LUND UNIVERSITY

Environmental Toxins and Essential Trace Elements at Delivery and ADHD

Ode, Amanda

2015

Link to publication

Citation for published version (APA):
Ode, A. (2015). *Environmental Toxins and Essential Trace Elements at Delivery and ADHD*. [Doctoral Thesis (compilation), Division of Occupational and Environmental Medicine, Lund University]. Division of Occupational and Environmental Medicine.

Total number of authors:
1

General rights
Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.
• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Download date: 04. May. 2026

"Epidemiology is called the 'basic science of public health,' but its contribution to this goal is constrained by a preoccupation with supposedly universal exposure-disease relationships that impedes consideration of the contexts in which exposures occur. … [Because] exposure-disease relationships are not self-contained, homogeneous or independent phenomena, they constitute an inadequate object of epidemiological science."

Wing, 1994, Medicine and Global Survival, 1, 74-86

Printed by Media-Tryck, Lund University 2015

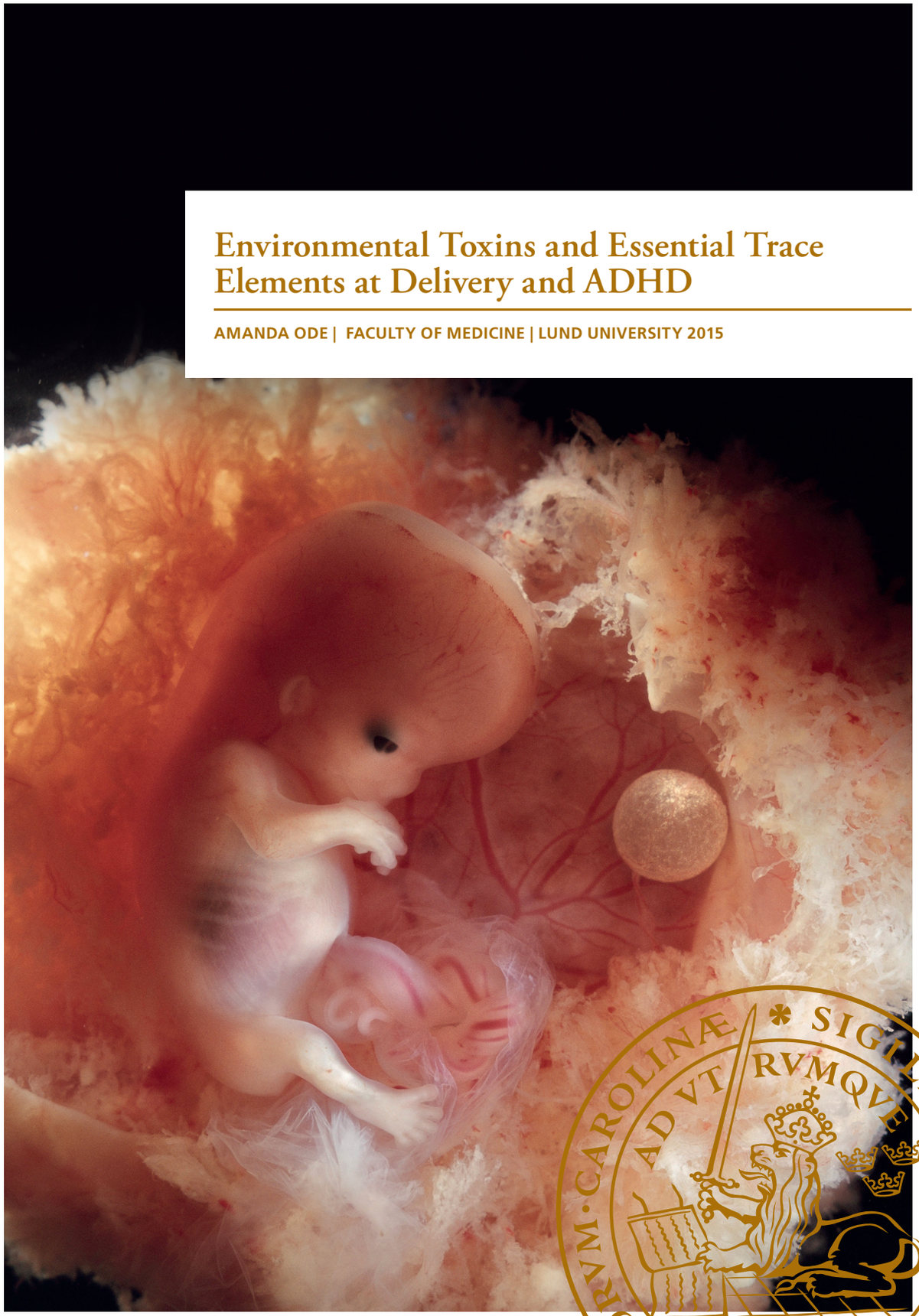
AMANDA ODE Environmental Toxins and Essential Trace Elements at Delivery and ADHD

Department of Occupational and Environmental Medicine

Faculty of Medicine
ISBN 978-91-7619-121-7
ISSN 1652-8220

Environmental Toxins and Essential Trace Elements at Delivery and ADHD

AMANDA ODE | FACULTY OF MEDICINE | LUND UNIVERSITY 2015



Environmental Toxins and Essential Trace Elements at Delivery and ADHD

Amanda Ode

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty Medicine, Lund University, Sweden.
To be defended at BMC, Segerfalksalen. Date 8th May 2015, at 09:00.

Faculty opponent
Carl-Gustaf Bornehag

Organization	Document name: DISSERTATION BOOK
LUND UNIVERSITY	
	Date of issue
Author(s): AMANDA ODE	Sponsoring organization

Title and subtitle: Environmental Toxins and Essential Trace Elements at Delivery and ADHD

Abstract

Perfluorinated compounds (PFCs), phthalates, manganese and selenium are ubiquitous in the environment and humans and influence fetal growth and development. Register and biobank based case-control studies were conducted to investigate the relationship between prenatal exposure to these chemicals and attention deficit hyperactivity disorder (ADHD) in childhood.

The study base comprised children born in Malmö, Sweden, between 1978 and 2000 that were followed up until 2005. Children with ADHD (n=206) were identified at the Department of Child and Adolescent Psychiatry in Malmö. Controls (n=206) were selected from the study base and were matched for year of birth and maternal country of origin. Concentrations of PFCs, phthalates, manganese, and selenium were measured in umbilical cord serum samples collected from a maternity unit biobank in Malmö.

No associations between prenatal exposure to PFCs, phthalates, and manganese and ADHD in childhood were found. Selenium, which was hypothesized to protect against ADHD, was found to be positively associated with ADHD diagnosis in childhood. These findings need to be replicated in other larger studies before definitive conclusions can be drawn.

Key words: PFCs, manganese, selenium, phthalates, ADHD, fetus		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language: English
ISSN and key title: 1652-8220		ISBN 978-91-7619-121-7
Recipient's notes	Number of pages 119	Price
	Security classification	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sourcespermission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Amanda Ode Date 2015-03-30

Environmental Toxins and Essential Trace Elements at Delivery and ADHD

Amanda Ode

LUND
UNIVERSITY

Copyright Amanda Ode

Faculty of Medicine
Department of Occupational and Environmental Medicine
ISBN 978-91-7619-121-7
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2015

Contents

Abstract 7

Svensk sammanfattning 9

List of papers 11

Abbreviations 12

Introduction 13

- Attention Deficit Hyperactivity Disorder (ADHD) 13
  - Overview 13
  - Characteristics and types of ADHD 14
  - Diagnosis 14
  - Causes and risk factors of ADHD 15
- Perfluorinated Compounds (PFCs) 17
  - Overview 17
  - Usage, exposure and metabolism 19
  - Prenatal exposure to PFCs 19
  - Effects on ADHD/ADHD symptoms 20
- Phthalates 21
  - Overview 21
  - Usage, exposure and metabolism 22
  - Prenatal exposure to phthalates 23
  - Effects on ADHD/ADHD symptoms 23
- Manganese 24
  - Overview 24
  - Usage, exposure and metabolism 25
  - Prenatal exposure to manganese 25
  - Effects on ADHD/ADHD symptoms 25
- Selenium 26
  - Overview 26
  - Usage, exposure and metabolism 27
  - Prenatal exposure to selenium 28

Effects on ADHD/ADHD symptoms 28
Aims and objectives 29
Materials and methods 31
Study population 31
Laboratory analyses 34
PFCs and cotinine 34
Phthalates 34
Manganese and selenium 34
Statistical analyses 35
Covariates 36
Confounders 36
Matching of ADHD children and controls 36
Power calculation 36
Results and discussions 37
Comparing the control group to the general population 37
PFCs 38
Phthalates 40
Manganese 41
Selenium 42
General discussion 42
Methodological considerations 42
Strengths 44
Generalizability 45
Conclusions 47
Main conclusions 47
Future studies 47
Acknowledgement 49
References 51

Abstract

Perfluorinated compounds (PFCs), phthalates, manganese and selenium are ubiquitous in the environment and humans and influence fetal growth and development. Register and biobank based case-control studies were conducted to investigate the relationship between prenatal exposure to these chemicals and attention deficit hyperactivity disorder (ADHD) in childhood.

The study base comprised children born in Malmö, Sweden, between 1978 and 2000 that were followed up until 2005. Children with ADHD (n=206) were identified at the Department of Child and Adolescent Psychiatry in Malmö. Controls (n=206) were selected from the study base and were matched for year of birth and maternal country of origin. Concentrations of PFCs, phthalates, manganese, and selenium were measured in umbilical cord serum samples collected from a maternity unit biobank in Malmö.

No associations between prenatal exposure to PFCs, phthalates, and manganese and ADHD in childhood were found. Selenium, which was hypothesized to protect against ADHD, was found to be positively associated with ADHD diagnosis in childhood. These findings need to be replicated in other larger studies before definitive conclusions can be drawn.

Svensk sammanfattning

Sambandet mellan exponering för miljögifter och mineraler i fosterlivet och risken att ha ADHD diagnos i barndomen

ADHD (uppmärksamhetsstörning med hyperaktivitet) är en neuropsykiatrisk funktionsnedsättning som drabbar barn och kan fortsätta till vuxen ålder. Antalet barn som diagnosticerats med ADHD har ökat under senare år. Om detta beror på att man fått mer kunskap om ADHD eller att man blivit bättre på att diagnosticera är svårt att uttala sig om. ADHD är relativt vanligt (i en vanlig skolklass finns det minst ett barn med ADHD) och varje riskfaktor som kan identifieras och som sedan kan elimineras/reduceras kan därför ha stor betydelse. De senaste åren har man i djurstudier funnit att djur exponerade för t.ex. bly eller polyklorerade bifenyler (PCBs) har uppvisat ADHD-liknande symtom. Man kan då spekulera över om även andra miljögifter kan orsaka ADHD. I detta avhandlingsarbete har vi studerat betydelsen av perfluorerade ämnen (PFCs) och ftalater. Dessa ämnen är s.k. hormonstörande ämnen, dvs. ämnen som kan påverka våra normala hormonfunktioner.

PFCs finns framförallt i produkter som avvisar vatten, fett och smuts; såsom kläder, livsmedelsförpackningar, möbler, och brandsläckningsskum. Ftalater används huvudsakligen i golvbeläggningar av plast inomhus men finns även i andra produkter som lim, färg, tapeter och kabel. Ftalater används också som mjukgörare i plast och därför finns de i diverse produkter som sandaler, pennskrin, suddgummin och i PVC-tryck på tröjor. Eftersom PFCs och ftalater finns i många produkter som vi dagligen använder har dessa kemikalier stor spridning i miljön och i människor.

För att kroppen skall fungera optimalt behöver människan mineraler i måttliga mängder. Överskott av till exempel mangan har kopplats till negativa effekter på hjärnan, särskilt på fosterhjärnan som är extremt känslig för påverkan av gifter. Nya rön visar dock att goda selennivåer skulle kunna minska den negativa effekten av höga mangannivåer. I detta avhandlingsarbete har vi därför även studerat sambandet mellan mangan- och selennivåerna i serum vid förlossningen och risken för ADHD-diagnos i barndomen.

I diagnosregistret vid Barn- och ungdomspsykiatriska kliniken i Malmö identifierades 206 barn med ADHD födda mellan 1978 och 2000. För varje barn med ADHD valdes ett kontrollbarn utan denna diagnos. Från kvinnoklinikens biobank i Malmö hämtades sparade navelsträngsprover samt blodprover från mödrar och i dessa analyserades nivåerna av PFC, ftalater, mangan och selen.

I delarbete I undersökte vi vad som påverkar variationen av PFC, dvs. varför vissa individer har låga nivåer medan andra har höga nivåer. Vi såg indikationer på att omföderskor har lägre nivåer av PFCs än förstföderskor och att kvinnor födda i nordiska länder hade högre PFC-halter jämfört med de som var födda någon annanstans. Generellt förklarade vi dock väldigt lite av variationen av PFC, vilket visar att vi inte kan förlita oss på t ex frågeformular utan faktiskt måste mäta i serum för att veta vilka nivåer en individ har.

I delarbetena II-IV studerade vi sambandet mellan PFCs, ftalater, mangan och selen och risken för ADHD-diagnos i barndomen. Resultaten visar inga samband mellan exponering för miljögifterna och ADHD däremot visar det sig att höga halter av selen i navelsträngsblod var positivt associerade med ADHD-diagnos. Trots att studier på människor tyder på att selen är en antioxidant och endast är länkad till god hälsa har djurstudier visat att höga halter av det goda selenet kan skada hjärnan. Våra studier talar alltså inte för några starka samband mellan PFC, ftalater eller mangan och risken för ADHD. För att utesluta även svagare samband krävs det dock betydligt större studier.

List of papers

Ode A, Rylander L, Lindh CH, Källén K, Jönsson BA, Gustafsson P, Olofsson P, lvarsson SA, Rignell-Hydbom A. Determinants of maternal and fetal exposure and temporaltrends of perfluorinated compounds. Environ Sci Pollut Res lnt. 2013;20(11):7970-8.

Ode A, Gustafsson P, Rylander L, Källén K, Jönsson BA, Olofsson P, lvarsson SA, Lindh CH, Rignell-Hydbom A. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS One. 2014:9(4):e95891.

Ode A, Rylander L, Källén K, Gustafsson P, Jönsson BA, Olofsson P, lvarsson SA, Lindh CH, Rignell-Hydbom A. Prenatal exposure to phthalate metabolites and ADHD in Childhood. Submitted.

Ode A, Rylander L, Gustafsson P, Lundh T, Källén K, Olofsson P, lvarsson SA, Rignell-Hydbom, A. Manganese and selenium concentrations in umbilical cord serum and attention deficit hyperactivity disorder in childhood. Environ Res. 2015:137:373-81.

Abbreviations

ADHD	Attention deficit hyperactivity disorder
BMI	Body mass index
CI	Confidence interval
DAT	Dopamine transporter
DBP	Di-butyl phthalate
DEHP	Di-2-ethyl-hexyl phthalate
DiNP	Di-isononyl phthalate
DRD	Dopamine receptor D
DSM	Diagnostic and statistical manual
LOD	Limit of detection
MCiOP	Mono-carboxy-iso-octyl phthalate
MECPP	Mono-2-ethyl-5-carboxypentyl phthalate
MEHHP	Mono-2-ethyl-5-hydroxyhexyl phthalate
MMUSB	Malmö maternity unit serum biobank
OR	Odds ratio
PCBs	Polychlorinated biphenyls
PFAS	Perfluoroalkyl sulfonates
PFCA	Perfluorocarboxylic acids
PFCs	Perfluorinated compounds
PFHxS	Perfluorohexane sulphonate
PFOA	Perfluorooctanoic acid
PFOS	Perfluoroocate sulfonate
SMBR	Swedish medical birth register

Introduction

Children today are at risk of exposure to 3000 new synthetic chemicals that come onto the market every year [1]. The majority of those chemicals that go through tests to determine toxicity are shown to have negative impact on health. Perfluorinated compounds (PFCs) and phthalates are examples of those chemicals that have been produced in huge amounts for more than fifty years. Children are also exposed to natural existing elements that are vital for good health but overdose or deficiency causes negative health effects. Examples of these trace elements are manganese and selenium. Experimental studies provide evidence that prenatal exposure to PFCs, phthalates, manganese and selenium has an impact on the developing brain of fetuses and small children. Recent studies, mainly on children, have linked those chemicals and trace elements to some neuropsychiatric disorders or symptoms, including attention deficit hyperactivity disorder (ADHD). The developing fetuses are extremely vulnerable to toxic chemicals and human studies on the impact of these chemicals on the risk of developing ADHD are very limited or lacking. It is therefore this thesis was conducted to add to the weak knowledge base within this field.

Attention Deficit Hyperactivity Disorder (ADHD)

Overview

ADHD is one of the most common neuropsychiatric conditions that affect about 5.29% of children and adolescents [2,3]. The onset of some symptoms of ADHD is usually before age 7 and tends to persist throughout childhood. Adult follow-up studies suggest that somewhere between one and two thirds (30–60%) of children treated for ADHD continue to have ADHD as adults [4,5]. Adults with history of this disorder have typically lower income, poor academic performance and underemployment, as well as higher school dropout rates, criminality, and substance abuse [5]. Furthermore, about 50–80% of individuals with ADHD have comorbid psychiatric disorders, such as learning disorders, depressive disorders, and anxiety disorders [5-7]. Thus, ADHD has a detrimental effect on individuals, their families and society as a whole.

Characteristics and types of ADHD

The three principal characteristics of ADHD are inattention, hyperactivity, and impulsivity [2]. Depending on which characteristics predominate, individuals with ADHD show different signs and symptoms.

The term "attention-deficit disorder" (ADD) was introduced in the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-III; American Psychiatric Association [APA], 1980) [8,9]. The DSM-III distinguished two types of ADD: with and without hyperactivity. DSM-III included a group of 14 symptoms and required 8 symptoms for a diagnosis. In the revised version of the DSM-III (DSM-III-R; APA, 1987), ADD was renamed to ADHD and the majority of the symptoms described hyperactivity and impulsivity with no possibility of an attentional disorder in the absence of hyperactivity or impulsivity [9,10]. The DSM-III-R diagnostic criteria, however, contained a set of criteria of severity. The subsequent editions of the DSM (DSM-IV and DSM-IV-R; APA, 1994 and 2000, respectively), defined three subtypes of ADHD: predominately inattentive type, predominately hyperactive-impulsive type, and combined type characterized by symptoms from both categories [9,11,12]. Criteria from each DSM-IV subtype required at least six of nine symptoms in each respective category. The combined subtype is the most commonly represented subtype accounting for from 50% to 75% of all ADHD individuals, followed by the inattentive subtype (20%–30%), and the hyperactive-impulsive subtype (less than 15%) [13]. In the next version of DSM (DSM-5; APA, 2013), the age onset criteria was increased from 7 years of age in DSM-IV to 12 years of age to reduce false negative diagnosis in adults [14]. To be diagnosed with ADHD, children up to the age 16 should have at least six symptoms while adolescents and adults only need five of the DSM-5 symptoms [14]. This change in the criteria may generate an increase in the prevalence of all subtypes of ADHD.

Diagnosis

Because there is no test or exam that can identify ADHD, a diagnosis depends on a complete evaluation. The assessment includes physical examination, parent and teacher report on development and behavior, clinical parent interviews, a measure of the cognitive functioning, and measures of sustained attention [15]. To be diagnosed with the disorder, a child must have symptoms for six or more months and to a degree that is greater than other children of the same age [15]. When a child meets the criteria listed in the DSM, a diagnosis is made.

Many researchers declare that ADHD diagnosis rates have been increased during the last decade. Increasing rates of the diagnosis may reflect increasing awareness and access to treatment, or changing in diagnosis criteria and clinical practices. There is no

evidence to suggest an increase in the number of children in the population who meet criteria for ADHD when standardized diagnostic procedures are followed [16].

ADHD diagnosis is not a reflection of the etiology of the disorder, but rather lists of symptoms with considerable overlaps with other psychiatric diagnoses. This may cause confusion among researchers. Although ADHD diagnosis is reliable, its diagnostic criteria do not have developmentally sensitive definitions that help doctors to distinguish between developmentally healthy levels of inattention, impulsivity, and hyperactivity from ADHD symptoms [2]. The diagnostic criteria do not provide guidelines to integrate diagnostic data gathered from parents, teachers, or the teenager with the disorder [2].

Causes and risk factors of ADHD

Although much research has been done on neuropsychological, neurochemical, neuroanatomical, environmental and genetic bases of ADHD, the exact cause of ADHD is not fully understood. ADHD is best described as "the final common pathway" of a number of possible etiological events [17]. Family, twin and adoption studies have provided evidence that genes play a substantial role in ADHD, with a hereditability estimate of 76%, ranging from 60% to 95% [5,18]. Molecular genetic research has identified genes that may be involved in etiology of ADHD. The catecholaminergic gene variants, particularly genes involved in dopamine and norepinephrine metabolism and signaling are shown to have an impact on the development of ADHD. Some of these genes are dopamine receptor genes (DRD2, DRD4, DRD5), dopamine transporter gene (DAT1), dopamine β-hydroxylate gene (DBH), catechol-o-methyl transferace gene (COMT), and monoamine oxidase A gene (MAOA) [19-21]. Gene variations of the noradrenergic and the serotonergic systems have also been linked to ADHD. Examples are variations in the noradrenergic transporter gene (NET), serotonin receptor genes (HTR1B), serotonin transporter genes (5-HTT), and tryptophan hydroxylase gene (TPH) [19-21]. Other candidate genes for ADHD are the acetylcholine and glutamate receptor genes, and snaptosomal-associated protein 25 gene (SNAP 25) [19-21]. Alterations in the catecholaminergic, noradrenergic, acetylcholinergic, and glutamatergic neurons affect diverse cognitive, motor, and endocrine functions with considerable overlaps as shown in Figure 1.

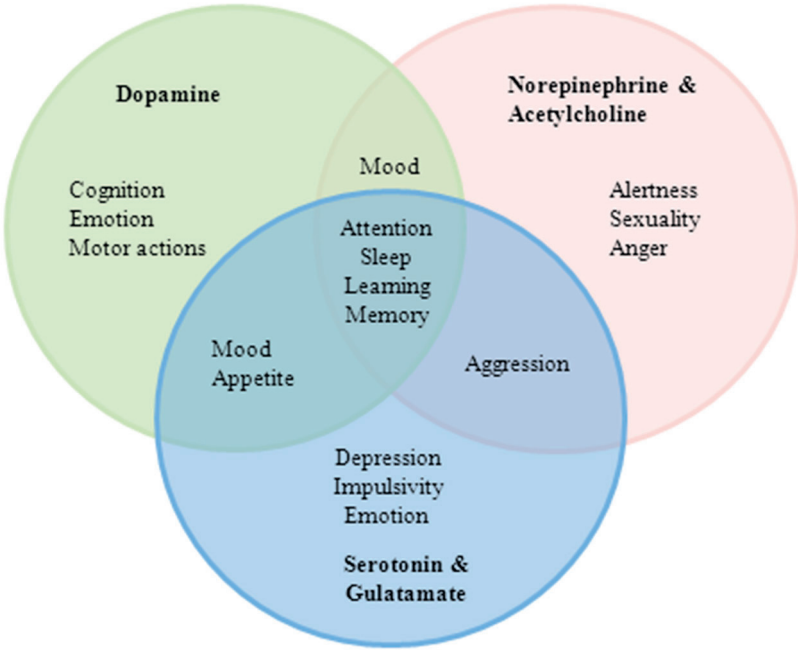


Figure 1
Overlap between functions of neurotransmitters in ADHD

Despite the large genetic basis of ADHD, twin studies indicate that pre- and postnatal environmental factors account for 12%–40% of the variance in twin ADHD scores [22]. Findings linking prenatal environmental risk factors to ADHD in humans are supported by animal studies. For example, prenatal exposure to toxins, drugs, maternal stress, and poor maternal diet has been linked to ADHD or ADHD symptoms in humans and experimental animals [23,24]. During pregnancy and the first years of the postnatal life, the developing brain is extremely vulnerable to toxins [25]. Animal studies have also shown that many toxins, such as nicotine, alcohol, and polychlorinated biphenyls (PCBs) can pass the placenta to the fetus and disrupts normal brain development [23]. The nature and severity of that disruption depend on the type of the toxin, as well as the level, duration, and timing of exposure. Prenatal exposure to nicotine, for example, disturbs neuronal cell proliferation and differentiation, and cause lesions in the catecholinergic and cholinergic systems [23,24]. Exposure to alcohol during pregnancy causes neuronal loss and neurodegeneration. PCBs pose a risk to the fetal brain by altering the thyroid functioning, neurotransmitter levels and dopamine metabolism [23,24]. Thus, early toxic effects on brain cells and their ability to perform specialized functions may lead to lifelong effects on learning, behavior, and health. Low birth weight, preterm birth, and maternal age at birth have been identified as risk factors for ADHD in numerous studies. About 13.8% of all

ADHD children have been suggested to be attributed to low birth weight [26]. Preterm births have been shown to be at 2.64-fold higher risk of having ADHD [27]. Both young and old maternal age are risk factors of ADHD [28] and teenage childbirth (<20 years) has been associated with 78% increased risk of ADHD [29]. Poverty, low paternal education and low income, as well as family size and structure, and birth order are examples of important postnatal psychosocial risk factors of ADHD [30,31]. Some of these factors may not cause ADHD but influence how symptoms are expressed, especially in those who are genetically susceptible [32]. Being a male is associated with higher risk of having ADHD which is probably because of ADHD being less disruptive in girls than in boys leading to under-diagnosis in girls [2].

The etiology of ADHD also involves the interplay of multiple genetic and environmental factors [23,33], which means that the disorder may be triggered by exposure to an environmental factor. For example, children carrying risk alleles of DAT1 or DRD4 gene are more likely to show higher levels of hyperactive-impulsive symptoms or DSM-IV ADHD when exposed to maternal smoking during pregnancy [33]. Other studies have suggested interactions of genes with indicators of psychosocial adversity, such as low income and adverse parental practices [34].

Perfluorinated Compounds (PFCs)

Overview

Perfluorinated compounds (PFCs) are a large group of commercially important man-made chemicals that have been produced at large amounts since the late 1940s [35]. PFCs contain only fluorinated carbon atoms, except for the carbon atom in the functional head group [35]. Depending on the functional group in the carbon chain, PFCs include perfluoroalkyl sulphonates (PFAS) and perfluorocarboxylic acids (PFCA) [35]. Perfluorooctane sulfonate (PFOS) (Figure 2) and perfluorooctanoic acid (PFOA) (Figure 3) are the most detected and studied PFAS and PFCA, respectively [36]. PFCs do not degrade under any condition, including treatment with chemicals, such as strong acids, alkalis, or oxidizing agents, photolysis, and microbial degradation [35]. Their resistance to degradation is due to the extremely strong bond between carbon and fluorine atoms, the presence of three pairs of nonbonding electrons around each fluorine atom, and the effective shielding of carbon by the fluorine atoms [35]. The structure of these chemicals makes them very stable, hydrophobic (water-repelling), and oleophobic (oil-repelling) [35].

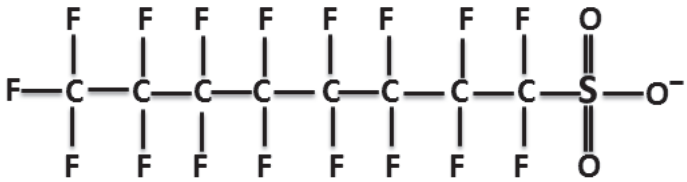


Figure 2
Structure of perfluorooctane sulfonate (PFOS)

Production of PFCs was mainly located to USA, Japan, and Europe [37]. The 3M Company was the major manufacturer of PFCs with the highest production between 1970 and the late 1990s [38,39].

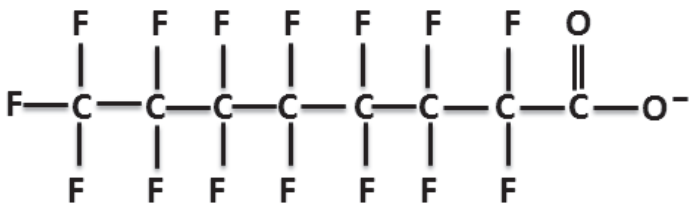


Figure 3
Structure of perfluorooctanoic acid (PFOA)

Organic fluorine was first detected in human serum in 1969 [40] and this finding was confirmed in 2001 after the development of a sensitive method for measurement of PFCs [41]. PFCs, mainly PFOS, were then detected in relatively high levels in the environment, wildlife, and in serum of non-occupationally exposed humans throughout the world [42]. In 2002, the 3M Company phased out its production of PFOS and related compounds because of concerns about their persistence in the environment and long-term health and environmental effects. To replace PFOS, other companies start manufacturing these compounds or other fluorinated compound to meet market demand for them [36].

Today there is limited information on the sources of emission and circulation of PFCs in the environment. Direct and indirect sources of PFCA emissions to the environment have been suggested [43]. Direct emissions result from the manufacture and use of commercial products containing PFCAs, while indirect sources in the environment include PFCAs as by-products from the manufacturing of other PFCs, or substances that may degrade to form PFCAs. Presence of PFCs in areas far away from sources of emissions, such as the Arctic, suggests that the compounds and their volatile precursors

are transported over long distances through atmospheric and aquatic transport pathways [43].

In Sweden, studies on guillemot eggs have shown that PFOS has been found in the Swedish environment since the 1960s and that the amount has increased since then, with a general increasing trend of between 7 and 11% per year [44]. Surveys of fish, such as perch, burbot, brown trout, whitefish and salmon, show that PFOS is widespread in relatively low concentrations in lakes and along the coast of Sweden [45]. PFOS is however also found in relative high levels in Swedish otters and minks [46].

Usage, exposure and metabolism

PFCs are widely used in many types of products that exploit the substances' surface active properties and high stability to form smooth, water-, grease-and dirt-repellent surface coatings for textiles, carpets, paper, tiles, and leather products, in detergents, in firefighting foams, in fat repellent paper, flooring, and many other products [35]. PFOA is mainly used in the manufacturing of polytetrafluoroethylene (PTFE, e.g. Teflon) [47]. PFOS related substances were the most prominent in the waterproofing and cleaning products. In Sweden PFCs have been mainly used in impregnating agents for textiles and leather and in detergents [37].

The human exposure routes to PFCs have been considered to be via inhalation of PFCs in outdoor and indoor air, and in household dust, through oral absorption of contaminated water or food, and PFCs in food packaging and PTFE-cookware, and via dermal contact with product containing PFCs [42]. Several studies suggest that consumption of fish from contaminated waters may be a source of PFOS exposure even for the Swedish population [45].

When entered the body, PFCs are distributed mainly to the blood and liver where they bind to proteins, mainly serum albumin and liver β-lipoproteins or fatty acid binding proteins [48]. In humans, PFCs are not metabolized, poorly eliminated, and therefore have serum half-lives up to 9 years [49,50].

Concentrations of PFOS and PFOA in the Swedish general population are generally comparable to the measured levels in other countries such as USA, Canada, Australia, and Japan. However, they are higher compared to countries such as Italy, India, and Belgium [42].

Prenatal exposure to PFCs

PFCs have been widely detected in pregnant women, in umbilical cord blood, and in amniotic fluid suggesting that the developing fetus can be exposed to those chemicals [51,52]. However, findings between studies vary. In several studies, PFOS and PFOA

could be detected in up to 100% of blood samples collected from pregnant women and from umbilical cord [53]. In another study conducted in Japan, the levels of maternal blood PFOS were highly correlated with the levels in cord blood while PFOA was detected in maternal but not in umbilical cord samples [52]. Correlations between maternal and fetal exposure to PFCs also vary across studies.

Some studies on humans have found associations between prenatal exposure to PFOS or PFOA and adverse birth outcomes, such as low birth weight, reduced birth length, decreased head circumference, and smaller abdominal circumference [51,53-55]. However, other studies did not find an association between prenatal PFC exposure and birth weight [56]. PFC blood levels detected in those studies were comparable to levels in the general population. Animal studies show similar findings, though typically at much higher levels than everyday human exposure [57,58].

Levels of PFCs in maternal or cord blood can be affected by several maternal or fetal factors. Some studies have investigated the relationship between ethnicity, maternal active smoking, mother's age and body mass index (BMI) at pregnancy, the number of previously born children (parity), infant sex, and gestational age and PFCs levels in pregnant women or newborns [52-55]. However, there are inconsistencies between results from different studies. Thus, more research on possible determinants of maternal and fetal exposure to PFCs is needed.

Effects on ADHD/ADHD symptoms

Animal data have revealed that PFCs cross the blood-brain barrier and accumulate in the developing brain [59-61]. The mechanism behind passage of PFCs through the blood-brain barrier is not extensively investigated. In a new study, PFOS was shown to trigger the opening of tight junctions in cells isolated from the human brain tissue that are responsible for formation of the blood-brain barrier [62]. Brain uptake of PFCs may occur even before the blood-brain barrier is formed [60].

Interest on high-lightning of the possible neural effects of PFCs on the developing brain has increased during the last years. Animal data suggest that PFCs are neurotoxic and exposure to them may affect neurobiological mechanisms of ADHD. Neonatal exposure to low doses of PFCs induced irreversible neurotoxic effects in adult mice and caused changes in behavior and habituation by altering the dopaminergic and cholinergic system [63,64]. PFCs also alter levels of neural proteins that are important for the formation and growth of the synapses [65] and induce apoptosis of neuronal cells [66].

In humans, a few studies performed so far found no or inverse associations between exposure to PFCs and ADHD or ADHD symptoms in school-age children. Two of those studies were cross-sectional based on parent-reported ADHD diagnosis [67,68]. One study found a positive relationship between ADHD and PFC levels in the blood

of children between 12 and 15 years [67] whereas the other study found an association with perfluorohexane sulfonate (PFHxS) [68]. A positive association was found between PFC levels and children's impulsivity [69]. Since prenatal exposure to PFCs caused behavioral problems in animals, limited human studies have been performed on this issue recently [51,70]. One study found that prenatal PFOS levels were weakly associated with delayed motor development in the first two years of life [51]. However, another study found that estimated prenatal exposure to PFOA was associated with increased IQ and decreased in characteristics of ADHD [71]. Thus, the potential relationship between prenatal PFC levels and ADHD need to be further investigated.

Phthalates

Overview

Phthalates are a family of high production organic chemicals produced at huge amounts since the 1930s [72]. These chemicals are mainly used as a lubricants or plasticizers [72]. Phthalates are classified into two groups depending on their molecular weights; high molecular weight phthalates (such as di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), and di-2-ethyl-hexyl phthalate (DEHP)) and low molecular weight phthalates (such as di-butyl phthalate (DBP), di-methyl phthalate (DMP), and di-ethyl phthalate (DEP)) [73]. The most commonly used plasticizers are DEHP, DiNP, and DiDP, which constitute more than 75% of phthalates used in Europe [74]. About 5000 to 6000 tons of phthalates are used in Sweden every year [75]. The generic chemical structure of phthalates is shown in Figure 4.

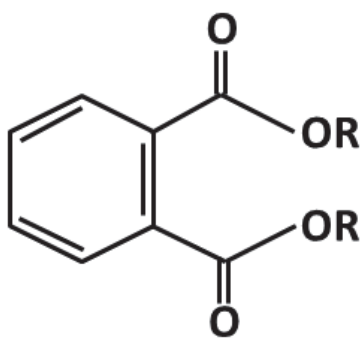


Figure 4
Generic chemical structure of the phthalates. R is an alkyl and/or aryl group: CH_3 (di-methyl phthalate), CH_2CH_3 (di-ethyl phthalate), $(CH_2)_3CH_3$ (dibutyl phthalate), $CH_2CH(CH_2CH_3)(CH_2)_3CH_3$ (di-2-ethyl-hexyl phthalate).

