TESI DOCTORAL UPF / 2014

Universitat Pompeu Fabra Barcelona

Effects of Endocrine Disrupting Chemicals on Childhood Growth and Obesity

Damaskini Valvi

Effects of Endocrine Disrupting Chemicals on Childhood Growth and Obesity

Evidence from the Spanish INMA Birth Cohort Study

Damaskini Valvi

UPF PhD Thesis 2014

Director: Dr. Martine Vrijheid

Centre for Research in Environmental Epidemiology (CREAL)

Co-Director: Dr. Michelle Ann Mendez

Gillings School of Public Health, UNC-Chapel Hill

To my Little Sister
and
all the Children of the World

(Younger and Older...)

Acknowledgments

This PhD project was financed by the Spanish Public Research Entity Institute of Health Carlos III (ISCIII, PFIS 2010, Register No: FI10/00399). The funding sources of the INMA birth cohort study are listed in the manuscripts that form the basis of this thesis. I would like to gratefully acknowledge sources of funding for making environmental epidemiologic research feasible. I am deeply grateful to all the participants of the INMA birth cohort study for their generous collaboration and for making research in this field possible.

This project started in 2010 in the Centre for Research in Environmental Epidemiology (CREAL) in Barcelona. Since then many people have contributed to the project design and execution in many different ways, some of them not always being fully aware of it. Although words and a couple of pages are not enough, I wish to express my deep gratitude to them in the next few lines.

I am deeply grateful to my supervisor Dr. Martine Vrijheid for all her help, support, encouragement and guidance during these years. If I had to start a PhD again, I would have wished it to be under her supervision. Working together has always been an unlimited source of motivation and inspiration, and I really feel I have learned so much by her side!

I am very much grateful to my co-supervisor Dr. Michelle Ann Mendez particularly for her guidance in the field of childhood obesity and for the unique opportunity she has given me to do as part of my PhD training a 4-month stay at the Gillings School of Public Health in the University of North Carolina (UNC)-Chapel Hill. The autumn days of Chapel Hill while working together helped me to grow fast not only as a professional but also as a person.

I would like to thank very much Rosa Ventura and Nuria Monfort from IMIM laboratory for our excellent and fruitful collaboration in the phthalate project and all my colleagues at the UNC in particular Beth Horton, Dave Pritchard and Amy Herring for helping me to extend my knowledge in the challenging field of advanced statistical methods.

I am very grateful to the Principal Investigators Maties Torrent, Ferran Ballester, Jesús Ibarluzea for their efforts to coordinate and reassure the continuity of the INMA birth cohorts that provided data for this project, and in particular to Jordi Sunyer further for always supporting and providing valuable input to my work.

I deeply thank all my dearest colleagues and friends from the INMA team (in its past and current forms), and in particular Maribel Casas, David Martinez, Raquel Garcia-Esteban, Keren Agayshay and Mireia Gascon for the many hours of brainstorming we have spent together during these years.

I would like to thank all my other friends and past and current colleagues at CREAL and in particular Alex Albert, Mar Alvarez, Laura Argenté, Xavier Basagaña, Alicia Borràs, Mariona Bustamante, Alejandro Caceres, Anne-Elie Carsin, Tom Cole, Payam Dadvand, David Donaire, Ivan Dueñas, Nadia Espejo, Paco Fernandez, Mar Ferrer, Maria Foraster, Judit Garcia, Stefano Guerra, Nadine Kubesch, Vanessa Marti, Monica Millan, Mark Nieuwenhuijsen, Lila Papadopoulou, Kyriaki Papantoniou, Marie Pedersen, Mariona Pinart, Joana Porcel, David Rojas, Dora Romaguera, Eleni Sazakli, Anna Schembari, Margarita Triguero, Michelle Turner, Marina Vafeiadi, Javier Vila, Diana van Gent, Nadia Vilahur and Manolis Kogevinas.

Special thanks to Juan Pablo Loyola for his patient, encouragement, and all the fruit juices, to Guillermo Rizzotto whose music has been such an endless source of inspiration during the past months while

writing the thesis, and to my dearest lifelong friends all over the world for being always interested to hear the latest news about environmental obesogens and the reason why I can always dream of a better world (in order of time appearance in my life): Δέσποινα Οικονομοπούλου, Ελένη Ροδιτάκη, Γιώργος Τσαπλές, Αλέξης Ασκητόπουλος, Σάββας Γερμάνης, Άρης Νασσίκας, Δημήτρης Νασσίκας, Γεωργία Ανδρουτσοπούλου, Βάσω Μπιμπή, Γιάννης Τσαλαφούτας, Μάρθα Καραμολέγκου, Miguel Angel Soto, Lucila Ciocoletto, Eino Nylund, Kristin Hjorth, Oscar Miranda, Ηρώ Λαγού, Φίλιππος Βαρδάκας, Victoria Correa-Luna, Rodrigo Muñoz Lillo, Carlos Zata, Thomas Gaeraert, Claudia Robles, Jürgen Martin, Ευάγγελος Ζηρδέλης, Max Heimansberg, Steve Hankey, Αριστοτέλης Κοτρωνούλας, James Crosthwaite, Eloiza Montaña, Guillermo *Gong*, Laura Sole, Laura McQuinn, Gemma Odena, Carmen Piernas and Mark Andrews.

Last but not least, I am deeply grateful for all the unconditional love and support to my family and especially to my parents and my grandmother Damaskini without whom I would have never learned as much.

Dania Valvi
Barcelona, June 3rd 2014

In the next page, "My second home during the past six years",
Photo of the CREAL team in 2011,

Made by

Dr. Payam Dadvand ©

Abstract

The Environmental Obesogen Hypothesis was recently suggested as a possible explanation contributing in the Global Obesity Epidemic that has been noted world widely over the past 30 years. Exposure to environmental pollutants with endocrine disrupting properties is hypothesised to alter the molecular pathways that underlie the hormonal and epigenetic regulation of adipose tissue development and energy homeostasis and thus, to increase individuals susceptibility towards obesity. The fetal and early postnatal life may be particularly sensitive periods in the effects of chemical exposures as it is when the development of tissues mostly occurs. Despite the growing body of evidence from experimental studies supporting this hypothesis, human evidence is still scarce and largely relies on cross-sectional data. The main scope of the present thesis was to prospectively evaluate the influences of prenatal exposures to persistent organic pollutants (POPs) and the non-persistent organic pollutants, BPA and phthalates, on childhood growth and obesity. The thesis was performed within the population-based INMA-"INfancia and Medio Ambiente" Birth Cohort Project in Spain. Prenatal low-level exposures to the POPs, DDE and HCB, were associated with child growth and increased obesity risk since the first year of life up to 7 years of age. Prenatal exposures to PCBs and DDT were less clearly associated with increased risk of obesity at 7 years of age. Effects on growth and obesity of at least some of these POP exposures may differ according to child sex. Prenatal exposure to BPA was weakly associated with increased body mass index and waist circumference at 4 years of age, but not with growth and obesity outcomes at earlier ages. Prenatal exposure to high molecular weight phthalates was negatively associated with early weight gain and obesity risk in boys up to 7 years of age, while a suggestion of positive associations was shown in girls. Prenatal exposure to low molecular weight phthalates did not influence childhood growth and obesity in either sexes. Although

further research is required to elucidate the role of environmental chemical exposures on the obesity epidemic, given the rising number of studies suggesting hazardous effects of early-life exposures on child growth as well as on other child health outcomes such as neurodevelopment, reproductive and respiratory health, the actual environmental regulations should be reconsidered and behaviour changes should be encouraged to reduce the levels of exposure in the general population.

Resumen

La Hipótesis de los "Obesógenos" Ambientales ha sido propuesta recientemente como una posible explicación a la Epidemia de Obesidad Global que se ha observado en los últimos 30 años a nivel mundial. Se cree que la exposición prenatal a contaminantes capaces de interrumpir el sistema endocrino podría alterar las vías moleculares de los procesos de regulación hormonal y epigenética que están involucrados en el desarrollo del tejido adiposo y la homeostasia energética, lo que incrementaría la susceptibilidad de los individuos a ser obesos. La vida fetal y los primeros meses de vida pueden ser periodos excepcionalmente vulnerables a los efectos de la exposición a contaminantes porque es cuando el desarrollo de los tejidos tiene lugar de forma más importante. Aunque la Hipótesis de los "Obesógenos" Ambientales sigue acumulando evidencia a través de estudios experimentales, la evidencia en humanos es escasa y se basa mayoritariamente en datos transversales. El objetivo principal de esta tesis fue evaluar los efectos de la exposición prenatal a compuestos orgánicos persistentes (COPs) y a los compuestos orgánicos no persistentes, BPA y ftalatos, en el crecimiento y la obesidad durante la infancia. La tesis se realizó en el marco del estudio poblacional de cohortes de nacimiento en España "Infancia y Medio Ambiente"-INMA. La exposición prenatal a los COPs, DDE and HCB, se asoció con el crecimiento del niño y el aumento del riesgo de obesidad desde el primer año de vida hasta los 7 años de edad. La exposición prenatal a los PCBs y el DDT se asoció de forma menos evidente, con un aumento del riesgo de la obesidad a los 7 años. Los efectos en el crecimiento y la obesidad de por lo menos algunos de estos COPs podrían estar modificados por el sexo del niño. La exposición prenatal a BPA se asoció débilmente con un incremento del índice de masa corporal y la circunferencia de cintura del niño a la edad de 4 años, pero no con el crecimiento y la obesidad del niño en edades más tempranas. La exposición prenatal a ftalatos de alto peso molecular se asoció negativamente con la ganancia de peso en la vida temprana y el riesgo de obesidad hasta la edad de 7 años en niños. Por el contrario, en niñas encontramos asociaciones más bien positivas. La exposición prenatal a ftalatos de bajo peso molecular no influenció el crecimiento o la obesidad en ninguno de los sexos.

Aunque se requiere de más investigación para aclarar el papel de la exposición a contaminantes ambientales en la epidemia de la obesidad, dado el creciente número de estudios que sugieren efectos adversos de estas exposiciones durante la vida temprana en el crecimiento como también en otros aspectos de la salud del niño tales como el neurodesarollo y la salud reproductiva y respiratoria, las regulaciones ambientales actuales se deberían reconsideran y promover cambios en el comportamiento para reducir los niveles de exposición en la población general.

Preface

Extensive multidisciplinary research efforts during the last 30 years have led successfully to the recognition of the impact that early-life environmental influences, starting *in utero* or even before at preconception, may have in establishing life-long patterns of human health and disease – The nowadays widely recognised concept of *Developmental Origins of Health and Disease (DOHaD)*. Early-life environmental influences, such as nutrition, chemical exposures and stress are suggested to interact with the genetic predisposition and later environmental influences of individuals determining the risks for the development (or the lack) of severe chronic diseases later in life, such as obesity, diabetes and cardiovascular disease. Living in the era of the Global Obesity Epidemic, recent, ongoing and future research conducted under the umbrella of the DOHaD concept is critical as it can provide important insights into new, more effective, strategies for obesity prevention.

The present PhD thesis gives insight into the role of prenatal exposures to environmental pollutants capable to disrupt the endocrine system on child growth and obesity from early infancy to childhood and provides prospective human evidence supporting the *Environmental Obesogen Hypothesis*. Considering the current state of evidence in this emerging field of research, the findings of this thesis contribute to: 1) understand the effects of low-level prenatal exposure to persistent organic pollutants (POPs), such as "DDT" on childhood growth and obesity, 2) elucidate the role of prenatal exposure to the non-persistent environmental pollutants, bisphenol A (BPA) and phthalates - currently widely used as plasticizers - on childhood growth and obesity, 3) identify groups of children that may be more susceptible to the obesogenic effects of chemical exposures and, 4) determine the variability and predictors of exposures to BPA and phthalates in pregnant women.

The thesis consists of a compilation of 6 articles (4 published and 2 under review) based on data from the population-based INMA-"INfancia and Medio Ambiente" Birth Cohort Project in Spain. The first 2 papers focused on evaluating the associations between prenatal exposure to POPs and childhood growth and obesity. The next 2 papers focused on evaluating predictors of BPA exposure in pregnant women and the association between prenatal exposure to BPA and childhood growth and obesity. The latest two papers focused on evaluating the reproducibility and predictors of phthalate exposure in pregnant women and further the association between prenatal phthalate exposure and childhood growth and obesity. A general introduction justifying the research scope and providing a general view of important aspects of environmental epidemiology linked to this project is enclosed. A general discussion of methodological aspects, the current state of evidence in this research field and the public health implications of findings, are provided. Author conclusions and thoughts for future research are further discussed. During the execution of the PhD thesis, the author coordinated the phthalate measurement project linked to the thesis and the field work on obesity outcomes at the latest follow-up of the INMA birth cohort study and participated actively in many national and international projects and conferences. A summary of the research activity of the author during the development of the thesis is provided in the Annex.

Contents

1

Introduction

1.1 Epidemiology of Obesity

Obesity is a complex chronic disorder characterised by abnormal or excessive fat accumulation that may lead to severe impairment of human health. According to the latest published data by the International Association for the Study of Obesity (IASO, 2012), around 475 million adults all over the world are estimated to be obese, almost 1 billion adults are overweight, while more than 200 million school-age children are overweight or obese making "this generation the first predicted to have a shorter lifespan than their parents". The rising trends in the prevalence of adult and child overweight and obesity during the last few decades are a serious public health problem recognised today as the "Global Obesity Epidemic". Over the past 30 years, the prevalence of obesity has more than doubled or tripled in children of all age groups and adolescents in developed countries including Spain, where almost 1 in every 3 girls and 1 in every 2 boys of elementary school age are currently estimated to be overweight or obese (IASO, 2012).

Overweight and obese children have increased risks of obesity later in adult life and the development of serious chronic diseases such as dyslipidemia, insulin resistance and diabetes, hypertension, ischemic heart disease, certain types of cancers and psychological disorders (Han et al. 2010). The increased morbidity and mortality due to the health consequences of obesity has important societal costs (Dee et al. 2014; Fry and Finley 2005; Hollingworth et al. 2012), and given the facts that obesity is a condition hard to reverse and that children who are obese will likely remain obese later in

adulthood (Freedman et al. 2009; Guo et al. 2002), the early prevention of the disease is critical.

