs) were used to select the confounders included in the final models using DAGitty software (Textor et al. 2011). All final multivariate models were adjusted for maternal age at birth (continuous, years), parity (dichotomous, 1st child/ 2nd child or more), maternal smoking

during pregnancy (dichotomous, serum cotinine ≤ 10 / > 10 ng/ml), maternal education (dichotomous, unskilled/skilled) and maternal pre-pregnancy BMI (continuous, kg/m^2). The models of WHtR were additionally adjusted for child age (continuous, weeks) and sex. The pooled analysis where furthermore adjusted for country. As missing data would cause a risk of selection bias as well as loss of power, missing covariate and outcome data was imputed using chained multiple imputation, generating 100 complete datasets (Donders et al. 2006; Sterne et al. 2009).

Effect modification of the association between the exposures and outcomes by sex was evaluated using stratified analysis and by adding interaction terms in the model. As birth weight may influence the risk of overweight later in life (Schellong et al. 2012), we restricted our analysis to children with normal birth weight (2,500-4,000 g, n=370 in Greenland and n = 465 in Ukraine) in a sensitivity analysis. Further, sugar intake and physical activity of the child was added to the model as these are strongly related to the outcomes. Also, both exposures were added in the same model to allow mutual adjustment in the Greenlandic and Ukrainian populations. Further, we analysed the association between PFAS and RR of overweight and WHtR > 0.5 in children with no missing data (complete cases). Finally, we explored the robustness of the imputation model by running the above mentioned analysis on 20 and 150 imputed data sets. All statistical analysis was performed using STATA 13.1 (Stata Corporation, College Station, TX, USA) and the significance level was set at $p < 0.05$.

Results

Levels of PFOA and PFOS in average and parental BMI did not differ for those excluded (n = 159) and those included in the study (n = 1,022) (See Supplemental Material, Table S1). Spearman's rank correlation between PFOA and PFOS was 0.49 in Greenland and 0.51 in Ukraine. The characteristics of the study population have previously been presented in detail

(Hoyer et al. 2014). Briefly, in comparison with children from Greenland, children from Ukraine had lower birth weight, were younger at follow-up and had a smaller BMI z-score at 5–9 years. Mothers from Ukraine had a lower pre-pregnancy BMI, were more often primiparous, and less often smokers than mothers from Greenland (Table 1).

The country specific medians, ranges and tertiles of PFOA and PFOS are presented in Table 2. Median exposure levels were highest in Greenland (median (range) PFOA 1.8 ng/ml (0.5, 5.1)), and PFOS (median (range) 20.3 ng/ml (4.1, 87.3)). Missing information is presented in Table 3, indicating the highest number of missing information on the outcome WHtR of 25.6%.

The results of adjusted RR of overweight in relation to pregnancy levels of PFOA and PFOS are presented in Table 4. For PFOA, the overall adjusted RR (95% CI) of being overweight in Greenland was 1.11 (0.82, 1.53) per log-unit increase. In girls, there was an increased risk of overweight comparing the highest PFOA tertile to the lowest (RR (95% CI) 1.81 (1.04, 3.17)), whereas no associations were evident in boys (p for interaction = 0.15). In Ukraine, the overall RR (95% CI) tended to be higher (1.38 (0.91, 2.10) comparing medium with low PFOA exposed children, whereas the high versus low PFOA exposed tended to be of lower risk (RR (95% CI) (0.78 (0.47, 1.29))). The same was seen among girls in Ukraine, but not as evident in boys. Pooled analysis of Greenlandic and Ukrainian populations showed increased risk of overweight of 7 to 15% across the pooled and sex stratified sample in relation to one log unit increase in continuous prenatal PFOA exposure. No clear association was found between prenatal PFOS exposure and offspring overweight. Crude RRs of overweight in relation to PFOA and PFOS were generally similar to the adjusted RRs (See Supplemental Material, Table S2).