In recent years, some phthalates have raised concerns internationally and as a result some have been banned in Europe and in the United States [76].

Usage, exposure and metabolism

High molecular weight phthalates make plastics flexible, and therefore are widely used in products like vinyl flooring, medical devices, wall coverings, and food containers. Low molecular weight phthalates are often found in personal care products that carry a scent, such as cosmetics, lotions and perfumes [77]. Because they are not chemically bound to the plastics, phthalates can leach into the surrounding environment. As a result they are routinely found in indoor air [78] and dust [79] as well as in food and water [80]. This leads to exposure via inhalation, ingestion, and dermal contact [74].

Once phthalates enter the body, they become metabolized in two steps [76]. In the first step, phthalates are hydrolyzed to their respective monoesters in the intestines or other tissues. High molecular weight phthalates undergo further oxidative metabolism to become more polar and easier to excrete by the renal system. These hydrolyzed monoesters and oxidative metabolites can be excreted in the urine and feces unchanged or undergo the second step of metabolism, called conjugation, to form the hydrophilic glucuronide conjugate which are easily excreted into urine within 24 hours. Phthalates are therefore not stored in the body.

Phthalate metabolites could be detected in human fluids such as urine, blood, and breast milk [72]. Infants may experience higher exposure to phthalates than adults with respect to their body weight [73].

Prenatal exposure to phthalates

Phthalate metabolites cross the placenta and have been found in amniotic fluid [81], placental tissue [82], cord blood [83] and neonatal meconium [84]. When studying health effects of prenatal exposure to phthalates, researchers usually measure the metabolites in the urine of pregnant women due to the relatively low levels detected in other biological fluid samples [83,85]. However, some studies measured phthalate metabolite levels in cord plasma or serum but found no or weak correlations with corresponding maternal plasma or serum [86].

Animal and human studies show that prenatal exposure to phthalates has negative impact on reproductive health, gestational length, birth size, and neurodevelopment [77]. However, human data is limited.

Effects on ADHD/ADHD symptoms

Because fetuses and infants are more sensitive to toxic effects of chemicals and infants may experience higher exposures to phthalates than adults, prenatal and neonatal exposure phthalates disturbs brain development, even at levels lower than their No Observed Adverse Effect Level (NOAEL) or Low Observed Adverse Effect Level (LOAEL) [87].

Animal data show that exposure to phthalates causes hyperactivity reminding of the clinical picture of ADHD as observed in humans [88,89]. Prenatal exposure to phthalates, such as DBP and DEHP, led to adverse neurobehavioral effects in mice and rat offspring [90,91]. These neurobehavioral effects in animal offspring could be caused by hippocampal neuron loss and structural alternation, as well as change in the expression patterns of the DRD4 and DAT1 in the brain [90,92]. Upon DEHP exposure, several neurodegenerative areas in rat brain have been identified [93]. DEHP metabolites have been shown to disturb neurodevelopment by suppressing cell proliferation and promote cell differentiation in neurocytes *in vitro* [94]. Male pups appeared to be more susceptible to DBP exposure than females [95].

Human data show that some phthalate metabolites are associated with ADHD, ADHD symptoms, ADD, learning disability (LD), intelligence scores in school-aged children [96,97]. Prenatal exposure to phthalate metabolites has been associated with poor birth outcomes [98], neurological outcomes [99], behavioral problems [100], decreases in psychomotor and mental development [101], reduced masculine play [102], nonoptimal reflexes [103], and social impairment [104] in infants or children. Many of those finding are sex specific [100,101,103]. A recent study has identified an association between urine phthalate metabolite levels and ADHD children with evidence of DRD4 gene-phthalate interaction [105]. The potential relationship between prenatal phthalates and ADHD diagnosis has not been investigated yet and research within this issue would be appreciated.

Manganese

Overview

Manganese is a naturally occurring metal and an essential trace element comprising about 0.1% of the earth's crust (Figure 5) [106]. Manganese does not occur in the environment as a pure metal, but combined with other substances such as oxygen, sulfur, and chlorine. As with other elements, manganese does not break down in the environment. Manganese is found in the air, bedrock, and soil, and may be leached into the groundwater. The highest water levels of manganese are generally in wells [106].

As an essential nutrient, manganese is necessary for human health and is involved in many metabolic functions in the body, is required for the formation of healthy cartilage and bone and the urea cycle, plays a key role in wound-healing, and is included in certain protection against free radicals [107,108]. Although sufficient levels of manganese intake are essential for human health, both deficiency and overexposure are associated with negative health effects [109]. The primary target of manganese toxicity is the central nervous system [106].

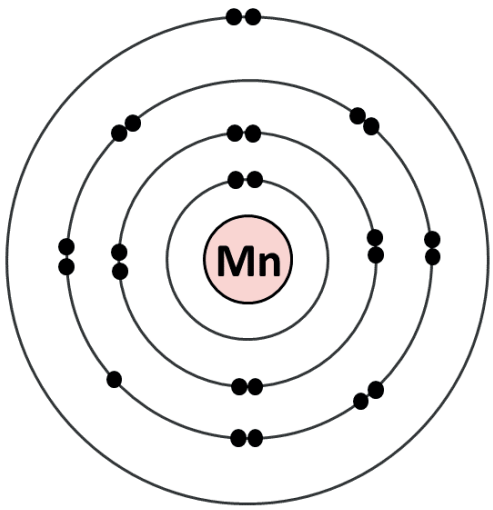


Figure 5
Manganese

Usage, exposure and metabolism

Because manganese is a natural component of the environment, humans are exposed to low levels of it in water, air, soil, and food [106]. Manganese is also used in many industrial and commercial applications, such as in production of steel, dry-cell batteries, fireworks, porcelain, and glass-bonding materials [106,110]. It is also found in the fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT) and in fungicides [110]. Emissions from the manufacture and release from usage of manganese-containing products are responsible for the additional manganese in air, soil, and water [106].

Once entering the body, manganese-containing chemicals break down to release manganese. As an element, manganese cannot be broken down and most manganese is eliminated from the body through the bile and only a small fraction leaves the body in urine and feces within a few days [106].

Manganese has been detected in biological samples including blood, urine, breast milk, and hair [106,111].

Prenatal exposure to manganese

During pregnancy, manganese in maternal blood crosses the placenta to maintain normal development of the fetus [112,113]. Manganese has been measured in cord blood plasma or serum of premature and full-term infants and their mothers [113]. Gestationally older infants have higher levels of manganese than preterm infant, suggesting that manganese levels may rise slightly as the fetus approaches birth [114]. Manganese is suggested to be actively transported through placenta because manganese levels in fetuses are usually higher than the corresponding maternal levels [112,115].

Epidemiological studies conducted on prenatal exposure to manganese have found negative effects of low and high levels of this nutrient on fetal growth, development, and survival [116,117].

Effects on ADHD/ADHD symptoms

Manganese is neurotoxic at high levels. This has been observed in occupationally exposed people inhaling high airborne manganese levels [106]. Chronic exposure to high levels of manganese results in a permanent neurological disorder known as manganism with symptoms that include tremors, difficulty in walking, and facial muscle spasms [106].

Manganese has been shown to cross the blood-brain barrier [118]. Epidemiological studies indicate that oral exposure to manganese, especially from contaminated water

sources, may lead to neurological effects in children, including poor school performance, impaired cognitive function, decreasing intelligence scores, abnormal performance on neurobehavioral tests, and increased oppositional behavior and hyperactivity [119-122]. There is cross-sectional evidence that high manganese blood levels in school-age children are associated with ADHD, but provide inadequate evidence to establish a causal relationship with this disorder [123].

Experimental and animal studies may indicate that the neurotoxicity effect of manganese could be due to selective destruction of dopaminergic neurons, and alteration in the brain expression of dopamine, dopamine receptors and dopamine transporter proteins [124-127]. Manganese may also trigger autoxidation or turnover of dopamine, leading to increased production of free radicals and other cytotoxic metabolites as well as depletion of cellular antioxidant defense mechanisms [128,129].

Because fetal brain is susceptible to injury by neurotoxins, it is of importance to study the effects of prenatal manganese exposure and neurobehavioral outcomes at later age.

Selenium

Overview

Selenium is a naturally occurring element that is widely distributed in the earth's crust (Figure 6) [130]. However, elemental selenium is seldom found naturally, but, as in the case of manganese, is usually combined with other substances [130]. It is commonly found in rocks and soil. Natural weathering of rocks and soils releases selenium in water, which may be taken up by plants. Weathering and volcanic eruptions release selenium into the air. Burning coal or oil also releases selenium into the air. Airborne selenium can enter the soil or surface water [130].

Selenium is an essential trace element needed for healthy body growth and function. It is a biologically active part of about 30 important proteins and enzymes, named selenoproteins, involved in antioxidant defense mechanisms, thyroid hormone metabolism, and redox control of intracellular reactions [131,132]. Low levels of selenium in the body have been associated with negative health effects, including increased risk of cancer [130,132].

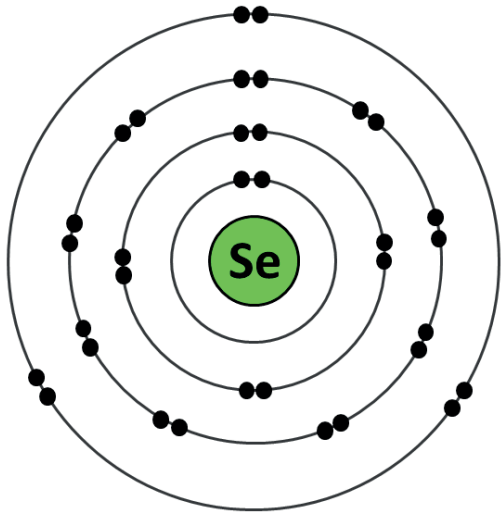


Figure 6
Selenium

Usage, exposure and metabolism

Selenium is ubiquitous element in the environment and can occur from both natural and anthropogenic sources [130]. Selenium is used in some photographic devices, plastics, paints, anti-dandruff shampoos, fungicides, vitamin and mineral supplements, and red and black glass [130,133].

The major intake of selenium in the general population is from diet, and to a lesser extent, from water intake and air [130]. There is a potential for exposure to higher-than-normal levels of selenium in people living in areas with naturally high selenium levels in the soil, or in people who occupationally exposed to selenium [130]. People may also be exposed to selenium from industrial sources [130].

In the body, selenium is metabolized via different pathways to selenide [131]. Selenide is then used either for selenoprotein biosynthesis or for biomethylation to metabolites that can be released in the breath and or excreted in urine [132]. The biological half-life of selenium in human body is estimated to be 100 days [134].

Selenium is usually measured in plasma or serum, but selenium metabolites have also been measured in urine [130].

Prenatal exposure to selenium

Selenium is needed for proper health and development of the fetus and therefore is transferred to the developing fetus through placenta. This trace element has been measured in pregnant women and their fetuses [135-137].

Effects of prenatal selenium on health of infants and children are not well explored, but there is some evidence that infants to selenium-deficient mothers are preterm and small for gestational age as well as having decreased cognitive function [138,139].

Effects on ADHD/ADHD symptoms

Although low selenium intake has detrimental effects on proper brain function in humans [140], acute oral exposure to selenium compounds causes aches and pains and irritability, as well as chills and tremors [130].

Several experimental studies show that selenium protects neonates from manganese neurotoxicity, reversed manganese induced impairment of dopaminergic neurotransmission in mice, and prevented the increased permeability of the blood-brain-barrier to toxins induced by free radicals [141-143]. An epidemiological study conducted on human fetuses found a high level of umbilical cord serum manganese increased the risk of low Neonatal Behavioral Neurological Assessment (NBNA) scores in neonates while high cord serum selenium reduced that risk [144]. However, neurotoxic effects of selenium have also been shown in laboratory studies as summarized in Vinceti et al. [145], including behavioral and neurological manifestations in mice, increase in brain dopamine levels and metabolites , alteration of cholinergic signaling and degeneration of cholinergic neurons, inhibition of glutamate uptake, induction of apoptosis in cultured mouse cortical neurons even at very low concentrations, and reduction of locomotor activity. These experimental studies may, or may not, necessarily be relevant to humans.

Because of the inconsistencies between experimental and human studies, and because effects of prenatal selenium on neurodevelopment and ADHD have not been investigated, research on this issue is needed.

Aims and objectives

PFCs, manganese, selenium, and phthalates have been shown to pass the placental and blood-brain barriers and exert diverse effects on the developing brain. Prenatal and postnatal exposure to environmental toxins, such as lead and PCBs has been shown to be associated with ADHD and this may be valid for other toxins with similar properties. PFCs, manganese, and phthalates have been linked to impaired behavior, decreased attention, and increased impulsivity and hyperactivity and therefore they could be even linked to ADHD. Selenium is known to be neuro-protective and recent studies show that it protects from manganese-induced neurotoxicity. For those reasons, we hypothesized that prenatal exposure to PFCs, manganese, and phthalates lead to ADHD diagnosis in childhood and prenatal selenium protects against ADHD that may be caused by manganese.

The main aim of the present studies was to investigate the associations between prenatal exposure to PFCs, manganese, selenium, and phthalates and ADHD diagnosis in childhood. Case-control studies based on registries and a biobank were performed to investigate the hypotheses. The secondary aim of the studies was to explore the associations between PFC, phthalate, manganese and selenium concentrations in maternal and cord serum, where we hypothesized positive correlations. Determinants of maternal and fetal exposure to PFCs and time trends of these compounds were also explored.

Materials and methods

Study population

Case-control studies were performed to investigate the hypotheses. At the Department of Child and Adolescent Psychiatry in the city of Malmö, 419 children born and living in Malmö between 1978 and 2000 with ADHD diagnosis were identified and were followed up until 2005. During the study period, the children with ADHD were diagnosed by one of ten experienced clinicians at the department using the DSM criteria. A child with suspected attention difficulties, hyperactivity and/or difficulties with impulse control is usually referred to the child and adolescent psychiatry by a special teacher and a school psychologist or by the parents. The assessment begins with gathering information about the child's general medical health condition and the child's development from birth until the present time. The school psychologist or the psychologist at the psychiatric clinic performs a cognitive testing with the Wechsler Intelligence Scale (WISC). The parents and the teacher are asked to fill in questionnaires like the Swanson, Nolan and Pelham IV (SNAP-IV), Conner's questionnaire or the 5-15 questionnaire which all cover the symptoms of ADHD. Parents are usually asked to fill in the Behaviour Rating Inventory of Executive Function (BRIEF) questionnaire concerning the child's executive functions in every-day life. Sometimes a member of the team at the clinic observes the child at school. The child's ability to concentrate is tested with the Test of Everyday Attention for Children (TEA-Ch) or with a computerized test of attention such as QB-Tech or the Integrated Visual and Auditory (IVA+). The child psychiatrist performs a paediatric examination with assessment of neurological soft-signs. The child's behaviour in different test situations and at the visits at the clinic is observed and registered. When all parts of the assessment have been performed, a team consisting of doctor, psychologist and sometimes a social worker meet and discuss the findings to come to a consensus decision concerning the diagnosis using DSM criteria. The DSM criteria DSM-III-R_{11} and DSM-IV_{12} were used before 1994 and from 1994 and onwards, respectively. Age at the time of diagnosis varied between 5 and 17 years, with most children being diagnosed between the ages of 8 and 12 years.

Using the personal identification numbers, children with ADHD were linked to the Swedish Medical Birth Register (SMBR) which contains demographic and obstetric information on nearly all (99%) the mothers and the infants in Sweden. Standardized record forms are used at all antenatal clinics, delivery units, and pediatric examinations

of newborn infants. Copies of these forms are sent to the National Board of Health, where they are computerized. Nearly all pregnant women receive free antenatal care. The MBR is annually linked with Statistics Sweden to obtain information on, for example, the infant's identification numbers and parental citizenship. Umbilical cord serum samples for children with ADHD were collected from the Malmö Maternity Unit Serum Biobank (MMUSB) using the personal identification numbers. Nearly all deliveries in Malmö take place at the Malmö University Hospital Maternity Unit, where blood samples from the mother and from the umbilical cord of the newborn have been collected at the time of delivery since 1969. Maternal blood was collected during early labor by venous puncture in vacutainer tubes, and the cord blood has been decanted into sterile sample tubes immediately after birth. The sample tubes were stored overnight in a refrigerator at +8 °C for sedimentation. The next morning, the sera were collected and frozen in polypropylene plastic test tubes at -20 °C until analysis. There are approximately samples from 70 000 deliveries stored at the biobank.

Controls were selected into two phases. In the first phase, for each ADHD child with an available umbilical blood sample in the biobank, three next-baby-born with serum samples of the same sex were selected as controls. However, a new publication by Gustafsson and Källén [28] revealed the impact of maternal country of birth on the diagnosis of ADHD. Thus, the benefit of matching for the maternal country of birth completely overrode that from matching for the infant's sex. Therefore, in the second phase, a pool of ten eligible controls per ADHD case were collected from the SMBR and were matched to the ADHD children for year of birth (±12 months) and country of birth of the mother. One of the three next-baby-born serum samples from the first phase matched for year of birth and maternal country of birth was used if no newborn in that eligible pool of controls had an available umbilical blood sample in the biobank. The selection procedure for cases and controls is presented in Figure 7.

To explore the concentrations of the environmental toxins and trace elements and the correlations between them in maternal and cord serum, the corresponding maternal sera for the control children were collected from the same biobank using the personal identification numbers. However, in Paper I additional control children without ADHD and autism diagnoses and matched mothers were used.

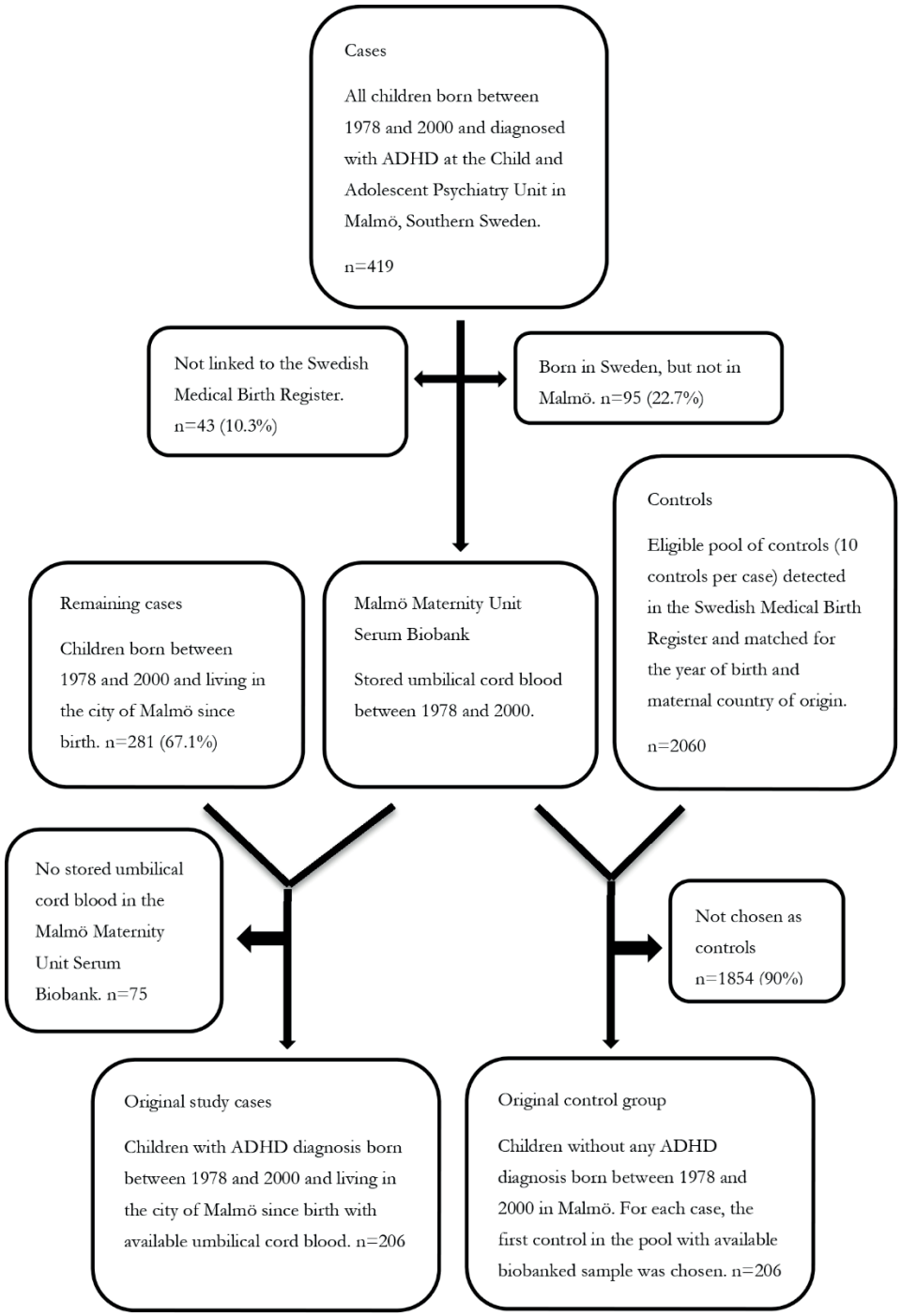


Figure 7
Flowchart for the selection procedure of the children with ADHD and controls.

Laboratory analyses

PFCs and cotinine

The analyses of PFHxS, PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), and cotinine were performed using a hybrid triple quadrupole linear ion-trap mass spectrometer (LC/MS/MS; UFLCXR, Shimadzu Corporation, Kyoto, Japan; QTRAP 5500; Sciex, Framingham, MA, USA) as described by Lindh et al. [146]. Only FPOS, PFOA, and PFNA could be analyzed in the serum samples. Usually we are able to analyze several more PFCs with the method but some factor, probably during the storage of the samples, resulted in a high background noise in the chromatograms making detection of some PFCs not possible. Although maternal serum contained only linear PFOS, both linear and branched PFOS were found in the cord serum. This could be due to that the cord blood had been contaminated with branched PFOS during sampling. Therefore, only results for linear PFOS were reported.

Phthalates

Phthalate metabolites were analyzed in serum samples by liquid chromatography tandem mass spectrometry (LC-MS) as described by Specht et al. [147]. Analyses included the secondary oxidized metabolites of DEHP (mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-carboxypentyl phthalate (MECPP), and DiNP (mono-4-methyl-7-hydroxy-octyl phthalate (7OH-MMeOP), mono-carboxy-iso-octyl phthalate (MCiOP), and mono-4-methyl-7-carboxyheptyl phthalate (7cx-MMeOP)). However, only MEHHP, MECPP, and MCiOP could be detected in the samples.

Manganese and selenium

The concentrations of manganese and selenium were determined by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo X7; Thermo Elemental, Winsford, UK) according to Barany et al. [148]. Cord serum lead concentrations were also measured in the samples, but unfortunately the samples were suspected to be contaminated with lead and therefore we decided not to use lead levels in our study. The contamination source is most probably the original sample vials that were used by the Serum Biobank.

Statistical analyses

Concentrations of the environmental toxins and trace elements below the limit of detection (LOD) in individual samples were replaced with the value of LOD or LOD/√2.

To make sure that selection bias was not introduced among the controls, maternal and infant characteristics of the controls were compared to the regional data (region of Skåne, Sweden) obtained from the SMBR. Since information on maternal smoking were first recorded in the register in 1982, data before that date was not included.

The following statistical tests were used in the present studies and thesis:

Mann-Whitney U test and Kruskal-Wallis test were used to compare characteristics between pregnant women and their fetuses and to compare concentrations of the toxins and trace elements between ADHD children and controls.

Spearman's rank correlation coefficient was used to investigate the correlations of the toxins and trace elements between maternal and umbilical cord serum samples.

The Wilcoxon matched-pairs signed-ranks test was used to investigate differences in concentrations of the toxins and trace elements between maternal and cord serum, and between ADHD children and controls, respectively.

Linear regression analysis was performed to study the time trends for PFCs during the study period. In this thesis, this test was also used to explore the determinants of maternal and fetal exposure to PFCs, phthalates, manganese, and selenium. Determinants of exposure to those chemicals and trace elements were investigated in native Swedish women and their fetuses as there were only few non-native Swedish individuals per country group.

Conditional logistic regression analysis was performed for investigating the association between PFCs, manganese and selenium, or the sum of DEHP metabolites (MEHPP and MECPP) and the DiNP metabolite MCiOP (in nM) in cord serum and ADHD diagnosis. When the exposure was used as continuous variable, the odds ratio was calculated for every one unit increase (ng/mL for PFCs, μg/L for manganese and selenium, or nM for the sum of phthalate metabolites) in the umbilical cord concentrations of the environmental toxins and trace elements. Exposure was also used as categorical variable and the odds ratio was calculated for concentrations at different cut-offs that were arbitrary selected. PFCs and phthalates are synthetic chemicals and they exert negative effects on human health usually at high levels. Thus, the upper quartile was arbitrarily selected as the cut-off value for those chemicals. Manganese concentrations above the 90th percentile and selenium concentrations below the 10th or above the 90th percentile were arbitrary selected as cut-offs due to that both manganese and selenium are essential nutrients and we believe that the waste majority have concentrations within the normal range.

Covariates

From SMBR we obtained data on birth weight, gestational age, birth length, head circumference, Apgar scores, infant sex, parity, maternal smoking during pregnancy, mothers' age at pregnancy, BMI, mode of delivery, and country of birth of the mother.

Confounders

The confounder factors considered in the associations between the investigated environmental toxins and trace elements and ADHD diagnosis in childhood were based on previous findings on factors that affect exposure levels of the toxins and trace elements and ADHD, respectively. Covariates that were suspected to be in the causal pathway between the exposure and the outcome, such as birth weight, were not adjusted for.

Matching of ADHD children and controls

Controls were matched to ADHD children for year of birth and maternal country of birth to make control for confounding more efficient since the sample size is relatively small. In a previous study used the same ADHD children of our studies and a reference group consisted of all individuals without ADHD diagnosis whose mothers were living in Malmö between 1986 and 1996, higher odds of having an ADHD diagnosis for native Swedish children compared to children of mothers born outside Sweden was found [28]. Since the proportion of immigrants is relatively high in Malmö, no matching for the maternal country of birth might result in a false positive relationship between the investigated toxins and manganese and ADHD.

Diagnosis criteria for ADHD have been changed during the study period. The main exposure route to PFCs, phthalates, manganese, and selenium is suggested to be through diet. As we believe that, at least, diet styles may differ between countries, we matched for the year of delivery to remove the effect of those differences in exposure levels to those toxins and trace elements and diagnosis on the results.

Power calculation

The power calculation was based on 206 ADHD children and matched controls (1:1). With the current setting, we had an 80% chance of detecting a difference in the levels of 0.20 standard deviations, with α value of 0.05, between cases and controls. For the analysis of the threshold effect (above the upper quartile), with α value of 0.05 and β value of 0.80, the lowest detectable odds ratio was 1.8.

Results and discussions

Comparing the control group to the general population

We compared the maternal and the infant characteristics of the study controls to the general population in Skåne from the SMBR. After exclusion of individuals who gave birth to children before 1982, we had 204 (out of 206) mother and infant pairs. There were only slight differences between the study cohorts and the population in Skåne as shown in Table 1. However, those differences were based on small number of individuals in the respective categories and not believed to affect our results.

Table 1
Characteristics of the mothers and the infants compared to the regional data from the medical birth register between 1982 and 2000.

	Cohort prevalence n (%)	Regional prevalence from Medical Birth Register (%)
Maternal age (years)		
< 20	6 (2.9)	(2.6)
20–24	41 (20.1)	(19.9)
25–29	74 (36.3)	(37.2)
30–34	55 (27.0)	(27.9)
35–39	19 (9.3)	(10.6)
≥ 40	9 (4.4)	(1.8)
Maternal country of origin		
Sweden	169 (82.8)	(84.6)
Country other than Sweden	35 (17.2)	(15.4)
Parity		
1	104 (51.0)	(41.8)
2	69 (33.8)	(36.1)
≥3	31 (15.2)	(22.1)
Smoking during pregnancy		
Unknown	6 (3.0)	(7.3)
Non-smoker	136 (66.7)	(75.5)
1–9 cigarettes	36 (17.6)	(15.1)
>9 cigarettes	26 (12.7)	(9.4)
Birth weight (grams)		
< 2500	5 (2.5)	(4.3)
2500–4000	149 (73.0)	(78.6)
>4000	50 (24.5)	(17.1)
Gestational age (weeks)		
<37	7 (3.4)	(5.9)
≥37	197 (96.6)	(94.1)

PFCs

In paper I, potential determinants that could influence PFC concentrations in mothers and their fetuses were investigated. The results revealed that parity, maternal country of birth, and gestational age were the potential determinants of maternal and fetal exposure to PFCs. We found positive correlations between cord PFOS, PFOA, and PFNA concentrations and gestational age. Maternal PFOS and PFOA concentrations

were higher in primiparous than multiparous women. These findings suggest that pregnancy might serve as a body purifier for PFCs through transmission of these compounds to the fetus during pregnancy and to the newborn through breastfeeding. There were also obvious differences in PFC levels between different countries of origin. Native Swedish women had higher levels of PFCs as compared to women originating from other countries but living in Sweden. This could be due to that non-native Swedish women were exposed to low levels of PFCs in their own countries before moving to Sweden and they have been living in Sweden for a short time. Unfortunately, we lack data on how long they have been living in Sweden. Other reasons for the differences in the levels might be differences in sources, distribution, exposure routes, genetic susceptibility, diets, and lifestyles between women with Swedish and non-Swedish origin. When linear regression analysis was performed to investigate the determinants of exposure to PFCs in native Swedish women and their fetuses PFOA, but not PFOS, was still higher in primiparous (β=0.7, p=0.001, adjusted R^2=0.071) than multiparous women. Cord PFOA showed the same trend, although not statistically significant (β=2.1, p=0.07, R^2=0.011). The results from the linear regression analysis also showed that maternal and cord serum PFOS increased with age (maternal serum: β=0.65, p<0.05, adjusted R^2=0.043; cord serum: 2.01, <0.05, 0.030). Since the investigated determinants only explained about 1% to 7% of the variation in maternal and cord PFC concentrations, we need to study the determinants of exposure in a larger study base before big conclusions are drawn.

In agreement with previous findings, maternal PFC concentrations were higher than umbilical cord PFC concentrations in our population. We examined the temporal trends of PFCs in native Swedish women and found that the serum PFC levels were roughly unchanged between 1978 and 2001 except for PFOS which increased with time. This finding is in accordance with a recent study in Swedish women for the period between 1987 and 2007 [149].

In paper II, we found no statistically significant associations between exposure to PFCs during pregnancy and ADHD diagnosis during childhood (Table 2), although the measured umbilical cord concentrations of PFOS were high compared to other countries [150-152]. A sub-analysis including only boys showed similar estimates as when both sexes were included (Crude ORs [95% CI] for every 1ng/mL increase in PFOS and PFOA were 0.96 [0.89–1.02] and 0.97 [0.91–1.03], respectively; and for the categorical PFOS and PFOA (>75th percentile) were 0.71 [0.4–1.2] and 0.97 [0.91–1.03], respectively). The negative finding in the present study could be due to that the statistical power was not strong enough to find weaker associations. A recent Danish study found no associations between prenatal exposure to PFCs and ADHD [153]. After adjustment for potential confounders, the highest quartile of maternal PFOS and PFHxS tend to be "protective" against having a child diagnosed with ADHD. When the authors mutually adjusted for all the PFCs in the same regression model, PFOS and PFHxS remained to be inversely associated with ADHD, but PFOA and PFNA became positively associated with ADHD. We found the same trend after including PFOA and

PFOA in the same model (Table 2). Whether this pattern occurs due to chance or multi-co-linearity bias, or it may reflect a true interactive or mixture effects, it is worth to pay attention. One might speculate that PFCs with the functional group sulfonate are negatively associated, and those with carboxylic acid are positively associated with ADHD. In a subsequent study on that Danish cohort, the authors suggested that the weak inverse association between prenatal exposure to PFCs and ADHD may appear if PFCs do not cause ADHD but have negative effect on fetal survival [154]. Thus, researchers should be aware of bias as a result from conditioning on live births in cohorts with high rates of perinatal death. For our population, this issue is not believed to affect our results due to the low perinatal death rates in Sweden.

Table 2
The crude and adjusted odds ratio with 95% confidence interval of attention deficit hyperactivity disorder (ADHD) and exposure to perfluorinated compounds.

	ADHD diagnosis		
	Crude OR (95% CI)	**Adjusted OR (95% CI)[a]**	**Adjusted OR (95% CI)[b]**
PFOS			
< 75th percentile	1	1	1
≥ 75th percentile	0.82 (0.51–1.3)	0.81 (0.50–1.3)	0.78 (0.44–1.3)
PFOA			
< 75th percentile	1	1	1
≥ 75th percentile	1.03 (0.65–1.6)	1.07 (0.67–1.7)	1.3 (0.73–2.2)

OR, odds ratio; CI, confidence interval; [a]Adjusted for smoking during pregnancy parity, and gestational age; [b]Adjusted for all covariates in a) and PFCs

Phthalates

In paper III, phthalate metabolites could be found in umbilical cord serum with good correlation with maternal serum phthalate metabolite concentrations, but no association between prenatal exposure to phthalate metabolites and ADHD in childhood was found. These findings did not change when male infants were restricted to the analyses (OR [95% CI] for each 1nM increase in ∑DEHP metabolites was 0.99 [0.94–1.04] and MCiOP OR=0.98 [0.94–1.02], for categorical ∑DEHP (>75th percentile) OR=1.1 [0.62–1.9], and MCiOP OR=0.86 [0.49–1.5]). Phthalates have short biological half-lives in the body tissues and the levels of phthalate metabolites are usually lower in serum than urine. Misclassification of exposure to phthalate metabolites may be present in this study since we had a single serum sample collected at delivery. In addition, phthalate metabolites in the urine show within-subject

variability and exposure assessment should therefore not be based on one point measurement [155]. However, if sources and daily patterns of exposure remain relatively unchanged, a single measurement may reflect a typical measurement at any time during pregnancy. As with the other studies of this thesis, the present study is also limited by its modest sample size, and consequently the limited statistical power to detect subtle effects.

Results from linear regression analysis showed that MECPP increased in fetuses to second-hand smokers (β=0.66, p=0.003, adjusted R^2=0.030) while MCiOP decreased in fetuses to non-smokers (β=-1.1, p=0.045, adjusted R^2=0.017) compared to fetuses to smoking women. These results have not been seen by other investigators before and could be due to chance findings.

Manganese

Paper IV showed no associations between fetal exposure to manganese and ADHD diagnosis in childhood. When the analyses were restricted to male infants, the estimates remained relatively unchanged (OR [95% CI] for each 1μg/L increase in manganese was 1.01 [0.94–1.1], and for categorical manganese (>90th and ≤90th (ref) percentile) was 1.1 [0.51–2.3]).

Serum samples were the only available biological matrix, since the study design is based on a historical serum biobank. Anyhow, serum levels along with urine levels are the least recommended for assessing manganese exposure since about 66% of manganese is bound to hemoglobin in the erythrocytes and is also found inside leukocytes as cofactor of several enzymes [156], with a short half-life of about 40 days [157]. Maybe that was the reason that no association was observed with manganese levels, associated with fact of the relatively small sample size. This issue is even more important when we opted by stratifying in 90th percentiles which resulted in few fetuses with the highest manganese levels in the upper percentile, which is not the case in the study performed by Yang et al. [144].