The aetiology of obesity is complex and not yet fully understood. Multiple environmental, societal, psychological and behavioural factors may interact at different degrees with the genetic and biological predisposition of individuals leading to an excessive fat accumulation and the development of obesity. These factors include influences that may start even *in utero* such as maternal nutrition, gestational weight gain, sleep alterations, stress, factors related to the built environment and chemical exposures during pregnancy (Brisbois et al. 2012; Robinson et al. 2012; Symonds et al. 2013; Trasande et al. 2009; Weng et al. 2012). Intervention studies for the prevention and treatment of obesity have been mainly focused so far on improving the dietary habits and increasing the physical activity levels in the population demonstrating few short-term beneficial results and low effectiveness in maintaining behaviour or weight changes over long periods (Stephens et al. 2014). Thus, the identification of other modifiable risk factors that may influence the balance between energy storage and energy expenditure, such as could be environmental chemical exposures, and a more in-depth understanding of the interrelationships between the different obesity drivers is critical for developing more efficient strategies for the prevention and treatment of obesity in the future.

1.2 The Environmental Obesogen Hypothesis

A potential link between chemical exposures and the obesity epidemic was firstly suggested by Baillie-Hamilton (2002) about a decade ago. The hypothesis was based on a few earlier experimental studies showing an association between low-dose chemical exposures and weight gain and on the observation of an ecological study showing that the rising trends in the production of synthetic organic chemicals, noted after the beginning of the 20th century,

were followed a few decades later by rising trends in adult overweight in the United States (Baillie-Hamilton 2002). Ecological study designs, although useful for generating new hypotheses, have limited value in establishing causal relationships as they rely on group- and not individual-level data and they are subject to ecological fallacy, an important limitation that can be overcome in observational studies with individual data available. Thus, the evaluation of the associations between different chemical exposures and obesity using other study designs, such as prospective cohort studies, started at that time to be required.

During the last decade, a rapidly cumulating number of laboratory studies has identified more than 20 chemical classes that are known or suspected to increase the risk of weight gain especially when exposure occurs during the critical window of tissue development (Barouki et al. 2012). This continuously increasing list of environmental chemicals that potentially promote obesity in humans, defined as "obesogens" by Blumberg and colleagues (Grun and Blumberg 2006), includes among others, tobacco- and traffic-related pollutants, pharmaceutical agents such as diethylstilbestrol (DES), plasticizers, such as phthalates and bisphenol A (BPA), other industrial chemicals, such as organotins and flame retardants, and several pesticides (reviewed in Casals-Casas and Desvergne 2011; Holtcamp 2012; La Merrill and Birnbaum 2011; Neel and Sargis 2011; Newbold 2010; Thayer et al. 2012). These environmental "obesogens" are part of a larger class of environmental chemicals, known as "endocrine disrupting chemicals (EDCs)" or "endocrine disruptors" (Colborn and Clement 1992; Diamanti-Kandarakis et al. 2010) i.e. chemical substances introduced in the environment by human activity that can alter the mechanisms of endocrine systems in wildlife and humans by mimicking or blocking the action of natural hormones that regulate the homeostasis, reproduction, development and/or behaviour of an organism.

By the time the objectives of this research project were developed, studies evaluating the Environmental Obesogen Hypothesis in humans were scarce and mainly focused on the effects of tobacco smoking on weight homeostasis. Numerous studies conducted in different settings had suggested that maternal smoking during pregnancy may reduce fetal growth and thus program the child towards to an elevated risk for obesity development later in life (reviewed in Behl et al. 2013; Oken et al. 2008). The obesogenic effects of smoking provided at that time the "proof-of-principle" for the role of developmental exposures to environmental chemicals on the development of obesity later in life (Heindel and vom Saal 2009). Few prospective studies in relatively small populations and with wide differences in the exposure range had further evaluated the association between the persistent organic pollutants (POPs), dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs), and obesity, but these demonstrate contradictory findings (reviewed in Chapter 6, subsection 6.3.1). The associations between other chemicals with a well-known potential for promoting weight gain in animal models, such as BPA and phthalates, had not yet been explored in humans. Future prospective human studies where needed to fill the research gap.

1.3 Early Developmental Origins of Obesity

1.3.1 The DOHaD Paradigm

In mid 1980's, the observation of a geographic association between ischemic heart mortality risk with increased newborn mortality rates previously shown in different regions of England and Wales by Barker and Osmond (1986) set the basis for the "fetal origins of adult disease", a concept also known as the Barker´s hypothesis. Further studies in the next few years revealed an association between low birth weight and ischemic heart disease. Epidemiologic studies across many countries over the last 30 years

confirmed that early human development influences the risk of non-communicable diseases later in life and have importantly contributed in advancing and expanding the original concept into the "Developmental Origins of Health and Disease" (DOHaD) (Gluckman and Hanson 2006a; Barker 2007). The DOHaD paradigm is nowadays a widely-accepted and multi-disciplinary field of research. The goal is to elucidate whether and how environmental influences during critical periods of developmental plasticity[1], prenatally and also postnatally, such as nutrition and chemical exposures, may increase disease risk in later life by inducing permanent structural and physiologic changes in tissues and organs and by altering the adaptation mechanisms of the organism to cope with future environmental influences (Barouki et al. 2012). The non-communicable diseases suggested to be linked to developmental exposures include obesity, diabetes, cardiovascular, respiratory and immune diseases, hormone-dependent types of cancer and neurodevelopmental, neurodegenerative and reproductive disorders.

Critical periods of developmental plasticity are different for each tissue and may extend from preconception to early childhood and puberty and perhaps further beyond (Barouki et al. 2012). Fetal and early postnatal life is considered a particularly sensitive period in the effects of environmental exposures as it is when cell division and differentiation and tissue development mostly occur. Further, in the fetus and neonate protective mechanisms such as detoxifying and DNA repair mechanisms have not fully matured (Newbold 2010) and their exposure levels per unit of body weight or body surface can be higher compared to adults (Landrigan et al. 1998) which could further increase their susceptibility to the effects of

[1]Developmental plasticity is the ability of a single genotype to change its developmental processes and phenotypic outcomes in response to different surrounding environments. It is sugested that if the resulting phenotype matches to its environment, the organism remains healthy, while if there is a mismatch, the individual's ability to respond to environmental challenges may be inadequate and the risk of disease may increase (Gluckman and Hanson, 2006b).

chemical exposures. Several studies, with best known the studies of the Dutch Famine (Painter et al. 2005), highlight now the influence of *in utero* environmental exposures, mainly of maternal nutrition and tobacco smoke, to increased susceptibility for obesity and cardiometabolic diseases later in life (Low et al. 2011). The next subsection describes the mechanisms that have been suggested to underlie the influences of chemical exposures during critical developmental periods on the development of obesity and metabolic dysfunction later in life.

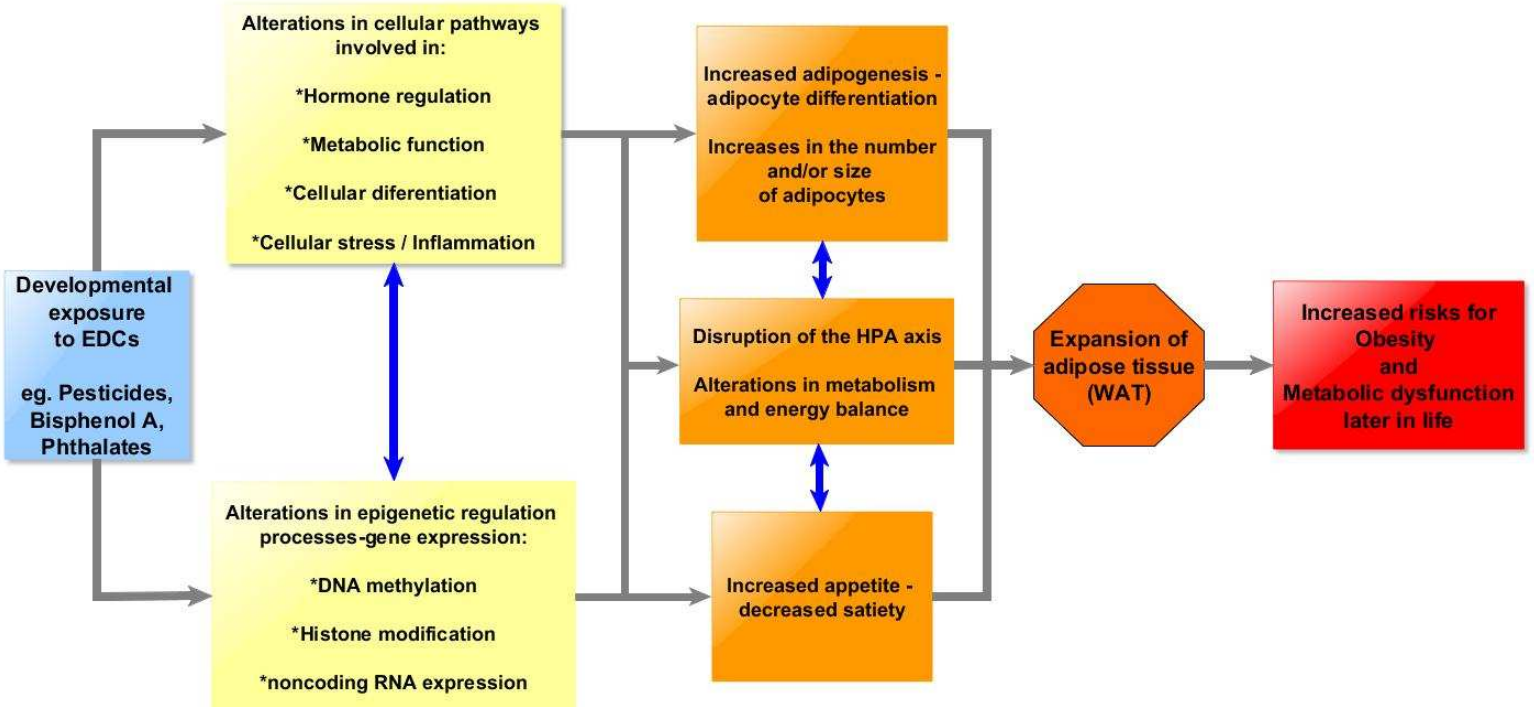
1.3.2 Developmental Effects of EDCs on Obesity Later in Life

Obesity is the result of increased adipogenesis or hyperplasia of adipose tissue that leads to a greater number of adipocytes and of a prolonged disturbance in the homeostatic regulation of energy metabolism that promotes lipid storage and adipocyte hypertrophy (i.e. greater size of adipocytes). Despite much progress in findings from *in vitro* and *in vivo* studies during the past 20 years, the developmental origins of adipocytes and the mechanisms underlying human adipogenesis and adipocyte differentiation are not yet well understood. However, adipogenesis in humans, similar to other species, is thought to start very early in pregnancy as mesechymal stem cell pericytes capable to differentiate into adipocytes, chondrocytes, osteoclasts and myocytes (Gregoire et al. 1998) are detected in human fetal blood, liver and bone marrow even in the first trimester of pregnancy (Campagnoli et al. 2001). Adipocyte numbers increase during early development and an adipose tissue expansion is shown to take place rapidly after birth as a result of increases in both the number and size of adipocytes (Gregoire et al. 1998). The increase in the number of adipocytes reaches a plateau later in early adulthood after which adipose tissue growth is thought to be mainly hypertrophic i.e. it is characterised by increases in the size rather than the number of adipocytes (Spalding et al. 2008). Thus, the number of adipocytes appears to be

largely determined at birth (Janesick and Blumberg 2011) suggesting that fetal exposures to obesogens may have a great impact in "programming" individuals towards obesity.

The mechanisms that are currently hypothesised to explain the obesogenic effects of early-life exposure to EDCs are summarised in **Figure 1.1**. A variety of tissues are involved in adipocyte differentiation and the homeostatic regulation of energy metabolism, including the adipose tissue, the hypothalamus, the liver, the pancreas and the gastrointestinal tract. EDCs may disrupt in target tissues the molecular pathways that underlie hormonal regulation, metabolism, cellular plasticity and cellular stress signals such as oxidative stress. This may lead to alterations in epigenetic regulation processes, including DNA methylation, histone modification and noncoding RNA expression and alter gene expression. In addition, epigenetic alterations induced by EDCs may exacerbate in turn the alterations in molecular pathways underlying hormonal regulation, metabolism, cellular plasticity and stress processes (Barouki et al. 2012).These mechanisms may involve the inappropriate targeting by EDCs of many nuclear receptors that play a key role in the development of adipose tissue and energy homeostasis. These include positive regulators of adipogenesis such as the peroxisome proliferator-activated receptors (PPARs), the retinoic X receptors, the steroid hormone receptors, the glycorticoid receptors and the liver X receptor and further negative regulators of adipogenesis such as the thyroid hormone and the vitamin D receptors (Janesick and Blumberg 2011 and 2012). Several laboratory studies have shown that EDCs may perturbate target nuclear receptor signalling pathways in mesechymal stem cells and preadipocytes and in this way stimulate adipogenesis, adipocyte differentiation and lipid storage in adipocytes leading to increases in the number and/or size of adipocytes (Grun and Blumberg 2007). Another potential mechanism may be the disruption by EDCs of appetite regulation and energy balance that is controlled and set early in life in the

Figure 1.1 Possible mechanisms underlying the influence of developmental exposure to EDCs on obesity and metabolic dysfunction in childhood and adulthood



hypothalamus and influenced by several hormones including sex steroid hormones and adipokines (i.e. hormones secreted by the adipose tissue) such as leptin and ghrelin (Berthoud 2012; Zheng et al. 2009; Ross and Desai 2013). For example, EDCs are shown to alter the expression of sex steroid receptors and aromatase that play a major role in appetite regulation and energy balance as well as in adipogenesis and alter the levels of sex steroid and metabolic hormones in peripheral blood. Estrogen and androgen receptors targeted by EDCs may alter the programming of energy balance in the developing brain since very early in life. Further, EDCs may bind to sensitive sensors in the digestive track, adipose tissue and developing brain disrupting some of the numerous monoaminoergic, peptidergic and endocannabinoid signals that regulate the function of the hypothalamic-pituitary-adrenocortical (HPA) axis leading to altered expression of key neurotransmitters that stimulate appetite such as is neuropeptide Y (Grun and Blumberg 2009; Meaney et al. 2007).