The crude results of the continuous BMI z-scores showed positive associations in relation to PFOA in Greenland (See Supplemental Material, Table S3). The adjusted associations were

positive, but not statistically significant. No associations were observed between crude or adjusted models of PFOA and BMI z-score in Ukraine or between PFOS and BMI z-score in the two countries.

The RR of having WHtR > 0.5 in relation to prenatal PFOA and PFOS exposures is presented in Table 5. The overall RR of having WHtR > 0.5 in relation to PFOA was elevated in both countries and in the pooled analysis (pooled, overall RR (95% CI) 1.30 (0.97, 1.74)). The overall RR of having WHtR > 0.5 in relation to continuous PFOS was increased in both countries, and in the pooled analysis, the RR (95% CI) was 1.38 (1.05, 1.82). In Greenland, the overall continuous model showed a RR (95 % CI) of 1.31 (0.97, 1.77) in relation to PFOS. In Ukraine, the risk was also increased.

When stratified by sex, Greenlandic girls tended to have a higher risk of having WHtR > 0.5 in relation to PFOS than boys but there was no evidence of an interaction between PFOS and sex in neither Greenland (*p* interaction between 0.08 and 0.57) nor Ukraine (*p* interaction between 0.32 and 0.41), whereas this was evident in the pooled estimate of Greenland and Ukraine (*p* interaction between 0.01 and 0.06). The overall crude RRs were in the same direction (See Supplemental Material, Table S2).

PFOA in relation to continuous WHtR showed weak positive associations in crude and adjusted models in Greenland (See Supplemental Material, Table S3). No other associations were observed in Ukraine in relation to PFOA or PFOS.

Including both pollutants in the model did not change results notably (data not shown), but the statistically significant result of prenatal continuous PFOS exposure and WHtR > 0.5 in the pooled analysis of Greenlandic and Ukrainian children was slightly weakened (RR (95%CI) 1.30 (0.95, 1.79)).

Restricting analysis to normal birth weight children and including sugar intake and physical activity of the child generally showed results similar to the main analysis (data not shown).

Complete case results were similar to imputation based results (See Supplemental Material, Table S4), and the two sensitivity analyses on the imputation model proved the imputation model robust, since the results did not materially change (data not shown).

Discussion

Higher prenatal exposure to PFOA and PFOS was associated with increased risk of WHtR > 0.5 in a pooled analysis of Greenlandic and Ukrainian children of 5 to 9 years of age and these associations showed no heterogeneity between the two countries. The associations were stronger in girls than in boys. Little or no association was observed for prenatal PFOA and PFOS exposure and risk of overweight.

Suggested mechanisms that could explain the current findings are not well established. However, animal studies suggest that PFAS may act as endocrine disruptors affecting oestrogen concentrations in the body (Shi et al. 2009) but also effects through an activation of peroxisome proliferator-activated receptor alpha (PPARα) that interact with liver estrogen receptor has been reported (Rosen et al. 2008; Wolf et al. 2008). Furthermore, experimental studies have found that endocrine disruptors could change the metabolic pathways and cause permanent changes in body weight (Newbold et al. 2008).

In one study authors reported a positive association between prenatal PFOA and overweight and central obesity (i.e. waist circumference) in young females adults (Halldorsson et al. 2012), which we also found consistent indications of among the girls in Greenland, but our findings were not consistent between countries. The lack of a statistically significant association among the girls in Ukraine in our study compared to the aforementioned study could be due to lower PFOA levels in the current study. Further, it is possible that the

sensitivity of the measure of childhood adiposity based on BMI z-score cut-offs in relation to POPs, is not as good as the WHtR. In addition, WHtR has been suggested to be equal or superior to BMI marker for cardio-metabolic morbidity later in life in children (Maffeis et al. 2008; Mokha et al. 2010), although there is not a general consensus on this yet (Bluher et al. 2013). Another study investigating the association between prenatal exposure to PFAS and offspring overweight reported no association between prenatal PFOA and BMI z-scores and overweight at age 7 years in a random sample from the DNBC (Andersen et al. 2013). Their null-finding on PFOA and overweight is in line with our study but it is not consistent with the aforementioned study on 20 year old females.