In this paper we also found that manganese concentrations were higher in cord than maternal serum, which is in accordance with previous studies. This suggests that manganese is actively transported through placenta to satisfy the fetus´s need of it. Among the investigated factors that could influence manganese levels in native Swedish women and their fetuses, only infants born between 37 and 41 weeks had lower cord manganese levels compared to those born after week 41 (β=-2.5, p=0.006, adjusted R^2=0.013). Manganese levels have been found to increase with advanced gestational age in other studies [50,114,115], which indicates that high manganese demands of the developing fetus during pregnancy result in increased blood manganese levels. Human data show that it is difficult to estimate past exposure to manganese by analysis of

manganese levels in blood, urine, or tissues due to several factors [106]. First, manganese is found in all human tissues and fluids as humans are mainly exposed to it through diet. Therefore, above average exposure is a result of an increase over a variable baseline. Second, manganese has a short half-life and is rapidly excreted in the bile, feces, and very little in the urine. Third, manganese metabolism is affected by homeostatic regulation, so above average exposures may induce small changes in fluid or tissue levels [106]. Manganese concentrations in our population are therefore representative for the third trimester of the pregnancy.

Selenium

In paper IV, high selenium concentrations surprisingly increased the odds of having an ADHD diagnosis in childhood. Odds ratios increased were roughly unchanged when analyses were restricted to male infants (OR [95% CI] for each 1μg/L increase in selenium was 1.02 [0.99–1.05]; for fetuses with selenium levels <10th percentile (10-90th percentile as ref) OR=1.8 [0.78–4.49]; for those with levels >90th percentile OR=3.7 [1.5–8.8]). Although selenium has been suggested to be neuroprotective in humans and animals, neurotoxic effects due to high levels of selenium have been seen in experimental studies mentioned earlier in this thesis. Thus, it is of importance to investigate the effects of selenium on neurodevelopment in humans before any conclusions can be drawn. As mentioned earlier, selenium is present in about 30 selenoproteins [131]. For this study, total selenium was measured and therefore it is not possible to determine whether the positive relationship between selenium and ADHD is attributed directly to this trace element per se or to one of the selenoproteins.

We found higher maternal serum selenium levels compared to cord serum levels, a finding reflects the literature to date. Among the investigated factors that were suspected to influence serum selenium levels, only decreased parity was negatively correlated with maternal serum levels (β=-8.5, p=0.01, adjusted R^2=0.055). This finding is not in agreement with two previous studies that showed no association between plasma selenium concentrations in pregnant women and parity [158,159].

General discussion

Methodological considerations

Some types of bias may be introduced in our studies. First, selection bias might be presented in our studies since we did not find umbilical cord samples for all children with ADHD in the biobank or when the amount of the serum was not enough to

measure the levels of the toxicants in some samples. However, we don´t believe that those cases with no cord serum in the biobank or with small amount of serum have the highest levels of the toxicants as our population of pregnant women were not aware of the study purpose prior to collection of their blood samples.

Second, design bias may affect the statistical power of the studies. During our study period, the clinical diagnostic criteria for ADHD were changed from the definition in DSM-III-R to the definition in DSM-IV, where DSM-IV is regarded as more inclusive and therefore yield a higher prevalence of ADHD [160]. About 93%–97.5% individuals who fulfill a diagnosis of ADHD according to DSM-III-R also fulfill the diagnostic criteria according to DSM-IV [161-163]. In addition, 85% and 60% of individuals with ADHD according to DSM-IV that also fulfilled diagnostic criteria according to DSM-III-R [161,163]. Thus the overlap between ADHD diagnoses according to DSM-III-R and DSM-IV is considerable. The more inclusive diagnosis used in the latter part of the study probably includes some less severe cases which might slightly weaken possible statistical associations between exposure to the chemicals and trace elements and having an ADHD diagnosis.

Third, our studies may be subject to problems with misclassifications of the exposure and the outcome. We might have misclassification of the exposure for those toxins with short biologic half-lives, such as phthalate metabolites and manganese, which could result in huge variation in the levels. Therefore, it is hard to establish causality in the relationship between prenatal exposure to those toxins and ADHD. It should be emphasized that there is no systematic differences related to the exposure as this problem is presented among the children with ADHD and the controls. This misclassification might result in decreased differences between the cases and the controls and, in our case, led to underestimation of the hypothesized association between the exposure and ADHD.

There is a possibility that some of the control children could have been diagnosed with ADHD elsewhere than Malmö during the study period, which would lead to misclassification of the outcome. There is also a possibility that some of the control children had ADHD symptoms but not been identified. A population-based study in Malmö found a frequency of ADHD in children between five and ten years of about 4% as cited in Gustafsson and Källén [28]. The frequency of ADHD in our population of children with this disorder was about 0.7% [28]. The low frequency of ADHD in our population may be due to underdiagnosing, which indicates that families coping with the most severe cases seek professional psychological help. Therefore, the children who have been diagnosed have been accurately assessed and represent only one-fifth of all the children with ADHD. Thus, children with ADHD not identified should constitute no more than 4% to 5% of the children in the reference group. Assuming that 5% of the reference group that moved outside Malmö after birth was misclassified regarding the outcome, the results observed in our studies were not believed to be affected by the extremely few numbers of those individuals.

We also had a sex bias with six times more males than females. A lower diagnosis rate among females in childhood has been shown in many studies because girls with ADHD are less likely to show obvious problems since they usually have the inattentive form of ADHD compared with boys [164]. Since girls with ADHD are harder to spot than boys, almost exclusively boys were diagnosed with ADHD using DSM-III before 1994. The more inclusive diagnosis used in the latter part of the study probably includes some less severe cases and included a small number of girls. However, the skewed sex distribution in our studies did not affect our results when analyses were restricted to male infants.

We did not have information about the paternal genetic component of ADHD and the socioeconomic status (SES). ADHD was not a well-defined disorder for Swedish child psychiatry before the 1970s and the parental history of the ADHD disorder was therefore not available for our study population. Although we could not adjust for SES, we had information about self-reported maternal smoking and we also measured cotinine levels in the maternal and cord blood. We could therefore have an idea about the maternal SES since the latter and smoking are often inversely associated with each other [164]. Although both genetics and SES are risk factors for ADHD, information about their relationship to the investigated toxins and trace elements is not available. Thus, evaluating these factors as potential confounders is not a significant concern for our studies.

Strengths

Our studies have several important strengths. Most of the previous findings on the relationship between PFCs, phthalates, and manganese were based on questionnaires or tests covering some of ADHD symptoms or parent and/or teacher reported ADHD diagnosis or symptoms. Our studies are more reliable in the sense that it is based on clinical diagnosis of ADHD made at the same psychiatric clinic by the same doctors through the whole study period.

The present studies are based on analyzed blood samples from the prenatal period, which we believe is the most susceptible exposure window, whereas previous findings on this issue were based on cross-sectional study design with difficulties in inferring causality. These serum samples were analyzed by specially trained staff in a national and international reference laboratory using state-of-the-art equipment to assure consistent and high quality testing.

We also had access to a high quality register, the SMBR, with extensive data on potential confounders. Although, additional data on maternal alcohol intake and socioeconomic status, that are currently not available in the register, would be appreciated.

It should be noted that our population is relatively homogenous in the sense that the pregnant women are from the same geographical area. Thus, we were not concerned about the differences in the background exposure to those toxins and trace elements that may otherwise arise between different geographical areas.

Generalizability

Generalizability is already difficult in research on ADHD because of the great heterogeneity among the children with this disorder and the diversity of the psychological (i.e., cognitive and behavioral) features associated with it. Children with severe ADHD symptoms are easier to identify are more likely to be referred to psychiatric clinics than those with a mild form of the disorder. These children are usually of the male sex. Although we lack information on the subtypes of ADHD for the children in the present studies, the children are more likely to have the severe form of the disorder. During the early years of the study period, ADHD was not fully recognized by the Swedish child psychiatry service and the diagnostic criteria mainly emphasized hyperactivity and impulsivity. When DSM-IV criteria were introduced in 1994, some children with the inattentive forms of ADHD might be presented in our population. Still, hyperactive and impulsive children were believed to be the most prominent children in our studies even after 1994 due to the fact that the inattentive children may go unnoticed and undiagnosed. Our studies may be therefore more applicable for children with severe ADHD symptoms.

Conclusions

Main conclusions

The present studies show that pregnant women and their fetuses are exposed to PFCs, phthalates, manganese and selenium but reveal no support for associations between prenatal exposure to PFCs, phthalates, and manganese and ADHD diagnosis in childhood. On the other hand, selenium was associated with higher odds of having ADHD, which was against our hypothesis that selenium would protect against having ADHD diagnosis. These findings, especially for selenium, need to be replicated in larger studies before any definitive conclusions can be drawn.

Future studies

We had the unique opportunity to investigate the relationship between prenatal exposure to environmental toxins and trace elements in biobanked serum samples and clinical ADHD diagnosis. However, we have learned that our studies may not be the optimal way to study this relationship due to several factors discussed throughout this thesis. Ideal future research would be that based on prospective, longitudinal birth cohorts (with the possibility of combining data from different cohorts) and using repeated measurements of the environmental toxins and trace elements beginning in pregnancy and continuing throughout childhood. By performing such studies it would be a good way to determine if pre- or postnatal exposure is most relevant for having ADHD. The most optimal way to study the effect of environmental toxins on ADHD is probably to conduct adoption or family studies to control for the genetic contribution to ADHD. Studies on gene-environment interactions are also likely to be of importance for understanding the effect of environmental exposures to toxins in genetically susceptible individuals. Since we are exposed to these toxins and trace elements in daily life and these have been linked to many health outcomes in humans and animals, toxicological studies are needed to generate dose-response curves for estimation of the toxicity for each chemical.

Acknowledgement

This dissertation is an outcome of a four-year research guided and supported by lovely people. It is a great pleasure for me to acknowledge the contributions of those people in making this dissertation possible.

I do not have enough words to express my deepest gratitude and sincere appreciation to my main supervisor, Anna Rignell-Hydbom. I would not have been able to achieve learning and complete this thesis in the same manner without the help, support, solid knowledge, and valuable guidance from her.

I have been fortunate to have the best co-supervisors I could have hoped for. I am deeply grateful for Lars Rylander who supported and encouraged me to believe in my abilities throughout this project. Without him I would not learn statistical concepts and methods in the same way as I do now. I am gratefully indebted to Karin Källén for her enthusiasm, and immense knowledge helping me to develop my insignificant background in statistics. Her stimulating suggestions, challenging questions, and insightful comments helped me to bring this research project to fruition. Special thanks to Peik Gustafsson for the support and encouragement and for responding to my questions and queries so promptly whenever I was in need.

Besides my supervisors, I would like to thank the other members of this project, Per Olofsson, Sten Ivarsson, and Nils Haglund for the stimulating meetings during the past four years. The thesis has greatly benefited from the valuable comments and insightful suggestions made by Per and Sten who, beside my supervisors, have read through the manuscripts.

Special and warm thanks to Bo Jönsson, Thomas Lindh, and Christian Lundh as well as Åsa Amilon, Agneta Kristensen, and Anna Akantis and at the laboratory of AMM for analyzing the serum samples. I am so grateful to Bosse and Thomas for the professional insights while reading my manuscripts. My research would not have been possible without their help.

While talking about the manuscripts, I must express my very profound gratitude to Robert Williams for the professional language editing making my papers much better.

I would like to express my unrestrained appreciation to Kristina Jakobsson and Jonas Björk for the stimulating discussions and valuable suggestions during my final seminar which have contributed greatly to the improvement of the thesis. I would also like to extend my sincerest thanks to Jonas for his help in many occasions and for sharing his great knowledge during courses.

Special thanks to Professor Tom McNeil for his concern, encouragement, and for the nice conversations during these four years. I am so happy to know such a wonderful person like him.

I would like to give my profound gratitude to my lovely roommate, Hanna Weimann for her concern, support, and encouragement. One of the best outcomes from these past four years is finding such a wonderful friend like her.

I have been blessed with a friendly and cheerful group of people in my daily work. I deeply thank Emilie, Kristoffer, Kristina M., Carita, Ebba, Zoli, Andrea, Anna Lindgren and Anna Larsson, Tahir, Theo, Yiyi, Kristin, Anna J., Kerstin, Jenny, Gert-Åke, Ewa, Catarina, Lisbeth, Jens, Håkan, Maria, Lothy, Camilla, and Jonatan. Special thanks to Ralf for his help making my computer and other technical equipment work properly. Many thanks go to lovely Yulia for the administrative help.

I would also like to express the profound gratitude and heartfelt love to my beloved mother, sisters and brothers, and all my close relatives for their love, their spiritually and materially support and for their encouragement with the best wishes throughout my life.

I would also like to thank all of my friends outside the academic world. My life is delighted by them.

This thesis is dedicated to my beloved family, William and Alice for always being there cheering me up and standing by my side through the good and bad times. This accomplishment would not have been possible without them. I am eternally grateful to them.

References

1. Landrigan PJ (1998) Environmental hazards for children in USA. Int J Occup Med Environ Health 11: 189-194.
2. Biederman J, Faraone SV (2005) Attention-deficit hyperactivity disorder. Lancet 366: 237-248.
3. Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA (2007) The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 164: 942-948.
4. Lara C, Fayyad J, de Graaf R, Kessler RC, Aguilar-Gaxiola S, et al. (2009) Childhood predictors of adult attention-deficit/hyperactivity disorder: results from the World Health Organization World Mental Health Survey Initiative. Biol Psychiatry 65: 46-54.
5. Wu J, Xiao H, Sun H, Zou L, Zhu LQ (2012) Role of dopamine receptors in ADHD: a systematic meta-analysis. Mol Neurobiol 45: 605-620.
6. Biederman J, Newcorn J, Sprich S (1991) Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am J Psychiatry 148: 564-577.
7. Spencer T, Biederman J, Wilens T (1999) Attention-deficit/hyperactivity disorder and comorbidity. Pediatr Clin North Am 46: 915-927.
8. American Psychiatry Association (1980) Diagnostic and statistical manual of mental disorders (DSM-III), 3rd ed. American Psychiatric Association, Washington DC.
9. Bernfeld J (2012) ADHD and factor analysis: are there really three distinct subtypes of ADHD? Appl Neuropsychol Child 1: 100-104.
10. American Psychiatry Association (1987) Diagnostic and statistical manual of mental disorders (DSM-III-R), 3rd ed. Revised. American Psychiatric Association, Washington DC.
11. American Psychiatry Association (1994) Diagnostic and statistical manual of mental disorders, 4th ed. American Psychiatric Association, Washington DC.
12. American Psychiatry Association (2000) Diagnostic and statistical manual of mental disorders, 4th ed. Text revision. American Psychiatric Association, Washington DC.
13. Spencer TJ, Biederman J, Mick E (2007) Attention-deficit/hyperactivity disorder: diagnosis, lifespan, comorbidities, and neurobiology. J Pediatr Psychol 32: 631-642.
14. American Psychiatry Association (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th ed. American Psychiatric Association, Arlington, VA.
15. Goldman LS, Genel M, Bezman RJ, Slanetz PJ (1998) Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. Council on Scientific Affairs, American Medical Association. JAMA 279: 1100-1107.

16. Polanczyk GV, Willcutt EG, Salum GA, Kieling C, Rohde LA (2014) ADHD prevalence estimates across three decades: an updated systematic review and meta-regression analysis. Int J Epidemiol 43: 434-442.
17. Jensen PS (2000) Current concepts and controversies in the diagnosis and treatment of attention deficit hyperactivity disorder. Curr Psychiatry Rep 2: 102-109.
18. Freitag CM, Rohde LA, Lempp T, Romanos M (2010) Phenotypic and measurement influences on heritability estimates in childhood ADHD. Eur Child Adolesc Psychiatry 19: 311-323.
19. Faraone SV, Khan SA (2006) Candidate gene studies of attention-deficit/hyperactivity disorder. J Clin Psychiatry 67 Suppl 8: 13-20.
20. Franke B, Neale BM, Faraone SV (2009) Genome-wide association studies in ADHD. Hum Genet 126: 13-50.
21. Gizer IR, Ficks C, Waldman ID (2009) Candidate gene studies of ADHD: a meta-analytic review. Hum Genet 126: 51-90.
22. Langley K, Holmans PA, van den Bree MB, Thapar A (2007) Effects of low birth weight, maternal smoking in pregnancy and social class on the phenotypic manifestation of Attention Deficit Hyperactivity Disorder and associated antisocial behaviour: investigation in a clinical sample. BMC Psychiatry 7: 26.
23. Mill J, Petronis A (2008) Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (ADHD): the potential role of epigenetic processes in mediating susceptibility. J Child Psychol Psychiatry 49: 1020-1030.
24. Froehlich TE, Anixt JS, Loe IM, Chirdkiatgumchai V, Kuan L, et al. (2011) Update on environmental risk factors for attention-deficit/hyperactivity disorder. Curr Psychiatry Rep 13: 333-344.
25. Grandjean P, Landrigan PJ (2006) Developmental neurotoxicity of industrial chemicals. Lancet 368: 2167-2178.
26. Mick E, Biederman J, Prince J, Fischer MJ, Faraone SV (2002) Impact of low birth weight on attention-deficit hyperactivity disorder. J Dev Behav Pediatr 23: 16-22.
27. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ (2002) Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. JAMA 288: 728-737.
28. Gustafsson P, Källén K (2011) Perinatal, maternal, and fetal characteristics of children diagnosed with attention-deficit-hyperactivity disorder: results from a population-based study utilizing the Swedish Medical Birth Register. Dev Med Child Neurol 53: 263-268.
29. Chang Z, Lichtenstein P, D'Onofrio BM, Almqvist C, Kuja-Halkola R, et al. (2014) Maternal age at childbirth and risk for ADHD in offspring: a population-based cohort study. Int J Epidemiol 43: 1815-1824.
30. Carballo JJ, Garcia-Nieto R, Alvarez-Garcia R, Caro-Canizares I, Lopez-Castroman J, et al. (2013) Sibship size, birth order, family structure and childhood mental disorders. Soc Psychiatry Psychiatr Epidemiol 48: 1327-1333.
31. Froehlich TE, Lanphear BP, Epstein JN, Barbaresi WJ, Katusic SK, et al. (2007) Prevalence, recognition, and treatment of attention-deficit/hyperactivity disorder in a national sample of US children. Arch Pediatr Adolesc Med 161: 857-864.

32. Thapar A, Cooper M, Jefferies R, Stergiakouli E (2012) What causes attention deficit hyperactivity disorder? Arch Dis Child 97: 260-265.
33. Thapar A, Harold G, Rice F, Langley K, O'Donovan M (2007) The contribution of gene-environment interaction to psychopathology. Dev Psychopathol 19: 989-1004.
34. Nigg J, Nikolas M, Burt SA (2010) Measured gene-by-environment interaction in relation to attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49: 863-873.
35. Kissa E (2001) Fluorinated Surfactants and Repellants. NY, USA: Marcel Dekker Inc.
36. Lindström AB, Strynar MJ, Libelo EL (2011) Polyfluorinated compounds: past, present, and future. Environ Sci Technol 45: 7954-7961.
37. Kärrman A, van Bavel B, Jarnberg U, Hardell L, Lindstrom G (2006) Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere 64: 1582-1591.
38. Haug LS, Thomsen C, Becher G (2009) Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ Sci Technol 43: 2131-2136.
39. Olsen GW, Huang H-Y, Helzlsouer KJ, Hansen KJ, Butenhoff JL, et al. (2005) Historical Comparison of Perfluorooctanesulfonate, Perfluorooctanoate, and Other Fluorochemicals in Human Blood. Environ Health Perspect 113: 539-545.
40. Taves DR (1968) Evidence that there are two forms of fluoride in human serum. Nature 217: 1050-1051.
41. Hansen KJ, Clemen LA, Ellefson ME, Johnson HO (2001) Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. Environ Sci Technol 35: 766-770.
42. Fromme H, Tittlemier SA, Volkel W, Wilhelm M, Twardella D (2009) Perfluorinated compounds--exposure assessment for the general population in Western countries. Int J Hyg Environ Health 212: 239-270.
43. Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH (2006) Sources, fate and transport of perfluorocarboxylates. Environ Sci Technol 40: 32-44.
44. Holmstrom KE, Jarnberg U, Bignert A (2005) Temporal trends of PFOS and PFOA in guillemot eggs from the Baltic Sea, 1968--2003. Environ Sci Technol 39: 80-84.
45. Berger U, Glynn A, Holmstrom KE, Berglund M, Ankarberg EH, et al. (2009) Fish consumption as a source of human exposure to perfluorinated alkyl substances in Sweden - analysis of edible fish from Lake Vattern and the Baltic Sea. Chemosphere 76: 799-804.
46. Butt CM, Berger U, Bossi R, Tomy GT (2010) Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci Total Environ 408: 2936-2965.
47. Lehmler HJ (2005) Synthesis of environmentally relevant fluorinated surfactants--a review. Chemosphere 58: 1471-1496.
48. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, et al. (2007) Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 99: 366-394.

49. Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, et al. (2007) Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115: 1298-1305.
50. Spencer A (1999) Whole blood manganese levels in pregnancy and the neonate. Nutrition 15: 731-734.
51. Fei C, McLaughlin JK, Lipworth L, Olsen J (2008) Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environ Health Perspect 116: 1391-1395.
52. Inoue K, Okada F, Ito R, Kato S, Sasaki S, et al. (2004) Perfluorooctane Sulfonate (PFOS) and Related Perfluorinated Compounds in Human Maternal and Cord Blood Samples: Assessment of PFOS Exposure in a Susceptible Population during Pregnancy. Environ Health Perspect 112: 1204-1207.
53. Apelberg BJ, Goldman LR, Calafat AM, Herbstman JB, Kuklenyik Z, et al. (2007) Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. Environ Sci Technol 41: 3891-3897.
54. Fei C, McLaughlin JK, Tarone RE, Olsen J (2007) Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect 115: 1677-1682.
55. Washino N, Saijo Y, Sasaki S, Kato S, Ban S, et al. (2009) Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect 117: 660-667.
56. Hamm MP, Cherry NM, Chan E, Martin JW, Burstyn I (2010) Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 20: 589-597.
57. Butenhoff JL, Kennedy GL, Jr., Frame SR, O'Connor JC, York RG (2004) The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196: 95-116.
58. Grasty RC, Wolf DC, Grey BE, Lau CS, Rogers JM (2003) Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-Dawley rat. Birth Defects Res B Dev Reprod Toxicol 68: 465-471.
59. Austin ME, Kasturi BS, Barber M, Kannan K, MohanKumar PS, et al. (2003) Neuroendocrine Effects of Perfluorooctane Sulfonate in Rats. Environ Health Perspect 111: 1485-1489.
60. Butenhoff JL, Ehresman DJ, Chang SC, Parker GA, Stump DG (2009) Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: developmental neurotoxicity. Reprod Toxicol 27: 319-330.
61. Cui L, Zhou QF, Liao CY, Fu JJ, Jiang GB (2009) Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch Environ Contam Toxicol 56: 338-349.
62. Wang X, Li B, Zhao WD, Liu YJ, Shang DS, et al. (2011) Perfluorooctane sulfonate triggers tight junction "opening" in brain endothelial cells via phosphatidylinositol 3-kinase. Biochem Biophys Res Commun 410: 258-263.

63. Johansson N, Fredriksson A, Eriksson P (2008) Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology 29: 160-169.
64. Slotkin TA, MacKillop EA, Melnick RL, Thayer KA, Seidler FJ (2008) Developmental neurotoxicity of perfluorinated chemicals modeled in vitro. Environ Health Perspect 116: 716-722.
65. Johansson N, Eriksson P, Viberg H (2009) Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicol Sci 108: 412-418.
66. Lee HG, Lee YJ, Yang JH (2012) Perfluorooctane sulfonate induces apoptosis of cerebellar granule cells via a ROS-dependent protein kinase C signaling pathway. Neurotoxicology 33: 314-320.
67. Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM (2010) Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environ Health Perspect 118: 1762-1767.
68. Stein CR, Savitz DA (2011) Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5-18 years of age. Environ Health Perspect 119: 1466-1471.
69. Gump BB, Wu Q, Dumas AK, Kannan K (2011) Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environ Sci Technol 45: 8151-8159.
70. Fei C, Olsen J (2011) Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environ Health Perspect 119: 573-578.
71. Stein CR, Savitz DA, Bellinger DC (2014) Perfluorooctanoate Exposure in a Highly Exposed Community and Parent and Teacher Reports of Behaviour in 6-12-Year-Old Children. Paediatr Perinat Epidemiol 2:146-156.
72. Latini G (2005) Monitoring phthalate exposure in humans. Clin Chim Acta 361: 20-29.
73. Braun JM, Sathyanarayana S, Hauser R (2013) Phthalate exposure and children's health. Curr Opin Pediatr 25: 247-254.
74. Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K (2006) What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal 26: 803-824.
75. Högberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, et al. (2008) Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. Environ Health Perspect 116: 334-339.
76. Ventrice P, Ventrice D, Russo E, De Sarro G (2013) Phthalates: European regulation, chemistry, pharmacokinetic and related toxicity. Environ Toxicol Pharmacol 36: 88-96.
77. Hauser R, Calafat AM (2005) Phthalates and human health. Occup Environ Med 62: 806-818.
78. Rudel RA, Perovich LJ (2009) Endocrine disrupting chemicals in indoor and outdoor air. Atmos Environ (1994) 43: 170-181.

79. Bornehag CG, Lundgren B, Weschler CJ, Sigsgaard T, Hagerhed-Engman L, et al. (2005) Phthalates in indoor dust and their association with building characteristics. Environ Health Perspect 113: 1399-1404.
80. Shi W, Hu X, Zhang F, Hu G, Hao Y, et al. (2012) Occurrence of thyroid hormone activities in drinking water from eastern China: contributions of phthalate esters. Environ Sci Technol 46: 1811-1818.
81. Jensen MS, Norgaard-Pedersen B, Toft G, Hougaard DM, Bonde JP, et al. (2012) Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: temporal trends and timing of amniocentesis in pregnancy. Environ Health Perspect 120: 897-903.
82. Mose T, Mortensen GK, Hedegaard M, Knudsen LE (2007) Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod Toxicol 23: 83-91.
83. Yan X, Calafat A, Lashley S, Smulian J, Ananth C, et al. (2009) Phthalates Biomarker Identification and Exposure Estimates in a Population of Pregnant Women. Hum Ecol Risk Assess 15: 565-578.
84. Kato K, Silva MJ, Needham LL, Calafat AM (2006) Quantifying phthalate metabolites in human meconium and semen using automated off-line solid-phase extraction coupled with on-line SPE and isotope-dilution high-performance liquid chromatography--tandem mass spectrometry. Anal Chem 78: 6651-6655.
85. Hines EP, Calafat AM, Silva MJ, Mendola P, Fenton SE (2009) Concentrations of phthalate metabolites in milk, urine, saliva, and Serum of lactating North Carolina women. Environ Health Perspect 117: 86-92.
86. Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, et al. (2003) Exposure to Di(2-ethylhexyl)phthalate in Humans during Pregnancy. Biology of the Neonate 83: 22-24.
87. Tanida T, Warita K, Ishihara K, Fukui S, Mitsuhashi T, et al. (2009) Fetal and neonatal exposure to three typical environmental chemicals with different mechanisms of action: mixed exposure to phenol, phthalate, and dioxin cancels the effects of sole exposure on mouse midbrain dopaminergic nuclei. Toxicol Lett 189: 40-47.
88. Ishido M, Masuo Y, Sayato-Suzuki J, Oka S, Niki E, et al. (2004) Dicyclohexylphthalate causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurochem 91: 69-76.
89. Masuo Y, Morita M, Oka S, Ishido M (2004) Motor hyperactivity caused by a deficit in dopaminergic neurons and the effects of endocrine disruptors: a study inspired by the physiological roles of PACAP in the brain. Regul Pept 123: 225-234.
90. Li X, Jiang L, Cheng L, Chen H (2014) Dibutyl phthalate-induced neurotoxicity in the brain of immature and mature rat offspring. Brain Dev 36: 653-660.
91. Tanaka T (2002) Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (DEHP) administered to mice in the diet. Food Chem Toxicol 40: 1499-1506.
92. Masuo Y, Ishido M, Morita M, Oka S (2004) Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats. Neural Plast 11: 59-76.

93. Dhanya CR, Indu AR, Deepadevi KV, Kurup PA (2003) Inhibition of membrane Na(+)-K+ Atpase of the brain, liver and RBC in rats administered di(2-ethyl hexyl) phthalate (DEHP) a plasticizer used in polyvinyl chloride (PVC) blood storage bags. Indian J Exp Biol 41: 814-820.
94. Chen T, Yang W, Li Y, Chen X, Xu S (2011) Mono-(2-ethylhexyl) phthalate impairs neurodevelopment: inhibition of proliferation and promotion of differentiation in PC12 cells. Toxicol Lett 201: 34-41.
95. Li Y, Zhuang M, Li T, Shi N (2009) Neurobehavioral toxicity study of dibutyl phthalate on rats following in utero and lactational exposure. J Appl Toxicol 29: 603-611.
96. Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, et al. (2010) Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ Health Perspect 118: 1027-1032.
97. Kim BN, Cho SC, Kim Y, Shin MS, Yoo HJ, et al. (2009) Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol Psychiatry 66: 958-963.
98. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, et al. (2008) Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 116: 1092-1097.
99. Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, et al. (2009) Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology 30: 522-528.
100. Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, et al. (2010) Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect 118: 565-571.
101. Whyatt RM, Liu X, Rauh VA, Calafat AM, Just AC, et al. (2012) Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect 120: 290-295.
102. Swan SH, Liu F, Hines M, Kruse RL, Wang C, et al. (2010) Prenatal phthalate exposure and reduced masculine play in boys. Int J Androl 33: 259-269.
103. Yolton K, Xu Y, Strauss D, Altaye M, Calafat AM, et al. (2011) Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. Neurotoxicol Teratol 33: 558-566.
104. Miodovnik A, Edwards A, Bellinger DC, Hauser R (2014) Developmental neurotoxicity of ortho-phthalate diesters: review of human and experimental evidence. Neurotoxicology 41: 112-122.
105. Park S, Kim BN, Cho SC, Kim Y, Kim JW, et al. (2014) Association between urine phthalate levels and poor attentional performance in children with attention-deficit hyperactivity disorder with evidence of dopamine gene-phthalate interaction. Int J Environ Res Public Health 11: 6743-6756.
106. Williams M, Todd GD, Roney N, Crawford J, Coles C, et al. (2012) Toxicological Profile for Manganese. Atlanta GA.
107. Wedler FC (1993) Biological significance of manganese in mammalian systems. Prog Med Chem 30: 89-133.
108. Santamaria AB, Sulsky SI (2010) Risk assessment of an essential element: manganese. J Toxicol Environ Health A 73: 128-155.

109. Keen CL, Ensunsa JL, Watson MH, Baly DL, Donovan SM, et al. (1999) Nutritional aspects of manganese from experimental studies. Neurotoxicology 20: 213-223.
110. Santamaria AB (2008) Manganese exposure, essentiality & toxicity. Indian J Med Res 128: 484-500.
111. Collipp PJ, Chen SY, Maitinsky S (1983) Manganese in infant formulas and learning disability. Ann Nutr Metab 27: 488-494.
112. Smargiassi A, Takser L, Masse A, Sergerie M, Mergler D, et al. (2002) A comparative study of manganese and lead levels in human umbilical cords and maternal blood from two urban centers exposed to different gasoline additives. Sci Total Environ 290: 157-164.
113. Wilson DC, Tubman R, Bell N, Halliday HL, McMaster D (1991) Plasma manganese, selenium and glutathione peroxidase levels in the mother and newborn infant. Early Hum Dev 26: 223-226.
114. Mora AM, van Wendel de Joode B, Mergler D, Cordoba L, Cano C, et al. (2014) Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in Costa Rica. Environ Sci Technol 48: 3467-3476.
115. Tholin K, Sandström B, Palm R, Hallmans G (1995) Changes in Blood Manganese Levels During Pregnancy in Iron Supplemented and non Supplemented Women. J Trace Elem Med Biol 9: 13-17.
116. Chung SE, Cheong HK, Ha EH, Kim BN, Ha M, et al. (2015) Maternal Blood Manganese and Early Neurodevelopment: The Mothers and Children's Environmental Health (MOCEH) Study. Environ Health Perspect doi: 10.1289/ehp.1307865.
117. Tsai MS, Liao KW, Chang CH, Chien LC, Mao IF, et al. (2015) The critical fetal stage for maternal manganese exposure. Environ Res 137: 215-221.
118. Yokel RA (2009) Manganese flux across the blood-brain barrier. Neuromolecular Med 11: 297-310.
119. Bouchard MF, Sauve S, Barbeau B, Legrand M, Brodeur ME, et al. (2011) Intellectual impairment in school-age children exposed to manganese from drinking water. Environ Health Perspect 119: 138-143.
120. Claus Henn B, Ettinger AS, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, et al. (2010) Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology 21: 433-439.
121. Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, et al. (2006) Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 114: 124-129.
122. Woolf A, Wright R, Amarasiriwardena C, Bellinger D (2002) A child with chronic manganese exposure from drinking water. Environ Health Perspect 110: 613-616.
123. Farias AC, Cunha A, Benko CR, McCracken JT, Costa MT, et al. (2010) Manganese in children with attention-deficit/hyperactivity disorder: relationship with methylphenidate exposure. J Child Adolesc Psychopharmacol 20: 113-118.

124. Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J, et al. (2000) Neurotoxicity of manganese chloride in neonatal and adult CD rats following subchronic (21-day) high-dose oral exposure. J Appl Toxicol 20: 179-187.
125. Kern CH, Stanwood GD, Smith DR (2010) Preweaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels. Synapse 64: 363-378.
126. Storch A, Ludolph AC, Schwarz J (2004) Dopamine transporter: involvement in selective dopaminergic neurotoxicity and degeneration. J Neural Transm 111: 1267-1286.
127. Tran TT, Chowanadisai W, Crinella FM, Chicz-DeMet A, Lonnerdal B (2002) Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels, and neurodevelopmental status. Neurotoxicology 23: 635-643.
128. Garner CD, Nachtman JP (1989) Manganese catalyzed auto-oxidation of dopamine to 6-hydroxydopamine in vitro. Chem Biol Interact 69: 345-351.
129. Verity MA (1999) Manganese neurotoxicity: a mechanistic hypothesis. Neurotoxicology 20: 489-497.
130. Risher J, McDonald AR, Citra MJ, Bosch S, Amata RJ (2003) Toxicological profile for selenium. Atlanta GA.
131. Papp LV, Lu J, Holmgren A, Khanna KK (2007) From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 9: 775-806.
132. Roman M, Jitaru P, Barbante C (2014) Selenium biochemistry and its role for human health. Metallomics 6: 25-54.
133. Fishbein L (1983) Environmental selenium and its significance. Fundam Appl Toxicol 3: 411-419.
134. Griffiths NM, Stewart RD, Robinson MF (1976) The metabolism of [75Se]selenomethionine in four women. Br J Nutr 35: 373-382.
135. Miklavcic A, Casetta A, Snoj Tratnik J, Mazej D, Krsnik M, et al. (2013) Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. Environ Res 120: 7-17.
136. Osman K, Akesson A, Berglund M, Bremme K, Schutz A, et al. (2000) Toxic and essential elements in placentas of Swedish women. Clin Biochem 33: 131-138.
137. Schulpis KH, Karakonstantakis T, Gavrili S, Chronopoulou G, Karikas GA, et al. (2004) Maternal--neonatal serum selenium and copper levels in Greeks and Albanians. Eur J Clin Nutr 58: 1314-1318.
138. Skröder HM, Hamadani JD, Tofail F, Persson LA, Vahter ME, et al. (2014) Selenium status in pregnancy influences children's cognitive function at 1.5 years of age. Clin Nutr doi: 10.1016/j.clnu.2014.09.020.
139. Mistry HD, Broughton Pipkin F, Redman CWG, Poston L (2012) Selenium in reproductive health. American Journal of Obstetrics and Gynecology 206: 21-30.
140. Brauer AU, Savaskan NE (2004) Molecular actions of selenium in the brain: neuroprotective mechanisms of an essential trace element. Rev Neurosci 15: 19-32.