The above mentioned and other not yet elucidated mechanisms may result to an abnormal expansion of adipose tissue and importantly of subcutaneous and visceral white adipose tissue (WAT) and thus, increase the risks for obesity and metabolic dysfunction later in life. These effects may be enhanced on the background of environmental influences that occur later in life such as the continuous exposure to EDCs through the life course, the high fat and high sugar diets and the individual´s physical activity patterns later in life. These effects may further be sex specific as some of the mechanisms underlying adipogenesis, adipocyte differentiation and energy regulation, such as those involving steroid hormones, may differ in the two sexes (Bluin et al. 2009; Dieudonne et al. 2000; Walker et al. 2014). Further, effects on adipogenesis and energy regulation can be exhibited even at low doses of EDC exposure and may be different than those shown at higher levels of exposure as they disrupt pathways related to natural hormones and an important number of *in vivo* and *in vitro* studies now provides evidence that these effects

can be dose-dependent (Vandenberg et al. 2012; Vandenberg 2014). Finally, effects could also be partially inherited as epigenetic alterations induced by EDCs, such as DDT, BPA and certain phthalates have been recently suggested to be transmitted across generations (Janesick and Blumberg 2011; Manikkam et al. 2013; Skinner et al. 2013; Zhang et al. 2014), however data supporting this latest hypothesis are still scarce.

1.4 Endocrine Disrupting Chemicals Potentially Obesogenic

This section describes the features of the EDCs that have been studied within the scope of this project. These include the POPs: dichlorodiphenyltrichloroethylene (DDT) and its prime metabolite DDE, hexachlorobenzene (HCB) and PCBs, and the non-persistent organic pollutants: BPA and phthalates. Information on other environmental obesogens that are beyond the scope of the current thesis can be found elsewhere (eg Holtcamp 2012; La Merrill and Birnbaum 2011; Thayer et al. 2012).

1.4.1 Persistent Organic Pollutants

POPs are a wide class of carbon-based synthetic substances that have been widely used in the past mainly as pesticides (e.g. DDT and HCB) and industrial chemicals (e.g. PCBs). These pollutants are characterized by their capacity to persist in the environment for decades, to spread over long-range distances through air, water and soil and to bioaccumulate in human and animal fat tissues for several years due to their lipophilic properties. Because of the known neurotoxic and adverse reproductive effects induced by high-level exposure to these chemicals, their use started to be restricted or banned in developed countries since late ´70s. In Europe the use of DDT, HCB and most of PCBs was banned by the Stockholm Convention (2004). However, DDT is still used today in

developing countries for the vector control of malaria (van den Berg 2009). Due to their persistence in the environment, the general population nowadays is still exposed to lower levels of these pollutants mainly through diet and especially through the consumption of fatty fish and meat. Exposure in early life is also possible from the mother to the fetus through the bloodstream via placenta and later to the newborn via breast milk.

DDT has an estrogenic activity and bind to the estrogen receptor, while its prime metabolite, DDE, exhibits both estrogenic and anti-androgenic properties and binds to the androgen receptor (Li et al. 2008; Sonnenschein and Soto 1998). Despite their known endocrine disrupting properties and the available human evidence on the potentially obesogenic effects of DDT and DDE (reviewed in Chapter 6, subsection 6.3.1), data from *in vitro* and *in vivo* studies evaluating these pollutants as obesity promoters are so far scarce. However, DDT has been suggested to induce dose-dependent adipocyte differentiation through increasing the expression of PPARγ receptor (Moreno-Aliaga and Matsumura 2002). Howell and Mangum (2011) have further recently shown that DDE may increase the gene transcription of adiponectin and resistin and induce fatty acid accumulation in mature NIH2T3-L1 adipocytes leading to adipocyte hypertrophy. Further research is needed to elucidate the mechanisms that underlie these effects.

HCB effects on fetal growth (Basterrechea et al. 2014; Eggesbø et al. 2009; Lopez-Espinosa et al. 2011; Vafeiadi et al. 2014) and postnatal growth (Cupul-Uicab et al. 2013; Smink et al. 2008; Mendez et al. 2011) have been recently evaluated in few birth cohort studies with findings being inconsistent among studies. Since HCB is shown to be an antagonist of the androgen and the estrogen-related receptors (Li et al. 2008) effects on growth and obesity are suspected. However, the effects of HCB on weight homeostasis in experimental studies are unexplored and given the existing evidence

from human studies, although it is limited, further research is needed to clarify its potentially obesogenic effects.

PCBs are a chemical group of more than 200 congeners with different degrees of chlorination commonly used in several industrial or consumer applications as mixtures (e.g. Aroclors). The non-dioxin-like PCB congeners 138, 153, 170 and 180 are presented in detectable levels in human biological tissues and highly correlated to other PCB congeners and thus they are commonly determined in human biomonitoring studies (Crinnion 2010). Other PCB congeners measured, such as PCB 118, are shown to act through the aryl hydrocarbon receptor to exhibit the full range of toxic responses elicited by 2,3,7,8-tetrachlorodibenzo-p-dioxin, thus are referred to as the dioxin-like PCBs. PCBs may exhibit estrogenic, androgenic and/or anti-androgenic effects depending on the congener (Bonefeld-Jorgensen et al. 2001) and may alter thyroid hormone secretion and metabolism (Boas et al. 2006). Individual PCB congeners (Arsenescu et al. 2008; Hennig et al. 2005; Taxvig et al. 2012) and PCB mixtures (Branchi et al. 2002) have been shown to promote adipogenesis in *in vitro* and *in vivo* studies.

1.4.2 Non-Persistent Organic Pollutants

BPA and phthalates are high production volume carbon-based synthetic substances that have been used as plasticisers for more than 50 years. In contrast to POPs, these pollutants present a quick rate of biodegradation in the environment, they do not accumulate in wildlife and humans and they are quickly metabolised and secreted within few hours or days after exposure. However, human biomonitoring data show detectable levels of exposure to these chemicals in almost all (>95%) of the individuals tested (Vandenberg et al. 2007; Wittassek et al. 2011) suggesting that exposure in the general population is continuous.

BPA is used in the manufacture of plastic polymers such as polycarbonate plastics and epoxy resins, which are found in many consumer products (eg plastic toys, food and beverage containers, water supply pipes, medical tubing, cigarette filters). The main route for human exposure is considered to be dietary ingestion and especially the consumptions of packaged food and beverages, but there are currently limited data and routes of exposure need to be further explored (Vandenberg et al. 2013). In 2011, the European commission prohibited the use of BPA in the manufacture of polycarbonate infant feeding bottles, due to the potentially adverse health effects of low-level developmental exposure to BPA supported by many experimental and few epidemiologic data. However, its use in other consumer products is still allowed in all EU countries, with the exception of France where the use of BPA in food containers intended for infants was banned in 2013 and it is scheduled to be prohibited in all food containers in 2015.

BPA is one of best studied environmental obesogens in *in vitro* and *in vivo* studies. The estrogenic properties of BPA have been known since 1930 (vom Saal et al. 2012). More recently, BPA has been suggested to act as a PPARγ activator (Pereira-Fernandes et al. 2013). Evidence from studies in rodents suggests that developmental exposure to BPA at environmentally relevant doses may alter adipogenesis, triglyceride accumulation and to increase fat mass later in life. BPA effects on weight gain are suggested to be sex-specific and dose-dependent with stronger effects shown at lower exposure levels (Hugo et al. 2008; Rubin and Soto 2009; Somm et al. 2009; Wei et al. 2011).

Phthalates are used in the manufacture of many industrial materials and consumer products (Wittassek et al. 2011). High molecular weight phthalates such as di-2-ethylhexyl (DEHP), benzylbutyl (BBzP) and di-isononyl (DINP) phthalates are widely used in polyvinyl chloride (PVC) applications and found in building materials, cables and wires, clothing and food containers among

other consumer products. Low molecular weight phthalates such as di-ethyl (DEP) and di-n-butyl (DnBP) phthalates are commonly used in non-PVC products including adhesives, personal-care and household cleaning products and enteric-coated tablets and capsules (Meeker et al. 2009; Wittassek et al. 2011; Wormuth et al. 2006). Dietary ingestion is considered to be the major route of exposure to the high molecular weight phthalates, while personal-care product use and indoor air may be important sources of exposure to the low molecular weight phthalates in the general population (Adibi et al. 2008; Koch et al. 2013; Wittassek et al. 2011). Due to their potentially toxic effects on reproduction, the use of certain phthalates, such as DEHP and BBzP in children toy´s and childcare articles has been banned by the European commission since 2008. However, these and many other phthalates are currently used in several consumer products.

Parent phthalate and phthalate metabolites have been shown to exhibit anti-androgenic and both estrogenic and anti-estrogenic effects depending on the phthalate and metabolite tested (Miodovnik et al. 2014). Perinatal exposure to some phthalates and phthalate metabolites at relatively low levels has been shown to alter the expression and activate PPARs receptors in the adipose tissue promoting in this way adipogenesis and lipid storage in mice (Hao et al. 2012; Feige et al. 2010). Developmental exposure to phthalates has been further shown to influence the transcription of genes related to steroidogenesis and metabolism (Boberg et al. 2008). Phthalate effects on adipogenesis and energy homeostasis are suggested to be sex-specific and dose-dependent (Boberg et al. 2008; Hao et al. 2012; Feige et al. 2010).

1.5 Meusures of Obesity in Early Life

A variety of methods are available for measuring general and regional obesity with anthropometry being the most commonly used

for research and clinical purposes. Body mass index (BMI) i.e. weight divided by squared height in kg/m^2 is the most widely used measure in epidemiologic studies for the classification of overweight and obesity. Its validity in children as an indirect measure of adiposity is based on the fact that it associates with both body fat mass and obesity risk factors. Its practicality is based on the fact that is a non-invasive, cheap and quick to perform measurement. Because child weight and height varies according to age and sex, BMI age-and-sex specific z-scores should be calculated using internal standardization or external reference populations (national or international) with being the most commonly used those suggested by the World Health Organization (CDC 2000; de Onis et al. 2007 and 2009) and the International Obesity Task Force (Cole et al. 2000; Cole and Lobstein 2012). Elevated BMI as early as in preschool ages from 2 to 5 years and onwards has been consistently associated with adult obesity, central obesity, and early onset metabolic syndrome (Graversen et al. 2014; Mei et al. 2002). The validity of BMI in the first 2 years of life as a predictor of obesity risk later in life however, is subject to ongoing discussion as BMI relies on measurements of standing height and thus weight-for-length is recommended to be used instead of BMI for this age group (CDC 2000). Further, early growth has recently drawn much attention as a promising screening tool for later risks of obesity and chronic diseases. Birth weight and rapid weight gain in the first months after birth have been consistently associated with obesity risk later in childhood and adulthood (Druet et al. 2012; Kramer et al. 2014; Monteiro and Victora 2005). Despite their utility for tracking later risks of obesity and obesity-related diseases, the above mentioned and other similar methods that are based on weight and/or height measurements are just indirect measures of total body fatness and provide no information about body fat distribution. Central obesity (i.e. excess in abdominal fat) is more strongly associated with cardiometabolic risk than generalised obesity (Bastien et al. 2014). Waist circumference is a widely used anthropometric measurement of central obesity and a waist-to-

height ratio>0.50 was recently advocated as a predictor of obesity, diabetes and cardiovascular disease later in adulthood in children as it is in adults (Browning et al. 2010).

The measurement of regional body fatness is traditionally performed through measurements of skinfold thickness, which are shown to correlate well with body fatness (Mei et al. 2002). Bioelectrical impedance is a commonly used and relatively cheap method that estimates the body percentages of lean and fat mass based on total body water measurements, although is not the most reliable one for children (Silva et al. 2013). Other available methods for a more accurate direct estimation of generalised and regional body fatness compared to anthropometric-based measures include isotope dilution and air displacement plethysmography, underwater weighing, dual-energy X-ray absorptiometry (DXA), total body water, total-body electrical conductivity, and computed tomography. However, the use of these methods in epidemiologic settings is more limited because of their complexity and cost (Silva et al. 2013).

1.6 Pitfalls and Challenges of Environmental Epidemiology – The Example of EDCs and Obesity

1.6.1 Study Design

Epidemiologic studies aim to estimate population parameters and most frequently have two major quantitative objectives which are to estimate: 1) the frequency of disease occurrence in the population and 2) the effect of a given exposure on disease occurrence in the population (Rothman et al. 2008). The best suited design to answer the research hypothesis is largely determined by the scope of the study. In environmental epidemiologic studies aiming to estimate health hazards, not benefits, experimental study designs are usually

unsuitable due to ethical considerations and limited in preventive interventions. Thus, if the aim is to study a highly prevalent outcome in the population, such as obesity, prospective cohort study designs are the best suited for this purpose. Prospective cohort studies although costly, can reassure that disease occurrence was posterior to population recruitment and after exposure status was measured and thus, may infer causal relationships, a great advantage over cross-sectional studies that although useful for effect estimations, are susceptible to reverse causation (i.e. disease occurrence preceded the exposure). However, similar to all study designs, prospective cohort studies have also weaknesses, and importantly they are prone to selection attrition due to follow-up losses of participants that may lead to biased effect estimates.

1.6.2 Population Size

The population size required to achieve the statistical power to detect a significant association depends on the magnitude of the effect and the frequency of the outcome of interest. For many environmental pollutants, including the EDCs evaluated in this project, the excesses in individuals´ risk of disease are usually small (eg for POPs and elevated BMI relative risks are in the range 1.10-1.30). Thus, in environmental studies a sufficient sample size may require a particularly increased number of individuals (many hundreds or thousands) to be studied in order to detect any excess in disease risk. This can be exceptionally difficult for environmental cohort studies where participants are followed over long periods.