We found no association between PFOS and overweight, which is consistent with both prior studies (Andersen et al. 2013; Halldorsson et al. 2012).

One study reported no associations between prenatal PFOA and PFOS exposures and residuals of WHtR, but no estimates were reported (Andersen et al. 2013). In our study, PFOA and PFOS were associated with WHtR > 0.5 in Greenland and Ukraine, especially among girls. The differences according to sex was also reported by Halldorsson et al. in relation to overweight and waist circumference and may in part relate to possible changes in oestrogen levels in relation to the compounds (Halldorsson et al. 2012; Shi et al. 2009). In our study, the analyses of exposure*sex interaction most often did not indicate interaction. In the pooled analysis of Greenland and Ukraine, however, the interaction was evident. Hence, the sex stratified results of the pooled analysis of the Greenlandic and Ukrainian populations is the most reliable compared with the overall results. Future studies should investigate this further in different ethnic groups.

We acknowledge that this study has some limitations. Children were weighed using different weighing scales at the examination. However, we believe that any misclassification is non-differential. Also, levels of PFAS tend to decrease during pregnancy (Fei et al. 2007), and as

blood samples in the current study were collected throughout pregnancy there is a risk of exposure misclassification. We addressed this issue by adjusting for gestational age at blood sampling, although we recognize that this has not completely eliminated possible misclassification. We have no information on maternal fish and seafood intake during pregnancy, which has been reported as a source of exposure (Haug et al. 2010; Lindh et al. 2012), although not all studies observed high levels of PFAS in fish (Jorundsdottir et al. 2014). Further, a relation to child overweight is not obvious, but confounding by fish intake can not be completely ruled out. Moreover, it has been debated that weight change during pregnancy may play a role in the levels of organochlorines compounds (Verner et al. 2013). It is uncertain whether the same goes for the PFAS as the body storage of the compounds is different. Unmeasured confounding by this variable is possible, in our study, if indeed gestational weight gain is a confounder of the associations of interest (Govarts et al. 2014). Strengths of the study include the prospective follow-up for up till nine years in birth cohorts from the early 2000's when PFAS levels around the world was very high. The study population including mother-child pairs from a European and an Arctic area enabled evaluation of consistency of exposure-outcome association across ethnicity and regions.

Conclusions

Our results indicate that prenatal PFOA and PFOS exposure may be associated with child central obesity (WHtR > 0.5), but not unequivocally with overweight at 5-9 years. There were some indications that females may be more sensitive towards exposure than males.

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Table 1. Characteristics of mothers and their children

Characteristics	Greenland (n=531)[a]	Ukraine (n=491)[a]
Child characteristics		
Sex, no. (%)		
Male	286 (54)	260 (53)
Female	242 (46)	228 (47)
Gestational age, no. (%)		
≥ 37 weeks	503 (95)	479 (98)
< 37 weeks	25 (5)	9 (2)
Birth weight, grams [median (10^{th}–90^{th} percentile)]	3,600 (2,840-4,370)	3,285 (2,800-3,800)
Age at follow-up, years [median (10^{th}–90^{th} percentile)	8.3 (7.3-9.1)	7.0 (6.6-7.6)
Anthropometric measures at follow-up		
Weight, kg [Median (10^{th}–90^{th} percentile)]	29.0 (23.7-38.3)	24.0 (20.0.-30.0)
Height, cm [Median (10^{th}–90^{th} percentile)]	131 (122-138)	124 (117-131)
BMI, kg/m^2 [Median (10^{th}–90^{th} percentile)]	17.0 (14.9-20.6)	15.8 (13.9-18.3)
BMI z-scores [Median (10^{th}–90^{th} percentile)]	0.7 (-0.5-2.2)	0.2 (-1.1-1.5)
Waist-to-height ratio [Median (10^{th}–90^{th} percentile)]	0.5 (0.4-0.6)	0.4 (0.4-0.5)
Overweight (+1 SD), no. (%)	103 (19)	66 (13)
Obese (+2 SD), no (%)	49 (9)	29 (6)
Underweight (<-2 SD), no. (%)	2 (0.4)	14 (3)
Maternal characteristics		
Maternal age at pregnancy, years [Median (10^{th}–90^{th} percentile)]	26 (20-36)	24 (20-32)
Maternal pre-pregnancy BMI, kg/m^2 [Median (10^{th}–90^{th} percentile)]	24 (20-30)	21 (18-26)
Parity, no. (%)		
0	169 (32)	399 (81)
>0	362 (68)	92 (19)
Maternal smoking during pregnancy, no. (%)		
Yes (serum cotinine > 10 ng/ml)	297 (56)	76 (15)
No (serum cotinine ≤ 10 ng/ml)	234 (44)	415 (85)
Maternal educational level, no. (%)		
Unskilled	226 (43)	183 (37)
Skilled/professional	305 (47)	308 (63)