141. Khan HA (2010) Selenium partially reverses the depletion of striatal dopamine and its metabolites in MPTP-treated C57BL mice. Neurochem Int 57: 489-491.
142. Santos AP, Lucas RL, Andrade V, Mateus ML, Milatovic D, et al. (2012) Protective effects of ebselen (Ebs) and para-aminosalicylic acid (PAS) against manganese (Mn)-induced neurotoxicity. Toxicol Appl Pharmacol 258: 394-402.
143. Oztas B, Kilic S, Dural E, Ispir T (2001) Influence of antioxidants on the blood-brain barrier permeability during epileptic seizures. J Neurosci Res 66: 674-678.
144. Yang X, Bao Y, Fu H, Li L, Ren T, et al. (2014) Selenium protects neonates against neurotoxicity from prenatal exposure to manganese. PLoS One 9: e86611.
145. Vinceti M, Mandrioli J, Borella P, Michalke B, Tsatsakis A, et al. (2014) Selenium neurotoxicity in humans: bridging laboratory and epidemiologic studies. Toxicol Lett 230: 295-303.
146. Lindh CH, Rylander L, Toft G, Axmon A, Rignell-Hydbom A, et al. (2012) Blood serum concentrations of perfluorinated compounds in men from Greenlandic Inuit and European populations. Chemosphere 88: 1269-1275.
147. Specht IO, Toft G, Hougaard KS, Lindh CH, Lenters V, et al. (2014) Associations between serum phthalates and biomarkers of reproductive function in 589 adult men. Environ Int 66: 146-156.
148. Barany E, Bergdahl IA, Schütz A, Skerfving S, Oskarsson A (1997) Inductively Coupled Plasma Mass Spectrometry for Direct Multi-element Analysis of Diluted Human Blood and Serum. J Anal At Spectrom 12: 1005-1009.
149. Axmon A, Axelsson J, Jakobsson K, Lindh CH, Jonsson BA (2014) Time trends between 1987 and 2007 for perfluoroalkyl acids in plasma from Swedish women. Chemosphere 102: 61-67.
150. Beesoon S, Webster GM, Shoeib M, Harner T, Benskin JP, et al. (2011) Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. Environ Health Perspect 119: 1659-1664.
151. Gutzkow KB, Haug LS, Thomsen C, Sabaredzovic A, Becher G, et al. (2012) Placental transfer of perfluorinated compounds is selective--a Norwegian Mother and Child sub-cohort study. Int J Hyg Environ Health 215: 216-219.
152. Needham LL, Grandjean P, Heinzow B, Jorgensen PJ, Nielsen F, et al. (2011) Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 45: 1121-1126.
153. Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, et al. (2014) Attention Deficit/Hyperactivity Disorder and Childhood Autism in Association with Prenatal Exposure to Perfluoroalkyl Substances: A Nested Case-Control Study in the Danish National Birth Cohort. Environ Health Perspect doi: 10.1289/ehp.1408412.
154. Liew Z, Olsen J, Cui X, Ritz B, Arah OA (2015) Bias from conditioning on live birth in pregnancy cohorts: an illustration based on neurodevelopment in children after prenatal exposure to organic pollutants. Int J Epidemiol 44: 345-354.
155. Fromme H, Bolte G, Koch HM, Angerer J, Boehmer S, et al. (2007) Occurrence and daily variation of phthalate metabolites in the urine of an adult population. Int J Hyg Environ Health 210: 21-33.

156. Milne DB, Sims RL, Ralston NV (1990) Manganese content of the cellular components of blood. Clin Chem 36: 450-452.
157. Dobbs MR (2009) Clinical Neurotoxicology: Syndromes, Substances, Environments. USA: Saunders; Elsevier.
158. Wasowicz W, Wolkanin P, Bednarski M, Gromadzinska J, Sklodowska M, et al. (1993) Plasma trace element (Se, Zn, Cu) concentrations in maternal and umbilical cord blood in Poland. Relation with birth weight, gestational age, and parity. Biol Trace Elem Res 38: 205-215.
159. Gathwala G, Yadav OP, Singh I, Sangwan K (2000) Maternal and cord plasma selenium levels in full-term neonates. Indian J Pediatr 67: 729-731.
160. Skounti M, Philalithis A, Galanakis E (2007) Variations in prevalence of attention deficit hyperactivity disorder worldwide. Eur J Pediatr 166: 117-123.
161. Baumgaertel A, Wolraich ML, Dietrich M (1995) Comparison of diagnostic criteria for attention deficit disorders in a German elementary school sample. J Am Acad Child Adolesc Psychiatry 34: 629-638.
162. Biederman J, Faraone SV, Weber W, Russell RL, Rater M, et al. (1997) Correspondence between DSM-III-R and DSM-IV attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 36: 1682-1687.
163. Lahey BB, Applegate B, McBurnett K, Biederman J, Greenhill L, et al. (1994) DSM-IV field trials for attention deficit hyperactivity disorder in children and adolescents. Am J Psychiatry 151: 1673-1685.
164. Hasson R, Fine JG (2012) Gender differences among children with ADHD on continuous performance tests: a meta-analytic review. J Atten Disord 16: 190-198.

Paper I

Environ Sci Pollut Res (2013) 20:7970–7978
DOI 10.1007/s11356-013-1573-5

Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds

Amanda Ode · Lars Rylander · Christian H. Lindh · Karin Källén · Bo A. G. Jönsson · Peik Gustafsson · Per Olofsson · Sten A. Ivarsson · Anna Rignell-Hydbom

Received: 2 October 2012 / Accepted: 11 February 2013 / Published online: 24 February 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract In recent years, some perfluorinated compounds (PFCs) have been identified as potentially hazardous substances which are harmful to the environment and human health. According to limited data, PFC levels in humans could be influenced by several determinants. However, the findings are inconsistent. In the present study, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were measured in paired maternal and cord serum samples (N=237) collected between 1978 and 2001 in Southern Sweden to study the relationship between these and to investigate several potential determinants of maternal and fetal exposure to PFCs. Time trends of PFCs in Swedish women were also evaluated. The study is a part of the Fetal Environment and Neurodevelopment Disorders in Epidemiological Research project. PFOS, PFOA, and PFNA levels (median) were higher in maternal serum (15, 2.1, and 0.24 ng/ml, respectively) than in cord serum (6.5, 1.7, and 0.20 ng/ml, respectively). PFC levels were among the highest in women originating from the Nordic countries and the lowest in women from the Middle East, North Africa, and sub-Saharan Africa. Multiparous women had lower serum PFOA levels (1.7 ng/ml) than primiparous women (2.4 ng/ml). Maternal age, body mass index, cotinine levels, and whether women carried male or female fetuses did not affect serum PFC concentrations. Umbilical cord serum PFC concentrations showed roughly similar patterns as the maternal except for the gestational age where PFC levels increased with advancing gestational age. PFOS levels increased during the study period in native Swedish women. In summary, PFOS levels tend to increase while PFOA and PFNA levels were unchanged between 1978 and 2001 in our study population. Our results demonstrate that maternal country of origin, parity, and gestational age might be associated with PFC exposure.

Keywords Perfluorinated compounds · Fetal exposure · Determinants · Country of origin · Parity · Gestational age · Time trends

Responsible editor: Leif Kronberg

A. Ode (✉) · L. Rylander · C. H. Lindh · K. Källén · B. A. G. Jönsson · A. Rignell-Hydbom
Division of Occupational and Environmental Medicine, Lund University, 221 85, Lund, Sweden
e-mail: amanda.ode@med.lu.se

P. Gustafsson
Department of Child and Adolescent Psychiatry, Clinical Sciences, Lund University, 221 85, Lund, Sweden

P. Olofsson
Department of Obstetrics and Gynecology, Institution of Clinical Sciences, Skane University Hospital, Lund University, 205 02, Malmö, Sweden

S. A. Ivarsson
Department of Clinical Sciences, Unit of Pediatric Endocrinology, Lund University/Clinical Research Centre (CRC), 205 02, Malmö, Sweden

Introduction

In recent years, some perfluorinated compounds (PFCs), mainly perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been identified as global pollutants with a wide dissemination in the environment, wildlife, and in humans throughout the world. The unique physical

and chemical properties of PFCs make them widely used in industrial and consumer applications including polytetrafluoroethylene (e.g., Teflon) cookware, water and oil repellent surface coatings for textiles, carpets, paper, packaging, flooring, leather, and in firefighting foams (Kissa 2001; Schultz et al. 2003). PFCs have an exceptional stability which makes them persistent and resistant to degradation by strong acids and alkalis, oxidation and reduction, photolysis, and microbes (Kissa 2001; Schultz et al. 2003). Thus, PFCs have a potential to bioaccumulate and biomagnify in the environment (Giesy and Kannan 2001; Butt et al. 2010).

The human exposure routes to PFCs have been considered to be through consumption of contaminated water or food and through inhalation of contaminated air (Fromme et al. 2009). PFCs are distributed mainly to the liver and blood where they bind to serum proteins, mainly albumin (Han et al. 2003; Jones et al. 2003), and they are poorly eliminated in humans and have serum half-lives between 2 and 9 years (Lau et al. 2007; Olsen et al. 2007; Bartell et al. 2010; Brede et al. 2010; Seals et al. 2011).

PFCs have been shown to cross the placenta (Inoue et al. 2004; Liu et al. 2011; Gützkow et al. 2012) and thus have a potential to exert a direct influence on the developing fetus (Apelberg et al. 2007a; Fei et al. 2007). According to previous studies, the median PFC concentrations in cord blood ranged from 30 to 130 % of the maternal concentrations (Liu et al. 2011; Kim et al. 2011a; Gützkow et al. 2012). PFCs have also been shown to be transmitted to the neonate through breast milk (Kärrman et al. 2007; Sundström et al. 2011).

PFC levels in humans are affected by several determinants, including age, BMI, smoking, sex, race, and ethnicity. The potential determinants of maternal and fetal exposure to PFCs are poorly investigated and their potential to affect the concentrations of PFCs in pregnant women and fetuses is still unclear. Some studies have shown that PFOS levels are higher in black women than in white and Asian women (Apelberg et al. 2007b) and are also higher in nonsmokers than smokers (Fei et al. 2007; Washino et al. 2009). The PFOA levels decrease with increasing maternal age (Washino et al. 2009), BMI (Fei et al. 2007), and parity (Fei et al. 2007; Apelberg et al. 2007b; Washino et al. 2009). The findings on the effect of infant sex (Inoue et al. 2004; Apelberg et al. 2007b) and gestational age (Apelberg et al. 2007a, b; Nolan et al. 2009) on the levels of PFCs are limited. Many of those studies on the determinants have contradicting results. Thus, more research on possible determinants is needed.

The manufacturing of PFCs began in the late 1940s (Schultz et al. 2003) and was mainly located in USA, Japan, and Europe (Kärrman et al. 2006). The 3M Company was the major manufacturer of PFCs with the highest production between 1970 and 2002. When the 3M Company phased out its production of some of the PFCs in 2002, other companies start manufacturing these compounds to meet the market demand for them (Lindstrom et al. 2011). Several studies have assessed time trends of human serum PFC levels and found increasing levels of PFOS and PFOA from the early 1970s through the late 1990s, followed by leveling out and a decreasing trend right after the phase-out of the production (Olsen et al. 2005, 2011; Harada et al. 2007; Calafat et al. 2007; Haug et al. 2009). The same trends have been observed in the human breast milk (Kärrman et al. 2007; Sundström et al. 2011). On the other hand, PFOA and PFOS have been substituted by other PFCs which have increased in humans during the last decade (Calafat et al. 2007; Haug et al. 2009; Glynn et al. 2012; Olsen et al. 2011).

In the present study, serum concentrations for several PFCs were analyzed in paired maternal and umbilical cord samples. The aim of the study was to investigate the correlation between PFCs in maternal and umbilical cord serum samples and to evaluate maternal age, parity, BMI, smoking, and maternal country of origin as potential determinants of maternal exposure and gestational age and sex of newborns as potential determinants of fetal exposure to PFCs. Another aim was to investigate temporal trends of PFC body burdens in Swedish women.

Materials and methods

Study population

Nearly all deliveries in Malmö, a city with around 300,000 inhabitants situated in Southern Sweden, take place at the Malmö University Hospital maternity unit. Since 1969, venous blood samples from mothers and umbilical cord blood samples from the newborns have routinely been collected at delivery and stored in the Malmö Maternity Unit Serum Biobank (MMUSB). There are samples from approximately 70,000 deliveries stored in the MMUSB. Maternal blood has been collected during early labor by venous puncture in vacutainer tubes, and the cord blood has been decanted into sterile sample tubes immediately after birth. The sample tubes were stored overnight in a refrigerator at + 8 °C for sedimentation. The next morning, the sera were collected and frozen in polypropylene plastic test tubes at −20 °C until analysis.

In the present study, maternal and corresponding cord serum samples (N=263) from year 1978 to 2001 were collected from the MMUSB. These samples represent controls for an upcoming case–control study on the possible association between PFCs and neurodevelopment disorders. Since it is more common for males to have a diagnosis as

compared to females, the material has a skewed distribution with with75% male and 25 % female fetuses.

From the Swedish Medical Birth Register, which contains medical information on nearly all (98–99 %) deliveries in Sweden, we obtained data on the newborns' characteristics such as birth weight, gestational age, and the sex of the child, as well as maternal characteristics like mothers' age, parity, BMI, smoking habits during pregnancy, and country of origin.

At the booking visit in the Maternal Health Care System, the women were informed that the samples collected could be used for research purposes in the future and those who accepted gave their verbal informed consent. The study protocol followed the requirements of the Declaration of Helsinki and was approved by the Research Ethics Committee at Lund University, Sweden.

Analysis of PFCs and cotinine

The analyses of perfluorohexane sulfonate (PFHxS), PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), and cotinine were performed by a hybrid triple quadrupole linear ion-trap mass spectrometer (LC/MS/MS; UFLC^{XR}, Shimadzu Corporation, Kyoto, Japan; QTRAP 5500; Sciex, Framingham, MA, USA). The samples were analyzed according to the method of Lindh et al. (2012). Aliquots of 60 μl serum were added with internal standards for all evaluated compounds, and proteins were precipitated with 120 μl acetonitrile. An aliquot of 150 μl of sample was transferred to 96-well plates and further diluted with 150 μl water. The PFCs were analyzed using two C_{18} columns (4 μm, 2.1 mm i.d. × 20 mm; Genesis, Grace Vydac, Hesperia, CA, USA) connected in a 2D system. The mobile phase was 0.08 % ammonia (NH_3) in water (A) and 0.08 % NH_3 in acetonitrile (B). The results reported is the average of two measurements from the same sample worked up and analyzed on different days. In all sample batches, the quality of the measurements was controlled by analyzing chemical blanks and in-house quality control (QC) samples. The reproducibility, determined as the relative standard deviation, between measured duplicate samples was 11 % for PFOS, 12 % for PFOA, and 12 % for PFNA. The reproducibility in QC samples was 8 % for PFOS, 11 % for PFOA, and 8 % for PFNA. PFHxS, PFDA, PFUnDA, and PFDoDA could not be detected in the samples due to matrix effects. The analyses of PFOS and PFOA are part of the round robin intercomparison program (Professor Dr. med. Hans Drexler, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany) with results within the tolerance limits. Among the 263 paired maternal and cord serum samples, PFCs could only be assessed in 237 pairs. The reason for this was that there was not enough amount of serum to perform the analyses.

Statistical analyses

Spearman's rank correlation coefficient with $p<0.05$ defined as statistically significant was used to explore the correlations between the different PFCs in maternal samples, and for each PFC between maternal and cord serum samples. The transfer efficiencies of PFCs through placenta were calculated as well. The Wilcoxon signed-rank test was used to compare the levels between maternal and cord serum samples.

Spearman's rank correlation was used to test the correlation between the different PFCs and the investigated determinants. The Mann–Whitney *U* test and Kruskal–Wallis test were performed to determine if significant differences in the PFC levels exist between groups (e.g., maternal age, parity, BMI, smoking habits based on measured cotinine levels, country of origin, gestational duration, and newborns' sex). Maternal age was divided into three groups: <25, 25–35, and >35 years. BMI was classified according to the following standard values of the World Health Organization: underweight <18.5 kg/m^2, normal weight 18.5–24.9 kg/m^2, overweight 25–29.9 kg/m^2, and obese ≥30 kg/m^2. Underweight (N=3) and normal weight women (N=53) were merged together in one group due to the small number of samples for the underweight women. For the same reason, obese (N=6) and overweight women (N=24) were merged together in one group. Women whose cotinine levels were below the limit of detection (0.2 ng/ml) were defined as nonsmokers and those having a level higher than 15 ng/ml were defined as active smokers (George et al. 2006). Women with cotinine levels between 0.2 and 15 ng/ml were considered to be second-hand smokers. Infants born before 37 weeks of gestation were defined as preterm and those born at or after that week were considered term births. Countries of origin were merged together into the following groups based on shared geographical regions or cultural similarities: Sweden (N=164), Finland (N=2), Denmark (N=5), Norway (N=1), Western Europe and the USA (N=2), previous Eastern Europe (N=21), sub-Saharan Africa (N=6), Middle East and North Africa (N=22), East Asia (N=5), South America (N=5), and unknown (N=4). Country groups with less than five women were excluded from the analyses regarding the differences in PFC concentrations between different countries. PFC concentrations below the limit of detection (0.20 ng/ml) in individual samples were replaced with the value 0.20 ng/ml.

The association between the year of sampling and the measured PFC levels was tested for significance using

linear regression. Data analyses were carried out in IBM SPSS Statistics version 20 (IBM Corporation 1989, 2011).

Results

PFOS, PFOA, and PFNA were the only detectable PFCs in the samples. PFOS concentrations were above the limit of detection (LOD) in 100 % of maternal serum samples and in 99.6 % of cord serum samples. Maternal PFOA serum concentrations were all above the LOD, but in cord serum, PFOA was below the LOD in 1.3 % of the samples. For PFNA, 38 % of the maternal PFNA samples and 66 % of the cord PFNA samples were under LOD. In both maternal and cord sera, PFOS was found to be the most abundant PFC (Table 1). PFC levels were higher in maternal serum than in cord serum. The strongest correlation between maternal and cord serum PFC levels was found for PFOS, followed by PFOA and PFNA. The correlations between the different PFCs in maternal serum were significant (r=0.72 for PFOS and PFOA, r=0.35 for PFOS and PFNA, and r=0.37 for PFOA and PFNA), with p values <0.01. Median cord PFOS, PFOA, and PFNA serum concentrations were 43, 80, and 99 %, respectively, of the maternal concentrations (Table 1).

Maternal PFC levels between 1978 and 2001 are illustrated in Fig. 1. Linear regression analyses revealed a significant increase in maternal PFOS levels during the study period (β=0.40, p=0.020). For PFOA and PFNA, no obvious time trends were observed (β=0.02, p=0.40 and β=−0.003, p=0.64, respectively). Since the number of samples was highest between 1985 and 1996, we even performed the analyses for that period. The findings were the same for PFOA and PFNA. For PFOS, the results were no longer significant (p=0.071) but the slope was roughly the same (β=0.42).

No significant associations between maternal PFC levels and maternal age, BMI, cotinine levels, or gestational duration were found (Table 2). Multiparous women had lower PFOA concentrations than primiparous women, and women carrying male fetuses had higher levels of PFOA as compared to women with female fetuses (Table 2). Maternal PFC serum concentrations were strongly associated with the country of origin. PFC levels were among the highest in women and newborns originating from the two investigated Nordic countries and the lowest in women from the Middle East, North Africa, and sub-Saharan Africa (Table 2).

Umbilical cord serum PFC concentrations showed distributions similar to the distributions in maternal serum, with one exception. Cord PFNA levels were not associated with maternal country of birth (p=0.59).

Gestational age at birth was positively correlated (p< 0.05) with cord PFOS, PFOA, and PFNA levels (r=0.20, r=0.17, and r=0.14, respectively). No significant difference in cord serum PFC levels between preterm and term infants was found. When the analysis was restricted to Swedish women, preterm newborns (N=7) had lower cord serum PFOS levels than term newborns (N=157) (p=0.008).

In order to avoid confounding by parity, analysis was carried out to investigate the effect of maternal age on PFC levels among primiparous women. No significant correlation was seen between maternal age and PFC levels in primiparous women. Among the multiparous women, 25 % were born outside Western Europe. An analysis was carried out to investigate the effect of parity on PFC levels among the native Swedish women. Multiparous women still had lower PFOA concentrations than primiparous women (p<0.001).

There were many PFNA samples with values below the LOD. In order to be certain that the substitution of those values with LOD did not introduce a bias, all the analyses were even performed on matched maternal and cord serum samples with values over the LOD before substitution with the LOD (N=71). The results were roughly the same as for

Table 1 Mean (5 % percentile, median, 95 % percentile) concentrations of perfluorinated compounds (in nanogram per milliliter) in 237 matched maternal and umbilical cord serum samples from Southern Sweden

	Mean (5 %percentile, median, 95 % percentile)			Correlation[a]	Difference between maternal and cord PFC levels (p value)[b]
	Maternal	Cord	Transfer efficiency (%)		
PFOS	17 (5.7, 15, 31)	7.4 (2.3, 6.5, 15)	45 (24, 43, 72)	0.76*	<0.001
PFOA	2.3 (0.61, 2.1, 4.6)	2.8 (0.39, 1.7, 6.0)	130 (45, 80, 160)	0.74*	<0.001
PFNA	0.31 (0.20, 0.24, 0.55)	0.26 (0.20, 0.20, 0.55)	93 (46, 99, 140)	0.51*	<0.001

PFOS perfluorooctane sulfonate, *PFOA* perfluorooctanoic acid, *PFNA* perfluorononanoic acid

* $p \leq 0.01$ (correlations were significant at this level)

[a] Spearman's correlation coefficient between maternal and cord serum

[b] Comparison between maternal and cord serum by Wilcoxon signed-rank test

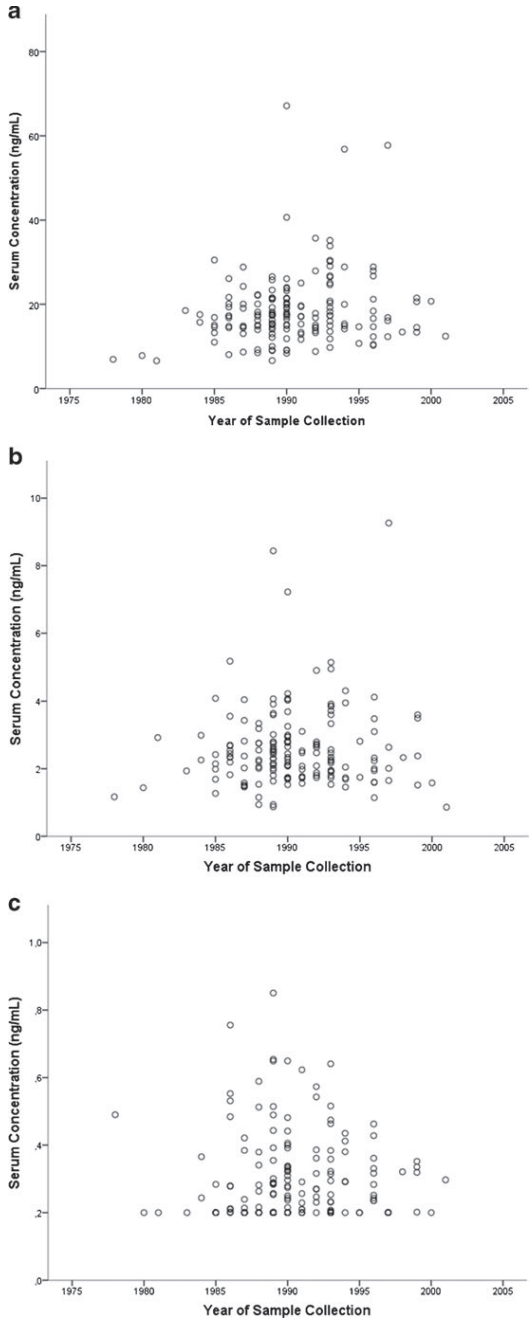


Fig. 1 Time trends of **a** perfluorooctane sulfonate, **b** perfluorooctanoic acid, and **c** perfluorononanoic acid in Swedish women (N=164) between 1978 and 2001. The extreme value for perfluorononanoic acid (3.4 ng/ml) is not shown in the figure

Table 2 Mean (5 % percentile, median, 95 % percentile) concentrations (in nanogram per milliliter) of perfluorinated compounds in maternal serum by maternal and fetal characteristics in 237 maternal samples from Southern Sweden

Characteristics	*N* (%)	Mean (5 % percentile, median, 95 % percentile)			Differences among groups (*p* value)[a]			Pairwise differences between groups (*p* value)[b]		
		PFOS	PFOA	PFNA	PFOS	PFOA	PFNA	PFOS	PFOA	PFNA
Age at delivery (years)					0.37	0.81	0.44			
<25	59 (25)	15 (3.5, 15, 29)	2.2 (0.45, 2.2, 4.6)	0.28 (0.20, 0.22, 0.55)				0.26	0.83	0.18
25–35	154 (65)	18 (5.7, 16, 31)	2.4 (0.61, 2.1, 4.9)	0.32 (0.20, 0.25, 0.54)				Reference	Reference	Reference
>35	24 (10)	20 (7.8, 16, 57)	2.2 (0.43, 1.9, 4.1)	0.32 (0.20, 0.24, 0.65)				0.92	0.51	0.81
Parity					–					
Primiparous	115 (48)	18 (5.6, 17, 31)	2.7 (0.75, 2.4, 5.1)	0.32 (0.20, 0.25, 0.55)				Reference	Reference	Reference
Multiparous	122 (52)	17 (5.7, 15, 29)	2.0 (0.43, 1.7, 3.9)	0.29 (0.20, 0.23, 0.55)				0.023	<0.001	0.26
Body mass index[c]					–					
Underweight and normal	56 (65)	19 (6.1, 16, 57)	2.4 (0.69, 1.9, 5.3)	0.29 (0.20, 0.25, 0.54)				Reference	Reference	Reference
Overweight and obese	30 (35)	19 (5.6, 14, 37)	2.4 (0.61, 1.6, 6.5)	0.26 (0.20, 0.21, 0.51)				0.23	0.19	0.22
Smoking status[d]					0.56	0.44	0.22			
Nonsmoker	113 (48)	17 (56, 16, 34)	2.2 (0.43, 2.0, 4.9)	0.30 (0.20, 0.24, 0.54)				Reference	Reference	Reference
Second-hand smoker	62 (26)	17 (6.0, 15, 27)	2.5 (0.65, 2.1, 4.2)	0.36 (0.20, 0.26, 0.65)				0.41	0.54	0.87
Active smoker	62 (26)	17 (6.7, 16, 30)	2.3 (0.92, 2.2, 4.1)	0.28 (0.20, 0.21, 0.57)				0.75	0.20	0.13
Country of origin					<0.001	<0.001	0.020			
Sweden	164 (72)	19 (9.0, 17, 30)	2.6 (1.4, 2.3, 4.2)	0.32 (0.20, 0.26, 0.59)				Reference	Reference	Reference
Denmark	5 (2.2)	31 (13, 22, 68)	3.9 (1.4, 2.7, 7.5)	0.25 (0.20, 0.25, 0.29)				0.31	0.62	0.59
Previous Eastern Europe	21 (9.2)	20 (5.6, 14, 30)	2.3 (0.68, 1.5, 5.3)	0.28 (0.20, 0.24, 0.45)				0.029	<0.001	0.51
Sub-Saharan Africa	6 (2.6)	9.1 (7.2, 8.5, 12)	1.1 (0.36, 1.1, 1.9)	0.26 (0.20, 0.20, 0.52)				<0.001	<0.001	0.23
Middle East	22 (9.6)	9.9 (3.1, 6.3, 29)	0.72 (0.22, 0.65, 1.4)	0.25 (0.20, 0.20, 0.51)				<0.001	<0.001	0.003
East Asia	5 (2.2)	13 (6.9, 12, 24)	1.5 (0.61, 1.0, 2.7)	0.48 (0.22, 0.33, 1.05)				0.075	0.041	0.12
South America	5 (2.2)	14 (7.3, 12, 24)	1.9 (0.75, 0.97, 3.7)	0.24 (0.20, 0.20, 0.35)				0.13	0.22	0.23
Gestational age					–					
Preterm	11 (5.0)	17 (6.0, 15, 37)	2.5 (1.1, 1.7, 6.5)	0.26 (0.20, 0.20, 0.46)				0.90	0.72	0.30
Term	226 (95)	17 (5.7, 15, 30)	2.3 (0.61, 2.1, 4.3)	0.31 (0.20, 0.24, 0.55)				Reference	Reference	Reference
Sex of newborns					–					
Male	177 (75)	17 (6.0, 16, 30)	2.3 (0.65, 2.2, 4.3)	0.32 (0.20, 0.24, 0.59)				Reference	Reference	Reference
Female	60 (25)	19 (5.3, 14, 54)	2.2 (0.37, 1.7, 6.9)	0.28 (0.20–0.24–0.50)				0.11	0.014	0.67

PFOS perfluorooctane sulfonate, *PFOA* perfluorooctanoic acid, *PFNA* perfluorononanoic acid

[a] Differences between groups obtained from Kruskal–Wallis test

[b] Pairwise difference to reference group obtained from Mann–Whitney *U* test

[c] Informations were available for 86 paired maternal and cord samples

[d] Results are based on measured cotinine concentrations in maternal serum

the matched 237 maternal and cord PFNA serum samples (data not shown).

Discussion

The present study is one of the largest studies that focused on the potential determinants of maternal and fetal exposure to PFCs. Our results showed that parity, maternal country of origin, and gestational duration were the potential determinants of maternal and fetal exposure to PFCs.

In the present study, we have applied a precise and accurate method that uses only 0.1 ml serum and is therefore suitable to for analyzing samples stored in serum biobanks. The coefficient of variation of duplicate samples worked up and analyzed on different samples was generally below 10 % for duplicate analysis of the same sample, analyzed in different sample batches on different days. Furthermore, a high accuracy of the method, at least for PFOS and PFOA, was proven by participation in an interlaboratory control program. The use of QCs and chemical blanks prevents drift and contamination during the analysis. However, we found both linear and branched PFOS but the branched PFOS was almost exclusively found in the cord blood. We see no reason why branched PFOS should be found in the blood of the fetus and not in the mother, and thus, we think that the cord blood has been contaminated during sampling. Therefore, we report only results for linear PFOS.

PFCs cross the placenta, but umbilical cord concentrations have in most studies been lower than those observed in maternal serum or plasma (Inoue et al. 2004; Fromme et al. 2010; Jensen et al. 2012; Gützkow et al. 2012). In the present study, we found higher levels of PFCs in maternal serum than in cord serum, which is consistent with previous studies (Monroy et al. 2008; Hanssen et al. 2010; Beesoon et al. 2011). Maternal and cord PFOS and PFOA concentrations were among the highest in Europe (Midasch et al. 2007; Fei et al. 2007; Needham et al. 2011) and even among the highest in the world (Liu et al. 2011; Kim et al. 2011a, b; Beesoon et al. 2011). Maternal and cord PFNA levels were lower than those measured in other countries (Monroy et al. 2008; Needham et al. 2011; Kim et al. 2011a; Beesoon et al. 2011).

The transfer efficiency (TE) of PFCs might be influenced by plasma volume expansion in pregnancy. The elimination rate of PFCs is extremely slow, and therefore, that would probably not affect the TE. Furthermore, our calculated transfer efficiencies mirror the literature to date.

We examined the temporal trends of PFCs in native Swedish women and found that the serum PFC levels were roughly unchanged between 1978 and 2001 except for PFOS which increased with time. Our samples were collected during the steady-state high-production period of PFCs, which probably explains the unchanged time trends for PFOA and PFNA. Use of PFOS in consumer and commercial products may be increased slightly in the country during the study period which might explain the increased trend for PFOS.

Consistent with previous studies (Fei et al. 2007; Apelberg et al. 2007b; Washino et al. 2009), our data confirm that increased parity is associated with lower levels of PFOA in both mother and fetus. The body burden of PFCs in multiparous women may decrease through transmission to the fetus during pregnancy and to the newborn through breastfeeding.

There was a strong heterogeneity between different countries for the investigated PFCs. Swedish and Danish women had higher levels of PFCs as compared to women originating from other countries but living in Sweden. One reason for the discrepancy might be that the women with non-Swedish origin come from countries with different background PFC exposure and have been living in Sweden for a short time. Unfortunately, we lack data on how long they have been living in Sweden. Another explanation for the discrepancy might be differences in sources, distribution, exposure routes, diets, and lifestyles between women with Swedish and non-Swedish origin. One might speculate that there is an interaction between genetic susceptibility and exposure to PFCs for women originating from different countries.

Studies on adult men (Eriksen et al. 2011) and pregnant women (Fei et al. 2007; Washino et al. 2009) have shown that nonsmokers have higher PFOS and PFOA levels than active smokers. The present study showed no such associations.

PFOS and PFOA levels were roughly the same across the maternal age groups, which is in accordance with the previous studies by Inoue et al. (2004) and Apelberg et al. (2007a). On the other hand, Washino et al. (2009) reported decreased maternal PFOS concentrations with increasing age, whereas Zhang et al. (2011) found a positive association between cord PFOS concentrations and mother's age.

In two previous studies, no associations between maternal BMI and maternal or cord serum PFC levels were found which are consistent with our results (Inoue et al. 2004; Rylander et al. 2009). Three other studies showed inverse associations between PFOS and PFOA levels and BMI in women or men (Fei et al. 2007; Eriksen et al. 2011; Lindh et al. 2012).

Pregnant women carrying male fetuses had higher PFOA levels compared to women with female fetuses. Consequently, higher levels of PFOA were measured in cord blood from male fetuses. Previous research on sex effect on PFC exposure is contradicting and has no reasonable explanation (Inoue et al. 2004; Apelberg et al. 2007a; Zhang et al. 2011). We believe that our finding is a chance finding and could be due to multiple testing.

In our study, we found a positive correlation between cord PFCs and gestational age. As for PFOS, we also found

in subgroup analyses comprising native Swedish women higher levels in term than in preterm newborns. This is not in accordance with other studies where no significant associations were found for gestational age (Apelberg et al. 2007a, b; Fei et al. 2007; Nolan et al. 2009). However, a new study by Chen et al. (2012) found an inverse association between PFOS levels and gestational age. PFC levels increase in fetuses as pregnancy progresses as a recent study by Jensen et al. (2012) showed that PFOS increased in amniotic fluid by gestational week. Advancing gestational duration might therefore result in decreased maternal body burden of PFCs.

In summary, maternal PFC concentrations were higher than umbilical cord PFC concentrations in our Swedish population who were from the southern parts of the country. PFOS levels tended to increase between 1978 and 2001, while PFOA and PFNA levels were unchanged during the same period. Women originating from Nordic countries had higher levels of PFCs than women from the rest of the world. To our best knowledge, this is the first study to report a positive association between PFCs and gestational age. Increasing cord PFCs and decreasing maternal PFOA concentrations with advancing gestational age and increased parity, respectively, suggest that pregnancy might serve as a body purifier for PFCs. Our findings are of special interest when studying the impact of perinatal PFC exposure on postnatal health outcomes.