1.6.3 Exposure Assessment

Exposure assessment is probably the greatest challenge in environmental epidemiology especially when the aim is to establish exposure-outcome relationships. Exposure misclassification is of particular concern in environmental epidemiology because of challenges in measuring the exposure to environmental

contaminants which can occur across multiple sources and routes of exposure and often at low levels. Given the usually small excesses in disease risk linked to environmental exposures, exposure assessment needs to be as optimal as possible so that a true health effect can be detected (Baker and Nieuwenhuijsen 2008). If misclassification of exposure is non-differential in terms of the health outcome, this biases effect estimates usually towards the *null* and always reduces the study power (Amstrong 1998) reducing in this way the chance of detecting true associations (false negative results, type II error).

For persistent chemicals that bioaccumulate in the human body, such as POPs, the measurement of levels of pollutants in tissues and fluids is considered to be an optimal tool for exposure assessment. Maternal serum determination of POPs is an indicator of cumulative internal exposure and concentrations are shown to correlate with POP concentrations determined in cord blood and placenta that are considered to be more relevant matrices for fetal exposure (Vizcaino et al. 2014). However, exposure biomonitoring of non-persistent chemicals that have a very short half-life in biological fluids, such as BPA and phthalates, is more challenging. BPA and phthalate assessment is commonly based on urine determinations that may only reflect recent exposure (in the last few hours). Repeated urine measurements may increase the accuracy of exposure assessment and reduce the risk of exposure misclassification. The integration of biomarker determinations with questionnaire data on lifestyle, food consumption and consumer product use and environmental monitoring estimations may further improve the accuracy of the assessment of exposure to these chemicals; however, these methods need to be better developed (Vandenberg et al. 2013; Wittassek et al. 2011). Human studies evaluating the variability and predictors of exposure may provide important input to improve the exposure assessment of these emerging pollutants in the future.

One more challenge for environmental studies that aim to assess in the population health risks of low-level widespread exposures to pollutants, such as BPA and phthalates, is the low between-subject variability. All individuals may be exposed to these pollutants and the exposure range may be narrow (there are no unexposed or "highly" exposed comparison groups) thus, underestimation of effect estimates becomes more likely.

1.6.4 Confounding

Environmental studies usually aim to estimate health effects that may be linked to multiple environmental exposures that are correlated (because they share common sources or predictors) and thus, are more prone to confounding bias. Even if a true effect exists and information on correlated exposures is available, eg several PCB congeners are measured, effect estimates cannot be determined precisely enough, may be biased (Pollack et al. 2013) and disentangling the effects between pollutants can be difficult or impossible (depending on the degree of correlation between pollutants). Other sources of error and bias that may influence effect estimates are common with those in every epidemiologic study (eg unmeasured and residual confounding) and thus, not described here.

1.6.5 Interpretation of Findings

Interpretation of findings largely depends on the consistency of the observed associations with those shown in previous laboratory or epidemiologic studies. The comparison of results for the associations between developmental exposure to EDCs and obesity among different studies may present particular difficulties due to the different windows of exposure susceptibility assessed (eg prenatal versus postnatal exposures), the different ages at outcome assessment as changes in the phenotype induced by early-life exposure to at least some EDCs may be apparent much later in life (Barouki et al. 2012) and the differences in the range of exposure

assessed as EDC health effects may be dose-dependent (Vandenberg et al. 2012). Further, extrapolation of findings from laboratory studies should be done with caution as some of the mechanisms underlying the obesogenic effects of EDCs in other species, such as rodents, have been shown *in vitro* not to apply in humans (Bastos Sales et al. 2013; Hao et al. 2012).

2

Rationale

Rapidly accumulating experimental evidence during the last decade has identified more than 20 chemical classes with endocrine disrupting properties, including POPs, BPA and phthalates, suggested to increase obesity risk, especially when exposure occurs during critical windows of development, such as fetal life. Developmental exposure to EDCs could alter adipogenesis, adipocyte differentiation and energy storage homeostasis very early in life by altering the molecular pathways that underlie hormonal regulation, metabolism and cellular plasticity and stress and/or by altering gene expression through epigenetic modifications, programming, in this way, individuals towards obesity. These effects are suggested to be sex-specific, dose-dependent and potentially enhanced on the background of environmental influences that occur later in life such as a high-fat, high-caloric diet. Despite the growing evidence from laboratory studies supporting the emerging Environmental Obesogen Hypothesis, human evidence on these effects is currently limited and largely based on cross-sectional data. Few prospective studies in relatively small populations and with wide differences in exposure ranges have evaluated so far the associations between prenatal exposure to POPs and obesity demonstrating contradictory findings. The associations between prenatal exposures to the currently high volume produced chemicals, BPA and phthalates, and obesity have scarcely been explored in prospective studies. Prospective studies evaluating the potentially obesogenic effects of these environmental pollutants in humans are thus much needed. Birth cohort studies are able to infer causal relationships as they are characterised by a clear temporal differentiation between the exposure and the disease occurrence and may importantly contribute in elucidating the role of early-life chemical exposures on the development of obesity and

further in identifying the most susceptible groups to these effects in the general population.

3

Objectives

The main scope of this PhD thesis was to examine the role of prenatal exposures to POPs, BPA and phthalates on early-life growth and childhood obesity. To address this main aim, the following specific objectives were developed:

POPs

- To evaluate the associations between prenatal exposure to POPs and infant early weight gain and subsequent obesity risk from infancy to childhood (ages 1 to 7 years). [Papers I and II]

BPA

- To evaluate sociodemographic, lifestyle and dietary predictors of BPA exposure in pregnant women. [Paper III]

- To evaluate the associations between prenatal exposure to BPA and infant early weight gain and subsequent obesity risk from infancy to early childhood (ages 1 to 4 years). [Paper IV]

Phthalates

- To evaluate the reproducibility of urine phthalate metabolite concentrations and sociodemographic, lifestyle and dietary predictors of exposure in pregnant women. [Paper V]

- To evaluate the associations between prenatal exposure to phthalates and infant early weight gain and subsequent obesity risk from infancy to childhood (ages 1 to 7 years). [Paper VI]

4

Methods

In this Chapter is provided a general overview of the methods applied with the aim to evaluate the research hypotheses linked with the present thesis. A summary of the study populations, the prenatal environmental chemical exposures assessed and the childhood growth and obesity outcomes studied in this thesis is available in **Table 4.1**. A more specific and detailed description of the methods followed is enclosed in the papers that are presented in the section of results (Chapter 5).

4.1 Description of the Study Birth Cohorts

The INMA-"INfancia y Medio Ambiente" (Environment and Childhood) project is a network of seven prospective population-based birth cohort studies in different Spanish regions that have followed a total of more than 3000 mother-child pairs from early pregnancy throughout childhood and adolescence (Guxens et al. 2012). The general scope of the project is to study the influences of early-life environmental exposures on child development and health. The wide range of exposures that are evaluated includes environmental pollutants in air, water and diet, behavioural (eg diet and physical activity), psychosocial, built environment and genetic factors. Health outcomes under study include prenatal and birth health events, fetal and postnatal growth, obesity, neurodevelopment, behavioural functioning, immunity and respiratory health.

The INMA birth cohorts encompass three older cohorts conducted in the geographical regions of Ribera d´Ebre, Menorca and Granada (recruitment period between 1997 and 2002) and 4 more recent

cohorts in the geographical regions of Asturias, Gipuzkoa, Sabadell and Valencia (recruitment period between 2003 and 2008) (**Figure 4.1**). The inclusion criteria for the mothers were age equal or above the 16 years, intention to give birth in the reference hospital, no communication problems, singleton pregnancy and not to have followed any program of assisted reproduction. For the purposes of this thesis we used data from the INMA-Menorca cohort and the INMA-new cohorts in the regions of Gipuzkoa, Sabadell and Valencia. The specific characteristics of the analysed cohorts are described in the next subsections.

Figure 4.1 Geographical locations of the seven INMA Birth Cohort Studies in Spain

Table 4.1 Summary of the environmental chemical exposures assessed in pregnancy and the obesity-related outcomes assessed at different child ages[1]

	Paper I	Paper II	Paper IV	Paper VI
Analysis population	INMA-Menorca (N=344)	INMA-new Gipuzkoa, Sabadell, Valencia (N=1285)	INMA-Sabadell (N=402)	INMA-Sabadell (N=391)
Prenatal exposure assessed (biological matrix)				
POPs [2]	✓ (cord serum)	✓ (maternal serum)		
BPA [3]			✓ (urine)	
Phthalates [4]				✓ (urine)
Child ages at outcome assessment				
0-6 months		✓	✓	✓
1 year		✓	✓	✓
4 years			✓	✓
7 years	✓			✓
Outcome assessed (age) [5]				
Weight gain Z-score / Rapid growth		✓ (0-6 months)	✓ (0-6 months)	✓ (0-6 months)
BMI Z-score / Overweight	✓ (7 years)	✓ (1 year)	✓ (1 and 4 years)	✓ (1-7 years)
Waist circumference Z-score / Waist-to-height ratio			✓ (1 and 4 years)	✓ (4-7 years)
Blood pressure Z-score				✓ (4-7 years)

[1]Papers III and V examined determinants of BPA and phthalate exposures, respectively, in the INMA-Sabadell birth cohort study. Because child growth and obesity outcomes were not analysed, these papers are not summarized in this table.
[2]POPs assessed include the congeners 28, 52, 101, 118, 138, 153 and 180 in the INMA-Menorca cohort and the congeners 138, 153 and 180 in the INMA-new cohorts.
[3]BPA measurements of total (free plus conjugated) concentrations were performed.
[4]Phthalate metabolites assessed include 5 high molecular weight phthalates (i.e. DEHP metabolites and MBzP) and 3 low molecular weight phthalates (MEP, MiBP and MnBP).
[5]All outcomes were standardized by child age and sex using international references (WHO references) or the population mean (i.e. for waist circumference and blood pressure). Blood pressure measurements were standardized also for child height.

4.1.1 The INMA-Menorca Birth Cohort Study

The INMA-Menorca birth cohort recruited 482 women seeking for antenatal care between April 1997 and June 1998 (participation rate: 98% of the eligible pregnant women). Women were interviewed around 20 weeks of gestation to collect information on environmental exposures and sociodemographic, lifestyle and behavioural factors. Mother-child pairs have been afterwards followed at birth and child ages of 6 months, 1, 2, 3, 4, 7, 10 and 14 years. Information on child feeding practices, diet and physical activity has been collected through questionnaires administered in postnatal follow-ups. Information on exact date of birth and repeated measurements of weight and height from birth to early childhood have been extracted from the medical records. Child weight and height at 4 years and later ages were measured by special trained personnel of the research team using standard protocols. Concentrations of POPs were determined in umbilical cord serum samples collected at birth and at child serum collected at 4 years of age.

4.1.2 The INMA-New Birth Cohort Studies

In the INMA-new birth cohorts mother-child pairs were recruited in the first prenatal visit (at 10-13 weeks of gestation) in the main public hospital or health centre of each study region. The recruitment periods were extended from May 2004 to July 2007 in Asturias (N=485, participation rate: 45% of the eligible pregnant women), from April 2006 to January 2008 in Gipuzkoa (N=638, participation rate: 68%), from July 2004 to July 2006 in Sabadell (N=657, participation rate: 60%) and from November 2003 to June 2005 in Valencia (N=855, participation rate: 54%). Mother-child pairs have been afterwards followed in the third trimester of pregnancy, at birth and at child ages of 6 months and 1, 4 and 7 years using the same study protocol in all cohorts. Interviewed-based questionnaires collected information about parental and child characteristics including information on environmental exposures

and sociodemographic, lifestyle and behavioural factors. Repeated weight and height measurements of the child in the first year of life were extracted from the medical records. Child weight, height, waist circumference and blood pressure were measured by special trained personnel of the research team at postnatal follow-ups. POP and lipid concentrations were measured in maternal serum samples collected between the 7^{th} and 26^{th} weeks of gestation. We did not include in our study the INMA-Asturias birth cohort as POP measurements in maternal serum were not yet available at the time of analyses. In the INMA-Sabadell birth cohort study, concentrations of BPA, phthalate metabolites and creatinine levels were measured in two maternal urine samples collected in the first trimester (around week 12 of gestation) and in the third trimester (around week 32 of gestation) of pregnancy. BPA and creatinine concentrations in this cohort study were further measured in serum samples collected from a small subgroup of children (N=130) at 4 years of age.

5

Results

POPs

5.1 Paper I - Prenatal Concentrations of Polychlorinated Biphenyls, DDE, and DDT and Overweight in Children: A Prospective Birth Cohort Study

5.2 Paper II - Prenatal Exposure to Persistent Organic Pollutants and Rapid Weight Gain and Overweight in Infancy

BPA

5.3 Paper III - Dietary and Sociodemographic Determinants of Bisphenol A Urine Concentrations in Pregnant Women and Children

5.4 Paper IV - Prenatal Bisphenol A Urine Concentrations and Early Rapid Growth and Overweight Risk in the Offspring

Phthalates

5.5 Paper V - Variability and Predictors of Urinary Phthalate Metabolites in Spanish Pregnant Women

5.6 Paper VI - Prospective Evidence on the Effects of Phthalate Exposure on Childhood Growth and Blood Pressure

5.1 Prenatal Concentrations of Polychlorinated Biphenyls, DDE, and DDT and Overweight in Children: A Prospective Birth Cohort Study

Authors: Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M

Published in: Environ Health Perspect 2012; 120(3):451-457

Full text: http://ehp.niehs.nih.gov/1103862/

Summary: In this study we examined whether cord-blood concentrations of PCBs, DDE and DDT are associated with child overweight at 7 years of age in 344 children from the INMA-Menorca birth cohort study. Because child sex and a high-fat diet have been suggested to modify these effects, we further explored the influence of these factors on the associations of interest. Findings suggest that prenatal PCB exposure may increase overweight risk in girls but not in boys, prenatal DDE exposure may increase overweight risk with somewhat higher risks shown in girls compared to boys, while prenatal DDT exposure may increase overweight risk only in boys and potentially only in children with fat intakes at or above the population median compared to children with fat intakes below the median. This is the first study that has aimed to evaluate the influence of child's high fat diet on the associations between prenatal POP exposures and obesity risk.

Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M. Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: a prospective birth cohort study. Environmental Health Perspectives. 2012; 120(3): 451-457. DOI 10.1289/ehp.1103862

5.2 Prenatal Exposure to Persistent Organic Pollutants and Rapid Weight Gain and Overweight in Infancy

Authors: Valvi D, Mendez MA, García-Esteban R, Ballestrer F, Ibarluzea J, Goñi F, Grimalt JO, Llop S, Santa Marina L, Vizcaino E, Sunyer J, Vrijheid M

Published in: Obesity 2014; 22:488-496

Full text: http://onlinelibrary.wiley.com/doi/10.1002/oby.20603/full

Summary: In this study we examined whether maternal serum DDE, HCB and PCB concentrations are associated with rapid weight gain in the first 6 months of life and subsequent infant overweight risk at 1 year of age. We analysed 1285 children from the INMA-new birth cohort studies conducted in the regions of Gipuzkoa, Sabadell and Valencia where levels of POP exposures are shown to be lower compared to the INMA-Menorca birth cohort study (Paper I). Findings suggest that prenatal exposures to DDE and HCB may increase rapid growth and subsequent overweight risk in early infancy, while no association was shown for prenatal exposure to PCBs. Some evidence was further shown that infant sex, exclusive breastfeeding duration may influence the associations of DDE and that maternal pre-pregnancy overweight may influence the associations of HCB and infant growth outcomes. This is the largest study so far that has evaluated the associations between relatively low-levels of prenatal POP exposure and childhood growth and obesity outcomes.

Valvi D, Mendez MA, Garcia-Esteban R, Ballester F, Ibarluzea J, Goñi F, Grimalt JO, Llop S, Marina LS, Vizcaino E, Sunyer J, Vrijheid M. Prenatal exposure to persistent organic pollutants and rapid weight gain and overweight in infancy. Obesity (Silver Spring). 2014 Feb;22(2):488-96. doi: 10.1002/oby.20603

5.3 Dietary and Sociodemographic Determinants of Bisphenol A Urine Concentrations in Pregnant Women and Children

Authors: Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin A, Fernandez M, Koch HM, Mendez MA, Sunyer J, Rubio S, Vrijheid M

Published in: Environ Int 2013; 56:10-18

Full text:
http://www.sciencedirect.com/science/article/pii/S0160412013000627

Summary: In this study we examined the associations between sociodemographic, lifestyle and dietary factors and BPA exposure levels in mothers during pregnancy (n=479) and in a small subgroup of children at 4 years of age (n=130) from the INMA-Sabadell birth cohort study. Using two spot-urine samples collected in pregnancy, we found that maternal younger age, lower education, smoking and second-hand tobacco smoke exposure during pregnancy are associated with increased BPA urine concentrations. From a wide list of food groups usually stored in plastic containers or cans evaluated, only the consumption of canned fish during pregnancy was associated with increased maternal BPA urine concentrations. Second-hand tobacco smoke exposure and potentially the consumption of canned fish were further shown to increase the BPA concentrations measured in a single spot-urine sample collected from children. This is the first study conducted in Spain and one of the few studies that have evaluated predictors of BPA exposure in pregnant women and children.

Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin AE, Fernandez MF, Koch HM, Mendez MA, Sunyer J, Rubio S, Vrijheid M. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int. 2013 Jun;56:10-8. doi: 10.1016/j.envint.2013.02.014

5.4 Prenatal Bisphenol A Urine Concentrations and Early Rapid Growth and Overweight Risk in the Offspring

Authors: Valvi D, Casas M, Mendez MA, Ballesteros-Gómez A, Luque N, Rubio S, Sunyer J, Vrijheid M

Published in: Epidemiology 2013; 24(6):791-799

Full text:
http://journals.lww.com/epidem/Abstract/2013/11000/Prenatal_Bisphenol_A_Urine_Concentrations_and.2.aspx

Summary: In this study we examined whether maternal BPA urine concentrations during pregnancy are associated with child rapid weight gain in the first 6 months of life and subsequent obesity outcomes (i.e. BMI and waist circumference Z-scores) at 1 and 4 years of age. We analysed 402 mother-child pairs from the INMA-Sabadell birth cohort study. Maternal BPA concentrations were measured in two spot-urine samples collected in the first and third trimesters of pregnancy and the average of the two measurements (in μg/g creatinine) was used as the main exposure variable. Findings suggest that prenatal BPA exposure may increase child BMI and waist circumference at 4 years of age, but no association was shown with rapid weight gain, BMI and waist circumference at earlier ages. This is one of the two prospective studies that have evaluated the effects of prenatal BPA exposure on child postnatal growth so far.

Valvi D, Casas M, Mendez MA, Ballesteros-Gómez A, Luque N, Rubio S, Sunyer J, Vrijheid M. Prenatal bisphenol A urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology. 2013 Nov;24(6):791-9. doi: 10.1097/EDE.0b013e3182a67822

5.5 Variability and Predictors of Urinary Phthalate Metabolites in Spanish Pregnant Women

Authors: Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, Vrijheid M

Submitted to: Int J Hyg Environ Health (since February 2014)

Summary: In this study we evaluated the reproducibility and determinants of phthalate metabolite concentrations measured in two maternal spot-urine samples collected in the first and third trimesters of pregnancy in the INMA-Sabadell birth cohort study (N=391). Concentrations of DEHP metabolites, MBzP, MEP, MiBP and MnBP phthalates were adjusted for creatinine concentrations to control for urine dilution. Potential determinants of exposure were selected based on previous literature and included sociodemographic, lifestyle and dietary factors and consumer product use. We found poor reproducibility (Intraclass correlation coefficient-ICC<0.25) for all phthalate metabolites. Phthalate metabolite urine concentrations were higher in overweight compared to normal weight women and in women who had been using household cleaning products (mainly bleach and oven cleaning sprays) at least once per week during pregnancy compared to women with a less frequent use. Spanish origin, lower education and social class, smoking and less frequent consumption of organic food during pregnancy were associated with increased concentrations of some of the phthalate metabolites tested. The consumptions of bottled-water and food groups usually stored in plastic containers or cans and the uses of plastic containers for heating food and cosmetics during pregnancy were not associated with phthalate urine concentrations. This is the largest study of the few studies that have assessed the reproducibility and determinants of phthalate exposure during pregnancy and the first study conducted in European pregnant women.

Highlights

- Data on urinary phthalate metabolite variability and predictors in pregnancy are scarce
- We measured phthalate metabolites of DEHP, BBzP, DEP, DiBP and DnBP in two spot urine samples from 391 Spanish pregnant women
- We found considerable variability between the two samples for all phthalate metabolites measured
- Consumption of bottled-water and foods usually stored in plastic containers or cans, use of plastic containers for heating food and cosmetics did not predict phthalate exposure in pregnancy
- Sociodemographics (overweight, Spanish origin, lower education and social class), lifestyle factors (smoking, less frequent consumption of organic food) and household cleaning product use may increase phthalate exposure in pregnancy

Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, Vrijheid M. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Int J Hyg Environ Health. 2015 Mar;218(2):220-31. doi: 10.1016/j.ijheh.2014.11.003

5.6 Prospective Evidence on the Effects of Phthalate Exposure on Childhood Growth and Blood Pressure

Authors: Valvi D, Casas M, Romaguera D, Monfort N, Ventura R, Martinez D, Sunyer J, Vrijheid M.

Submitted to: Environ Health Perspect (since June 2014)

Summary: In this study we examined the associations between prenatal phthalate exposure and child postnatal growth and cardiovascular risk outcomes up to 7 years of age. We analysed 391 mother-child pairs from the INMA-Sabadell birth cohort study. Maternal phthalate urine concentrations were measured in two spot-urine samples collected in the first and third trimesters of pregnancy and we used the averages of the two measurements (in μg/g creatinine) as the main exposure variables. Prenatal exposure to high molecular weight phthalates (i.e. the sum of DEHP metabolites and MBzP) was associated with decreased weight gain in the first 6 months of age and decreased BMI at later ages in boys. In girls we found little evidence that exposure to high molecular weight phthalates may increase BMI in childhood. Prenatal exposure to low molecular weight phthalates (i.e. the sum of MEP, MiBP and MnBP) was not associated with postnatal growth outcomes in either sexes. Prenatal exposures to high and low molecular weight phthalates were shown to decrease systolic blood pressure in girls but not in boys. No association was shown with diastolic blood pressure and waist-to-height ratio. Findings suggest that prenatal exposure to phthalates may be associated with postnatal growth and blood pressure and that these effects may be modified by child sex. This is the first prospective study evaluating these associations.

Article publicat finalment amb títol diferent

Valvi D, Casas M, Romaguera D, Monfort N, Ventura R, Martinez D, Sunyer J, Vrijheid M. Prenatal Phthalate Exposure and Childhood Growth and Blood Pressure: Evidence from the Spanish INMA-Sabadell Birth Cohort Study. Environ Health Perspect. 2015 Oct;123(10):1022-9. doi: 10.1289/ehp.1408887

6

Discussion

6.1 Main findings

The first part of this thesis focused on the associations between prenatal exposure to POPs and childhood growth and obesity. For this purpose we conducted two analyses. In the first analysis we used data from the INMA-Menorca cohort (n=344, outcome assessment at 7 years of age) where levels of POP exposure were relatively higher (Paper I) and in the second one we used data from the INMA-new cohorts (n=1285, outcome assessment at 0-1 year of age) in the regions of Gipuzkoa, Sabadell and Valencia (Paper II) where the levels of exposure are relatively lower. Prenatal exposure to DDE was associated with increased risk for rapid weight gain in the first 6 months of life and increased risk for overweight at the ages of 1 and 7 years. The association with rapid growth was only seen in boys, however child sex did not clearly modify the associations with overweight at later ages with only a small suggestion that the association with overweight at 7 years of age may be only seen in girls but not in boys. Prenatal exposure to HCB was associated with increased risks for rapid weight gain in the first 6 months of life and overweight at 1 year of age similarly to the previously published findings at 7 years of age (Smink et al. 2008). The associations between HCB and growth and obesity outcomes were not shown to be influenced by child sex. Prenatal low-level PCB exposure was not associated with early weight gain or overweight at 1 year of age, however prenatal PCB exposure was shown to increase overweight risk in girls but not in boys at the age of 7 years. Detectable levels of DDT were shown only in mothers of the INMA-Menorca cohort study and non-linearly associated with somewhat increased overweight risk in boys but not in girls at 7

years of age. Overall, our findings suggest that, at the levels of exposure assessed, prenatal exposures to DDE and HCB may influence child growth and increase obesity risk as early as in the first year of life, while prenatal exposure to PCBs may increase obesity risk later in life and potentially only in girls. Less clearly, low-levels of prenatal DDT exposure may be associated with increased risk for childhood obesity in boys only.

The second part of the thesis focused on evaluating the associations between prenatal exposure to BPA and childhood growth and obesity. For this purpose, we used data from the INMA-Sabadell cohort study. Because little is currently known about predictors of BPA exposure in pregnant women we first aimed to evaluate sociodemographic, lifestyle and dietary determinants of exposure in our population (Paper III, n=479). Using two spot-urine measurements, higher BPA concentrations were detected in mothers of younger age and lower educational level, who had smoked or were exposed to second-hand tobacco smoke during pregnancy. From a wide list of lifestyle habits (including type of consumed water and use of plastic containers for heating food) and dietary factors tested (consumption of foods usually stored/not stored in plastic containers and cans), only the consumption of canned fish during pregnancy was associated with increased levels of BPA concentrations in maternal urine. These findings provided valuable input in the second analysis in which we evaluated the associations between prenatal exposure to BPA and infant weight gain in the first 6 months of life and subsequent obesity risk at ages 1 and 4 years (Paper IV, n=402). Our findings suggest that prenatal exposure to BPA may be weakly associated with increased BMI and waist circumference Z-scores at 4 years of age, but no association was observed at earlier ages with infant weight gain, BMI or waist-circumference. Associations were not shown to be influenced by child sex.

The third part of the thesis focused on evaluating the associations between prenatal exposure to phthalates and childhood growth and obesity using data from the INMA-Sabadell cohort study. For this purpose, we first evaluated the reproducibility of phthalate metabolites and predictors of exposure using two spot-urine samples collected from the mothers during pregnancy (Paper V, n=391). We observed a poor reproducibility (ICC<0.25) for all phthalate metabolites measured. Higher phthalate metabolite urine concentrations were detected in overweight mothers and mothers of Spanish origin, of lower educational level and social class, who had been smoking, consuming less frequently organic food and using more frequently household cleaning products during pregnancy. The consumptions of bottled-water and food groups usually stored in plastic containers or cans, the use of plastic containers for heating food and the use of cosmetics were not associated with maternal phthalate metabolite urine concentrations. Because of the low reproducibility shown for all phthalate metabolites, we evaluated the associations between prenatal exposure to phthalates and child growth up to 7 years of age using the average of the two spot-urine phthalate measurements as a better proxy of exposure throughout pregnancy (Paper VI, n=391). Because it was recently suggested that phthalate exposure may increase systolic blood pressure in children aged 6-19 years (Trasande et al. 2013a) we further evaluated associations with repeated child blood pressure measurements at 4 and 7 years of age. Our findings suggest negative associations between the sum of high molecular weight phthalates (i.e. DEHP metabolites and MBzP) and weight gain in the first 6 months of life and repeated BMI Z-score measurements at later ages in boys while in girls we found some suggestion for positive associations with weight gain and BMI Z-scores. The sum of low molecular weight phthalates (i.e. MEP, MiBP and MnBP) was not associated with any of the obesity outcomes evaluated. Both high and low molecular weight phthalate sums were shown to decrease child systolic blood pressure, independently of BMI, while no clear association was shown with diastolic blood pressure.