Abbrieviations: BMI; body mass index

[a]Missing information is presented in Table 3

Table 2. Description of pregnancy PFOA and PFOS medians (ranges) and tertiles

PFOA	Median (range)	Low tertile	Medium tertile	High tertile
Greenland	1.8 (0.5, 5.1)	0.5, 1.5	1.5, 2.2	2.2, 5.1
Ukraine	1.0 (0.2, 9.8)	0.2, 0.8	0.8, 1.1	1.1, 9.8
PFOS	Median (range)	Low	Medium	High
Greenland	20.2 (4.1, 87.3)	4.1, 16.8	16.8, 23.9	23.9, 87.3
Ukraine	5.0 (0.7, 18.1)	0.7, 4.2	4.2, 5.9	5.9, 18.1

Abbreviations: PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate
[a] Exposure range of PFOS and PFOA was measured in ng/ml

Table 3. Number (%) of missing values according to country

Variables	Greenland (n=531) n (%)	Ukraine (n=491) n (%)
Child sex	3 (0.6)	3 (0.6)
Gestational age at birth	3 (0.6)	3 (0.6)
Birth weight	6 (1.1)	1 (0.2)
Child age at follow-up	10 (1.9)	0 (0.0)
Weight at follow-up	93 (17.5)	0 (0.0)
Height at follow-up	45 (8.5)	0 (0.0)
BMI at follow-up	98 (18.5)	0 (0.0)
BMI z-score	105 (19.8)	3 (0.6)
Waist-to-height ratio	136 (25.6)	1 (0.2)
Overweight	105 (19.8)	1 (0.2)
Obese	105 (19.8)	1 (0.2)
Underweight	136 (19.8)	1 (0.2)
Maternal age at baseline	38 (7.2)	24 (4.9)
Pre-pregnancy BMI	2 (0.4)	8 (1.6)

Abbreviations: BMI; body mass index

Table 4. Maternal PFOA and PFOS concentrations during pregnancy and adjusted relative risk (RR) of offspring overweight (WHO > 85th percentile (sex and age standardised) at 5 to 9 years