Acknowledgments We thank the Swedish Research Council and the Medical Faculty at Lund University for funding the project. Special thanks are also given to the laboratory technicians Åsa Amilon and Agneta Kristensen for analyzing the samples.

References

Apelberg BJ, Witter FR, Herbstman JB, Calafat AM, Halden RU, Needham LL, Goldman LR (2007a) Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect 115:1670–1676

Apelberg BJ, Goldman LR, Calafat AM, Herbstman JB, Kuklenyik Z, Heidler J, Needham LL, Halden RU, Witter FR (2007b) Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. Environ Sci Technol 41:3891–3897

Bartell SM, Calafat AM, Lyu C, Kato K, Ryan PB, Steenland K (2010) Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ Health Perspect 118:222–228

Beesoon S, Webster GM, Shoeib M, Harner T, Benskin JP, Martin JW (2011) Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. Environ Health Perspect 119:1659–1664

Brede E, Wilhelm M, Göen T, Müller J, Rauchfuss K, Kraft M, Hölzer J (2010) Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. Int J Hyg Environ Health 213:217–223

Butt CM, Berger U, Bossi R, Tomy GT (2010) Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci Total Environ 408:2936–2965

Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL (2007) Polyfluoroalkyl chemicals in the US population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. Environ Health Perspect 115:1596–1602

Chen MH, Ha EH, Wen TW, Su YN, Lien GW, Chen CY, Chen PC, Hsieh WS (2012) Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS One 7:e42474

Eriksen KT, Sørensen M, McLaughlin JK, Tjønneland A, Overvad K, Raaschou-Nielsen O (2011) Determinants of plasma PFOA and PFOS levels among 652 Danish men. Environ Sci Technol 45:8137–8143

Fei C, McLaughlin JK, Tarone RE, Olsen J (2007) Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect 115:1677–1682

Fromme H, Tittlemier SA, Volkel W, Wilhelm M, Twardella D (2009) Perfluorinated compounds—exposure assessment for the general population in western countries. Int J Hyg Environ Health 212:239–270

Fromme H, Mosch C, Morovitz M, Alba-Alejandre I, Boehmer S, Kiranoglu M, Faber F, Hannibal I, Benzel-Boroviczény O, Koletzko B, Völkel W (2010) Pre- and postnatal exposure to perfluorinated compounds (PFCs). Environ Sci Technol 44:7123–7129

George L, Granath F, Johansson ALV, Cnattingius S (2006) Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. Acta Obstet Gynecol Scand 85:1331–1337

Giesy JP, Kannan K (2001) Global distribution of perfluorooctane sulfonate in wildlife. Environ Sci Technol 35:1339–1342

Glynn A, Berger U, Bignert A, Ullah S, Aune M, Lignell S, Darnerud PO (2012) Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. Environ Sci Technol 46:9071–9079

Gützkow KB, Haug LS, Thomsen C, Sabaredzovic A, Becher G, Brunborg G (2012) Placental transfer of perfluorinated compounds is selective—a Norwegian Mother and Child sub-cohort study. Int J Hyg Environ Health 215:216–219

Han X, Snow TA, Kemper RA, Jepson GW (2003) Binding of perfluorooctanoic acid to rat and human plasma proteins. Chem Res Toxicol 16:775–781

Hanssen L, RollinH OJO, Moe MK, Sandanger TM (2010) Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. J Environ Monit 12:1355–1361

Harada K, Koizumi A, Saito N, Inoue K, Yoshinaga T, Date C, Fujii S, Hachiya N, Hirosawa I, Koda S, Kusaka Y, Murata K, Omae K, Shimbo S, Takenaka K, Takeshita T, Todoriki H, Wada Y, Watanabe T, Ikeda M (2007) Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere 66:293–301

Haug LS, Thomsen C, Becher G (2009) Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ Sci Technol 43:2131–2136

Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, Uno A, Saijo Y, Sata F, Yoshimura Y, Kishi R, Nakazawa H (2004) Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of

PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect 112:1204–1207

Jensen MS, Nørgaard-Pedersen B, Toft G, Hougaard DM, Bonde JP, Cohen A, Thulstrup AM, Ivell R, Anand-Ivell R, Lindh CH, Jönsson BA (2012) Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: temporal trends and timing of amniocentesis in pregnancy. Environ Health Perspect 120:897–903

Jones PD, Hu W, De Coen W, Newsted JL, Giesy JP (2003) Binding of perfluorinated fatty acids to serum proteins. Environ Toxicol Chem 22:2639–2649

Kärrman A, van Bavel B, Järnberg U, Hardell L, Lindström G (2006) Perfluorinated chemicals in relation to other persistent organic pollutants in human blood. Chemosphere 64:1582–1591

Kärrman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A, Lignell S, Lindström G (2007) Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996–2004, in Sweden. Environ Health Perspect 115:226–230

Kim SK, Lee KT, Kang CS, Tao L, Kannan K, Kim KR, Kim CK, Lee JS, Park PS, Yoo YW, Ha JY, Shin YS, Lee JH (2011a) Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environ Pollut 159:169–174

Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, Kim S, Park S, Hwang I, Jeon J, Yang H, Giesy JP (2011b) Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol 45:7465–7472

Kissa E (2001) Fluorinated surfactants and repellants, 2nd edn. Marcel Dekker, New York

Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J (2007) Perfluo-roalkyl acids: a review of monitoring and toxicological findings. Toxicol Sci 99:366–394

Lindh CH, Rylander L, Toft G, Axmon A, Rignell-Hydbom A, Giwercman A, Pedersen HS, Góalczyk K, Ludwicki JK, Zvyezday V, Vermeulen R, Lenters V, Heederik D, Bonde JP, Jönsson BA (2012) Blood serum concentrations of perfluorinated compounds in men from Greenlandic Inuit and European populations. Chemosphere 88:1269–1275

Lindstrom AB, Strynar MJ, Libelo EL (2011) Polyfluorinated compounds: past, present, and future. Environ Sci Technol 45:7954–7961

Liu J, Li J, Liu Y, Chan HM, Zhao Y, Cai Z, Wu Y (2011) Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environ Int 37:1206–1212

Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J (2007) Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health 80:643–648

Monroy R, Morrison K, Teo K, Atkinson S, Kubwabo C, Stewart B, Foster WG (2008) Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res 108:56–62

Needham LL, Grandjean P, Heinzow B, Jørgensen PJ, Nielsen F, Patterson DG Jr, Sjödin A, Turner WE, Weihe P (2011) Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 45:1121–1126

Nolan LA, Nolan JM, Shofer FS, Rodway NV, Emmett EA (2009) The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reprod Toxicol 27:231–238

Olsen GW, Huang HY, Helzlsouer KJ, Hansen KJ, Butenhoff JL, Mandel JH (2005) Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. Environ Health Perspect 113:539–545

Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, Zobel LR (2007) Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect 115:1298–1305

Olsen GW, Ellefson ME, Mair DC, Church TR, Goldberg CL, Herron RM, Medhdizadehkashi Z, Nobiletti JB, Rios GA, Reagen WK, Zobel LR (2011) Analysis of a homologous series of perfluorocarboxylates from American Red Cross adult blood donors, 2000–2001 and 2006. Environ Sci Technol 45:8022–8029

Rylander C, Phi DT, Odland JØ, Sandanger TM (2009) Perfluorinated compounds in delivering women from south central Vietnam. J Environ Monit 11:2002–2008

Schultz MM, Barofsky DF, Field JA (2003) Fluorinated alkyl surfactants. Environ Eng Sci 20:487–501

Seals R, Bartell SM, Steenland K (2011) Accumulation and clearance of perfluorooctanoic acid (PFOA) in current and former residents of an exposed community. Environ Health Perspect 119:119–124

Sundström M, Ehresman DJ, Bignert A, Butenhoff JL, Olsen GW, Chang S-C, Bergman Å (2011) A temporal trend study (1972–2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. Environ Int 37:178–183

Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, Ito R, Nakada A, Iwasaki Y, Saito K, Nakazawa H, Kishi R (2009) Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect 117:660–667

Zhang W, Lin Z, Hu M, Wang X, Lian Q, Lin K, Dong Q, Huang C (2011) Perfluorinated chemicals in blood of residents in Wenzhou, China. Ecotoxicol Environ Saf 74:1787–1793

Paper II

OPEN ACCESS Freely available online

PLOS ONE

Fetal Exposure to Perfluorinated Compounds and Attention Deficit Hyperactivity Disorder in Childhood

Amanda Ode[1]*, Karin Källén[1], Peik Gustafsson[2], Lars Rylander[1], Bo A. G. Jönsson[1], Per Olofsson[3], Sten A. Ivarsson[4], Christian H. Lindh[1], Anna Rignell-Hydbom[1]

1 Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden, **2** Child and Adolescent Psychiatry Unit, Department of Clinical Sciences, Lund University, Lund, Sweden, **3** Obstetrics and Gynecology Unit, Department of Clinical Sciences, Skåne University Hospital, Lund University, Malmö, Sweden, **4** Department of Clinical Sciences, Unit of Pediatric Endocrinology, Lund University/Clinical Research Centre (CRC), Malmö, Sweden

Abstract

Background: The association between exposure to perfluorinated compounds (PFCs) and attention deficit hyperactivity disorder (ADHD) diagnosis has been sparsely investigated in humans and the findings are inconsistent.

Objectives: A matched case-control study was conducted to investigate the association between fetal exposure to PFCs and ADHD diagnosis in childhood.

Methods: The study base comprised children born in Malmö, Sweden, between 1978 and 2000 that were followed up until 2005. Children with ADHD (n = 206) were identified at the Department of Child and Adolescent Psychiatry. Controls (n = 206) were selected from the study base and were matched for year of birth and maternal country of birth. PFC concentrations were measured in umbilical cord serum samples. The differences of the PFC concentrations between cases and controls were investigated using Wilcoxon's paired test. Possible threshold effects (above the upper quartile for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and above limit of detection [LOD] for perfluorononanoic acid (PFNA)) were evaluated by conditional logistic regression.

Results: The median umbilical cord serum concentrations of PFOS were 6.92 ng/ml in the cases and 6.77 ng/ml in the controls. The corresponding concentrations of PFOA were 1.80 and 1.83 ng/ml. No associations between PFCs and ADHD were observed. Odds ratios adjusted for smoking status, parity, and gestational age were 0.81 (95% confidence interval [CI] 0.50 to 1.32) for PFOS, 1.07 (95% CI 0.67 to 1.7) for PFOA, and 1.1 (95% CI 0.75 to 1.7) for PFNA.

Conclusions: The current study revealed no support for an association between fetal exposure to PFOS, PFOA, or PFNA and ADHD.

Citation: Ode A, Källén K, Gustafsson P, Rylander L, Jönsson BAG, et al. (2014) Fetal Exposure to Perfluorinated Compounds and Attention Deficit Hyperactivity Disorder in Childhood. PLoS ONE 9(4): e95891. doi:10.1371/journal.pone.0095891

Editor: Aimin Chen, University of Cincinnati, United States of America

Received December 16, 2013; **Accepted** March 31, 2014; **Published** April 23, 2014

Copyright: © 2014 Ode et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The project was funded by the Swedish Research Council (via SIMSAM Early Life) (web page: www.vr.se/) and the Medical Faculty at Lund University (web page: www.med.lu.se). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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

* E-mail: amanda.ode@med.lu.se

Introduction

Emission of pollutants from densely populated areas and industries is a growing environmental problem. Contaminants present in the environment can have a negative impact on both human health and environment. Perfluorinated compounds (PFCs) are extremely stable and persistent man-made organic chemicals that have been identified as environmental pollutants. The unique properties of PFCs have made them highly useful in numerous industrial and consumer applications such as lubricants, firefighting foams, cleaning agents, and in surface coating for paper, food packaging, textiles, furniture, carpets and cookware [1–3].

PFCs, particularly perfluorooctane sulfonate (PFOS) and perfluorooctanic acid (PFOA) have been widely detected in the environment, wildlife, and humans [4–8]. Humans are exposed to PFCs through consumer products as well as contaminated air, water, and food [1]. In recent years, studies have revealed that PFCs cross the placenta and accumulate in the fetus [9–11]. The fetal brain is immature and is therefore susceptible to injury caused by toxic agents [12]. Animal data have indicated that PFCs accumulate in the brain both before and after the blood-brain barrier is formed [13–16].

Animal studies have shown that neonatal exposure to low doses of PFCs induced irreversible neurotoxic effects in adult mice and caused changes in behavior and habituation by altering the dopaminergic and cholinergic system [17,18]. PFCs also alter levels of neural proteins that are important for the formation and growth of the synapses [19]. Defects in the dopamine transporters and receptors have been suggested to be the most significant neurobiological problem in attention deficit hyperactivity disorder (ADHD) [20,21].

ADHD is a neurodevelopmental disorder defined by inattention, hyperactivity and impulsivity [22,23]. The disorder has its

onset in childhood, and persists into adolescence and into adulthood in some cases [24,25]. The genetic factor is believed to play the major role in the development of ADHD [22,26,27]. In addition, exposure to environmental toxins, such as lead, mercury, and persistent chlorinated biphenyls, has also been related to ADHD [26,28,29].

Two cross-sectional studies based on parent-reported ADHD diagnosis have investigated the potential association between PFC levels in school-age children and ADHD [30,31]. The study by Hoffman et al. [30] found a positive relationship between ADHD and PFC levels in the blood of children between 12 and 15 years, whereas an association with only perfluorohexane sulfonate (PFHxS) was found in the study by Stein and Savitz [31]. In another cross-sectional study, PFC exposure was associated with impulsivity in children [32]. Other studies based on questionnaires investigated whether behavioral health and motor coordination as well as motor and mental developmental milestones were associated with maternal PFCs during pregnancy and found no such associations except for PFOS which was associated with delayed motor development in the first two years of life [33,34].

The frequency of children receiving an ADHD diagnosis has increased in recent years [35]. Improved diagnostic criteria might be responsible for the increased detection of ADHD cases. Increased exposure to environmental pollutants might also contribute to the high prevalence of ADHD. Since the human brain is susceptible to disturbance by environmental pollutants during the fetal period, it is of importance to investigate the association between exposure to these pollutants during the sensitive period of fetal development and ADHD.

The objective of this study is to investigate the association between fetal exposure to PFCs and ADHD diagnosis in childhood. Unlike previous studies, this case-control study is based on clinical ADHD diagnosis and PFCs are measured in umbilical cord serum samples which reflect the PFC concentrations in the fetus. The study is a part of the Fetal Environment and Neurodevelopment Disorders in Epidemiological Research project (the FENDER project).

Material and Methods

Participants

The selection procedure of the children with ADHD diagnosis has been previously described by Gustafsson and Kallen [36]. Briefly, at the Department of Child and Adolescent Psychiatry in the city of Malmö, 419 children born and living in Malmö between 1978 and 2000 with ADHD diagnosis were identified and were followed up until 2005. During the study period, the children with ADHD were diagnosed by one of ten experienced clinicians at the department using the Diagnostic and Statistical Manual of Mental Disorders (DSM). A child with suspected attention difficulties, hyperactivity and/or difficulties with impulse control is usually assessed to the child and adolescent psychiatry by a special teacher and a school psychologist or by the parents. The assessment begins with gathering information about the child's general medical health condition and the child's development from birth until the present time. The school psychologist or the psychologist at the psychiatric clinic performs a cognitive testing with the Wechsler Intelligence Scale (WISC). The parents and the teacher are asked to fill in questionnaires like SNAP-IV, Conner's questionnaire or the 5–15 questionnaire which all cover the symptoms of ADHD. Parents are usually asked to fill in the BRIEF-questionnaire concerning the child's executive functions in every-day life. Sometimes a member of the team at the clinic observes the child at school. The child's ability to concentrate is tested with TEA-Ch or with a computerized test of attention such as QB-Tech or IVA+. The child psychiatrist performs a paediatric examination with assessment of neurological soft-signs. The child's behaviour in different test situations and at the visits at the clinic is observed and registered. When all parts of the assessment have been performed, a team consisting of doctor, psychologist and sometimes a social worker meet and discuss the findings to come to a consensus decision concerning the diagnosis using DSM criteria. The DSM criteria DSM-III-R_{11} and DSM-IV_{12} were used before 1994 and from 1994 and onwards, respectively. Age at the time of diagnosis varied between 5 and 17 years, with most children being diagnosed between the ages of 8 and 12 years.

Using the personal identification numbers, children with ADHD were linked to the Swedish Medical Birth Register (SMBR) which contains demographic and obstetric information on nearly all (99%) the mothers and the infants in Sweden. Umbilical cord serum samples for children with ADHD were collected from the Malmö Maternity Unit Serum Biobank (MMUSB) using the personal identification numbers. Nearly all deliveries in Malmö take place at the Malmö University Hospital Maternity Unit, where blood samples from the mother and from the umbilical cord of the newborn have been collected at the time of delivery and stored at $-20^{\circ}C$ at the MMUSB since 1969. Controls were selected into two phases. In the first phase, for each ADHD case with an available umbilical blood sample in the biobank, the next-baby-born with serum sample of the same sex was selected as a control. However, a new publication by Gustafsson and Kallen [36] revealed the impact of maternal country of birth on the diagnosis of ADHD. Thus, the benefit of matching for the maternal country of birth completely overrode that from matching for the infant's sex. Therefore, in the second phase, a pool of ten eligible controls per ADHD case were collected from the SMBR and were matched to the cases for year of birth (± 12 months) and country of birth of the mother. The sample of the next-baby-born from the first phase was used if no newborn in that eligible pool of controls had an available umbilical blood sample in the biobank. The selection procedure for cases and controls is presented in Figure 1.

Ethics statements

At the Maternity Unit, the women were informed that the umbilical cord serum sample collected could be used for research purposes in the future and those who accepted gave their verbal informed consent that was documented in the medical records. During the study period only verbal informed consent was obtained. The written informed consent has been implemented in 2005 and therefore could not be considered for the current study. The data were analysed anonymously. The study protocol followed the requirements of the Declaration of Helsinki and the study, together with the consent procedure, was approved by the Research Ethics Committee at Lund University, Sweden.

Analysis of perfluorinated compounds and cotinine in umbilical cord serum

The analyses of PFHxS, PFOS, PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), and cotinine were performed as previously described [37]. Briefly, aliquots of 100 μL sera were added with isotopically labeled internal standards, the proteins were precipitated by acetonitrile and centrifugation, and analysis was then performed using a hybrid triple quadrupole linear ion-trap mass spectrometer (LC/MS/MS; UFLCXR, Shimadzu Corporation, Kyoto, Japan; QTRAP 5500; Sciex, Framingham, MA, USA). The limits of detections for the detected PFCs and

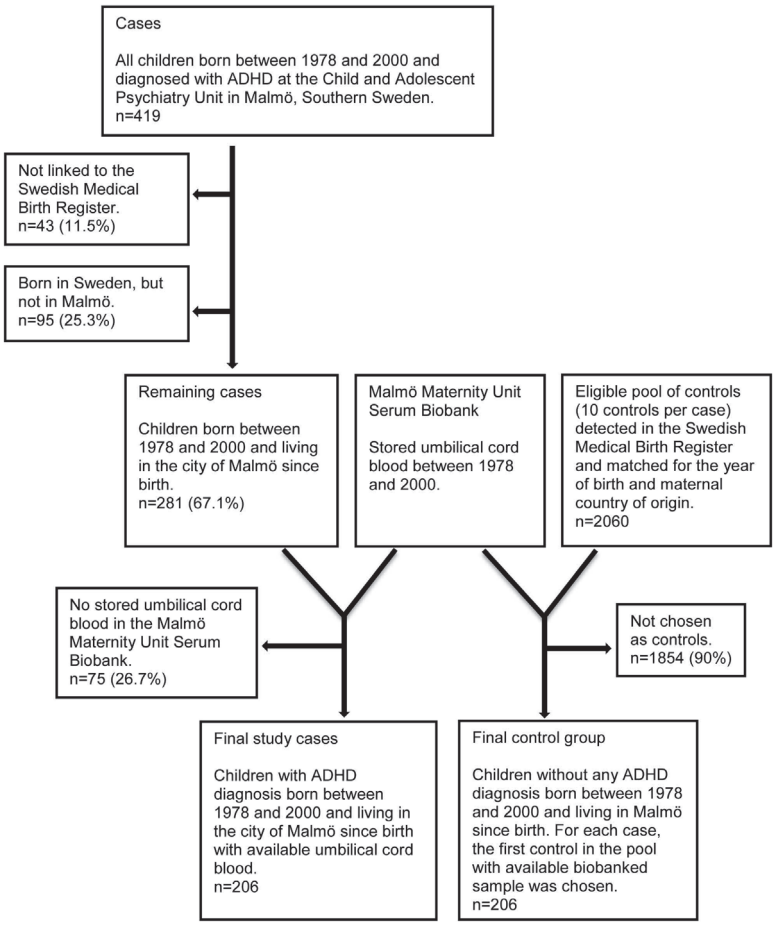


Figure 1. Flowchart for the selection procedure of the children with attention deficit hyperactivity disorder and controls.
doi:10.1371/journal.pone.0095891.g001

cotinine were 0.2 ng/ml. To increase the accuracy, the result reported is the average of two measurements from the same sample worked up and analyzed on different days. In all sample batches, the quality of the measurements was controlled by analyzing chemical blanks and in-house quality control (QC) samples. The reproducibility, determined as the relative standard deviation, between measured duplicate samples was 11% for PFOS, 12% for PFOA, 12% for PFNA, and 9% for cotinine. The reproducibility in QC samples was 8% for PFOS, 11% for PFOA, 8% for PFNA, and 5% for cotinine. Usually we are able to analyze several more PFCs with the method but some factor, probably during the storage of the samples, resulted in a high background noise in the chromatograms making detection impossible. Thus, PFHxS, PFDA, PFUnDA, and PFDoDA could not be detected in the samples due to this effect. On the other hand, due to the high correlation between PFOS and other PFCs often only PFOS and PFOA are reported in studies of PFCs. Although contamination of samples during collection is believed to be minimal, field blanks could not be provided to control for eventual contamination of the samples with PFCs. The analyses of PFOS and PFOA are part of the round robin intercomparison program (Professor Dr. med. Hans Drexler, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany) with results within the tolerance limits.

Statistical analyses

The Wilcoxon's paired test was used to compare the PFC concentrations between ADHD cases and controls. Conditional logistic regression analysis was used to assess the association between fetal exposure to PFCs and ADHD. The odds ratio was

Table 1. Median concentration (in nanograms/milliliters) of perfluorinated compounds by the maternal and infant demographic characteristics.

Characteristics	Children with ADHD			Control group		
	n (%)	PFOS	PFOA	n (%)	PFOS	PFOA
Group (cases/controls)	203 (49.8%)	6.92	1.80	205 (50.2%)	6.77	1.83
Year of delivery						
1978–1981	2 (1.0)	2.66	0.45	2 (1.0)	8.70	0.85
1982–1985	13 (6.4)	5.69	1.50	10 (4.9)	6.49	1.71
1986–1989	63 (31.0)	6.96	2.0	66 (32.2)	6.71	1.82
1990–1993	86 (42.4)	7.08	1.78	87 (42.4)	6.74	1.82
1994–1997	34 (16.7)	6.65	1.69	35 (17.1)	7.44	1.87
1998–2000	5 (2.5)	7.68	1.64	5 (2.4)	8.11	1.86
Maternal age (years)						
<20	8 (3.9)	6.67	1.57	6 (2.9)	8.65	2.16
20–34	172 (84.7)	6.94	1.81	171 (83.4)	6.74	1.82
≥35	23 (11.3)	6.34	1.64	28 (13.7)	7.05	1.81
Parity						
0 [nulliparous]	97 (47.8)	7.00	2.01	106 (51.7)	7.56	2.13
1	71 (35.0)	6.60	1.55	68 (33.2)	6.22	1.55
≥2	35 (17.2)	6.80	1.68	31 (15.1)	6.17	1.42
Maternal country of origin						
Sweden	168 (83.3)	7.02	1.85	170 (82.9)	7.06	1.89
Other Nordic countries[a]	7 (3.4)	4.28	2.13	7 (3.4)	6.18	1.69
Rest of Europe[b]	8 (3.9)	7.47	1.60	9 (4.4)	5.48	1.48
Sub-Saharan Africa	2 (1.0)	4.23	0.72	2 (1.0)	2.10	0.45
Middle East and North Africa	13 (6.4)	4.42	0.85	12 (5.9)	2.76	0.47
East Asia	1 (0.5)	6.83	1.71	2 (1.0)	9.36	1.43
South America	2 (1.0)	7.58	2.89	2 (1.0)	7.63	13.5
Unknown	1 (0.5)	2.96	0.46	1 (0.5)	2.60	1.70
Maternal body mass index (kg/m^2)[c]						
Not available	141 (69.5)	6.85	1.89	142 (69.3)	6.67	1.83
<18.5 (Underweight)	1 (0.5)	10.1	2.64	3 (1.5)	8.75	2.39
18.5–24.9 (Normal)	37 (18.2)	6.83	1.63	42 (20.5)	7.50	1.72
25–29.9 (Overweight)	16 (7.9)	7.27	1.36	14 (6.8)	7.58	2.02
≥30 (Obese)	8 (3.9)	6.06	2.09	4 (2.0)	6.40	2.31
Smoking during pregnancy[d]						
Non-smoker	65 (32.0)	6.54	1.83	85 (41.5)	6.82	1.86
Second-hand smoker	57 (28.1)	7.08	1.71	57 (27.8)	6.91	1.86
Active smoker	81 (39.9)	7.49	1.82	63 (30.7)	6.37	1.72
Infant sex						
Male	180 (88.7)	6.97	1.76	163 (79.5)	6.87	1.84
Female	23 (11.3)	6.32	1.99	42 (20.5)	6.51	1.64
Birth weight (grams)						
<1500	4 (2.0)	5.73	2.31	0		
<2500	9 (4.4)	4.85	1.44	5 (2.4)	6.37	1.84
2500–4000	166 (81.8)	7.12	1.84	152 (74.1)	6.63	1.82
>4000	24 (11.8)	6.41	1.67	48 (23.4)	7.25	1.94
Gestational age (weeks)						
<32	5 (2.5)	4.77	1.44	1 (0.5)	4.71	1.05
<37	6 (3.0)	4.36	1.09	6 (2.9)	4.74	1.97
37–42	176 (86.7)	7.12	1.88	178 (86.8)	6.73	1.82
>42	16 (7.9)	6.54	1.63	20 (9.8)	8.37	1.77

Table 1. Cont.

Characteristics	Children with ADHD			Control group		
	n (%)	PFOS	PFOA	n (%)	PFOS	PFOA
SD scores (for gestational age)						
<−2 (small for gestational age)	9 (4.4)	6.69	2.26	14 (6.8)	7.92	1.94
−2 to −1.1	37 (18.2)	6.60	1.53	29 (14.1)	6.56	1.94
−1.1 to 1	138 (68.0)	7.01	1.79	126 (61.5)	6.67	1.77
1.1 to 2	15 (7.4)	5.48	2.00	32 (15.6)	7.79	2.02
>2 (large for gestational age)	4 (2.0)	7.30	2.40	4 (2.0)	4.05	1.20
Apgar scores						
0–6	5 (2.5)	4.28	1.80	2 (1.0)	10.2	2.44
≥7	198 (97.5)	6.94	1.79	203 (99.0)	6.74	1.82

Abbreviations: ADHD, attention deficit hyperactivity disorder; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; Parity, number of previous pregnancies.
[a]Finland, Denmark, and Norway.
[b]Western Europe and former Eastern Europe.
[c]Body mass index was classified according to the standard values of the World Health Organization.
[d]Maternal smoking is based on measured cotinine concentrations in the umbilical cord serum.
doi:10.1371/journal.pone.0095891.t001

calculated for both 1 unit increase (nanogram per milliliter) in the concentrations of PFOS and PFOA and for comparisons between concentrations above and below the 75th percentile for the control group. For PFNA the concentrations above the limit of detection (LOD) were compared to those below LOD (0.2 ng/ml).

The potential confounding variables that were considered in the present study were smoking during pregnancy, parity, and gestational age at birth, since they have been found to be associated with both PFC exposure and ADHD [11,38–46].

Smoking during pregnancy was determined by cotinine levels in umbilical cord serum. Cotinine levels below the LOD (0.2 ng/ml) were related to nonsmoking pregnant women, cotinine levels higher than 15 ng/ml were related to active smokers and levels between 0.2 and 15 ng/ml were related to second-hand smokers [47]. Parity was divided into three groups according to number of previously born children (0 [i.e. nulliparous], 1, or ≥2 children). Gestational age was entered in the analyses as class variable divided into three groups; <37, 37–42, and >42 weeks of pregnancy.

The odds ratios were calculated in paired samples (n = 202) using Egret for Windows 2.0 (Cytel Software Corporation). The rest of the analyses were performed in IBM SPSS Statistics version 20 (IBM Corporation 1989, 2011).

The power calculation was based on 206 cases and matched controls. With the current setting, we had an 80% chance of detecting a difference in the levels of 0.20 standard deviations, with α value of 0.05, between cases and controls. For the analysis of the threshold effect, with α value of 0.05 and β value of 0.80, the lowest detectable odds ratio was 1.8.

Results

PFOS and PFOA concentrations were above the LOD in 98% of the samples, whereas for PFNA about 12% were above the LOD. PFOS and PFOA concentrations below the LOD in individual samples (n = 2 for each) were replaced with 0.2 ng/ml.

The demographic characteristics and the umbilical cord PFC concentrations of the study population are presented in Table 1.

Figure 2 shows the distribution of PFOS and PFOA levels in the ADHD cases and the controls. The median concentrations of PFNA above LOD for cases and controls were 0.31 and 0.28 ng/ml, respectively. Wilcoxon's paired test revealed no differences in cord serum PFC concentrations between children with ADHD diagnosis and controls ($p = 0.72$, 0.44, and 0.48 for PFOS, PFOA, and PFNA respectively).

Conditional logistic regression analyses revealed no significant associations between umbilical cord concentrations of PFCs and ADHD (Table 2). The result did not change after adjusting for smoking during pregnancy, parity, and gestational age at birth.

Discussion

The present study found no statistically significant associations between exposure to PFCs during pregnancy and ADHD diagnosis during childhood, although the measured umbilical cord concentrations of PFOS were among the highest in Europe [42,48,49] and even among the highest in the world [9,50–52]. For PFOA, the levels were higher than those measured in Norway and other non-European countries but lower than those in Danmark and Faraoe Islands [9,42,48,49].

Animal data revealed that neonatal mice that were exposed to high doses of PFOS and PFOA showed behavioral defects which ranged from slight effects at the anxiety level [53] to reduced habituation and hyperactivity in adult mice [17]. It has been suggested that PFOS and PFOA act as developmental neurotoxicants that mediate their effects on normal brain development, with consequences for cognitive and behavioral functions, through different mechanisms. Examples of those mechanisms are alteration in the dopaminergic system [17,18], elevated levels of proteins important for normal neuronal survival, growth and synaptogenesis, such as CaMKII, GAP-43, synaptophysin and tau, in the brain [19], and induction of apoptosis of neuronal cells [54]. Although most of these findings were obtained from experiments on mice or rat derived cell lines that were exposed to extremely high levels of PFOS and PFOA compared to the low levels measured in the present study, other studies found that PFCs were detrimental to neurodevelopment at levels comparable to those observed in humans [17,19].

Our study is primarily comparable to the study by Fei and Olsen [34] because both studies used measures of prenatal rather than postnatal exposure to PFCs. Fei and Olsen [32] found higher levels of PFOS and PFOA compared to those seen among

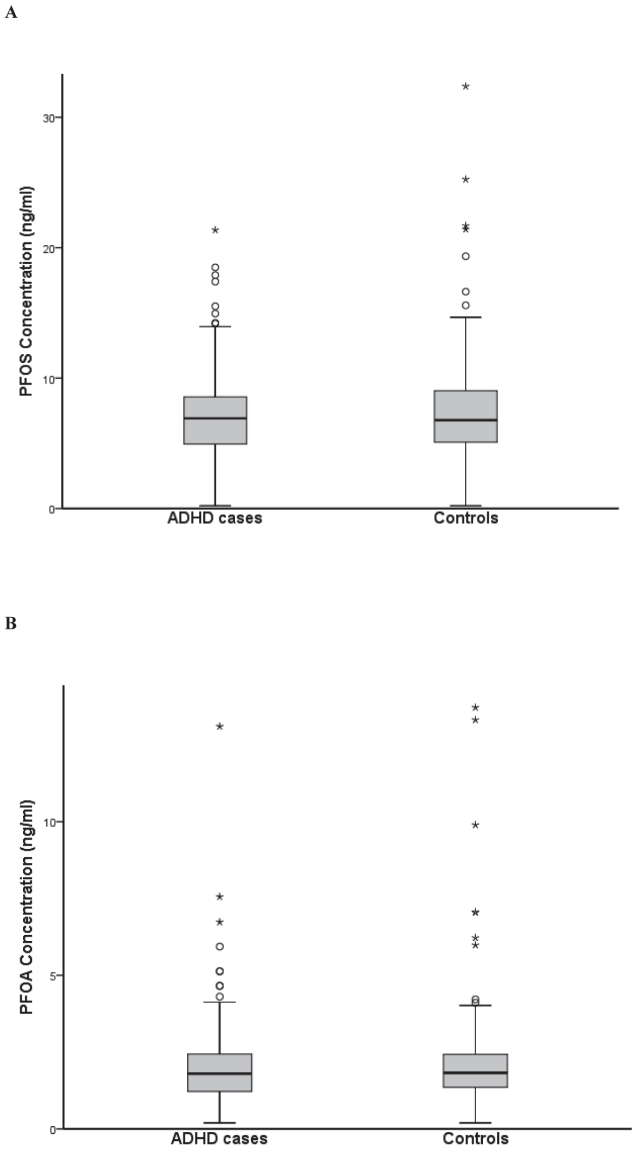


Figure 2. Boxplot of the umbilical cord concentrations of perfluorooctane sulfonate (PFOS) (a) and perfluorooctanoic acid (PFOA) (b) in cases having attention deficit hyperactivity disorder and controls. The extreme values of perfluorooctanoic acid for the ADHD cases, 48 and 36 ng/ml, and for the controls, 66, 49, 31, and 23 ng/ml, are not presented in the boxplot.
doi:10.1371/journal.pone.0095891.g002

pregnant women in other countries including the Nordic countries [11,48]. Consistent with that study, our results provide further indication that fetal exposure to PFCs at the present levels do not play a major role in having ADHD diagnosis at later age. Hoffman et al. [30] found an association between PFC serum concentrations and ADHD in children aged 12 to 15 years. Another study

Table 2. The crude and adjusted odds ratio with 95% confidence interval of attention deficit hyperactivity disorder and exposure to perfluorinated compounds.

	ADHD Diagnosis	
	Crude	**Adjusted[a]**
PFOS[b]	0.98 (0.92–1.03)	0.98 (0.92–1.04)
PFOA[b]	0.98 (0.94–1.02)	0.98 (0.94–1.02)
PFOS[c]		
$<75^{th}$ percentile	1	1
$\geq 75^{th}$ percentile	0.82 (0.51–1.31)	0.81 (0.50–1.32)
PFOA[c]		
$<75^{th}$ percentile	1	1
$\geq 75^{th}$ percentile	1.03 (0.65–1.6)	1.07 (0.67–1.7)
PFNA[d]		
<LOD	1	1
≥LOD	1.1 (0.72–1.6)	1.1 (0.75–1.7)

Abbreviations: ADHD, attention deficit hyperactivity disorder; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; LOD, limit of detection.