The following sections of the discussion include general aspects related to the findings of this thesis rather than more specific issues that have been already discussed in the papers. General considerations about methodological issues, the current state of evidence in this field and the public health implications of findings are discussed. Further, some important considerations for future studies in this field that are discussed in the next sections are summarised in **Table 6.1**.

6.2 Methodological issues

6.2.1 Study Design

The use of the population-based INMA birth cohort studies is one of the major strengths of this thesis. The prospective design minimises the risk of reverse causation between the exposures and the outcomes of interest and is a great advantage over the existing literature that is mainly based on cross-sectional data. Further, the continuous follow-ups conducted from as early in life as in the first trimester of pregnancy and onwards in infancy and childhood have permitted to collect information on the exposures, the outcomes and additional covariates at different time-points. This has enabled us to control for postnatal POP exposure (Paper I), to collect and analyse repeated measurements of BPA and phthalate metabolites (Papers III-VI), to assess growth and obesity outcomes at different ages from early infancy to childhood (Papers II, IV and VI) and to account for a wide list of potential confounders (Papers I-VI). However, similar to all study designs, prospective cohorts have also limitations that are discussed in the subsection of bias.

6.2.2 Population Size and Statistical Power

Power is a major consideration for all studies but it is of particular concern when the aim is to conduct stratified analysis and test for

effect-measure modifications. Beside the relatively small magnitude of the associations studied overall, the population size enabled us to detect statistically significant associations in unstratified analyses in all papers with the exception of Paper IV in which associations between prenatal BPA and obesity outcomes at the age of 4 years using both the outcome and exposure variables on a continuous scale were of borderline statistical significance. However, in stratified analyses by the main hypothesised effect modifiers (Papers I by sex, high-fat diet; II by sex, exclusive breastfeeding, maternal pre-pregnancy BMI; IV by sex, exclusive breastfeeding, maternal age, education, smoking; and VI by sex) only few of the associations reached the level of statistical significance even when it was adequate to use the outcome and the main exposure variables on a continuous scale to gain more power. This may be because these factors do not truly modify the associations of interest or because our studies were underpowered to detect biological interactions. Except for the population size and the effect magnitude, the accuracy of the measured exposure variables is another issue that may have influenced the power of the studies conducted, as it is discussed in the next subsections.

6.2.3 Exposure Assessment

6.2.3.1 Assessment of Exposure to Environmental Pollutants

Exposure misclassification in the studies evaluating the effects of prenatal POP exposure on childhood growth and obesity (Papers I and II) is less likely compared to studies assessing exposures to non-persistent environmental pollutants, as POPs have a long half-life in human tissues (years) and thus, one single blood measurement of concentrations is considered to reflect fetal exposure throughout pregnancy (Longnecker et al. 1999). At recent years, POP exposure predominantly occurs through diet and given the well-known limitations in the accuracy of dietary assessment methods due to the highly variable dietary patterns between- and

within- subjects in the population, the use of POP biomarkers is advantageous as it captures the substantial variations in exposure through diet and other potential sources (eg occupational exposure). Nevertheless, an important consideration in studies that assess exposure to environmental pollutants using biomarkers is the interindividual differences in metabolism that may have influenced the levels of the biomarker measured (Savitz 2014). The physiological and metabolic changes during pregnancy may be another source of variation in the POP concentrations measured in blood (Adetona et al. 2013). If a common biological determinant exists (eg maternal metabolism) between the concentrations of the environmental pollutants measured during pregnancy in the mothers and the obesity outcomes of the child, then our findings could be explained by reverse caution. This is of particular concern in the studies evaluating the potentially obesogenic effects of highly lipophilic chemicals, such as POPs, that are stored in the adipose tissue. Although the mechanisms are not yet understood, some evidence exists that POP metabolism may differ in obese compared to lean subjects with extended half-lives of at least some POPs suggested in obese subjects (La Merrill and Birnbaum 2011; Lee et al. 2011). The facts that not all POP exposures evaluated in our studies were shown to be similarly associated with the childhood growth and obesity outcomes evaluated, that some of these associations were shown to differ between boys and girls and that maternal overweight was not shown to substantially influence the associations of interest, strengthen our confidence that findings are not explained by reverse causation. However, we cannot be sure whether and at what extent interindividual variation in maternal metabolism and physiological changes during pregnancy may have influenced the associations between prenatal POP exposures and childhood growth and obesity. A more in-depth understanding of POP storage and metabolism in biological tissues is required to be able to better explain these associations in the future as it has been also recently suggested by La Merrill et al (2013).

Another important consideration related to the assessment of POP exposure is that because fat mass in obese subjects is greater compared to lean subjects, POP concentrations in blood may be lower in obese persons due to dilution (Porta et al. 2009). For this reason, POP concentrations are often normalized to blood lipid content to account for this variation. However, this may be inadequate as if some POPs cause both obesity and dyslipidemia through a common biological pathway, then adjusting or correcting POP concentrations for the lipid content would be an overadjustment and would attenuate associations towards the null. Information on blood lipid concentrations was not available in the INMA-Menorca cohort (Paper I). However, lipid-adjusted and unadjusted POP concentrations in the analyses of the INMA-new cohorts (Paper II) were shown to be similarly associated to the childhood growth and obesity outcomes evaluated. In the absence of a consensus about whether lipid adjustment is adequate or not, the evaluation of associations using POP concentrations both adjusted and unadjusted for lipid content is recommended (La Merrill et al. 2013; Porta et al. 2009).

Exposure misclassification is a major limitation in our studies evaluating the effects of the non-persistent pollutants, BPA and phthalates, on child health outcomes (Papers IV and VI). Similar to the findings from studies conducted in many populations in different countries, concentrations of BPA and phthalate metabolites using two spot-urine samples collected in pregnancy were poorly correlated (Pearson r=0.19 for BPA and in the range 0.06-0.24 for phthalate metabolites, see Papers III and V) and presented a very low reproducibility (ICC<0.25 for BPA and all phthalate metabolites, see Papers IV and V). For this reason, we have used the average of the two spot-urine concentrations, instead of the single spot-urine concentrations, as a potentially better proxy of exposure throughout pregnancy. Despite this, exposure misclassification is still very likely due to the high within-subject variability observed for both BPA and phthalate metabolites and

even two measurements may not be enough to adequately classify exposure. The measurement error in BPA and phthalate exposure assessment reduces the study power and is likely to be non-differential in relation to other covariates analysed including the outcomes, as there is no reason to believe otherwise and thus, it has probably attenuated the associations biasing effect estimates towards the null. Optimizing exposure assessment of these environmental pollutants with the use of more than two urine measurements and potentially by combining biomarker data with exposure estimations based on questionnaire and environmental monitoring data is an important consideration for future studies. To optimise exposure assessment, further research is needed to determine the minimum sufficient number of urine samples required and to detect predictors, sources and pathways of exposure in different subgroups of the general population, such as pregnant women and children.

Underestimation of effect estimates in particular for BPA and phthalates (Papers IV and VI) is also possible due to the narrow range of exposure assessed and the lack of defining an adequate comparison group of unexposed mother-child pairs. Almost all of the mothers had detectable levels of exposure (values below LOD ≤1%, see Papers IV and VI) to these currently widespread used pollutants and the interquartile increases of the urine concentrations measured were relatively small (1.6-fold increase for BPA, 1-fold increase for the sum of high molecular weight phthalates and 2.3-fold increase for the sum of low molecular weight phthalates). In Paper IV, with the aim to increase the contrast between the comparison groups we classified mother-child pairs into categories of "consistently low" (both spot-urine BPA measurements in the lowest exposure tertile or one in the lowest and one in medium), "consistently high" (both spot-urine BPA measurements in the highest tertile or one in the highest and one in medium) and "medium" exposure (all others). However, using this exposure classification instead of using the average of the two spot-urine

BPA measurements in tertiles did not influence effect estimates. The comparison of risks defining more extreme groups of exposure was not possible in our study due to sample size restrictions.

6.2.3.2 Assessment of Predictors of BPA and Phthalate Exposures

Exposure misclassification is also of important concern in our studies aiming to evaluate predictors of BPA and phthalate urine concentrations (Papers III and V). BPA and phthalates are quickly metabolised and excreted from the human body within few hours or days after exposure. Information about potential predictors that present a high day-to-day and within-day variation in the population, such as diet and the use of cosmetics, was collected through questionnaires that assessed predictors on average during long pregnancy periods and were not especially designed to capture predictors of exposure to these non-persistent pollutants. This may explain, at least partially, why we did not observe any association between the consumption of food groups usually stored in plastic containers and cans or the use of cosmetics and phthalate urine concentrations, and why only canned fish consumption but not other packaged-foods was related to urine BPA concentrations. Future studies aiming to evaluate highly variable predictors of exposure to these environmental pollutants should consider the use of short-term (few hours or days) recall diaries to increase the accuracy of measurements.

6.2.4 Outcome Assessment

Growth and obesity outcome assessment in all studies (Papers I, II, IV and VI) was based on medical registries (repeated weight measurements in the first year of life) and on measurements conducted by special trained personnel of the INMA team (BMI and waist circumference measures at different child ages). This is an advantage over studies using self-reported anthropometric data as mothers have been shown to importantly misreport theirs child

weight and height especially at preschool ages (Dubois and Girad 2007). Further, the outcomes assessed (early weight gain in the first 6 months of life, and BMI [or weight-for-length in early infancy] and waist circumference in infancy and childhood) are all shown to fairly predict fat mass in validation studies using DXA (Koo et al. 2000; Wohlfahrt-Veje et al. 2014) and are valid measures for predicting obesity risk and obesity-related diseases later in life (Druet et al. 2012; Graversen et al. 2014; Mei et al. 2002; Monteiro and Victora 2005). Similar to our studies, the outcome assessment in other studies that have evaluated the associations between prenatal exposures to POPs, BPA and phthalates and childhood obesity was based on child anthropometry. One birth cohort study further assessed associations with triceps skinfold thickness in adolescents (Gladen et al. 2000) and one other birth cohort study has performed more direct measures of fatness using bioelectrical impedance in children aged 9 years (Harley et al. 2013; Warner et al. 2014) additionally to anthropometry. In these previous studies, the direction and the statistical significance of the associations between prenatal exposure to POPs and skinfolds (Gladen et al. 2000) and between BPA and fat mass measures (Harley et al. 2013) did not differ compared to the associations shown with child BMI and waist circumference. In the study by Warner et al. (2014), prenatal exposures to DDT and DDE were associated with increased BMI at 9 years of age in boys, however the associations with % fat mass, although were in the same direction, did not reach the level of statistical significance. The use of more direct measures of fatness commonly used in other research fields, such as bioelectrical impedance and preferable DXA, although more costly and less practical compared to child anthropometry, is an important consideration for future follow ups in the INMA birth cohort studies and other future studies in this field. Further, the associations between the exposure to these environmental pollutants and biomarkers of metabolic function have been explored in previous adult studies (Lee et al. 2007 and 2011; Pereira-Fernandes et al. 2014) however in children these association are unexplored. The

integration of biomarkers of metabolic function, such as lipids and adipokines, in future studies may contribute importantly in elucidating the mechanisms that underlie the associations shown.

Although early accelerated growth defined as a weight Z-score difference between two time points in the first year of life (eg from 0 to 6 months, as in our studies) is related to later obesity and chronic disease risks, many different time windows for assessing growth can be found in the literature (eg 0-3 months, 6-12 months, 0-2 years) and it is not clear yet which time window better predicts later obesity risk. Further, the cut-off of +0.67 SD that is commonly used to define rapid growth based on weight or weight-to-length z-score differences during a time window (Monteiro and Victora 2005) is quite arbitrary, thus longitudinal growth trajectories have been suggested alternatively as a more precise predictor for later obesity risk if repeated anthropometric measures are available (Howe et al. 2014; Slining et al. 2013). Different statistical approaches have been recently proposed for modelling growth data and distinguishing groups of children that follow different shaped trajectories including mixed effect models (Chivers et al. 2009), latent class growth analysis (LCGA) (Slining et al. 2013), latent class growth mixture modelling (LCGMM) (Muthen B and Muthen LK 2000) and linear spline multilevel modelling (Howe et al. 2014). The influence of prenatal exposures to environmental pollutants on longitudinal growth trajectories is an interesting field for future research.

6.2.5 Confounding, Measurement Error and Bias

6.2.5.1 Confounding due to Correlated Exposures

Confounding due to correlated exposures is likely in environmental studies similar to ours. The POP exposures studied (i.e. DDT, DDE, HCB and sum of PCB congeners) were moderately correlated to each other (r ranged from 0.27 to 0.43). We assessed potential

confounding due to colinearity by evaluating associations with growth and obesity outcomes in multipollutant models adjusted for all the prenatal POP exposures measured and this did not substantially influence the effect estimates (Papers I and II). Similarly, the simultaneous adjustment for prenatal exposure to the sums of high and low molecular phthalate metabolites (r=0.18) did not change the effect estimates of the associations of interest (Paper VI).

Postnatal exposures to environmental pollutants may also confound the associations between prenatal exposures and growth and obesity outcomes. This is of particular interest in studies evaluating the effects of prenatal exposure to POPs as maternal exposure during pregnancy may correlate with child cumulative exposure in the first years of life through fetal exposure and breastfeeding. We have been able to evaluate confounding by postnatal exposures only in Paper I where POP concentrations at child serum collected at 4 years of age had been measured for a subgroup of children (76% of the main analysis population). Controlling in the models for postnatal POP exposure did not influence effect estimates for the associations between prenatal exposure to POPs and the outcomes assessed. However, prospective studies so far have mainly focused on evaluating the effects of prenatal exposures on childhood growth and obesity therefore, future prospective studies should aim to elucidate the role of postnatal exposures and to evaluate any potential synergies between prenatal and postnatal exposure on the risk of obesity. Recognising though that traditional regression models simultaneously adjusted for correlated exposures are susceptible to overadjustment bias and estimated coefficients may be unstable even when model convergence is achieved, future studies should consider the use of more sophisticated models suggested for dealing with multicolinearity such as hierarchical Bayesian regression methods (MacLehose et al. 2007). Unlike to POPs, child postnatal exposures to BPA and phthalates may less likely have confounded the association between prenatal exposure

and growth and obesity outcomes. In a subgroup of children from the INMA-Sabadell cohort (n=113) child urine BPA concentrations at 4 years of age were not correlated with maternal urine concentrations measured in pregnancy (Paper V) as it has been also shown elsewhere (Harley et al. 2013). Relatively low correlations ($r<0.25$) have been further shown between prenatal and postnatal exposure to phthalates in other settings (Frederiksen et al. 2013). However, the potential influence of postnatal exposures to these environmental pollutants on childhood growth and obesity is an interesting field for future research.