	n	Overall Adjusted[a] RR (95% CI)	n	Girls Adjusted[a] RR (95 % CI)	n	Boys Adjusted[a] RR (95 % CI)	P interaction Exposure*sex
Greenland							
PFOA Low	177	1.00	82	1.00	96	1.00	
Medium	177	1.24 (0.89, 1.75)	82	1.56 (0.90, 2.72)	95	1.02 (0.67, 1.57)	0.10
High	177	1.23 (0.87, 1.74)	81	1.81 (1.04, 3.17)	95	1.03 (0.66, 1.59)	0.15
Continuous[b]	531	1.11 (0.82, 1.53)	245	1.34 (0.82, 2.19)	286	0.98 (0.66, 1.46)	0.22
PFOS Low	177	1.00	82	1.00	96	1.00	
Medium	177	0.95 (0.72, 1.26)	82	1.30 (0.83, 2.03)	95	0.72 (0.48, 1.07)	0.03
High	177	0.84 (0.61, 1.14)	81	1.09 (0.66, 1.79)	95	0.75 (0.51, 1.10)	0.23
Continuous[b]	531	0.91 (0.69, 1.20)	245	1.15 (0.76, 1.74)	286	0.74 (0.50, 1.11)	0.15
Ukraine							
PFOA Low	164	1.00	77	1.00	87	1.00	
Medium	164	1.38 (0.91, 2.10)	77	1.29 (0.63, 2.66)	87	1.43 (0.85, 2.40)	0.90
High	163	0.78 (0.47, 1.29)	77	0.40 (0.13, 1.19)	86	1.04 (0.60, 1.80)	0.11
Continuous[b]	491	1.02 (0.72, 1.44)	231	0.62 (0.32, 1.20)	260	1.34 (0.89, 2.02)	0.04
PFOS Low	164	1.00	77	1.00	87	1.00	
Medium	164	0.91 (0.60, 1.40)	77	0.60 (0.27, 1.33)	87	1.07 (0.64, 1.80)	0.14
High	163	0.89 (0.57, 1.37)	77	0.89 (0.43, 1.85)	86	0.85 (0.48, 1.48)	0.86
Continuous[b]	491	1.10 (0.75, 1.60)	231	0.84 (0.42, 1.69)	260	1.22 (0.78, 1.91)	0.22
Greenland & Ukraine[c]							
PFOA Continuous[b]	1,022	1.11 (0.88, 1.38)	476	1.07 (0.76, 1.52)	546	1.15 (0.86, 1.53)	0.31
PFOS Continuous[b]	1,022	0.97 (0.78, 1.21)	476	1.06 (0.74, 1.51)	546	0.92 (0.69, 1.23)	0.06

Abbreviations: CI, confidence interval; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; RR, Relative risk

[a]Adjusted for maternal age at birth, maternal pre-pregnancy body mass index, smoking during pregnancy, maternal education and parity

[b] Continuous PFOA and PFOS are natural logarithm transformed

[c] In addition adjusted for country

Table 5. Maternal PFOA and PFOS concentrations during pregnancy and adjusted relative risk (RR) of offspring waist-to-height-ratio >0.5 at 5 to 9 years

	n	Overall Adjusted[a] RR (95% CI)	n	Girls Adjusted[a] RR (95 % CI)	n	Boys Adjusted[a] RR (95 % CI)	P interaction Exposure*sex
Greenland							
PFOA Low	177	1.00	82	1.00	96	1.00	
Medium	177	1.32 (0.92, 1.90)	82	1.93 (1.15, 3.24)	95	0.92 (0.52, 1.61)	0.06
High	177	1.18 (0.80, 1.74)	81	1.65 (0.94, 2.89)	95	1.11 (0.65, 1.90)	0.30
Continuous[b]	531	1.28 (0.91, 1.82)	245	1.49 (0.95, 2.33)	286	1.10 (0.66, 1.84)	0.27
PFOS Low	177	1.00	82	1.00	96	1.00	
Medium	177	1.14 (0.80, 1.63)	82	1.64 (1.01, 2.66)	95	0.83 (0.48, 1.43)	0.08
High	177	1.22 (0.86, 1.74)	81	1.63 (0.99, 2.68)	95	1.06 (0.63, 1.77)	0.30
Continuous[b]	531	1.31 (0.97, 1.77)	245	1.44 (0.98, 2.11)	286	1.19 (0.72, 1.97)	0.57
Ukraine							
PFOA Low	164	1.00	77	1.00	87	1.00	
Medium	164	1.33 (0.61, 2.89)	77	4.14 (0.81, 21.29)	87	0.80 (0.27, 2.34)	0.11
High	163	1.11 (0.48, 2.57)	77	1.04 (0.12, 8.98)	86	1.24 (0.48, 3.22)	0.89
Continuous[b]	491	1.50 (0.83, 2.72)	231	1.06 (0.44, 2.55)	260	1.71 (0.74, 3.92)	0.65
PFOS Low	164	1.00	77	1.00	87	1.00	
Medium	164	1.43 (0.63, 3.25)	77	0.55 (0.10, 2.99)	87	1.85 (0.72, 4.80)	0.32
High	163	1.44 (0.62, 3.31)	77	1.78 (0.44, 7.18)	86	0.98 (0.33, 2.92)	0.41
Continuous[b]	491	1.67 (0.81, 3.47)	231	2.45 (0.62, 9.60)	260	1.36 (0.57, 3.26)	0.33
Greenland & Ukraine[c]							
PFOA Continuous[b]	1,022	1.30 (0.97, 1.74)	476	1.41 (0.97, 2.05)	546	1.25 (0.80, 1.95)	0.06
PFOS Continuous[b]	1,022	1.38 (1.05, 1.82)	476	1.54 (1.06, 2.23)	546	1.24 (0.82, 1.87)	0.01