[a]Adjusted for maternal active smoking, parity, and gestational age at birth.

[b]Odds ratio is calculated for 1 ng/ml increase in umbilical cord serum concentration.

[c]Odds ratio is calculated for PFOS and PFOA concentrations at or above the 75^{th} percentile (75^{th} percentile for PFOS and PFOA were 9,1 ng/ml and 2,4 ng/ml, respectively).

[d]Odds ratio is calculated for PFNA concentrations at or above the LOD (0.2 ng/ml).

doi:10.1371/journal.pone.0095891.t002

by Stein and Savitz [31] on the relationship between self-reported ADHD and PFC levels in children in the same age range as for those in the study of Hoffman et al. [30] showed an association with PFHxS but not with the other PFCs even though both ADHD prevalence and exposure levels for PFCs were higher in the study by Stein and Savitz. Exposure to PFCs tends to be higher among newborns, toddlers, and children due to high uptake via food consumption, hand-to-mouth transfer of the PFCs from carpets, and through ingestion of dust [55]. If the positive association between PFC exposure and self-reported ADHD found in the study by Hoffman et al. [30] was not due to a chance finding, that might indicate that postnatal exposure to PFCs, rather than prenatal exposure, is associated with ADHD.

The present study has some limitations. Unfortunately, while 419 children with ADHD diagnosis were identified at the Department of Child and Adolescent Psychiatry, there were significant losses to get to the final study sample. The study was restricted to children born in Malmö with available obstetric and demographic information from the SMBR and stored cord blood samples in the biobank. About 50% of the identified children met these two inclusion criteria and were included in the study. The second limitation of the current study is the small number of ADHD cases. Although we would be able to detect an odd ratio of 1.8 and a difference in PFC levels of at least 0.20 standard deviations between cases and controls, it should be emphasized that the statistical power was not high enough to detect some minor associations and we could not accordingly rule out small effects. In addition, we lack information about other exposures that are significant for ADHD, such as exposure to mercury and lead. The PFC levels measured here might also be too low to trigger undesirable effects on the brain development.

During our study period, the clinical diagnostic criteria for ADHD were changed from the definition in DSM-III-R to the definition in DSM-IV, where DSM-IV is regarded as more inclusive. Thus, DSM-IV criteria yield a higher prevalence of ADHD [56]. Most individuals (93%–97.5%) who fulfill a diagnosis of ADHD according to DSM-III-R also fulfill the diagnostic criteria according to DSM-IV [57-59]. Individuals with ADHD according to DSM-IV that also fulfilled diagnostic criteria according to DSM-III-R were 85% [57] and 60% [59]. Thus the overlap between ADHD diagnoses according to DSM-III-R and DSM-IV is considerable. The more inclusive diagnosis used in the latter part of the study probably includes some less severe cases which might slightly weaken possible statistical associations between exposure to PFCs and having an ADHD diagnosis.

The study has also several important strengths. First, unlike most of the previous studies on the associations between PFC levels and ADHD, our prospective study design is more reliable in the sense that it is based on clinical diagnosis of ADHD made at the Department of Child and Adolescent Psychiatry. Children were diagnosed at the same psychiatric clinic through the whole study period.

Second, the present study is based on analyzed blood samples from the fetal period, which we believe is the most susceptible exposure window, whereas in other studies, which were of a cross-sectional nature, blood samples were collected from school-age children [30,31].

Third, we were able to account for important covariates; smoking during pregnancy, parity, and gestational age at birth that have been found to be associated with both PFC levels in pregnant women or infants [11,38,42,46] and ADHD diagnosis or symptoms [39–41,43–45]. Epidemiological findings have shown that prevalence of ADHD is higher among males [20]. It has also previously been shown that infant sex has no effect on the concentrations of PFCs [11,42,60]. Thus, infant sex was not considered as a potential confounder in the current data set.

In a previous study, we found that fetuses of mothers originating from a country other than Sweden, especially those from Middle East and sub-Saharan Africa, had lower PFC levels in the cord blood than fetuses of native Swedish mothers [11]. Another study

found higher odds of having an ADHD diagnosis for native Swedish children compared to children of mothers born outside Sweden [36]. Since the proportion of immigrants is relatively high in Malmö, no matching for the maternal country of birth might result in a false positive relationship between PFCs and ADHD.

Human serum PFC levels showed an increasing pattern from the early 1970s through the late 1990s, followed by leveling out and a decreasing trend right after the phase-out of the production of PFOS and PFOS related compounds in 2002 [8,61–64]. Diagnosis criteria for ADHD have been changed during the study period. We matched for the year of delivery to remove the effect of those differences in PFC levels and diagnosis on the results.

According to our findings, fetal exposure to PFOS, PFOA and PFNA was not associated with ADHD diagnosis in childhood.

Acknowledgments

We would like to thank the laboratory technicians Åsa Amilon and Agneta Kristensen for analyzing the samples.

Author Contributions

Conceived and designed the experiments: KK PG LR SAI PO ARH AO. Performed the experiments: BAGJ CHL. Analyzed the data: AO KK LR ARH. Contributed reagents/materials/analysis tools: BAGJ CHL. Wrote the paper: AO. Wrote the section about the laboratory analysis: BAGJ CHL. Reviewed the manuscript and made comments on it before submission: ARH LR KK PO PG SAI.

References

1. Fromme H, Tittlemier SA, Volkel W, Wilhelm M, Twardella D (2009) Perfluorinated compounds—exposure assessment for the general population in Western countries. Int J Hyg Environ Health 212: 239–270.
2. Kissa E (2001) Fluorinated Surfactants and Repellants. NY, USA: Marcel Dekker Inc.
3. Lemal DM (2004) Perspective on fluorocarbon chemistry. J Org Chem 69: 1–11.
4. Butt CM, Berger U, Bossi R, Tomy GT (2010) Levels and trends of poly- and perfluorinated compounds in the arctic environment. Sci Total Environ 408: 2936–2965.
5. Giesy JP, Kannan K (2001) Global distribution of perfluorooctane sulfonate in wildlife. Environ Sci Technol 35: 1339–1342.
6. Giesy JP, Kannan K (2002) Perfluorochemical surfactants in the environment. Environ Sci Technol 36: 146A–152A.
7. Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, et al. (2004) Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environmental Science & Technology 38: 4489–4495.
8. Olsen GW, Huang H-Y, Helzlsouer KJ, Hansen KJ, Butenhoff JL, et al. (2005) Historical Comparison of Perfluorooctanesulfonate, Perfluorooctanoate, and Other Fluorochemicals in Human Blood. Environmental Health Perspectives 113: 539–545.
9. Beesoon S, Webster GM, Shoeib M, Harner T, Benskin JP, et al. (2011) Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer. Environ Health Perspect 119: 1659–1664.
10. Inoue K, Okada F, Ito R, Kato S, Sasaki S, et al. (2004) Perfluorooctane Sulfonate (PFOS) and Related Perfluorinated Compounds in Human Maternal and Cord Blood Samples: Assessment of PFOS Exposure in a Susceptible Population during Pregnancy. Environmental Health Perspectives 112: 1204–1207.
11. Ode A, Rylander L, Lindh CH, Kallen K, Jonsson BA, et al. (2013) Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds. Environ Sci Pollut Res Int 20: 7970–7978.
12. Grandjean P, Landrigan PJ (2006) Developmental neurotoxicity of industrial chemicals. Lancet 368: 2167–2178.
13. Austin ME, Kasturi BS, Barber M, Kannan K, MohanKumar PS, et al. (2003) Neuroendocrine Effects of Perfluorooctane Sulfonate in Rats. Environmental Health Perspectives 111: 1485–1489.
14. Butenhoff JL, Ehresman DJ, Chang SC, Parker GA, Stump DG (2009) Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+ PFOS) in rats: developmental neurotoxicity. Reprod Toxicol 27: 319–330.
15. Cui L, Zhou QF, Liao CY, Fu JJ, Jiang GB (2009) Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch Environ Contam Toxicol 56: 338–349.
16. Greaves AK, Letcher RJ, Sonne C, Dietz R (2013) Brain region distribution and patterns of bioaccumulative perfluoroalkyl carboxylates and sulfonates in east greenland polar bears (Ursus maritimus). Environ Toxicol Chem 32: 713–722.
17. Johansson N, Fredriksson A, Eriksson P (2008) Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology 29: 160–169.
18. Slotkin TA, MacKillop EA, Melnick RL, Thayer KA, Seidler FJ (2008) Developmental neurotoxicity of perfluorinated chemicals modeled in vitro. Environ Health Perspect 116: 716–722.
19. Johansson N, Eriksson P, Viberg H (2009) Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicol Sci 108: 412–418.
20. Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, et al. (2005) Molecular Genetics of Attention-Deficit/Hyperactivity Disorder. Biological Psychiatry 57: 1313–1323.
21. Gizer IR, Ficks C, Waldman ID (2009) Candidate gene studies of ADHD: a meta-analytic review. Hum Genet 126: 51–90.
22. Faraone SV, Khan SA (2006) Candidate gene studies of attention-deficit/hyperactivity disorder. J Clin Psychiatry 67 Suppl 8: 13–20.
23. Gustafsson P, Thernlund G, Ryding E, Rosen I, Cederblad M (2000) Associations between cerebral blood-flow measured by single photon emission computed tomography (SPECT), electro-encephalogram (EEG), behaviour symptoms, cognition and neurological soft signs in children with attention-deficit hyperactivity disorder (ADHD). Acta Paediatr 89: 830–835.
24. Faraone SV, Biederman J, Mick E (2006) The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol Med 36: 159–165.
25. Lara C, Fayyad J, de Graaf R, Kessler RC, Aguilar-Gaxiola S, et al. (2009) Childhood predictors of adult attention-deficit/hyperactivity disorder: results from the World Health Organization World Mental Health Survey Initiative. Biol Psychiatry 65: 46–54.
26. Banerjee TD, Middleton F, Faraone SV (2007) Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatr 96: 1269–1274.
27. Thapar A, Cooper M, Jefferies R, Stergiakouli E (2012) What causes attention deficit hyperactivity disorder? Arch Dis Child 97: 260–265.
28. Braun JM, Kahn RS, Froehlich T, Auinger P, Lanphear BP (2006) Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children. Environ Health Perspect 114: 1904–1909.
29. Eubig PA, Aguiar A, Schantz SL (2010) Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. Environ Health Perspect 118: 1654–1667.
30. Hoffman K, Webster TF, Weisskopf MG, Weinberg J, Vieira VM (2010) Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environ Health Perspect 118: 1762–1767.
31. Stein CR, Savitz DA (2011) Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5–18 years of age. Environ Health Perspect 119: 1466–1471.
32. Gump BB, Wu Q, Dumas AK, Kannan K (2011) Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environ Sci Technol 45: 8151–8159.
33. Fei C, McLaughlin JK, Lipworth L, Olsen J (2008) Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environ Health Perspect 116: 1391–1395.
34. Fei C, Olsen J (2011) Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environ Health Perspect 119: 573–578.
35. Pastor PN, Reuben CA (2008) Diagnosed attention deficit hyperactivity disorder and learning disability: United States, 2004–2006. Vital Health Stat 10: 1–14.
36. Gustafsson P, Kallen K (2011) Perinatal, maternal, and fetal characteristics of children diagnosed with attention-deficit-hyperactivity disorder: results from a population-based study utilizing the Swedish Medical Birth Register. Dev Med Child Neurol 53: 263–268.
37. Lindh CH, Rylander L, Toft G, Axmon A, Rignell-Hydbom A, et al. (2012) Blood serum concentrations of perfluorinated compounds in men from Greenlandic Inuit and European populations. Chemosphere 88: 1269–1275.
38. Apelberg BJ, Goldman LR, Calafat AM, Herbstman JB, Kuklenyik Z, et al. (2007) Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland. Environ Sci Technol 41: 3891–3897.
39. Carballo JJ, Garcia-Nieto R, Alvarez-Garcia R, Caro-Canizares I, Lopez-Castroman J, et al. (2013) Sibship size, birth order, family structure and childhood mental disorders. Soc Psychiatry Psychiatr Epidemiol 48: 1327–1333.
40. Chu SM, Tsai MH, Hwang FM, Hsu JF, Huang HR, et al. (2012) The relationship between attention deficit hyperactivity disorder and premature infants in Taiwanese: a case control study. BMC Psychiatry 12: 85.
41. Desrosiers C, Boucher O, Forget-Dubois N, Dewailly E, Ayotte P, et al. (2013) Associations between prenatal cigarette smoke exposure and externalized behaviors at school age among Inuit children exposed to environmental contaminants. Neurotoxicol Teratol 39C: 84–90.

42. Fei C, McLaughlin JK, Tarone RE, Olsen J (2007) Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect 115: 1677–1682.
43. Kotimaa AJ, Moilanen I, Taanila A, Ebeling H, Smalley SL, et al. (2003) Maternal smoking and hyperactivity in 8-year-old children. J Am Acad Child Adolesc Psychiatry 42: 826–833.
44. Langley K, Holmans PA, van den Bree MB, Thapar A (2007) Effects of low birth weight, maternal smoking in pregnancy and social class on the phenotypic manifestation of Attention Deficit Hyperactivity Disorder and associated antisocial behaviour: investigation in a clinical sample. BMC Psychiatry 7: 26.
45. Perricone G, Morales MR, Anzalone G (2013) Neurodevelopmental outcomes of moderately preterm birth: precursors of attention deficit hyperactivity disorder at preschool age. Springerplus 2: 221.
46. Washino N, Saijo Y, Sasaki S, Kato S, Ban S, et al. (2009) Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect 117: 660–667.
47. George L, Granath F, Johansson AL, Cnattingius S (2006) Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. Acta Obstet Gynecol Scand 85: 1331–1337.
48. Gutzkow KB, Haug LS, Thomsen C, Sabaredzovic A, Becher G, et al. (2012) Placental transfer of perfluorinated compounds is selective—a Norwegian Mother and Child sub-cohort study. Int J Hyg Environ Health 215: 216–219.
49. Needham LL, Grandjean P, Heinzow B, Jorgensen PJ, Nielsen F, et al. (2011) Partition of environmental chemicals between maternal and fetal blood and tissues. Environ Sci Technol 45: 1121–1126.
50. Kim S, Choi K, Ji K, Seo J, Kho Y, et al. (2011) Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol 45: 7465–7472.
51. Kim SK, Lee KT, Kang CS, Tao L, Kannan K, et al. (2011) Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environ Pollut 159: 169–174.
52. Liu J, Li J, Liu Y, Chan HM, Zhao Y, et al. (2011) Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environ Int 37: 1206–1212.
53. Fuentes S, Vicens P, Colomina MT, Domingo JL (2007) Behavioral effects in adult mice exposed to perfluorooctane sulfonate (PFOS). Toxicology 242: 123–129.
54. Lee HG, Lee YJ, Yang JH (2012) Perfluorooctane sulfonate induces apoptosis of cerebellar granule cells via a ROS-dependent protein kinase C signaling pathway. Neurotoxicology 33: 314–320.
55. Trudel D, Horowitz L, Wormuth M, Scheringer M, Cousins IT, et al. (2008) Estimating consumer exposure to PFOS and PFOA. Risk Anal 28: 251–269.
56. Skounti M, Philalithis A, Galanakis E (2007) Variations in prevalence of attention deficit hyperactivity disorder worldwide. Eur J Pediatr 166: 117–123.
57. Lahey BB, Applegate B, McBurnett K, Biederman J, Greenhill L, et al. (1994) DSM-IV field trials for attention deficit hyperactivity disorder in children and adolescents. Am J Psychiatry 151: 1673–1685.
58. Biederman J, Faraone SV, Weber W, Russell RL, Rater M, et al. (1997) Correspondence between DSM-III-R and DSM-IV attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 36: 1682–1687.
59. Baumgaertel A, Wolraich ML, Dietrich M (1995) Comparison of diagnostic criteria for attention deficit disorders in a German elementary school sample. J Am Acad Child Adolesc Psychiatry 34: 629–638.
60. Hamm MP, Cherry NM, Chan E, Martin JW, Burstyn I (2010) Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 20: 589–597.
61. Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL (2007) Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. Environ Health Perspect 115: 1596–1602.
62. Harada K, Koizumi A, Saito N, Inoue K, Yoshinaga T, et al. (2007) Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere 66: 293–301.
63. Haug LS, Thomsen C, Becher G (2009) Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. Environ Sci Technol 43: 2131–2136.
64. Olsen GW, Ellefson ME, Mair DC, Church TR, Goldberg CL, et al. (2011) Analysis of a homologous series of perfluorocarboxylates from American Red Cross adult blood donors, 2000-2001 and 2006. Environ Sci Technol 45: 8022–8029.

Paper III

Prenatal exposure to phthalate metabolites and ADHD in Childhood

Amanda Ode[1], Lars Rylander[1], Karin Källén[1], Peik Gustafsson[2], Bo AG Jönsson[1], Per Olofsson[3], Sten A Ivarsson[4], Christian H Lindh[1], Anna Rignell-Hydbom[1]

[1]Lund University, Institute of Laboratory Medicine, Lund, Division of Occupational and Environmental Medicine, Sweden

[2]Lund University, Department of Clinical Sciences, Lund, Child and Adolescent Psychiatry, Sweden

[3]Lund University, Department of Clinical Sciences, Malmö, Department of Obstetrics and Gynecology, Sweden

[4]Lund University, Department of Clinical Sciences, Malmö, Pediatric Endocrinology Unit, Sweden

Corresponding author: Amanda Ode, Ph.D. student, Division of Occupational and Environmental Medicine, Lund University, HSC, Baravägen 3, SE-22240 Lund, Sweden, E-mail: amanda.ode@med.lu.se, Telephone: (46) 725892202

Abbreviations

ADHD, Attention Deficit Hyperactivity Disorder; BMI, Body Mass Index; CI, Confidence Interval; DEHP, di-2-ethylhexyl phthalate; DiNP, di-isononyl phthalate; DSM, Diagnostic and Statistical Manual of Mental Disorders; LOD, Limit of Detection; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MCiOP, mono-carboxy-iso-octyl phthalate; MMUSB, Malmö Maternity Unit Serum Biobank; OR, Odds Ratio; SD, Standard Deviation; SMBR, Swedish Medical Birth Register

Keywords

Fetus, Exposure, Phthalates, DEHP, MEHHP, DiNP, MCiOP, Attention Deficit Hyperactivity Disorder (ADHD), Epidemiology, Neurodevelopment.

Abstract

Background Phthalates are ubiquitous environmental pollutants that have been linked to adverse neurodevelopmental impairments. Effects of prenatal exposure to phthalates on neurodevelopment need to be further investigated.

Aim In the present study, the relationship between prenatal exposure to phthalate metabolites and attention deficit hyperactivity disorder (ADHD) diagnosis in childhood is explored.

Materials and methods Children born between 1978 and 2000 with ADHD (n=202) were identified at the Department of Child and Adolescent Psychiatry in Malmö, Southern Sweden. Controls (n=202), matched for year of birth and maternal country of birth, from the same region were selected from the Medical Birth Register. Phthalate metabolites were measured in umbilical cord serum collected at a biobank and the association between the sum of di-2-ethylhexyl phthalate (DEHP) or di-isononyl phthalate (DiNP) metabolites and ADHD was investigated.

Results The median cord serum concentrations of mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), and mono-carboxy-iso-octyl phthalate (MCiOP) in children with ADHD were 0.14, 1.2, and 0.29 ng/ml, respectively. The corresponding median serum concentrations of those metabolites in the controls were 0.13, 1.2, and 0.31, respectively. There were no differences in the phthalate metabolite serum concentrations between children with ADHD and controls (all p values > 0.3). The sum of DEHP metabolites or MCiOP in cord serum was not associated with ADHD diagnosis in childhood.

Conclusion The low cord serum concentrations of phthalate metabolites detected in the present study seem not to cause ADHD in later age.

Introduction

Phthalates are industrial chemicals which are found in numerous household and consumer products. They are mainly used as plasticizers, but even found in numerous consumer and commercial products, such as solvents, detergents, vinyl flooring, medical devices, wall coverings, personal care products, and food containers (Barr et al. 2003; Hauser and Calafat 2005; Hauser et al. 2007; Hernandez-Diaz et al. 2013). Because they are not chemically bound to the products, phthalates can leach into the environment, leading to exposure via inhalation of contaminated air or dust, ingestion of contaminated food, and dermal contact with care products (Afshari et al. 2004; Sathyanarayana 2008). After entering the body, phthalates break down into metabolites that are excreted in the urine and feces (Koch et al. 2006; Wormuth et al. 2006). Due to the ubiquitous presence in the environment, phthalates have drawn much public attention.

Phthalate metabolites have been found in biological samples such as urine (Langer et al. 2014; Wittassek et al. 2011), blood (Frederiksen et al. 2010; Wan et al. 2013), and breast milk (Fromme et al. 2011). Furthermore, fetuses are exposed to these compounds and their metabolites have been found in placental tissue, amniotic fluid, and cord blood (Jensen et al. 2012; Kato et al. 2006; Koch and Calafat 2009; Mose et al. 2007). Thus, phthalates or its metabolites seem to cross the placental barrier. Because fetuses and infants are more sensitive to toxic effects of chemicals (Landrigan 1998) and infants may experience higher exposures to phthalates than adults (Wormuth et al. 2006), prenatal and neonatal exposure to endocrine disrupters, including phthalates, disturbs brain development, even at levels lower than their No Observed Adverse Effect Level (NOAEL) or Low Observed Adverse Effect Level (LOAEL) (Tanida et al. 2009). Studies in rats have shown that exposure to phthalates causes hyperactivity reminding of the clinical picture of attention deficit hyperactivity disorder (ADHD) as observed in humans (Ishido et al. 2004; Masuo et al. 2004b).

ADHD is usually diagnosed during childhood, and while the exact cause of ADHD is unknown, both genetic and environmental factors, as well as their interactions may contribute to the development of ADHD (Archer et al. 2011). A recent study has identified an association between urine phthalate metabolite levels and poor attentional performance in ADHD children with evidence of dopamine gene-phthalate interaction (Park et al. 2014). There is also cross-sectional evidence that certain phthalates are associated with ADHD, attention deficit disorder (ADD), and both ADD and learning disability (LD) in school-aged children (Chopra et al. 2014; Kim et al. 2009). Furthermore, certain phthalates have been found to impair neurodevelopment both *in vivo* and *in vitro* (Chen et al. 2011; Lin et al. 2011; Smith et al. 2011).

Prenatal exposure to phthalates in relation to neurological and behavioral outcomes is poorly studied and has been exclusively restricted to measured levels in maternal urine (Engel et al. 2009; Engel et al. 2010; Miodovnik et al. 2014; Whyatt et al. 2012; Yolton et al. 2011). However, the effects of prenatal phthalate exposure on the development of ADHD have so far not been investigated.

In the present study, the association between umbilical cord serum concentrations of di-2-ethylhexyl phthalate (DEHP) and di-isononyl phthalate (DiNP) metabolites and ADHD diagnosis in childhood was explored. In addition, maternal and cord serum concentrations of these metabolites were compared.

Materials and methods

Study population

The selection of and diagnostic procedures for ADHD children have previously been described by Ode et al. (2014). Briefly, 419 children born in the city of Malmö between 1978 and 2000 were diagnosed with ADHD and were followed until 2005 at the Department of Child and Adolescent Psychiatry in Malmö. Age at the time of diagnosis varied between 5 and 17 years. The Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria DSM-III-R_{11} were used before 1994, and from 1994 onwards the DSM-IV_{12} criteria. Intelligence and ability to concentrate were tested with the Wechsler Intelligence Scale (WISC), TEA-Ch, and QB-Tech or IVA+. A pediatric examination with assessment of neurological soft signs was also performed. The school teacher and/or the parents were asked to fill in questionnaires such as the SNAP-IV, Conner's questionnaire or the 5-15 questionnaire, and the BRIEF- questionnaire. Behavior in school and at the visits to the clinic was observed and registered. A team of a psychiatrist, a psychologist and sometimes also a social worker met to achieve a consensus ADHD diagnosis using the DSM criteria.

Using the unique personal identification numbers, maternal and umbilical cord serum samples for children with ADHD were collected from the Malmö Maternity Unit Serum Biobank (MMUSB). The MMUSB has previously been described (Ode et al. 2014). Maternal and umbilical cord serum samples were collected at delivery and stored at -20ºC at the biobank. A pool of 10 controls per ADHD child matched for year of birth ± 12 months and country of birth of the mother was retrieved from the Swedish Medical Birth Register (SMBR). The first newborn in the pool of controls with an umbilical cord blood sample found in the MMSUB was chosen as control. If no cord serum was found among the 10 matched controls in the pool, the sample of the next-baby-born was used. Demographic and obstetric data were obtained from the SMBR. The selection procedure for cases and controls is presented in Figure 1.

The study protocol followed the requirements of the Declaration of Helsinki and was approved by the Research Ethics Committee at Lund University, Sweden.

Chemical analyses

Phthalate metabolites were analyzed in serum samples by liquid chromatography tandem mass spectrometry at the Department of Occupational and Environmental Medicine at Lund University, Sweden as described by Specht et al. (2014). Analyses included the secondary oxidized metabolites of DEHP (mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-carboxypentyl phthalate (MECPP), and DiNP (mono-4-methyl-7-hydroxy-octyl phthalate (7OH-MMeOP), mono-carboxy-iso-octyl phthalate (MCiOP), and mono-4-methyl-7-carboxyheptyl phthalate (7cx-MMeOP)). However, only MEHHP, MECPP, and MCiOP could be detected in the samples.

For the analysis of phthalate metabolites, aliquots of 100 µL serum were added with isotopically labeled internal standards for all evaluated compounds. The samples were digested with glucuronidase and the proteins were precipitated using acetonitrile. The samples were prepared in 96-well plates and analyzed using a triple quadrupole linear ion trap mass spectrometer (QTRAP 5500; AB Sciex, Foster City, CA, USA) coupled to a liquid chromatography system (UFLCXR, Shimadzu Corporation, Kyoto, Japan; LC/MS/MS) without any further clean-up. The analysis was performed in negative ion mode. All data acquisition was performed using Analyst 1.6.1 software and data processing was performed using Multiquant 2.1 (AB Sciex).

The quality of the analyses was checked by including chemical blank samples and an in-house quality control in all analyzed sample batches. Moreover, each sample was analyzed three times in three different analytical batches. The samples were analyzed in randomized order. The imprecision in the analyzed control sample was 8% for MEHHP, 18% for MECPP, and 19% for MCiOP. The LODs were 0.2 ng/mL for MEHHP, 0.1 ng/mL for MECPP, and 0.1 ng/mL for MCiOP. The laboratory is a European reference laboratory for urinary phthalate metabolite analyses for all the included compounds (www.eu-hbm.info/democophes).

Smoking during pregnancy, as a potential confounder, was determined by analysis of cotinine in umbilical cord serum as described by Ode et al. (2014). Women with cotinine concentrations above 15 ng/mL were classified as active smokers (George et al. 2006).

Statistical analyses

Mann-Whitney *U* test and Kruskal-Wallis test were used to compare characteristics between pregnant women and their fetuses and phthalate metabolites between ADHD children and controls. Spearman's rank correlation coefficient was used to investigate the correlations of the phthalate metabolites between maternal and umbilical cord serum samples. The Wilcoxon matched-pairs signed-ranks test was used to investigate differences in phthalate metabolite concentrations between maternal and cord serum, and between ADHD cases and controls, respectively. Concentrations below the LOD were replaced with the commonly used value LOD/√2.

Conditional logistic regression was performed for investigating the association between the sum of DEHP metabolites (MEHPP and MECPP) and the DiNP metabolite MCiOP in nM in cord serum and ADHD diagnosis. The odds ratio was calculated for every 1 nM increase in the umbilical cord phthalate concentrations (i.e. exposure used as continuous variable) or for concentrations at or above the 75th percentile (i.e. exposure used as categorical variable). As potential confounders we considered maternal age, smoking during pregnancy based on cord cotinine serum levels, and parity. Variables that were suspected to be in the causal pathway between exposure to phthalate metabolites and ADHD, such as birth weight and gestational age at birth were not included in the adjusted model.

The conditional regressions were performed with LogXact statistical software (Cytel Studio 10), while the rest of the analyses were performed with SPSS version 21 (SPSS Inc., Chicago, IL, USA).

Results

The DEHP metabolites (MEHHP and MCEPP) and the DiNP metabolite (MCiOP) could be analyzed in 193 matched maternal and umbilical cord serum samples of the controls. For children with ADHD and controls, the three metabolites could be analyzed in 202 matched umbilical cord serum samples. The detection frequencies of the metabolites in maternal and umbilical cord sera are presented in Table 1.

The demographic and obstetric characteristics of the study children are shown in Table 2. The median age of mothers to the cases and controls was 26 and 28 years, respectively. The majority of the mothers to ADHD children and controls were primiparous and of Swedish origin. Mothers to the ADHD children smoked more than those to the controls. Median birth weight was 3465 g for children with ADHD and 3600 g for controls. Gestational age, birth length, and head circumference were

almost identical for both groups. Mann-Whitney *U* test and Kruskal-Wallis test showed no differences between children with or without ADHD diagnosis except for smoking status and birth weight ($p<0.05$).

Concentrations of phthalate metabolites in maternal and cord serum of the controls are presented in Table 3. Phthalate metabolite concentrations in maternal serum were positively correlated with the corresponding metabolite in cord serum (Table 3). MEHHP concentrations were higher in maternal than in cord serum, whereas MECPP and MCiOP were higher in cord than in maternal serum.

Median cord serum concentrations of MEHHP, MECPP, and MCiOP in children with ADHD were 0.14, 1.2, and 0.29 ng/mL, respectively. The corresponding median serum concentrations of those metabolites in the controls were 0.13, 1.2, and 0.31 ng/mL, respectively (Table 4). There were no differences in the phthalate metabolite serum concentrations between the two groups ($p > 0.05$). DEHP and DiNP metabolites in cord serum were not associated with higher odds of having ADHD diagnosis in childhood, even after adjustment for potential confounders (Table 5).

Discussion

As phthalate metabolites cross the placental and the blood-brain barrier and have negative impact on fetal development, including neurodevelopment, we investigated the hypothesized relationship between prenatal exposure to phthalate metabolites and ADHD. Phthalate metabolites could be found in umbilical cord serum with good correlation with maternal serum phthalate metabolite concentrations, but no association between prenatal exposure to phthalate metabolites and ADHD in childhood was found.

In accordance with previous data, maternal serum phthalate metabolites cross the placenta and are transferred to the fetus. Phthalates are usually measured in urine of pregnant women where their levels are much higher compared to those found in blood or serum samples (Hines et al. 2009; Yan et al. 2009). Levels of phthalate metabolites measured in our samples were low compared to those measured in the analogous biological samples (de Cock et al. 2014; Latini et al. 2003a; Zhang et al. 2009). This might reflect different exposure patterns that arise among countries. There were modest to high correlations between maternal and cord serum phthalate metabolites in our samples compared to other studies that usually found no correlations between maternal and fetal samples (Latini et al. 2003b). The higher concentrations of MECPP and MCiOP in cord serum than in maternal serum might reflect bioaccumulations of these chemicals due to the immature secretory mechanisms

in the developing fetuses, or due to the possible negative effects of these phthalate metabolites on the secretory organs.

Experimental and epidemiological studies have found that phthalates have negative impact on neurodevelopment (Chen et al. 2011; Lin et al. 2011; Smith et al. 2011). Experimental studies revealed that exposure to higher levels of phthalates resulted in neurobehavioral impairments, such as hyperactivity, in mice and rats (Ishido et al. 2004; Masuo et al. 2004a; Masuo et al. 2004b; Tanaka 2002). Animal studies have also shown that neonatal and immature offspring animals were more vulnerable to phthalates during the developing stages of the brain (Adams et al. 2000; Vorhees 1994). Perinatal exposure to phthalates led to neurotoxicity and had negative effects on neurobehavioral development in neonate animals, but had no influence on mature offspring (Li et al. 2014; Li et al. 2009). In summary, these results suggest that infants and children can experience greater harm from phthalates than adults. However, exposure to low phthalate levels, as those detected in the present study, might have no effects on the developing brains of the fetuses.

In humans, there is cross-sectional evidence that certain phthalates are associated with ADHD-symptoms, increased odds of ADD and both ADD and LD, and decreased Full Scale IQ and Verbal IQ scores (Cho et al. 2010; Chopra et al. 2014; Kim et al. 2009). Other studies showed that phthalate metabolites during pregnancy were associated with decreased mental and physical development and with increased odds of psychomotor delay, increased motor performance, poorer orientation, attention problems, and more internalizing behaviors in children, and non-optimal reflexes in infants (Engel et al. 2009; Engel et al. 2010; Kim et al. 2009; Téllez-Rojo et al. 2013; Whyatt et al. 2012; Yolton et al. 2011). Sex-specific effects were observed in many of those studies (Engel et al. 2010; Téllez-Rojo et al. 2013; Whyatt et al. 2012; Yolton et al. 2011). Unfortunately, we were not able to investigate sex-phthalate metabolite interaction due to the small number of female infants in our data.

The present study has several limitations. Phthalates have short biological half-lives in the body tissues (Fennell et al. 2004; Koch et al. 2006). Since we had a single serum sample and we lack data on daily use of personal care products for the mothers, some misclassification of exposure to phthalate might be present in our data. However, if sources and daily patterns of exposure remain relatively unchanged, a single measurement may reflect a typical measurement at any time during pregnancy. The present study is also limited by its modest sample size, and consequently the limited statistical power to detect subtle effects.

To summarize, pregnant women were exposed to relatively low levels of phthalates and phthalate metabolites were transferred to the fetuses. The low phthalate metabolite levels detected in umbilical cord serum did not cause ADHD symptoms in later age. Since this is the first study investigating the association between exposure to phthalate metabolites during pregnancy and ADHD diagnosis, more research using repeated measurements of phthalate metabolites during pregnancy is needed to address possible associations with neurodevelopmental disorders.

References

Adams J, Barone S, Jr., LaMantia A, Philen R, Rice DC, Spear L, et al. 2000. Workshop to identify critical windows of exposure for children's health: Neurobehavioral work group summary. Environ Health Perspect 108 Suppl 3:535-544.

Afshari A, Gunnarsen L, Clausen PA, Hansen V. 2004. Emission of phthalates from pvc and other materials. Indoor Air 14:120-128.

Archer T, Oscar-Berman M, Blum K. 2011. Epigenetics in developmental disorder: Adhd and endophenotypes. J Genet Syndr Gene Ther 2.

Barr DB, Silva MJ, Kato K, Reidy JA, Malek NA, Hurtz D, et al. 2003. Assessing human exposure to phthalates using monoesters and their oxidized metabolites as biomarkers. Environ Health Perspect 111:1148-1151.

Chen T, Yang W, Li Y, Chen X, Xu S. 2011. Mono-(2-ethylhexyl) phthalate impairs neurodevelopment: Inhibition of proliferation and promotion of differentiation in pc12 cells. Toxicol Lett 201:34-41.

Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, et al. 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ Health Perspect 118:1027-1032.

Chopra V, Harley K, Lahiff M, Eskenazi B. 2014. Association between phthalates and attention deficit disorder and learning disability in u.S. Children, 6-15 years. Environ Res 128:64-69.

de Cock M, de Boer MR, Lamoree M, Legler J, van de Bor M. 2014. First year growth in relation to prenatal exposure to endocrine disruptors - a dutch prospective cohort study. Int J Environ Res Public Health 11:7001-7021.

Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, et al. 2009. Prenatal phthalate exposure and performance on the neonatal behavioral assessment scale in a multiethnic birth cohort. Neurotoxicology 30:522-528.

Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, et al. 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect 118:565-571.

Fennell TR, Krol WL, Sumner SC, Snyder RW. 2004. Pharmacokinetics of dibutylphthalate in pregnant rats. Toxicol Sci 82:407-418.

Frederiksen H, Jorgensen N, Andersson AM. 2010. Correlations between phthalate metabolites in urine, serum, and seminal plasma from young danish men determined by isotope dilution liquid chromatography tandem mass spectrometry. J Anal Toxicol 34:400-410.

Fromme H, Gruber L, Seckin E, Raab U, Zimmermann S, Kiranoglu M, et al. 2011. Phthalates and their metabolites in breast milk--results from the bavarian monitoring of breast milk (bambi). Environ Int 37:715-722.

George L, Granath F, Johansson AL, Cnattingius S. 2006. Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. Acta Obstet Gynecol Scand 85:1331-1337.

Hauser R, Calafat AM. 2005. Phthalates and human health. Occup Environ Med 62:806-818.

Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, et al. 2007. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. Hum Reprod 22:688-695.

Hernandez-Diaz S, Su YC, Mitchell AA, Kelley KE, Calafat AM, Hauser R. 2013. Medications as a potential source of exposure to phthalates among women of childbearing age. Reprod Toxicol 37:1-5.

Hines EP, Calafat AM, Silva MJ, Mendola P, Fenton SE. 2009. Concentrations of phthalate metabolites in milk, urine, saliva, and serum of lactating north carolina women. Environ Health Perspect 117:86-92.

Ishido M, Masuo Y, Sayato-Suzuki J, Oka S, Niki E, Morita M. 2004. Dicyclohexylphthalate causes hyperactivity in the rat concomitantly with impairment of tyrosine hydroxylase immunoreactivity. J Neurochem 91:69-76.

Jensen MS, Norgaard-Pedersen B, Toft G, Hougaard DM, Bonde JP, Cohen A, et al. 2012. Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: Temporal trends and timing of amniocentesis in pregnancy. Environ Health Perspect 120:897-903.

Kato K, Silva MJ, Needham LL, Calafat AM. 2006. Quantifying phthalate metabolites in human meconium and semen using automated off-line solid-phase extraction coupled with on-line spe and isotope-dilution high-performance liquid chromatography--tandem mass spectrometry. Anal Chem 78:6651-6655.

Kim BN, Cho SC, Kim Y, Shin MS, Yoo HJ, Kim JW, et al. 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol Psychiatry 66:958-963.

Koch HM, Preuss R, Angerer J. 2006. Di(2-ethylhexyl)phthalate (dehp): Human metabolism and internal exposure-- an update and latest results. Int J Androl 29:155-165; discussion 181-155.

Koch HM, Calafat AM. 2009. Human body burdens of chemicals used in plastic manufacture. Philos Trans R Soc Lond B Biol Sci 364:2063-2078.

Landrigan PJ. 1998. Environmental hazards for children in USA. Int J Occup Med Environ Health 11:189-194.

Langer S, Beko G, Weschler CJ, Brive LM, Toftum J, Callesen M, et al. 2014. Phthalate metabolites in urine samples from danish children and correlations with phthalates in dust samples from their homes and daycare centers. Int J Hyg Environ Health 217:78-87.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. 2003a. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. Environ Health Perspect 111:1783-1785.

Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. 2003b. Exposure to di(2-ethylhexyl)phthalate in humans during pregnancy. Biology of the Neonate 83:22-24.

Li X, Jiang L, Cheng L, Chen H. 2014. Dibutyl phthalate-induced neurotoxicity in the brain of immature and mature rat offspring. Brain Dev 36:653-660.

Li Y, Zhuang M, Li T, Shi N. 2009. Neurobehavioral toxicity study of dibutyl phthalate on rats following in utero and lactational exposure. J Appl Toxicol 29:603-611.

Lin CH, Chen TJ, Chen SS, Hsiao PC, Yang RC. 2011. Activation of trim17 by ppargamma is involved in di(2-ethylhexyl) phthalate (dehp)-induced apoptosis on neuro-2a cells. Toxicol Lett 206:245-251.

Masuo Y, Ishido M, Morita M, Oka S. 2004a. Effects of neonatal treatment with 6-hydroxydopamine and endocrine disruptors on motor activity and gene expression in rats. Neural Plast 11:59-76.

Masuo Y, Morita M, Oka S, Ishido M. 2004b. Motor hyperactivity caused by a deficit in dopaminergic neurons and the effects of endocrine disruptors: A study inspired by the physiological roles of pacap in the brain. Regul Pept 123:225-234.

Miodovnik A, Edwards A, Bellinger DC, Hauser R. 2014. Developmental neurotoxicity of ortho-phthalate diesters: Review of human and experimental evidence. Neurotoxicology 41:112-122.

Mose T, Mortensen GK, Hedegaard M, Knudsen LE. 2007. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod Toxicol 23:83-91.

Ode A, Kallen K, Gustafsson P, Rylander L, Jonsson BA, Olofsson P, et al. 2014. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS One 9:e95891.

Park S, Kim BN, Cho SC, Kim Y, Kim JW, Lee JY, et al. 2014. Association between urine phthalate levels and poor attentional performance in children with attention-deficit hyperactivity disorder with evidence of dopamine gene-phthalate interaction. Int J Environ Res Public Health 11:6743-6756.

Sathyanarayana S. 2008. Phthalates and children's health. Current Problems in Pediatric and Adolescent Health Care 38:34-49.

Smith CA, Macdonald A, Holahan MR. 2011. Acute postnatal exposure to di(2-ethylhexyl) phthalate adversely impacts hippocampal development in the male rat. Neuroscience 193:100-108.

Specht IO, Toft G, Hougaard KS, Lindh CH, Lenters V, Jonsson BA, et al. 2014. Associations between serum phthalates and biomarkers of reproductive function in 589 adult men. Environ Int 66:146-156.

Tanaka T. 2002. Reproductive and neurobehavioural toxicity study of bis(2-ethylhexyl) phthalate (dehp) administered to mice in the diet. Food Chem Toxicol 40:1499-1506.

Tanida T, Warita K, Ishihara K, Fukui S, Mitsuhashi T, Sugawara T, et al. 2009. Fetal and neonatal exposure to three typical environmental chemicals with different mechanisms of action: Mixed exposure to phenol, phthalate, and dioxin cancels the effects of sole exposure on mouse midbrain dopaminergic nuclei. Toxicol Lett 189:40-47.

Téllez-Rojo MM, Cantoral A, Cantonwine DE, Schnaas L, Peterson K, Hu H, et al. 2013. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Science of The Total Environment 461-462:386-390.

Wan HT, Leung PY, Zhao YG, Wei X, Wong MH, Wong CK. 2013. Blood plasma concentrations of endocrine disrupting chemicals in hong kong populations. J Hazard Mater 261:763-769.

Whyatt RM, Liu X, Rauh VA, Calafat AM, Just AC, Hoepner L, et al. 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect 120:290-295.

Wittassek M, Koch HM, Angerer J, Bruning T. 2011. Assessing exposure to phthalates - the human biomonitoring approach. Mol Nutr Food Res 55:7-31.

Vorhees CV. 1994. Developmental neurotoxicity induced by therapeutic and illicit drugs. Environ Health Perspect 102 Suppl 2:145-153.

Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in europeans? Risk Anal 26:803-824.

Yan X, Calafat A, Lashley S, Smulian J, Ananth C, Barr D, et al. 2009. Phthalates biomarker identification and exposure estimates in a population of pregnant women. Hum Ecol Risk Assess 15:565-578.

Yolton K, Xu Y, Strauss D, Altaye M, Calafat AM, Khoury J. 2011. Prenatal exposure to bisphenol a and phthalates and infant neurobehavior. Neurotoxicol Teratol 33:558-566.

Zhang Y, Lin L, Cao Y, Chen B, Zheng L, Ge RS. 2009. Phthalate levels and low birth weight: A nested case-control study of chinese newborns. J Pediatr 155:500-504.

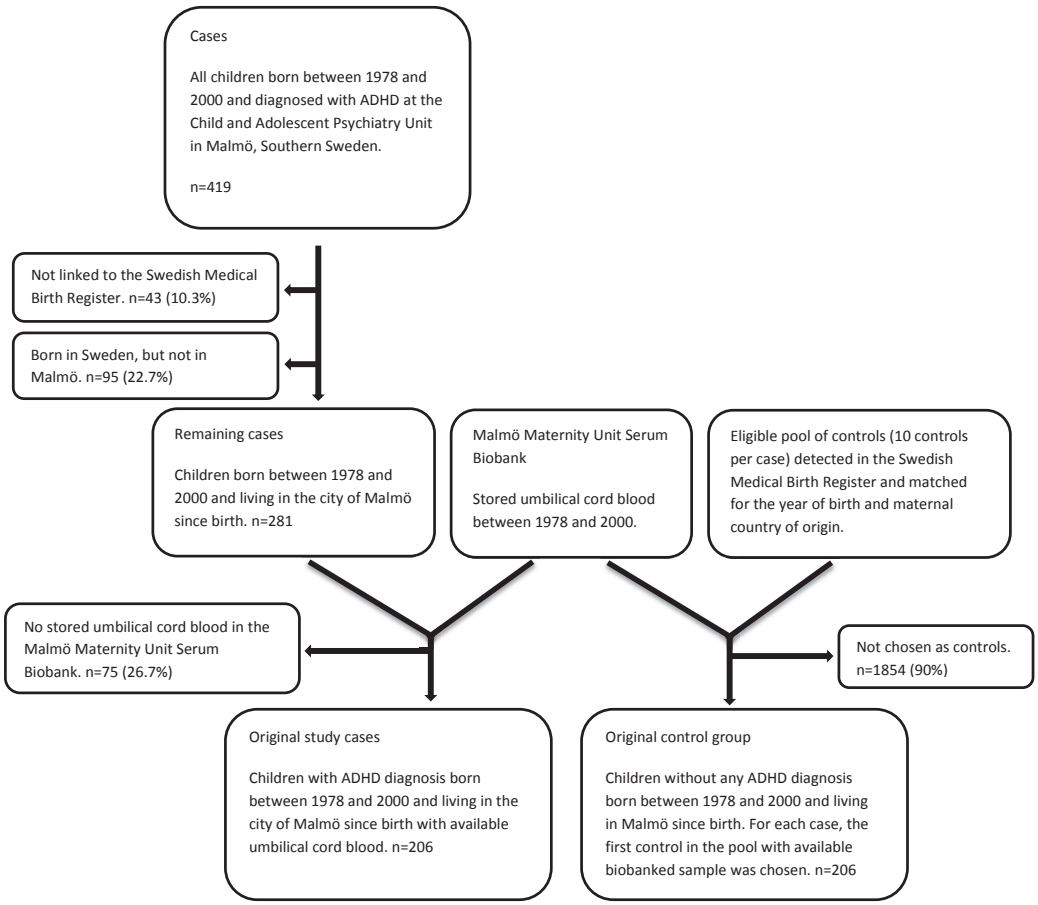


Fig. 1. Flowchart for the selection procedure of the children with attention deficit hyperactivity disorder (ADHD) and controls.

Table 1 Number of available maternal and umbilical cord serum samples for our population

Phthalate metabolite	Matrix	Available samples (N=206)	
		Samples with sufficient amount of serum (n)	**>LOD (%)**
MEHHP	MS	194	95.4
	CS Control	205	89.3
	CS ADHD child	203	84.9
MECPP	MS	194	100
	CS Control	205	100
	CS ADHD child	203	100
MCiOP	MS	194	83.0
	CS Control	205	93.7
	CS ADHD child	203	94.1

MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MCiOP, mono-carboxy-iso-octyl phthalate; MS, maternal serum; CS, cord serum; LOD, limit of detection.

Table 2 Demographic characteristic of the study children, n(%) or median (25^{th}, 75^{th} percentile)

	ADHD children (n=202)	Controls (n=202)
Maternal characteristics		
Age (years)	26 (23, 30)	28 (25, 31)
Parity		
Primiparous	97 (48.0)	105 (52.0)
Multiparous	105 (52.0)	97 (48.0)
Body mass index[a]		
Missing	141 (69.8)	141 (69.8)
Underweight	1 (0.5)	3 (1.5)
Normal	36 (17.8)	40 (19.8)
Overweight	16 (7.9)	14 (6.9)
Obese	8 (4.0)	4 (2.0)
Native Swedish	168 (83.2)	169 (83.7)
Active smoker[b]	81 (40.1)	62 (30.7)
Fetal characteristics		
Boy	179 (88.6)	161 (79.7)
Birth weight (grams)	3465 (3180-3810)	3600 (3200-3960)
Gestational age (weeks)	40 (39-40)	40 (40-41)
Birth length (cm)	50 (49-52)	51 (49-52)
Head circumference (cm)	35 (34-36)	35 (34-36)
At risk of neurodevelopmental deficits		
Low birth weight (<2500 grams)	13 (6.4)	5 (2.5)
Preterm birth (<37 weeks)	11 (5.4)	7 (3.5)

[a]Body mass index was classified according to the standard values of the World Health Organization.
[b]Maternal smoking is based on measured cotinine concentrations in the umbilical cord serum.

Table 3 Concentrations of phthalate metabolites in matched maternal and umbilical cord serum control samples

Phthalte metabolite	Serum sample	Mean	25% Percentile	Median	75% Percentile	*p*[a]	Spearman's correlation coefficient
MEHHP	MS	0.25	0.12	0.19	0.31	<0.0001	0.40**
	CS	0.25	0.09	0.13	0.26		
MECPP	MS	1.3	0.71	1.03	1.6	<0.0001	0.66**
	CS	1.6	0.90	1.2	1.8		
MCiOP	MS	1.2	0.12	0.20	0.51	0.017	0.83**
	CS	0.88	0.18	0.32	0.56		

MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MCiOP, mono-carboxy-iso-octyl phthalate; MS, maternal serum; CS, cord serum

[a] *p* value is obtained from the *Wilcoxon matched-pairs* signed-ranks *test*.

**Correlation is significant at the 0.01 level (2-tailed).

Table 4 Concentrations of phthalate metabolites in ng/mL as (mean±SD) and median (5^{th}, 95^{th} percentile) in umbilical cord serum of matched children (n=202) with and without ADHD diagnosis

Phthalate metabolite	ADHD cases	Controls	Difference (*p* value)[a]
MEHHP	0.21±0.23	0.24±0.48	0.76
	0.14 (0.04, 0.62)	0.13 (0.04, 0.67)	
MECPP	1.7±1.3	1.6±1.3	0.31
	1.2 (0.52, 4.2)	1.2 (0.65, 3.6)	
MCiOP	0.62±1.2	0.85±2.3	0.66
	0.29 (0.07, 1.9)	0.31 (0.07, 3.1)	

MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MCiOP, mono-carboxy-iso-octyl phthalate

[a] *p* value is obtained from the *Wilcoxon matched-pairs* signed-ranks *test*.

Table 5 Odds ratios and 95% confidence intervals for association of prenatal exposure to phthalate metabolites in children with and without attention deficit hyperactivity disorder (ADHD)

Phthalate metabolite (nM)		ADHD	
		Crude OR (95% CI)	**Adjusted OR (95% CI)[c]**
∑DEHP metabolites[a]		1.01 (0.96-1.05)	1.00 (0.96-1.05)
MCiOP[a]		0.98 (0.94-1.01)	0.97 (0.94-1.01)
∑DEHP metabolites[b]	**<75th P**	1	1
	≥75th P	1.03 (0.65-1.6)	0.98 (0.61-1.6)
MCiOP[b]	**<75th P**	1	1
	≥75th P	0.92 (0.57-1.5)	0.93 (0.58-1.5)

OR, odds ratio; CI, confidence interval; DEHP, di-2-ethylhexyl phthalate; MCiOP, mono-carboxy-iso-octyl phthalate
[a]Odds ratio is calculated for every 1 nM increase in the concentration.
[b]Odds ratio is calculated for categorized variables.
[c]Adjusted for maternal age, parity, and cotinine levels during pregnancy.

Paper IV

Environmental Research 137 (2015) 373–381

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Manganese and selenium concentrations in umbilical cord serum and attention deficit hyperactivity disorder in childhood

Amanda Ode [a,*], Lars Rylander [a], Peik Gustafsson [b], Thomas Lundh [a], Karin Källén [a], Per Olofsson [c], Sten A. Ivarsson [d], Anna Rignell-Hydbom [a]

[a] Institute of Laboratory Medicine, Division of Occupational and Environmental Medicine, Lund University, SE-22185 Lund, Sweden
[b] Institute of Clinical Sciences, Department of Child and Adolescent Psychiatry, Lund University, Lund, Sweden
[c] Department of Obstetrics and Gynecology, Skåne University Hospital, Lund University, Malmö, Sweden
[d] Pediatric Endocrinology Unit, Clinical Research Center, Lund University, Malmö, Sweden

ARTICLE INFO

Article history:
Received 31 October 2014
Received in revised form
2 January 2015
Accepted 3 January 2015

Keywords:
Fetal exposure
Manganese
Selenium
Attention deficit hyperactivity disorder (ADHD)
Epidemiology
Neurodevelopment

ABSTRACT

Existing evidence on the effects of manganese and selenium during fetal life on neurodevelopmental disorders is inadequate. This study aims to investigate the hypothesized relationship between fetal exposure to manganese and selenium and attention deficit hyperactivity disorder (ADHD) diagnosis in childhood. Children born between 1978 and 2000 with ADHD ($n=166$) were identified at the Department of Child and Adolescent Psychiatry in Malmö, Sweden. Controls from the same region ($n=166$) were selected from the Medical Birth Register and were matched for year of birth and maternal country of birth. Manganese and selenium were measured in umbilical cord serum. The median cord serum concentrations of manganese were 4.3 μg/L in the cases and 4.1 μg/L in the controls. The corresponding concentrations of selenium were 47 and 48 μg/L. When the exposures were analyzed as continuous variables no associations between cord manganese or selenium concentration and ADHD were observed. However, children with selenium concentrations above the 90th percentile had 2.5 times higher odds (95% confidence interval 1.3–5.1) of having ADHD compared to those with concentrations between the 10th and 90th percentiles. There was no significant interaction between manganese and selenium exposure ($p=0.08$). This study showed no association between manganese concentrations in umbilical cord serum and ADHD. The association between ADHD diagnoses in children with relatively high cord selenium was unexpected and should be interpreted with caution.

© 2015 Published by Elsevier Inc.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurobehavioral disorder with inattention, impaired impulse control, and hyperactivity as core symptoms (Faraone et al., 2006; Gustafsson et al., 2000). About five percent of children are diagnosed with ADHD and the problems often persist into adulthood (Faraone et al., 2006; Lara et al., 2009). The etiology of ADHD is not yet fully known, but both genetic and environmental factors are involved Swanson et al. (2007). Disturbance of the dopaminergic system is the main characteristic of ADHD (Faraone et al., 2005; Gizer et al., 2009). Exposure to environmental toxins, such as lead, mercury, and persistent chlorinated biphenyls (PCBs), has been linked to ADHD (Banerjee et al., 2007; Braun et al., 2006; Eubig et al., 2010).

Manganese is an essential trace element involved in many metabolic functions in the body (Santamaria and Sulsky, 2010). Exposures to high levels of manganese have been linked to hyperactivity and ADHD in children (Bouchard et al., 2006; Farias et al., 2010). Manganese has been suggested to mediate its toxic effect on the nervous system mainly by causing selective destruction of dopaminergic neurons, and by altering brain expression of dopamine, dopamine receptors and dopamine transporter proteins (Dorman et al., 2000; Kern et al., 2010; Storch et al., 2004; Tran et al., 2002). Manganese may trigger autoxidation or turnover of dopamine, resulting in increased production of free radicals and other cytotoxic metabolites, along with a depletion of cellular antioxidant defense mechanisms (Garner and Nachtman, 1989; Liccione and Maines, 1988; Parenti et al., 1988; Verity, 1999).

Abbreviations: ; ADHD, attention deficit hyperactivity disorder; BMI, body mass index; CI, confidence interval; DSM, Diagnostic and Statistical Manual of Mental Disorders; LOD, limit of detection; MMUSB, Malmö Maternity Unit Serum Biobank; OR, odds ratio; SD, standard deviation; SMBR, Swedish Medical Birth Register

* Corresponding author.
E-mail address: amanda.ode@med.lu.se (A. Ode).

http://dx.doi.org/10.1016/j.envres.2015.01.001
0013-9351/© 2015 Published by Elsevier Inc.

Selenium is an essential trace element with potent antioxidant properties (Battin et al., 2006) and has recently been found to protect from prenatal manganese neurotoxicity (Yang et al., 2014). In mice, manganese-induced impairment in dopaminergic neurotransmission is reversed by selenium treatment (Khan, 2010). The increased permeability of the blood–brain-barrier induced by free radicals is inhibited by selenium (Oztas et al., 2001).

Manganese and selenium both cross the placenta and the fetal blood–brain barrier (Michalke et al., 2009; Wilson et al., 1991; Yokel, 2009). The potential associations between manganese and selenium exposures during fetal life and later development of nervous system symptoms are relatively unexplored. The primary aim of the present case–control study was to investigate the possible associations between manganese and selenium concentrations in neonatal blood at birth and ADHD diagnosis in childhood. We then hypothesized that a high neonatal manganese concentration is associated with ADHD, and that a high selenium concentration imparts protection against ADHD. The secondary aim of the study was to explore the associations between manganese and selenium concentrations in maternal and fetal blood, where we hypothesized positive correlations.

2. Material and methods

2.1. Participants

The selection of and diagnostic procedures for ADHD children have previously been described (Ode et al., 2014). Briefly, 419 children born in the city of Malmö between 1978 and 2000 were diagnosed with ADHD and were followed until 2005 at the Department of Child and Adolescent Psychiatry in Malmö. Age at the time of diagnosis varied between 5 and 17 years, with most children being diagnosed between the ages of 8 and 12 years. The Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria DSM-III-R_{11} were used before 1994, and from 1994 onwards the DSM-IV_{12} criteria. Intelligence and ability to concentrate were tested with the Wechsler Intelligence Scale (WISC), TEA-Ch, and QB-Tech or IVA+. A pediatric examination with assessment of neurological soft signs was also performed. The school teacher and/or the parents were asked to fill in questionnaires such as the SNAP-IV, Conner's questionnaire or the 5–15 questionnaire, and the BRIEF-questionnaire. Behavior in school and at the visits to the clinic was observed and registered. A team of a psychiatrist, a psychologist and sometimes also a social worker met to achieve a consensus ADHD diagnosis using the DSM criteria.

Using the unique personal identification numbers, maternal and umbilical cord serum samples for children with ADHD were collected from the Malmö Maternity Unit Serum Biobank (MMUSB). The MMUSB has previously been described (Ode et al., 2014). A pool of 10 controls per ADHD child matched for year of birth ± 12 months and country of birth of the mother was retrieved from the Swedish Medical Birth Register (SMBR). The first newborn in the pool of controls with an umbilical cord blood sample found in the MMSUB was chosen as control. If no cord serum was found among the 10 matched controls in the pool, the sample of the next-baby-born was used. Demographic and obstetric data were obtained from the SMBR. The selection procedure for cases and controls is presented in Fig. 1. Manganese and selenium could be analyzed in 180 ADHD and 191 control (166 matched) umbilical cord samples.

The study protocol followed the requirements of the Declaration of Helsinki and was approved by the Research Ethics Committee at Lund University, Sweden.

2.2. Analyses of manganese, selenium, and cotinine

The concentrations of manganese and selenium were determined by inductively coupled plasma-mass spectrometry (ICP-MS; Thermo X7; Thermo Elemental, Winsford, UK). Aliquots of 100 μL serum were diluted 10 times with an alkaline solution according to Barany et al. (1997). The detection limit, calculated as 3 times the standard deviation (SD) of the blank was 0.01 μg/L for manganese and 1.3 μg/L for selenium. The analytical accuracy was checked against two different reference materials. For Seronorm Trace Elements Serum L-1 (lot 0903106; SERO AS, Billingstad, Norway) the results obtained for manganese and selenium were 16 ± 1.0 and 107 ± 6.3 μg/L (mean $\pm$ SD, $n=28$) vs. recommended 15 ± 0.9 and 107 ± 7 μg/L, respectively and for human serum reference samples from the Centre de Toxicologie du Québec, Canada (lot QMEQAS06S-06) the obtained values were 3.9 ± 0.26 and 286 ± 15 μg/L ($n=28$) vs. recommended 4.0 ± 1.05 and 287 ± 63 μg/L, respectively. All analyzed samples were prepared in duplicate and the method imprecisions (calculated as the coefficients of variation in measurements of duplicate preparations) were 9.4% and 6.6% for manganese and selenium, respectively.

The smoking status of the cases and controls was determined with analyses of the cotinine concentrations in umbilical cord serum as part of another study (Ode et al., 2013).

2.3. Statistical analyses

Spearman's rank correlation coefficient was used to investigate the correlations of the trace elements between maternal and umbilical cord serum samples and the Wilcoxon matched-pairs signed-ranks test to investigate differences in manganese and selenium concentrations between maternal and cord serum, and between ADHD cases and controls, respectively.

Conditional logistic regression was performed for calculating the odds ratio for every 1 ng/mL increase in the umbilical cord serum manganese or selenium concentrations and for the manganese/selenium ratio and ADHD diagnosis. Based on the distribution of the trace elements among the controls, we defined manganese concentrations above the 90th percentile (6.68 μg/L) and selenium concentrations below the 10th (36.3 μg/L) or above the 90th percentile (59 μg/L) as deviating. These cut-offs were arbitrary selected and the reason for the relatively high and low cut-offs were that both manganese and selenium are essential nutrients and we believe that the waste majority have concentrations within the normal range. For evaluation of potential interaction between the categorized manganese and selenium we included an interaction term in the model (i.e. manganese*selenium). We calculated crude as well as adjusted odds ratios (ORs). If the crude and the adjusted ORs did not differ by more than 10%, we only present the crude OR. As potential confounders we considered smoking during pregnancy, parity, and gestational age at birth. Women with a cotinine concentration below the detection limit (0.2 μg/L) were classified as non-smokers, women with a concentration above 15 μg/L as active smokers, and women with levels between 0.2 and 15 μg/L as second hand smokers (George et al., 2006). Parity was entered in the analysis as a class variable divided into two groups according to number of previously born children (0 [i.e. primiparous], or ≥ 1 children). Gestational age was divided into three groups: <37, 37–42, and >42 weeks of pregnancy.

The conditional regressions were performed with LogXact statistical software (Cytel Studio 10), while the rest of the analyses were performed with SPSS version 21 (SPSS Inc., Chicago, IL, USA).

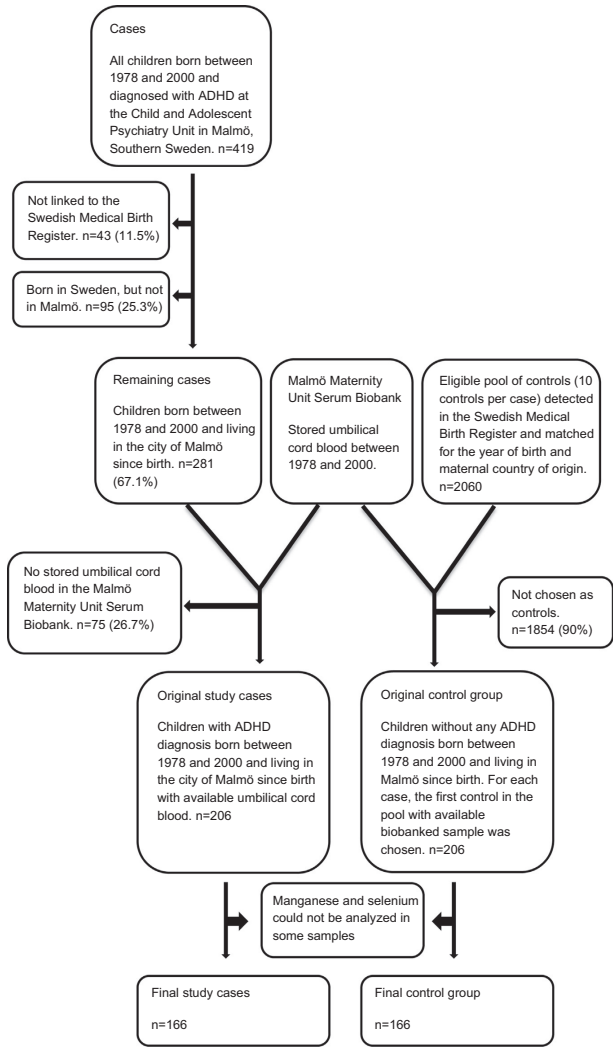


Fig. 1. Flowchart for the selection procedure of the children with attention deficit hyperactivity disorder (ADHD) and controls.

3. Results

For 180 cases and 191 controls, both manganese and selenium could be analyzed in umbilical cord serum samples. The number of the matched ADHD cases and controls where both manganese and selenium could be assessed was 166. The obstetric and demographic characteristics for the 166 matched ADHD children and controls are presented in Tables 1 and 2.

Out of the 206 original maternal serum samples for the control group, manganese and selenium could be analyzed in 193 samples. Concentrations of manganese and selenium in paired maternal and cord serum ($n=181$) are presented in Table 3 and were positively correlated with each other (Table 4).

Cord manganese concentrations were significantly higher than maternal manganese concentrations, whereas cord selenium concentrations were significantly lower than maternal selenium concentrations (p-values <0.0001).There were no significant differences in the manganese and selenium levels between matched ADHD cases and controls ($n=166$) with $p>0.05$.

Umbilical cord serum concentrations of manganese and

Table 1
Manganese concentrations (μg/L) in the umbilical cord serum of ADHD cases and controls.

Characteristics	Children with ADHD			Controls		
	N (%)	Mean ± SD	Median (min–max)	*N* (%)	Mean ± SD	Median (min–max)
Group						
Case/Control	166	4.7 ± 2.2	4.3 (1.7–16)	166	4.6 ± 3.4	4.1 (1.3–39)
Year of delivery						
1978–1985	14 (8.4)	4.1 ± 1.4	3.5 (2.2–6.8)	11 (6.6)	4.0 ± 1.5	4.3 (1.3–6.2)
1986–1993	125 (75.3)	4.7 ± 2.1	4.3 (1.7–16)	127 (76.5)	4.7 ± 3.8	4.1 (1.3–39)
1994–2000	27 (16.3)	5.1 ± 2.8	4.5 (2.2–15)	28 (16.9)	4.8 ± 1.6	4.4 (2.0–9.6)
Mother's age at pregnancy (years)						
< 20 years	8 (4.8)	5.4 ± 0.70	5.5 (4.4–6.5)	5 (3.0)	4.6 ± 1.3	4.3 (3.5–6.9)
20–35 years	138 (83.1)	4.7 ± 2.3	4.3 (1.7–16)	138 (83.1)	4.7 ± 3.6	4.1 (1.3–39)
> 35 years	20 (12.0)	4.7 ± 1.5	4.5 (2.2–8.9)	23 (13.9)	4.3 ± 2.0	3.8 (1.8–9.6)
Parity						
Primiparous	79 (47.6)	4.8 ± 2.3	4.4 (1.7–16)	89 (53.6)	4.5 ± 1.8	4.2 (1.3–11)
Multiparous	87 (52.4)	4.6 ± 2.1	4.3 (1.7–15)	77 (46.4)	4.8 ± 4.6	3.9 (1.6–39)
Maternal body mass index (kg/m^2)[a]						
Missing	117 (70.5)	4.9 ± 2.4	4.4 (1.7–16)	120 (72.3)	4.5 ± 3.8	4.0 (1.3–39)
Underweight (< 18.5)	1 (0.6)	4.0	4.0	2 (1.2)	4.0 ± 0.16	4.0 (3.9–4.1)
Normal (18.5–25)	31 (18.7)	4.1 ± 1.4	4.1 (1.9–9.2)	29 (17.5)	5.2 ± 1.9	4.9 (2.1–9.8)
Overweight (25–30)	12 (7.2)	4.8 ± 1.8	4.3 (2.2–8.9)	12 (7.2)	4.4 ± 1.3	4.1 (2.6–6.8)
Obese (≥ 30)	5 (3.0)	4.3 ± 1.7	4.7 (2.3–6.5)	3 (1.8)	5.4 ± 3.0	3.8 (3.5–8.8)
Maternal country of origin						
Sweden	140 (84.3)	4.8 ± 2.3	4.3 (1.7–16)	140 (84.3)	4.7 ± 3.6	4.2 (1.3–39)
Other Nordic countries[b]	6 (3.6)	3.4 ± 1.2	3.1 (2.3–5.3)	6 (3.6)	4.3 ± 1.2	4.0 (2.6–6.0)
The rest of Europe[c]	6 (3.6)	5.1 ± 2.0	4.7 (3.3–8.8)	6 (3.6)	4.8 ± 2.1	4.1 (3.2–8.9)
Sub-Saharan Africa	1 (0.6)	5.6	5.62	1 (0.6)	4.1	4.1
Middle East/North Africa	9 (5.4)	3.9 ± 1.3	4.0 (2.1–5.9)	9 (5.4)	3.8 ± 1.2	3.9 (1.6–5.1)
East Asia	1 (0.6)	3.1	3.1	1 (0.6)	1.3	1.3
North America	2 (1.2)	6.6 ± 2.8	6.6 (4.7–8.6)	2 (1.2)	5.0 ± 2.5	5.02 (3.2–6.8)
Unknown	1 (0.6)	4.5	4.5	1 (0.6)	3.1	3.1
Smoking status[d]						
Non-smoker	51 (30.7)	4.5 ± 1.7	4.3 (2.0–10)	70 (42.2)	4.2 ± 1.7	3.9 (1.6–9.8)
SHS	46 (27.7)	4.8 ± 2.5	4.4 (2.1–15)	44 (26.5)	5.0 ± 3.1	4.2 (1.3–21)
Active smoker	69 (41.6)	4.8 ± 2.4	4.3 (1.7–16)	52 (31.3)	4.9 ± 5.0	4.3 (2.1–39)
Infant sex						
Boy	149 (89.8)	4.8 ± 2.3	4.4 (1.7–16)	135 (81.3)	4.8 ± 3.7	4.1 (1.3–39)
Girl	17 (10.2)	4.0 ± 1.2	3.9 (2.6–7.1)	31 (18.7)	3.9 ± 1.2	3.9 (1.3–5.8)
Birth weight (g)						
< 2500	11 (6.6)	4.0 ± 2.0	4.1 (1.7–8.9)	5 (3.0)	4.3 ± 0.71	4.7 (3.2–4.8)
2500–4000	134 (80.7)	4.8 ± 2.3	4.4 (1.7–16)	124 (74.7)	4.8 ± 3.8	4.2 (1.3–39)
> 4000	21 (12.7)	4.5 ± 1.7	4.4 (2.1–9.2)	37 (22.3)	4.3 ± 1.8	4.0 (1.8–9.8)
Gestational age (weeks)						
< 37	9 (5.4)	3.6 ± 0.96	4.1 (1.9–4.5)	7 (4.2)	4.4 ± 1.5	4.7 (2.1–6.4)
37–41	146 (88.0)	4.8 ± 2.3	4.5 (1.7–16)	142 (85.5)	4.4 ± 2.2	4.1 (1.3–21)
≥ 42	11 (6.6)	4.5 ± 1.8	4.5 (2.6–8.9)	17 (10.2)	6.7 ± 8.5	4.1 (2.6–39)

Abbreviations: ADHD: attention deficit hyperactivity disorder; SD: standard deviation; parity, number of previous pregnancies.