6.2.5.2 Other Sources of Confounding and Measurement Error

The prospective design of the INMA studies has permitted the collection at different time points of numerous potential confounders for the associations of interest, including maternal, paternal and child characteristics. A thorough consideration of confounders, effect mediators and colliders (i.e. variables blocking the pathways between the exposure and the outcome variables) was made prior and in the first stages of data analysis. Potential confounders were considered based on previous evidence from other studies and on data driven associations between covariates shown in analyses conducted in the INMA populations. Despite our careful consideration thought, we cannot rule out that other confounders that were not measured or confounders that we are not aware of may partially explain the estimated associations, as it is discussed in more detail in the papers. Further, measurement errors in the potential confounders analysed may have compromised our ability to control for their effect, leaving “residual” confounding.

Confounders included in the final statistical models assessing the associations between prenatal exposure to environmental pollutants and child growth and obesity outcomes were selected using change-in-estimate procedures (i.e. backward and forward selection) (Papers I, II and IV) and in Paper VI, using a combination of directed acyclic graphs (DAGs) and change-in-estimate procedures.

DAGs permit to visualise the known or assumed relationships between the related to the research hypothesis covariates (measured or unmeasured) and to detect sources of confounding, overadjustment and unnecessary adjustment (Schisterman et al. 2009), measurement error (VanderWeele and Hernan 2012) and selection bias (Hernan et al. 2004) that may distort effect estimates. Thus, DAGs are a useful tool in epidemiologic studies for detecting sources of bias and/or loss of precision in effect estimation and for representing causal relationships between variables and, in this way, can help to build etiologic models based on prior knowledge. This is a great advantage over change-in-estimate statistical procedures as statistical inference does not necessarily imply causation. Although during the last 20 years the use of DAGs for confounder identification has become increasingly common in epidemiologic studies, the use of DAGs in environmental studies evaluating the role of prenatal exposures on childhood obesity is very limited. This is an important consideration as the aetiology of obesity is multi-factorial with many of the known obesity causes shown to be associated with the environmental exposures of interest (eg dietary factors, sociodemographic characteristics) and further because, in studies assessing postnatal growth during the life course, mediation effects (eg through gestational age, birth weight or postnatal growth outcomes assessed at earlier ages) are likely. Given the uncertainties rising due to the lack of evidence for the associations between the different covariates included in a DAG (eg uncertainties about predictors of environmental exposures that are also related to the outcome), many causal DAGs may be considered plausible under different assumptions for the same research hypothesis. For this purpose, researchers are encouraged to report the DAGs underlying their assumptions and by this permit the comparability between the plausible DAGs suggested in different settings. This can be a helpful first step to move the debate forward in areas where evidence supporting the causal assumptions between covariates included in the DAGs of environmental chemical exposures and obesity is still scarce.

6.2.5.3 Selection Bias and Results Generalizability

A main weakness of prospective cohort study designs are losses to follow-up due to censoring, lack of participation or migration. This may bias effect estimation if attrition is associated with the risk of the disease to a different extent in the exposed and unexposed groups (i.e. data are not missing at random). In our studies, mother-child pairs with complete exposure and outcome data included in the analysis were the 60-71% of the origin population at recruitment (Paper I: 71%; Paper II: 63%; Paper IV: 61%; Paper VI: 60%). Not all eligible pregnant women from the source populations agreed to participate in the INMA birth cohort studies which increases further the risk of bias. Selection bias is less likely in the studies evaluating the associations between prenatal POP exposures and child growth and obesity (Papers I and II) as included and excluded from analysis children did not differ with respect of many main characteristics including parental characteristics in pregnancy and child characteristics after birth. However, in the analyses of the INMA-Sabadell birth cohort study evaluating the effects of prenatal exposures to BPA and phthalates, analysed children differed in few main characteristics compared to those excluded, and importantly they were more likely of mothers with higher education and social class and less likely of mothers who had smoked during pregnancy (Papers IV and VI). In our population, higher education and social class are associated with decreased BPA and phthalate maternal urine concentrations in pregnancy while maternal smoking in pregnancy is related to increased maternal urine concentrations of these pollutants (Papers III and V). Thus, the most highly exposed children may have been excluded from our analyses. Because of this and further considering that BPA and phthalate effects on obesity (if any), have been suggested to be non-monotonic with different effects exhibited at lower and higher levels of exposure, our findings may not be generalizable to the least socially advantaged groups of the general population.

Table 6.1 Summary of Key Considerations for Future Studies Evaluating the Role of Developmental Exposures to POPs, BPA and Phthalates on Growth and Obesity[1]

	Persistent organic pollutants (POPs): DDT/DDE, HCB, PCBs	Non-persistent organic pollutants: BPA, Phthalates
Study Design	➢ Many cross-sectional studies available ➢ Prospective studies available for DDE and PCBs ➢ Few prospective studies available for DDT and HCB	➢ Almost all studies available are cross-sectional ➢ Two prospective studies available for BPA ➢ No prospective data published for Phthalates
Exposure Assessment	➢ Commonly based on biomarkers measured in blood or other biological matrices (eg breast milk) ➢ Exposure misclassification: Less likely due to the long half-life and the bioaccumulation in human tissues ➢ Reverse causation: Possible due to the high lipophility of POPs and their capacity to store in the adipose tissue ➢ Methodological aspects: Adjusting or correcting concentrations for the lipid content may be an overadjustment. Recommended to evaluate associations using POP concentrations both adjusted and unadjusted for lipids	➢ Commonly based on biomarkers measured in urine ➢ Exposure misclassification: Very likely due to the short half-life and the high within-subject variability ➢ Reverse causation: Possible due to variations in behaviour (eg packaged food and beverage consumption) between lean and obese subjects ➢ Methodological aspects: Urine concentrations should be corrected for urine dilution. Further research is needed to determine the minimum number of urine samples required and to identify sources and pathways of exposure in specific subgroups of the populations (eg pregnant women, children) with the aim to optimise exposure assessment accuracy
Outcome Assessment	➢ Almost exclusively based on child anthropometry (eg BMI, waist circumference) ➢ Direct measures of fat mass (eg DXA), longitudinal growth trajectories and the integration of metabolic function biomarkers should be considered in future studies ➢ Methodological aspects: Anthropometric measures in children and adolescents should be standardized by age and sex	

	Persistent organic pollutants (POPs): DDT/DDE, HCB, PCBs	Non-persistent organic pollutants: BPA, Phthalates
Confounding	➢ Possible confounding due to correlated environmental exposures (exposure to different pollutants at the same time and/or exposure to the same pollutant prenatally and postnatally). Advanced statistics methods should be applied to deal with muticollinearity ➢ The integration of DAG approaches can help to minimise bias and/or loss of precision in effect estimation and put forward the existing knowledge about the causal models underlying the associations of interest	
Current State Of Evidence	➢ Lack of *in vivo* and *in vitro* studies supporting the available evidence from epidemiologic studies – Studies evaluating the potentially obesogenic effects at lower and higher doses of exposure are required ➢ DDE may be associated with childhood growth and obesity in a dose-dependent manner with effects shown only at lower levels of exposure. Effects may be modified by sex ➢ PCB associations with childhood growth and obesity are inconsistent. Some suggestion exists that girls may be more susceptible to these effects, if indeed there are any ➢ DDT and HCB associations with childhood growth and obesity have been evaluated in very few studies so far and findings are inconsistent	➢ The effects of developmental exposure to BPA and some phthalates (mostly DEHP) on growth are well-documented in *in vivo* and *in vitro* studies – Future research is required to elucidate the mechanisms underlying these effects ➢ Findings from cross-sectional studies are inconclusive ➢ The inconsistencies in findings between previous cross-sectional studies and the first prospective studies conducted recently in this field highlight the need to evaluate these effects in prospective studies
	➢ Further research is required to elucidate the effect of postnatal exposures on childhood growth and obesity, any potential synergies between prenatal and postnatal exposures on the development of obesity and to detect groups in the population potentially more susceptible to these effects	

[1]The points that are summarised in this table are discussed in sections 6.2 and 6.3 of the thesis.

6.3 Contribution to the Current State of Evidence

This PhD thesis gives insight in the role of prenatal exposures to environmental chemicals with endocrine disrupting properties on child growth and obesity from early infancy to childhood and provides prospective human evidence supporting the Environmental Obesogen Hypothesis. Considering the current state of existing evidence in this emerging field of research, our findings contribute to: 1) understand the effects of low-level prenatal exposure to POPs on childhood growth and obesity, 2) elucidate the role of prenatal exposure to the widely currently used non-persistent environmental pollutants, BPA and phthalates, on childhood growth and obesity, 3) identify groups of children that may be more susceptible to the obesogenic effects of exposures to these environmental pollutants and 4) determine the variability and predictors of exposures to BPA and phthalates in pregnant women residing in Spain. The specific contributions of findings in the existing literature are discussed in the next subsections.

6.3.1 POPs and Childhood Growth and Obesity

The main findings of the prospective birth cohort studies that have evaluated so far the effects of prenatal exposure to DDE on childhood growth and obesity, including the studies presented in this thesis, are summarized in **Table 6.2** (*see page 227*). The exposure assessment in all studies was based on measurements of DDE concentrations in maternal serum samples collected at different time-points during pregnancy or cord serum samples collected at birth. Outcome assessment included weight and height measurements or reports at different ages from early pregnancy to puberty depending on the study and Z-scores for anthropometric measurements were calculated in all studies except from two (Gladen et al. 2000 and 2004). Further, one study estimated prenatal DDE exposure based on repeated maternal serum samples collected years after birth and assessed associations with adult BMI in female

offspring (Karmaus et al. 2009) Despite the differences in the populations assessed and the methods followed, a consistent dose-dependent pattern in associations with child BMI, weight and/or height can be observed among studies. Therefore, studies in Table 6.1 are ordered according to the median DDE concentrations assessed (from the lowest to the highest) to facilitate comparisons. Birth cohort studies evaluating relatively lower to moderate levels of DDE exposure (i.e. median <3000 ng/g lipid based on a rough calculation) including ours (n=9) have all suggested positive associations with obesity outcomes except from one Mexican study where null associations with child anthropometric measurements in the first year of life were shown (Garced et al. 2012) and the INUENDO study that suggested null association with child BMI at 5-9 years of age (Høyer et al. 2014). Further, all studies assessing low to moderate DDE levels of prenatal exposure have evaluated effect modification by child sex except from the INUENDO study (Høyer et al. 2014) and one other small study (Verhulst et al. 2009). In Paper II, which is the largest study conducted so far evaluating the effects of DDE at relatively low levels of exposure, we found that effects of DDE on rapid weight gain in the first 6 months of life are mainly seen in boys but not in girls, while no effect modification by child sex was observed for the association with child BMI at 1 year of age. In the INMA-Menorca study (Paper II), we found some evidence that the effects of DDE on child overweight at 7 years of age may be enhanced in girls compared to boys, however a suggestion of effect modification by child sex was only shown in the 2nd but not in the 3rd tertile of exposure. Two other studies assessing associations with child BMI at later ages suggested DDE effects to be mainly shown in boys but not in girls (Warner et al. 2014; Gladen et al. 2000). However, one small study suggested associations with waist-to-height to be seen only in girls but not in boys (Delvaux et al. 2014) and a positive association with BMI was also suggested in the study conducted in female adults. To the contrary, birth cohort studies (n=4) evaluating the associations between relatively higher levels of prenatal DDE exposure (i.e.

median >3000 ng/g lipid) and child anthropometric outcomes at different ages from early infancy to puberty have suggested null associations. Two of these studies found no evidence for effect modification by child sex (Cupul-Uicab et al. 2013; Jusko et al. 2006) while the other two included only males (Cupul-Uicab et al. 2010; Gladen et al. 2004). Overall, the existing evidence from prospective studies seem to suggest that relatively lower to moderate levels of prenatal exposure to DDE (eg <3000 ng/g lipid) may influence childhood growth and increase the risk of obesity in a sex-specific manner with effects being potentially enhanced in boys compared to girls while higher levels of exposure may not have an effect on childhood growth and obesity in either sexes. The effects of DDE on weight homeostasis have been only recently started to be explored in *in vivo* and *in vitro* studies and whether lower and higher doses of exposure may have different effects on weight homeostasis remains unclear. However, non-monotonic effects of both DDT and DDE have been suggested for other endpoints (Kiyosawa et al. 2008; Vandenberg et al. 2012). Experimental studies evaluating the developmental effects of DDE on obesity at different doses of exposure are much needed to further support the current state of evidence provided from epidemiologic studies.

It is a legitimate argument though, that cohort or generation effects (Keyes et al. 2010) may explain the different associations shown with obesity outcomes at lower versus higher levels of prenatal DDE exposure as almost all of the birth cohort studies with higher levels of exposure started before the early 70´s when DDT use started to be restricted, while birth cohort studies with lower to moderate levels of exposure are all posterior to late 70´s and the obesity prevalence during this period has been increasingly rising. However, the recent large birth cohort study of Mexican boys with high levels of DDE exposure and period of recruitment only 1 year prior to the recruitment period of the INMA-new birth cohort studies suggested null associations between DDE and BMI in the

2nd year of life (Cupul-Uicab et al. 2010) in contrary to our findings suggesting a positive association between low levels of prenatal exposure to DDE and BMI at 14 months of age (Paper II). The inconsistent findings between these two recent studies that have assessed obesity outcomes at similar ages provides some evidence that the differences in the ranges of exposure rather than a cohort effect could more likely explain the inconsistencies in findings observed between older and more recent birth cohort studies. Differential effects at lower and higher levels of exposure may be a plausible answer to doubts rising from the fact that obesity prevalence has been increasing while POP levels have been decreasing during the last decades. Substantial changes in factors that potentially modify the effects of POP exposures on obesity, such as the increases in fat and sugar intakes in the population during this period, may be an alternative answer to these concerns.