Abbreviations: CI, confidence interval; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; RR, Relative risk; WHtR, waist-to-height-ratio

[a] Adjusted for child sex, child age at follow-up, maternal age at birth, maternal pre-pregnancy body mass index, smoking during pregnancy, maternal education and parity

[b] Continuous PFOA and PFOS are natural logarithm transformed

[c] In addition adjusted for country

Supplemental Material, Table S1. Characteristics of participants and those lost to follow-up

	Greenland		Ukraine	
	Lost to follow-up[a] n=40	Participants[b] n=531	Lost to follow-up[c] n=119	Participants[d] n=491
PFOA [Median (range)]	1.7 (0.5, 3.3)	1.8 (0.5, 5.1)	0.9 (0.3, 2.6)	1.0 (0.2, 9.8)
PFOS [Median (10-90 percentile)]	19.4 (10.9, 67.7)	20.2 (4.1, 87.3)	5.1 (0.8, 13.3)	5.0 (15.0, 37.9)
Maternal pre-pregnancy BMI [Mean (SD)]	24.2 (4.6)	24.5 (4.5)	21.6 (2.9)	21.7 (3.3)
Paternal BMI [Mean (SD)]	25.8 (3.4)	26.8 (3.8)	24.3 (3.2)	24.2 (2.9)

[a] In the lost to follow-up group, 27.5 % had missing information on paternal BMI

[b] Among the participants, 0.4 % had missing information on maternal BMI and 25.0% had missing information on paternal BMI

[c] In the lost to follow-up group, 0.8 % had missing PFOA, PFOS and maternal BMI and 4.2 % had missing paternal BMI

[d] Among the participants, 1.6 % had missing information on maternal BMI and 3.3% had missing information on paternal BMI

Abbreviations: BMI, body mass index; PFOA, perfluorooctanoate acid; PFOS, perfluorooctane sulfonate

Supplemental Material, Table S2. Maternal PFOA and PFOS concentrations during pregnancy and crude relative risk (RR) of offspring overweight (WHO > 85th percentile (age and sex standardised)) and waist-to-height-ratio >0.5 at 5 to 9 years

		Overweight		WHtR >0.5
	n	**Crude RR (95 % CI)**	**n**	**Crude RR (95 % CI)**
Greenland				
PFOA Low	177	1.00	177	1.00
Medium	177	1.47 (1.07, 2.02)	177	1.34 (0.95, 1.89)
High	177	1.41 (1.02, 1.94)	177	1.12 (0.78, 1.59)
Continuous[a]	531	1.31 (0.99, 1.72)	531	1.21 (0.90, 1.63)
PFOS Low	177	1.00	177	1.00
Medium	177	1.04 (0.78, 1.38)	177	1.12 (0.79, 1.59)
High	177	0.89 (0.65, 1.22)	177	1.19 (0.85, 1.68)
Continuous[a]	531	0.95 (0.72, 1.24)	531	1.26 (0.94, 1.68)
Ukraine				
PFOA Low	164	1.00	164	1.00
Medium	164	1.34 (0.90, 2.04)	164	1.18 (0.54, 2.56)
High	163	0.78 (0.48, 1.27)	163	1.01 (0.45, 2.27)
Continuous[a]	491	1.02 (0.73, 1.42)	491	1.36 (0.79, 2.32)
PFOS Low	164	1.00	164	1.00
Medium	164	0.92 (0.60, 1.40)	164	1.45 (0.64, 3.31)
High	163	0.86 (0.56, 1.34)	163	1.45 (0.64, 3.31)
Continuous[a]	491	1.07 (0.74, 1.56)	491	1.70 (0.87, 3.32)