[a] Body mass index was classified according to the standard values of the World Health Organization.
[b] Finland, Denmark, and Norway.
[c] Western Europe and former Eastern Europe.
[d] Maternal smoking is based on measured cotinine concentrations in the umbilical cord serum.

selenium in children with or without ADHD diagnosis are illustrated in Figs. 2 and 3, respectively. Concentrations of manganese and selenium in umbilical cord serum of ADHD cases and controls in relation to maternal and infant obstetric and demographic characteristics are presented in Tables 1 and 2.

Manganese or selenium concentrations in cord serum were not associated with having an ADHD diagnosis in childhood when the exposures were analyzed as continuous variables (Table 5). The estimates did not change after adjusting for the potential confounders. In addition, manganese/selenium ratio was not associated with ADHD diagnosis ($p=0.81$).When the exposure variables were treated as categorized there was no significant association between manganese and ADHD (OR 1.3 [95% CI 0.64 to 2.6]), while selenium exposure was significantly associated with ADHD when > 90th percentile was compared to 10th–90th percentile (OR 2.5 [95% CI 1.3–5.1]) (Table 5). However, there was no significant interaction between manganese and selenium exposure ($p=0.08$). Table 6 shows the associations when manganese concentrations < 90th percentile and selenium concentrations between 10th and 90th percentile were used as the reference group. Children with manganese concentrations below the 90th percentile and selenium concentrations above the 90th percentile had 2.5 times higher odds of having an ADHD diagnosis compared to the control group ($p=0.015$). The odds ratio increased to 3.1 after adjustment for parity, gestational age, and cotinine concentrations ($p=0.008$).

4. Discussion

Manganese and selenium are essential metals that are required for proper functioning of the human body. However, neurotoxicity has been shown for manganese at high levels (Santamaria and Sulsky, 2010). A protective effect of selenium on neurotoxicity

Table 2
Selenium concentrations (μg/L) in the umbilical cord serum of ADHD cases and controls.

Characteristics	Children with ADHD			Controls		
	N (%)	Mean ± SD	Median (min–max)	*N* (%)	Mean ± SD	Median (min–max)
Group						
Case/Control	166	49 ± 12	47 (19–95)	166	48 ± 8.9	48 (25–73)
Year of delivery						
1978–1985	14 (8.4)	48 ± 9.6	48 (34–63)	11 (6.6)	48 ± 9.7	50 (28–62)
1986–1993	125 (75.3)	49 ± 12	47 (26–95)	127 (76.5)	47 ± 8.8	48 (25–74)
1994–2000	27 (16.3)	51 ± 13	50 (19–82)	28 (16.9)	51 ± 8.4	51 (35–66)
Mother's age at pregnancy (years)						
< 20 years	8 (4.8)	50 ± 14	47 (37–79)	5 (3.0)	49 ± 8.2	50 (40–58)
20–35 years	138 (83.1)	49 ± 12	48 (19–95)	138 (83.1)	47 ± 9.1	48 (25–66)
> 35 years	20 (12.0)	48 ± 13	45 (31–82)	23 (13.9)	49 ± 7.9	48 (37–74)
Parity						
Primiparous	79 (47.6)	50 ± 12	49 (28–93)	89 (53.6)	49 ± 9.5	49 (25–74)
Multiparous	87 (52.4)	47 ± 12	46 (19–95)	77 (46.4)	46 ± 7.9	46 (30–62)
Maternal body mass index (kg/m²)[a]						
Missing	117 (70.5)	48 ± 12	46 (28–95)	120 (72)	47 ± 8.7	47 (28–74)
Underweight (< 18.5)	1 (0.6)	62	62	2 (1.2)	39 ± 4.8	39 (35–42)
Normal (18.5–25)	31 (18.7)	52 ± 12	52 (19–82)	29 (17.5)	50 ± 9.4	51 (25–66)
Overweight (25–30)	12 (7.2)	48 ± 10	46 (33–73)	12 (7.2)	53 ± 7.6	53 (38–66)
Obese (≥ 30)	5 (3.0)	49 ± 14	55 (26–61)	3 (1.8)	54 ± 7.8	57 (46–60)
Maternal country of origin						
Sweden	140 (84.3)	50 ± 12	48 (26–95)	140 (84.3)	48 ± 8.8	49 (25–74)
Other Nordic countries[b]	6 (3.6)	47 ± 9.5	46 (33–62)	6 (3.6)	48 ± 6.8	46 (41–60)
The rest of Europe[c]	6 (3.6)	49 ± 11	51 (28–61)	6 (3.6)	43 ± 10	40 (30–60)
Sub-Saharan Africa	1 (0.6)	42	42	1 (0.6)	44	44
Middle East/North Africa	9 (5.4)	42 ± 11	44 (19–53)	9 (5.4)	44 ± 7.1	43 (33–54)
East Asia	1 (0.6)	61	61	1 (0.6)	29	29
North America	2 (1.2)	36 ± 2.6	36 (34–38)	2 (1.2)	55 ± 5.9	55 (51–59)
Unknown	1 (0.6)	33	33	1 (0.6)	34	34
Smoking status[d]						
Non-smoker	51 (30.7)	50 ± 13	48 (19–95)	70 (42.2)	49 ± 8.0	48 (31–66)
SHS	46 (27.7)	50 ± 9.4	49 (35–83)	44 (26.5)	48 ± 10	48 (28–74)
Active smoker	69 (41.6)	48 ± 14	46 (26–93)	52 (31.3)	46 ± 8.6	45 (25–66)
Infant sex						
Boy	149 (89.8)	49 ± 12	47 (19–95)	135 (81.3)	47 ± 8.5	48 (25–66)
Girl	17 (10.2)	50 ± 14	49 (28–93)	31 (18.7)	49 ± 10	49 (28–74)
Birth weight (g)						
< 2500	11 (6.6)	38 ± 11	37 (28–68)	5 (3.0)	47 ± 8.6	47 (32–54)
2500–4000	134 (80.7)	49 ± 12	48 (19–93)	124 (74.7)	47 ± 9.1	47 (25–74)
> 4000	21 (12.7)	54 ± 14	51 (37–95)	37 (22.3)	49 ± 8.0	48 (30–66)
Gestational age (weeks)						
< 37	9 (5.4)	34 ± 4.1	35 (28–40)	7 (4.2)	43 ± 8.9	40 (32–54)
37–41	146 (88)	50 ± 12	49 (19–95)	142 (85.5)	48 ± 9.0	48 (25–66)
≥ 42	11 (6.6)	50 ± 11	47 (35–73)	17 (10.2)	50 ± 7.8	48 (41–74)

Abbreviations: ADHD: attention deficit hyperactivity disorder; SD: standard deviation;
parity, number of previous pregnancies.

[a] Body mass index was classified according to the standard values of the World Health Organization.
[b] Finland, Denmark, and Norway.
[c] Western Europe and former Eastern Europe.
[d] Maternal smoking is based on measured cotinine concentrations in the umbilical cord serum.

Table 3
Manganese and selenium concentrations (μg/L) in matched (n = 181) maternal and umbilical cord serum.

	Manganese		Selenium	
	Mean (SD)	Median (min–max)	Mean (SD)	Median (min–max)
Maternal serum	2.9 (1.1)	2.7 (1.2–7.7)	72 (20)	68 (31–172)
Umbilical cord serum	4.5 (3.1)	4.1 (1.3–39)	48 (9.3)	48 (25–78)

SD: standard deviation.

Table 4
Spearman´s correlations and p values between manganese (Mn) and selenium (Se) in 181 pairs of maternal and cord serum.

	Cord Mn	Maternal Se	Cord Se
Maternal Mn	0.25 (0.001)	0.16 (0.038)	0.11 (0.15)
Cord Mn		0.012 (0.87)	0.15 (0.051)
Maternal Se			0.28 (< 0.001)

through its antioxidant effect has been reported (Khan, 2010; Yang et al., 2014). The hypotheses raised in the present study are that manganese is associated with ADHD diagnosis, while selenium has a protective function against having ADHD. The present study showed no associations between fetal exposure to manganese, but surprisingly, high selenium concentrations increased the odds of having an ADHD diagnosis in childhood.

Studies in children have suggested that exposure to high levels of manganese may be detrimental to neurodevelopment. Nervous system disturbances have been observed in children exposed to excess manganese, including poor school performance, impaired cognitive function, abnormal performance in neurobehavioral tests, and decreased mental development scores, as well as

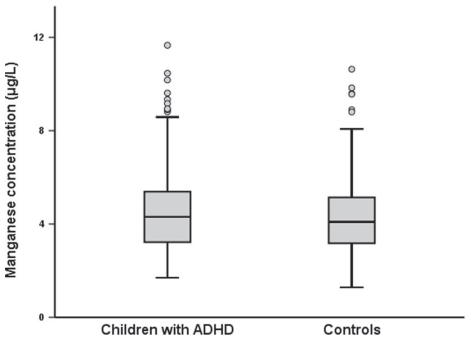


Fig. 2. Manganese concentrations (µg/L) in umbilical cord serum in children with attention deficit hyperactivity disorder (ADHD) diagnosis and controls. The extreme values of manganese are not shown in the boxplot.

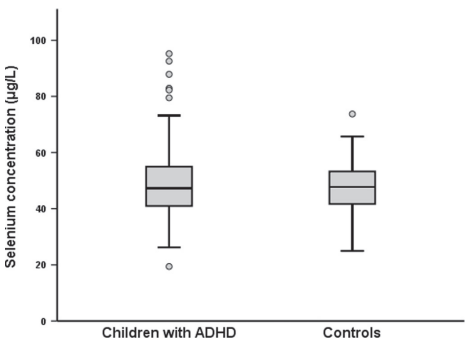


Fig. 3. Selenium concentrations (µg/L) in umbilical cord serum in children with attention deficit hyperactivity disorder (ADHD) diagnosis and controls.

Table 5
Odds ratios and 95% confidence intervals obtained from conditional logistic regressions for the association between umbilical cord serum manganese and selenium and ADHD.

		n[a]	ADHD diagnosis	
			Univariate model[b]	**Multivariate model**[b,c]
Mn[d]			1.01 (0.94, 1.1)	1.01 (0.94, 1.1)
Se[d]			1.01 (0.99, 1.3)	1.01 (0.99, 1.03)
Mn[e]	***P*** **≤ 90th**	296	1 (Reference)	1 (Reference)
	P **> 90th**	36	1.3 (0.64, 2.6)	0.91 (0.37, 2.2)
Se[e]	***P*** **< 10th**	38	1.8 (0.84, 4.0)	1.5 (0.63, 3.7)
	P **10th–90th**	246	1 (Reference)	1 (Reference)
	P **> 90th**	48	2.5 (1.3, 5.1)	2.6 (1.2, 5.5)

Abbreviations: ADHD: attention deficit hyperactivity disorder; Mn: manganese; Se: selenium; P: percentile.

[a] Number of subjects if the variable is dichotomous.

[b] The crude and the adjusted odds ratios did not differ by more than 10% and thereby only the crude estimates are presented.

[c] Mn and Se concentrations were included simultaneously and were entered in the analysis either as continuous or categorized variables.

[d] Odds ratio is calculated for every 1 µg/L increase in the concentration.

[e] Odds ratio is calculated for categorized concentrations.

Table 6
Odds ratios and 95% confidence intervals obtained from conditional logistic regressions for the association between umbilical cord serum manganese and selenium and ADHD.

		n	ADHD diagnosis	
			Crude OR (95% CI)	**Adjusted OR (95% CI)**[a]
Mn ≤ 90th P	**Se *P* < 10th**	31	1.5 (0.63, 3.7)	0.74 (0.27, 2.0)
	Se *P* 10th–90th	226	1 (Reference)	1 (Reference)
	Se *P* > 90th	39	2.5 (1.2, 5.5)	3.1 (1.4, 7.0)
Mn > 90th P	**Se *P* < 10th**	7	3.4 (0.62, 19)	4.2 (0.74, 24)
	Se *P* 10th–90th	20	0.91 (0.37, 2.2)	0.82 (0.32, 2.1)
	Se *P* > 90th	9	2.6 (0.61, 11)	3.6 (0.74, 18)

Abbreviations: ADHD: attention deficit hyperactivity disorder; OR: odds ratio; 95% CI: 95% confidence interval; Mn: manganese; Se: selenium; P: percentile.

[a] Adjusted for parity, gestational age, and cotinine concentrations in cord serum.

changes in behavior and decreases in the ability to learn and remember (Bouchard et al., 2011; Claus Henn et al., 2010; Wasserman et al., 2006; Woolf et al., 2002). Although the observed effects in these cross-sectional studies do not establish a causal relationship due to manganese exposure exclusively, they support the hypothesis that exposure to elevated manganese may have negative impacts on brain development.

In a previous pilot study by Bouchard et al. (2006), a statistically significant relationship between increased levels of manganese in drinking water and oppositional behaviors and hyperactivity was found in children aged between 6 and 15 years. However, this pilot study has several shortcomings described by Santamaria and Sulsky (2010), including small sample size; lack of control for potential confounders; self-selection of subjects; the exposure assessment not being blinded; and misclassification of exposure since most of the subjects (91%) drank bottled water at home. The findings from Farias et al. (2010) support the hyperactivity findings by Bouchard et al. (2006). The results from this cross-sectional study showed that students, aged between 7 and 15 years, with ADHD diagnosis had significantly elevated serum manganese (4.5 µg/L), compared with controls (3.5 µg/L). Children with ADHD have disruptive patterns of eating behavior and unfavorable nutrition status compared to healthy controls (Ptacek et al., 2014). Due to the cross-sectional nature of the study by Farias et al. (2010), changed nutrition status accompanied by ADHD diagnosis might be the reason for the positive relationship between manganese and ADHD found there. In the present study, the children were followed from birth until ADHD was diagnosed. In addition, manganese concentrations measured here represent the highest concentrations during the fetal life as manganese levels increase with increasing gestational age (Mora et al., 2014; Spencer, 1999; Tholin et al., 1995), although, no relationship was observed between manganese levels and ADHD diagnosis.

Manganese neurotoxicity may in part involve interactions with other minerals (Lai et al., 1999). In a developmental rat model of chronic manganese toxicity, chronic manganese treatment altered levels and distributions of iron, aluminum, selenium, copper, zinc, and calcium in various regions of the brain. Such interactions with electrolytes and minerals other than selenium might have a protective effect on the induction of destruction of dopaminergic neurons caused by manganese. This could be one explanation for the lack of association between manganese and ADHD diagnosis.

In animals, selenium protected neonates from manganese neurotoxicity, reversed manganese induced impairment of dopaminergic neurotransmission in mice, and prevented the increased permeability of the blood–brain-barrier to toxins induced by free radicals (Khan, 2010; Oztas et al., 2001; Santos et al., 2012). These

findings raised the hypothesis that selenium might protect against development of ADHD symptoms through protection against manganese neurotoxicity. The results from the current study contradict this hypothesis. In a recent study by Yang et al. (2014), a high level of umbilical cord serum manganese (≥ 9.1 μg/L) increased the risk of low Neonatal Behavioral Neurological Assessment (NBNA) scores in neonates and the risk was reduced with high cord serum selenium (≥ 63.1 μg/L). Manganese and selenium concentrations were lower in the present study than in the study by Yang et al. (2014) and stratifying concentrations at or above the 90th percentile resulted in fewer fetuses in the highly exposed group. This could weaken the power of detecting significant differences in the concentrations between ADHD and control children. There is no other reasonable explanation for our finding since there is an adequate literature suggesting a protective effect of selenium against neurotoxicity. However, a possible explanation is the multiple testing which has resulted in a chance finding.

Manganese concentrations in our population of pregnant women and fetuses were low compared to those measured in Sweden and in other countries (Abdelouahab et al., 2010; Rudge et al., 2009; Smargiassi et al., 2002; Wilson et al., 1991). On the other hand, manganese concentrations measured by us were analogous with those measured in China and Slovenia using the same analytical method (Krachler et al., 1999; Smargiassi et al., 2002; Yang et al., 2014). Manganese is an essential metal, and food consumption represents the main contribution to its daily intake (Barceloux, 1999; Santamaria and Sulsky, 2010). The current study does not distinguish between maternal manganese intake from the diet and from other sources such as environmental exposure to the gasoline additive methylcyclopentadienyl manganese tricarbonyl (MMT). The discrepancy in manganese concentrations observed between countries may reflect different dietary habits, different analytical methods and inter-laboratory variability, or variations in environmental exposures. The results of the present study confirm that umbilical cord serum manganese concentrations are higher than maternal concentrations at delivery. This finding suggests that there is an active transport of manganese across the placenta, which is also suggested by other investigators (Smargiassi et al., 2002; Tholin et al., 1995). Maternal and cord manganese serum levels were poorly correlated, a finding consistent with previous studies (Takser et al., 2004). About 66% of the manganese is located in erythrocytes Milne et al. (1990), and the erythrocyte count in cord blood is higher than that in the corresponding maternal blood (Dapper and Didia, 2006; Qaiser et al., 2013). This may explain the high manganese in umbilical cord compared to maternal serum.

Selenium concentrations in maternal serum were higher than those in umbilical cord serum analogous to previous findings. Maternal and cord serum selenium levels were comparable to those measured in Sweden, Denmark, India and Italy (Alimonti et al., 2000; Bro et al., 1988; Gathwala et al., 2000; Jariwala et al., 2014; Osman et al., 2000). The levels were however higher than those in Poland, Albania, and Northern Ireland (Schulpis et al., 2004; Wasowicz et al., 1993; Wilson et al., 1991) but lower than those measured in the Middle East, Iran, Israel, the Mediterranean area, the United States, South Africa, and Canada (Al-Saleh et al., 2004; Boskabadi et al., 2012; Butler Walker, 2006; Chen et al., 2014; Makhoul et al., 2004; Miklavcic et al., 2013; Rudge et al., 2009). The correlation between maternal and cord serum selenium was weak compared to previous studies (Dobrzynski et al., 1998; Sakamoto et al., 2010; Wilson et al., 1991), but comparable with that found in a Swedish population (Osman et al., 2000).

Manganese has a short half-life in blood and serum manganese concentration might therefore not be a perfect biomarker. However, Farias et al. (2010) found elevated serum manganese concentrations in ADHD cases compared to the controls. Serum manganese levels were also used in monitoring exposure to airborne manganese (Jarvisalo et al., 1992; Lu et al., 2005; Roels et al., 1987). Many researchers use hair content of manganese as an indicator of chronic exposure when investigating the relationship between manganese and neurological and behavioral outcomes (Bouchard et al., 2011; Menezes-Filho et al., 2014; Riojas-Rodriguez et al., 2010). Though, use of hair is problematic for several reasons since manganese hair levels may be affected by exogenous contamination, hair color, physical activity, and hair growth and loss (Stauber et al., 1987; Sturaro et al., 1994; Zaitseva et al., 2014).

The insufficient amount of umbilical cord serum to perform the analyses in some samples and the matching led to a loss of cases and controls, resulting in the relatively small sample size. This loss of cases, along with the few subjects in the upper percentiles, might weaken the statistical power to detect minor associations and to rule out small effects. We do not think that there has been a selective loss of cases since the probability of the excluded children having the highest or lowest levels of manganese or selenium is likely to be minimal.

5. Conclusions

ADHD diagnosis in childhood was not associated with manganese levels during pregnancy. Manganese concentrations measured here might be too low to trigger undesirable effects on brain development. Selenium intake during pregnancy may not protect against ADHD diagnosis in the offspring. The results regarding the increased odds of having ADHD with relatively low manganese and high selenium concentrations should be interpreted with caution. The results of this study should preferably to be replicated by other investigators before any conclusions can be drawn.

Competing financial interests

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

Acknowledgments

The project was funded by the Swedish Research Council (via SIMSAM Lund) and the Medical Faculty at Lund University.

References

Abdelouahab, N., Huel, G., Suvorov, A., Foliguet, B., Goua, V., Debotte, G., et al., 2010. Monoamine oxidase activity in placenta in relation to manganese, cadmium, lead, and mercury at delivery. Neurotoxicol. Teratol. 32, 256–261.
Al-Saleh, E., Nandakumaran, M., Al-Shammari, M., Al-Falah, F., Al-Harouny, A., 2004. Assessment of maternal–fetal status of some essential trace elements in pregnant women in late gestation: relationship with birth weight and placental weight. J. Matern. Fetal Neonatal Med. 16, 9–14.
Alimonti, A., Petrucci, F., Laurenti, F., Papoff, P., Caroli, S., 2000. Reference values for selected trace elements in serum of term newborns from the urban area of Rome. Clin. Chim. Acta 292, 163–173.
Banerjee, T.D., Middleton, F., Faraone, S.V., 2007. Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatr. 96, 1269–1274.
Barany, E., Bergdahl, I.A., Schütz, A., Skerfving, S., Oskarsson, A., 1997. Inductively coupled plasma mass spectrometry for direct multi-element analysis of diluted human blood and serum. J. Anal. Atomic Spectrom. 12, 1005–1009.
Barceloux, D.G., 1999. Manganese. J. Toxicol. Clin. Toxicol. 37, 293–307.
Battin, E.E., Perron, N.R., Brumaghim, J.L., 2006. The central role of metal coordination in selenium antioxidant activity. Inorg. Chem. 45, 499–501.
Boskabadi, H., Maamouri, G., Rezagholizade Omran, F., Mafinejad, S., Tara, F., Rayman, M.P., et al., 2012. Effect of prenatal selenium supplementation on cord blood selenium and lipid profile. Pediatr. Neonatol. 53, 334–339.
Bouchard, M., Laforest, F., Vandelac, L., Bellinger, D., Mergler, D., 2006. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ. Health Perspect. 115, 122–127.

Bouchard, M.F., Sauve, S., Barbeau, B., Legrand, M., Brodeur, M.E., Bouffard, T., et al., 2011. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ. Health Perspect. 119, 138–143.
Braun, J.M., Kahn, R.S., Froehlich, T., Auinger, P., Lanphear, B.P., 2006. Exposures to environmental toxicants and attention deficit hyperactivity disorder in U.S. children. Environ. Health Perspect. 114, 1904–1909.
Bro, S., Berendtsen, H., Norgaard, J., Host, A., Jorgensen, P.J., 1988. Serum selenium concentration in maternal and umbilical cord blood. Relation to course and outcome of pregnancy. J. Trace Elem. Electrolytes Health Dis. 2, 165–169.
Butler Walker, J., 2006. Houseman J, Seddon L, McMullen E, Tofflemire K, Mills C, et al. Maternal and umbilical cord blood levels of mercury, lead, cadmium, and essential trace elements in arctic Canada. Environ. Res. 100, 295–318.
Chen, Z., Myers, R., Wei, T., Bind, E., Kassim, P., Wang, G., et al., 2014. Placental transfer and concentrations of cadmium, mercury, lead, and selenium in mothers, newborns, and young children. J. Expo. Sci. Environ. Epidemiol. 5, 537–544.
Claus Henn, B., Ettinger, A.S., Schwartz, J., Tellez-Rojo, M.M., Lamadrid-Figueroa, H., Hernandez-Avila, M., et al., 2010. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology 21, 433–439.
Dapper, D.V., Didia, B.C., 2006. Haemorheological parameters of umbilical cord blood of nigerian newborns: correlations with maternal parameters. West Afr. J. Med. 25, 226–230.
Dobrzynski, W., Trafikowska, U., Trafikowska, A., Pilecki, A., Szymanski, W., Zachara, B.A., 1998. Decreased selenium concentration in maternal and cord blood in preterm compared with term delivery. Analyst 123, 93–97.
Dorman, D.C., Struve, M.F., Vitarella, D., Byerly, F.L., Goetz, J., Miller, R., 2000. Neurotoxicity of manganese chloride in neonatal and adult cd rats following subchronic (21-day) high-dose oral exposure. J. Appl. Toxicol. 20, 179–187.
Eubig, P.A., Aguiar, A., Schantz, S.L., 2010. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. Environ. Health Perspect. 118, 1654–1667.
Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., et al., 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biol. Psychiatry 57, 1313–1323.
Faraone, S.V., Biederman, J., Mick, E., 2006. The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. Psychol. Med. 36, 159–165.
Farias, A.C., Cunha, A., Benko, C.R., McCracken, J.T., Costa, M.T., Farias, L.G., et al., 2010. Manganese in children with attention-deficit/hyperactivity disorder: relationship with methylphenidate exposure. J. Child Adolesc. Psychopharmacol. 20, 113–118.
Garner, C.D., Nachtman, J.P., 1989. Manganese catalyzed auto-oxidation of dopamine to 6-hydroxydopamine in vitro. Chem. Biol. Interact. 69, 345–351.
Gathwala, G., Yadav, O.P., Singh, I., Sangwan, K., 2000. Maternal and cord plasma selenium levels in full-term neonates. Indian J. Pediatr. 67, 729–731.
George, L., Granath, F., Johansson, A.L., Cnattingius, S., 2006. Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. Acta Obstet. Gynecol. Scand. 85, 1331–1337.
Gizer, I.R., Ficks, C., Waldman, I.D., 2009. Candidate gene studies of ADHD: a meta-analytic review. Hum. Genet. 126, 51–90.
Gustafsson, P., Thernlund, G., Ryding, E., Rosen, I., Cederblad, M., 2000. Associations between cerebral blood-flow measured by single photon emission computed tomography (SPECT), electro-encephalogram (EEG), behaviour symptoms, cognition and neurological soft signs in children with attention-deficit hyperactivity disorder (ADHD). Acta Paediatr. 89, 830–835.
Jariwala, M., Suvarna, S., Kiran Kumar, G., Amin, A., Udas, A.C., 2014. Study of the concentration of trace elements fe, Zn, Cu, Se and their correlation in maternal serum, cord serum and colostrums. Indian J. Clin. Biochem. 29, 181–188.
Jarvisalo, J., Olkinuora, M., Kiilunen, M., Kivisto, H., Ristola, P., Tossavainen, A., et al., 1992. Urinary and blood manganese in occupationally nonexposed populations and in manual metal arc welders of mild steel. Int. Arch. Occup. Environ. Health 63, 495–501.
Kern, C.H., Stanwood, G.D., Smith, D.R., 2010. Preweaning manganese exposure causes hyperactivity, disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels. Synapse 64, 363–378.
Khan, H.A., 2010. Selenium partially reverses the depletion of striatal dopamine and its metabolites in MPTP-treated c57bl mice. Neurochem. Int. 57, 489–491.
Krachler, M., Rossipal, E., Micetic-Turk, D., 1999. Trace element transfer from the mother to the newborn—investigations on triplets of colostrum, maternal and umbilical cord sera. Eur. J. Clin. Nutr. 53, 486–494.
Lai, J.C., Minski, M.J., Chan, A.W., Leung, T.K., Lim, L., 1999. Manganese mineral interactions in brain. Neurotoxicology 20, 433–444.
Lara, C., Fayyad, J., de Graaf, R., Kessler, R.C., Aguilar-Gaxiola, S., Angermeyer, M., et al., 2009. Childhood predictors of adult attention-deficit/hyperactivity disorder: results from the World Health Organization World Mental Health survey initiative. Biol. Psychiatry 65, 46–54.
Liccione, J.J., Maines, M.D., 1988. Selective vulnerability of glutathione metabolism and cellular defense mechanisms in rat striatum to manganese. J. Pharmacol. Exp. Ther. 247, 156–161.
Lu, L., Zhang, L.L., Li, G.J., Guo, W., Liang, W., Zheng, W., 2005. Alteration of serum concentrations of manganese, iron, ferritin, and transferrin receptor following exposure to welding fumes among career welders. Neurotoxicology 26, 257–265.
Makhoul, I.R., Sammour, R.N., Diamond, E., Shohat, I., Tamir, A., Shamir, R., 2004. Selenium concentrations in maternal and umbilical cord blood at 24–42 weeks of gestation: basis for optimization of selenium supplementation to premature infants. Clin. Nutr. 23, 373–381.
Menezes-Filho, J.A., de Carvalho-Vivas, C.F., Viana, G.F., Ferreira, J.R., Nunes, L.S., Mergler, D., et al., 2014. Elevated manganese exposure and school-aged children's behavior: a gender-stratified analysis. Neurotoxicology 45, 293–300.
Michalke, B., Grill, P., Berthele, A., 2009. A method for low volume and low Se concentration samples and application to paired cerebrospinal fluid and serum samples. J. Trace Elem. Med. Biol. 23, 243–250.
Miklavcic, A., Casetta, A., Snoj Tratnik, J., Mazej, D., Krsnik, M., Mariuz, M., et al., 2013. Mercury, arsenic and selenium exposure levels in relation to fish consumption in the Mediterranean area. Environ. Res. 120, 7–17.
Milne, D.B., Sims, R.L., Ralston, N.V., 1990. Manganese content of the cellular components of blood. Clin. Chem. 36, 450–452.
Mora, A.M., van Wendel de Joode, B., Mergler, D., Cordoba, L., Cano, C., Quesada, R., et al., 2014. Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in costa rica. Environ. Sci. Technol. 48, 3467–3476.
Ode, A., Rylander, L., Lindh, C.H., Kallen, K., Jonsson, B.A., Gustafsson, P., et al., 2013. Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds. Environ. Sci. Pollut. Res. Int. 20, 7970–7978.
Ode, A., Kallen, K., Gustafsson, P., Rylander, L., Jonsson, B.A., Olofsson, P., et al., 2014. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS One 9, e95891.
Osman, K., Akesson, A., Berglund, M., Bremme, K., Schutz, A., Ask, K., et al., 2000. Toxic and essential elements in placentas of Swedish women. Clin. Biochem. 33, 131–138.
Oztas, B., Kilic, S., Dural, E., Ispir, T., 2001. Influence of antioxidants on the blood–brain barrier permeability during epileptic seizures. J. Neurosci. Res. 66, 674–678.
Parenti, M., Rusconi, L., Cappabianca, V., Parati, E.A., Groppetti, A., 1988. Role of dopamine in manganese neurotoxicity. Brain Res. 473, 236–240.
Ptacek, R., Kuzelova, H., Stefano, G.B., Raboch, J., Sadkova, T., Goetz, M., et al., 2014. Disruptive patterns of eating behaviors and associated lifestyles in males with ADHD. Med. Sci. Monit. 20, 608–613.
Qaiser, D.H., Sandila, M.P., Omair, A., Ghori, G.M., 2013. Correlation of routine haematological parameters between normal maternal blood and the cord blood of healthy newborns in selected hospitals of Karachi. J. Coll. Physicians Surg. Pak. 23, 128–131.
Riojas-Rodriguez, H., Solis-Vivanco, R., Schilmann, A., Montes, S., Rodriguez, S., Rios, C., et al., 2010. Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. Environ. Health Perspect. 118, 1465–1470.
Roels, H., Lauwerys, R., Buchet, J.P., Genet, P., Sarhan, M.J., Hanotiau, I., et al., 1987. Epidemiological survey among workers exposed to manganese: effects on lung, central nervous system, and some biological indices. Am. J. Ind. Med. 11, 307–327.
Rudge, C.V., Rollin, H.B., Nogueira, C.M., Thomassen, Y., Rudge, M.C., Odland, J.O., 2009. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. J. Environ. Monit. 11, 1322–1330.
Sakamoto, M., Murata, K., Kubota, M., Nakai, K., Satoh, H., 2010. Mercury and heavy metal profiles of maternal and umbilical cord RBCs in Japanese population. Ecotoxicol. Environ. Saf. 73, 1–6.
Santamaria, A.B., Sulsky, S.I., 2010. Risk assessment of an essential element: manganese. J. Toxicol. Environ. Health A 73, 128–155.
Santos, A.P., Lucas, R.L., Andrade, V., Mateus, M.L., Milatovic, D., Aschner, M., et al., 2012. Protective effects of ebselen (EBS) and para-aminosalicylic acid (PAS) against manganese (Mn)-induced neurotoxicity. Toxicol. Appl. Pharmacol. 258, 394–402.
Schulpis, K.H., Karakonstantakis, T., Gavrili, S., Chronopoulou, G., Karikas, G.A., Vlachos, G., et al., 2004. Maternal–neonatal serum selenium and copper levels in Greeks and Albanians. Eur. J. Clin. Nutr. 58, 1314–1318.
Smargiassi, A., Takser, L., Masse, A., Sergerie, M., Mergler, D., St-Amour, G., et al., 2002. A comparative study of manganese and lead levels in human umbilical cords and maternal blood from two urban centers exposed to different gasoline additives. Sci. Total Environ. 290, 157–164.
Spencer, A., 1999. Whole blood manganese levels in pregnancy and the neonate. Nutrition 15, 731–734.
Stauber, J.L., Florence, T.M., Webster, W.S., 1987. The use of scalp hair to monitor manganese in aborigines from groote eylandt. Neurotoxicology 8, 431–435.
Storch, A., Ludolph, A.C., Schwarz, J., 2004. Dopamine transporter: Involvement in selective dopaminergic neurotoxicity and degeneration. J. Neural Transm. 111, 1267–1286.
Sturaro, A., Parvoli, G., Doretti, L., Allegri, G., Costa, C., 1994. The influence of color, age, and sex on the content of zinc, copper, nickel, manganese, and lead in human hair. Biol. Trace Elem. Res. 40, 1–8.
Swanson, J.M., Kinsbourne, M., Nigg, J., Lanphear, B., Stefanatos, G.A., Volkow, N., et al., 2007. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. Neuropsychol. Rev. 17, 39–59.
Takser, L., Lafond, J., Bouchard, M., St-Amour, G., Mergler, D., 2004. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. Environ. Res. 95, 119–125.
Tholin, K., Sandström, B., Palm, R., Hallmans, G., 1995. Changes in blood manganese levels during pregnancy in iron supplemented and non-supplemented women. J. Trace Elem. Med. Biol. 9, 13–17.
Tran, T.T., Chowanadisai, W., Lonnerdal, B., Le, L., Parker, M., Chicz-Demet, A., et al., 2002. Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. Neurotoxicology 23, 645–651.

Verity, M.A., 1999. Manganese neurotoxicity: a mechanistic hypothesis. Neurotoxicology 20, 489–497.
Wasowicz, W., Wolkanin, P., Bednarski, M., Gromadzinska, J., Sklodowska, M., Grzybowska, K., 1993. Plasma trace element (Se, Zn, Cu) concentrations in maternal and umbilical cord blood in poland. Relation with birth weight, gestational age, and parity. Biol. Trace Elem. Res. 38, 205–215.
Wasserman, G.A., Liu, X., Parvez, F., Ahsan, H., Levy, D., Factor-Litvak, P., et al., 2006. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ. Health Perspect. 114, 124–129.
Wilson, D.C., Tubman, R., Bell, N., Halliday, H.L., McMaster, D., 1991. Plasma manganese, selenium and glutathione peroxidase levels in the mother and newborn infant. Early Hum. Dev. 26, 223–226.
Woolf, A., Wright, R., Amarasiriwardena, C., Bellinger, D., 2002. A child with chronic manganese exposure from drinking water. Environ. Health Perspect. 110, 613–616.
Yang, X., Bao, Y., Fu, H., Li, L., Ren, T., Yu, X., 2014. Selenium protects neonates against neurotoxicity from prenatal exposure to manganese. PLoS One 9, e86611.
Yokel, R.A., 2009. Manganese flux across the blood–brain barrier. Neuromol. Med. 11, 297–310.
Zaitseva, I.P., Skalny, A.A., Tinkov, A.A., Berezkina, E.S., Grabeklis, A.R., Skalny, A.V., 2014. The influence of physical activity on hair toxic and essential trace element content in male and female students. Biol. Trace Elem. Res..