Abbreviations: CI, confidence interval; PFOA, perfluorooctanoate acid; PFOS, perfluorooctane sulfonate; RR, Relative risk; WHtR, waist-to-height-ratio

[a] Continuous PFOA and PFOS are natural logarithm transformed

Supplemental Material, Table S3. Maternal PFOA and PFOS concentrations during pregnancy and crude and adjusted associations with BMI z-scores (sex and age standardised) and WHtR in offspring at 5 to 9 years

		BMI z-score			Waist-to-height ratio	
	n	**Crude β (95 % CI)**	**Adjusted[a] β (95% CI)**	**n**	**Crude β (95 % CI)**	**Adjusted[b] β (95 % CI)**
Greenland						
PFOA Low	177	reference	reference	177	reference	reference
Medium	177	0.23 (-0.03, 0.49)	0.09 (-0.18, 0.35)	177	0.01 (0.00, 0.02)*	0.01 (0.00, 0.02)
High	177	0.30 (0.05, 0.56)*	0.19 (-0.09, 0.46)	177	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)
Continuous[c]	531	0.25 (0.01, 0.49)**	0.11 (-0.14, 0.37)	531	0.01 (0.00, 0.02)**	0.01 (0.00, 0.02)**
PFOS Low	177	reference	reference	177	reference	reference
Medium	177	0.00 (-0.25, 0.26)	-0.06 (-0.31, 0.19)	177	0.01 (0.00, 0.02)	0.01 (0.00, 0.02)
High	177	-0.11 (-0.37, 0.15)	-0.15 (-0.41, 0.10)	177	0.01 (-0.01, 0.02)	0.00 (0.00, 0.02)
Continuous[c]	531	-0.05 (-0.28, 0.19)	-0.07 (-0.31, 0.16)	531	0.00 (0.00, 0.01)	0.00 (0.00, 0.02)
Ukraine						
PFOA Low	164	reference	reference	164	reference	reference
Medium	164	0.21 (-0.03, 0.46)	0.28 (0.03, 0.52)*	164	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)
High	163	-0.01 (-0.25, 0.24)	0.07 (-0.18, 0.32)	163	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)
Continuous[c]	491	0.03 (-0.17, 0.23)	0.02 (-0.19, 0.23)	491	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)
PFOS Low	164	reference	reference	164	reference	reference
Medium	164	-0.01 (-0.25, 0.23)	-0.03 (-0.27, 0.22)	164	0.00 (-0.01, 0.01)	0.00 (-0.01, 0.01)
High	163	-0.01 (-0.25, 0.24)	0.03 (-0.22, 0.28)	163	0.01 (0.00, 0.02)*	0.01 (0.00, 0.02)
Continuous[c]	491	0.03 (-0.19, 0.25)	0.04 (-0.19, 0.26)	491	-0.01 (-0.02, 0.01)	0.00 (0.00, 0.01)

Abbreviations: CI, confidence interval; PFOA, perfluorooctanoate acid; PFOS, perfluorooctane sulfonate; WHtR, waist-to-height-ratio

[a] Adjusted for maternal age at birth, maternal pre-pregnancy body mass index, smoking during pregnancy, maternal education and parity

[b] Adjusted for child sex, child age at follow-up, maternal age at birth, maternal pre-pregnancy body mass index, smoking during pregnancy, maternal education and parity

[c] Continuous PFOA and PFOS are natural logarithm transformed and β is the change in outcome for one log unit of exposure

* Statistically significantly different compared to low exposed children, $p < 0.05$

** Statistically significantly different from 0 for one log unit increase in exposure, $p < 0.05$

Supplemental Material, Table S4. Complete-case-analysis of maternal PFOA and PFOS concentrations during pregnancy and crude relative risk (RR) of offspring overweight (WHO > 85 percentile (sex and age standardised)) and waist-height-ratio >0.5 at 5 to 9 years.

	Overweight				WHtR >0.5			
	Cases/ n	**Crude RR (95% CI)**	**Cases/ n**	**Adjusted[b] RR (95% CI)**	**Cases/ n**	**Crude RR (95% CI)**	**Cases/ n**	**Adjusted[c] RR (95 % CI)**
Greenland								
PFOA Low	38/143	1.00	35/135	1.00	35/130	1.00	43/117	1.00
Medium	57/136	1.57 (1.12, 2.19)	55/127	1.40 (0.98, 2.01)	49/126	1.44 (1.01, 2.07)	36/114	1.50 (1.02, 2.21)
High	57/147	1.47 (1.05, 2.06)	53/133	1.27 (0.88, 1.84)	40/139	1.07 (0.73, 1.57)	37/122	1.20 (0.80-1.82)
Continuous[a]	152/426	1.35 (1.01, 1.79)	143/395	1.13 (0.82, 1.57)	124/395	1.16 (0.85, 1.57)	116/353	1.28 (0.90-1.82)
PFOS Low	51/139	1.00	48/132	1.00	32/122	1.00	32/112	1.00
Medium	55/143	1.05 (0.78, 1.42)	51/132	0.93 (0.69, 1.27)	42/130	1.23 (0.84, 1.82)	38/116	1.18 (0.80, 1.74)
High	46/144	0.87 (0.63, 1.20)	44/131	0.85 (0.61, 1.18)	50/143	1.33 (0.92, 1.94)	46/125	1.31 (0.90, 1.92)
Continuous[a]	152/426	0.96 (0.73, 1.27)	143/395	0.93 (0.79, 1.25)	124/395	1.35 (1.00, 1.83)	116/353	1.47 (1.08, 2.00)
Ukraine								
PFOA Low	31/164	1.00	30/155	1.00	11/164	1.00	10/155	1.00
Medium	40/161	1.31 (0.87, 1.99)	39/150	1.37 (0.89, 2.10)	13/164	1.18 (0.55, 2.56)	12/150	1.41 (0.62, 3.21)
High	24/163	0.81 (0.50, 1.31)	23/156	0.79 (0.48, 1.32)	11/162	1.01 (0.45, 2.27)	10/155	1.13 (0.47, 2.72)
Continuous[a]	95/488	1.04 (0.75, 1.45)	92/461	1.00 (0.70, 1.41)	35/490	1.36 (0.79, 2.32)	32/460	1.43 (0.78, 2.63)
PFOS Low	35/164	1.00	34/153	1.00	9/164	1.00	8/153	1.00
Medium	31/163	0.89 (0.58, 1.37)	31/157	0.89 (0.58, 1.37)	13/163	1.45 (0.64, 3.31)	13/156	1.66 (0.71, 3.87)
High	29/161	0.87 (0.56, 1.35)	27/151	0.86 (0.55, 1.34)	13/163	1.45 (0.64, 3.31)	11/151	1.42 (0.58, 3.46)
Continuous[a]	95/488	1.08 (0.74, 1.58)	92/461	1.09 (0.74, 1.59)	35/490	1.70 (0.87, 3.31)	32/460	1.67 (0.80, 3.47)

Abbreviations: CI, confidence interval; PFOA, perfluorooctanoate acid; PFOS, perfluorooctane sulfonate; RR, relative risk; WHtR, waist-to-height-ratio

[a] Continuous PFOA and PFOS are natural logarithm transformed

[b] Adjusted for maternal age at birth, maternal pre-pregnancy body mass index, smoking during pregnancy, maternal education and parity

[c] Adjusted for child sex, age at follow-up, maternal age at birth, maternal pre-pregnancy body mass index, smoking during pregnancy, maternal education and parity