Prospective studies evaluating the effects of prenatal exposure to DDT on childhood obesity outcomes have generally shown null associations (Cupul-Uicab et al. 2010 and 2013; Gladen et al. 2004; Jusko et al. 2006). Only one previous study with relatively lower levels of exposure suggested positive associations between prenatal exposure to both *o,p´*-DDT and *p,p´*-DDT and obesity outcomes (i.e. overweight and elevated waist circumference) in boys, in agreement with the small evidence we found in the INMA-Menorca birth cohort study that prenatal DDT exposure may be associated with increased risk for overweight at age 7 years in boys only (Paper I). Further research is required to elucidate whether prenatal low-level exposure to DDT may influence childhood growth and obesity and whether boys may be more susceptible to these effects compared to girls.

The potential effects of prenatal exposure to HCB on childhood growth and obesity have been evaluated so far only in one large US birth cohort study (Cupul-Uicab et al. 2013), the INMA-birth cohort studies (Mendez et al. 2011; Smink et al. 2008; Paper II) and a

small birth cohort study of Flanders (Delvaux et al. 2014). Although levels of prenatal HCB exposure are similarly low in all studies, in the US and Flanders studies null associations were shown with child BMI Z-scores and other anthropometric obesity measures evaluated at 7-9 years of age in contrary to the positive associations shown with rapid weight gain in the first 6 months of life and overweight risks at 1 (Paper II) and 7 years of life (Smink et al. 2008) in the INMA-birth cohort studies. Differences in the population characteristics (eg 55% of children were African Americans in the US study) or the restricted sample size in the Flanders study may explain at least partially the inconsistencies in findings. Our findings suggest that the effects of prenatal exposure to HCB, similarly to DDE, on child growth may be apparent very early in life and require these associations to be further explored in other settings.

The main findings of the prospective birth cohort studies that have evaluated so far the effects of prenatal exposure to PCBs on growth and obesity including the studies presented in this thesis are summarized in **Table 6.3** (*see page 234*). PCB congeners may exhibit different endocrine-disrupting effects (Bonefeld-Jorgensen et al. 2001), however disentangling their effects in human population is particularly hard due to the high correlation observed among congeners, and thus all studies including ours have assessed exposure using PCB mixtures (Aroclors), sums of the measured congeners or surrogates of PCB exposure (eg PCB-153). The comparison of levels of exposure between studies is complex due to the different PCB congeners measured in the different settings. Further, findings within birth cohorts prior to ´70s and within more recent birth cohorts are shown to be inconsistent. Thus, studies in Table 6.2 are ordered according to the child age at outcome assessment (from the youngest to the oldest) to facilitate comparisons between studies evaluating growth outcomes at the same age. Six studies including one ours, with two of them being the largest conducted so far, have suggested null associations

between prenatal PCB exposure and rapid weight gain in the first 6 months of life, BMI Z-score at 1 year (Paper II), weight-for-length Z-score at 2 years (Jackson et al. 2010), BMI Z-scores at 5-9 years (Høyer et al. 2014) and BMI Z-scores or overweight and obesity at 7-9 years of life (Cupul-Uicab et al. 2013; Devaux et al. 2014) and BMI in female adults (Karmaus et al. 2009). Four studies including one ours have suggested positive associations with BMI Z-scores from 1 to 3 years of life (Verhulst et al. 2009), weight and height Z-scores at 5 years of age (Hertz-Picciotto et al. 2005), overweight at 7 years of age (Paper I) and weight-adjusted-for-height at 10-16 years (Gladen et al. 2000). All four studies found evidence that associations may be only seen in girls but not in boys, except from one very small study in which effect modification by sex did not reach the level of statistical significance (Verhulst et al. 2009). However, four other studies have suggested negative associations with weight Z-score gain in the first 3 months of life, but not at later age windows assessed up to 4 years of age (Patandin et al. 1998), with weight at 4 years of age (Jacobson et al. 1990), with repeated weight and height measures at 4, 7 and 17 years (Lamb et al. 2006) and with weight-adjusted-for-height at 5-24 years (Blanck et al. 2002). All four studies except from the one conducted in early infancy (Patandin et al. 1998) suggested that the negative associations are shown in girls, but not in boys. An important consideration regarding the studies that have shown negative associations between prenatal PCB exposure and childhood growth is that three of them (Blanck et al. 2002; Jacobson et al. 1990; Lamb et al. 2006) assessed anthropometric outcomes not-standardized for child age, two of which further relied on self-reported data and thus, measurement errors in the outcome assessment are more likely in these studies compared to others. The direction of a potential bias is hard to predict, as outcome misclassification when the outcome is evaluated in a continuous scale, as in these studies, may bias effect estimates upwards or downwards from the null (Rothman et al. 2008). Overall, the current state of prospective evidence on the association between prenatal PCB exposure and childhood growth

and obesity is inconclusive. There seem to be some suggestion though, that associations, if any, may be only seen in girls, however, the direction of the associations need to be further explored. Recent findings evaluating the potential effects on fetal growth provide further evidence that prenatal low-level PCB exposure may impair growth. Prenatal exposure to PCB-153 (as a surrogate for total PCB exposure) has been associated with decreased birth weight in a meta-analysis of 12 European cohorts (Govarts et al. 2012) and pooled analysis of these data recently showed that the negative association with birth weight may be more prominent in girls compared to boys (Casas et al. submitted). Children or at least girls with restricted fetal growth induced by prenatal PCB exposure may experience rapid catch-up growth in the first years of life and/or be at increased risk to become obese later in life. Whether this is a plausible scenario should be further explored in future studies conducted in larger populations.

Little efforts have been made so far to detect groups of the population potentially more susceptible to the effects of prenatal POP exposures on childhood growth and obesity. Except from child sex, the evaluation of other potential modifiers in previous studies has been scarce. We found little evidence that the effects of DDE on overweight at 1 years of life may be only seen in children exclusively breast-fed for shorter periods (≤4 months) compared to children breast-fed for longer periods and that the effects of HCB on rapid weight gain in the first 6 months of life may be only seen in children of mothers with normal pre-pregnancy BMI status compared to those of overweight mothers (Paper II). Further, in the INMA-Menorca cohort we found a small suggestion, that prenatal DDT exposure may increase overweight risk at 7 years of age only in children with fat intakes at or above the median compared to children with fat intakes below the median. These findings should be replicated in other settings as some of them could be chance findings due to the multiple comparisons tested. Future studies should be conducted in larger populations improving in this way the

power for detecting effect modifiers. The elucidation of whether some groups of the population are more susceptible than others to the effects of prenatal POP exposures on childhood growth and obesity can have important implications for public health as it may provide in the future recommendations related to effect modifiers (eg breastfeeding duration, high-fat diet) that could prevent the development of obesity in children prenatally exposed to POPs.

6.3.2 BPA, Phthalates and Childhood Growth and Obesity

The effects of prenatal exposure to BPA and phthalates on growth and obesity have only recently started to be evaluated in humans. Our study on the effects of prenatal BPA exposure on childhood growth and obesity is one of the two studies published so far (Paper IV; Harley et al. 2013) while no published prospective data are currently available on the effects of prenatal phthalate exposure on childhood growth and obesity. Thus, our studies (Papers IV and VI) contribute importantly in the lack of prospective evidence in this emerging field of research.

A recent systematic review of human studies evaluating the potentially obesogenic effects of BPA concluded that almost all studies conducted so far in both children and adult populations have relied on one single measure of BPA concentrations in blood or urine samples and demonstrate large inconsistencies in findings (LaKind et al. 2014). From the 35 studies representing independent data sources reviewed, only 7 evaluated associations between BPA exposure and obesity outcomes in children and/or adolescents: 2 studies used US NHANES survey data (Bhandari et al. 2013; Trasande et al. 2012), 3 more studies were conducted in US populations (Harley et al. 2013; Wolff et al. 2007 and 2010) and 2 studies were conducted in Chinese populations (Li et al. 2013; Wang et al. 2012). All cross-sectional studies assessed associations between single spot-urine BPA concentrations and BMI measurements standardised for age and sex. The two NHANES and

the two Chinese studies suggested positive associations with BMI in children and adolescents (Bhandari et al. 2013; Trasande et al. 2012; Li et al. 2013; Wang et al. 2012) while the other two US studies suggested null associations at school-aged children (Wolff et al. 2007 and 2010). The study conducted by Harley et al. (2013) found in cross-sectional analysis a positive association between BPA single spot-urine measurements and child BMI, waist circumference and % fat mass at 9 years of age in a US Latina population. However, in the same population maternal BPA urine concentrations during pregnancy, using the average of two spot-urine measurements, similarly to our methods, were negatively associated with BMI Z-scores from 2 to 9 years of age and with % fat mass at 9 years of age in girls while no associations with obesity outcomes was shown in boys. To the contrary, in our population with higher levels of BPA exposure compared to those detected in the US Latina mothers, we found weak positive associations between prenatal BPA exposure and child BMI and waist circumference Z-scores at 4 years of age and associations were not shown to be modified by child sex. Whether prenatal BPA exposure at different ranges influences childhood growth and obesity needs to be further explored in future prospective studies conducted in larger populations and assessing exposure using multiple spot-urine samples. The inconsistencies in findings between the cross-sectional and prospective analyses conducted by Harley et al. (2013) provide evidence that conclusions about the potential effect of prenatal BPA exposure on child growth and obesity cannot be driven based on cross-sectional data.

Current human evidence on the associations between phthalate exposure and obesity is similarly inconsistent as for BPA. A recent systematic review (Goodman et al. 2014) identified 7 cross-sectional studies conducted in children, out of which 1 was conducted in a Chinese population (Wang et al. 2013), 1 in a German population (Kasper-Sonnenberg et al. 2012), 1 in a Danish population (Boas et al. 2010) and 4 studies in US populations

(Hatch et al. 2008; Teitelbaum et al. 2012; Trasande et al. 2013b; Wolff et al. 2010). All studies assessed exposure measuring phthalate metabolites in one single spot-urine sample and evaluated associations with BMI, waist circumference, overweight and/or obesity status based on BMI at different ages from early childhood to late puberty. The sum of high molecular weight phthalates has been associated with increased BMI in Chinese children and adolescents in one previous study (Wang et al. 2013) but null associations have been suggested in three other US studies (Teitelbaum et al. 2012; Trasande et al. 2013b; Wolff et al. 2010). Similarly, inconsistent results are observed between studies for the sum of DEHP metabolites or MEHP studied separately. The sum of low molecular weight phthalates has been positively associated with obesity outcomes in three of these studies (Trasande et al. 2013b; Teitelbaum et al. 2012; Wang et al. 2013) while MEP was negatively correlated to child BMI in the Danish study and null associations have been shown between the sum of low molecular weight phthalates (Wolff et al. 2010) or MEP separately (Hatch et al. 2010) and child BMI in other studies. In our study (Paper VI), we evaluated prenatal exposure using the average of two spot-urine measurements and we found negative associations between the sum of high molecular weight phthalates (including DEHP metabolites and MBzP) and weight gain in the first 6 months of life and repeated BMI Z-score measurements up to 7 years of age in boys while some suggestion for positive associations with BMI Z-scores was shown in girls. The sum of low molecular weight phthalates (including MEP, MiBP and MnBP) was not associated with any of the obesity outcomes evaluated. Because it was recently suggested that phthalate exposure may increase systolic blood pressure in children aged 6-19 years (Trasande et al. 2013a) we further evaluated associations with repeated child blood pressure measurements at 4 and 7 years of age. Both high and low molecular weight phthalate sums were shown to decrease child systolic blood pressure in girls but not in boys, while null associations were shown with diastolic blood pressure in either sexes (Paper VI). Our

findings need to be replicated in other prospective studies however, the inconsistencies in findings between our study and previous studies provide further evidence that cross-sectional data have limited value in elucidating the effects of prenatal exposure to phthalates and child health outcomes.

6.3.3 Variability and Predictors of BPA and Phthalate Exposure in Pregnant Women

In the last few years, a growing number of studies conducted in different countries has assessed the reproducibility and determinants of exposure to BPA and phthalates due to the concerns raised about the potential health adverse effects linked to the exposure to these environmental pollutants. However, studies conducted in pregnant women are very few. This is an important consideration because maternal exposure during pregnancy may be particularly hazardous for the fetus and further because within-individual variability may differ in pregnant compared to non-pregnant women due to changes in diet, habits (eg smoking, time spend in indoor environments) and the use of consumer products (eg personal-care products) and/or physiological changes during gestation that could influence the metabolism of these environmental pollutants. Further, exposure patterns may differ in the different populations due to variations in sources of exposure and variations in individuals' behaviour. Thus, the reproducibility of measurements and determinants of exposure shown in one population may not always be generalizable to other populations. This is supported by our findings related to predictors of exposure to BPA and phthalates in pregnant women residing in Spain (Papers III and V). Although we found some consistent results about BPA predictors (mainly sociodemographic characteristics) compared to those shown in previous studies, we have also detected differences in the associations shown across countries. For example, although the reproducibility of the urine measurements of BPA and most of the phthalate metabolites measured were poor in agreement with the findings shown in

previous pregnancy cohort studies, the reproducibility of urine measurements of MEP and MnBP was shown to be substantially lower in our population compared to other non-European pregnant women populations (Adibi et al. 2008; Braun et al. 2012; Cantonwine et al. 2014). This finding suggests that the reproducibility of measurements at least for some of these biomarkers may be population-specific. An other example, related to exposure predictors is the evidence we found that the consumption of canned fish during pregnancy may increase the levels of BPA exposure in pregnant women in contrast to the findings previously shown in other settings (LaKind and Naiman 2011). Canned fish consumption and especially the consumption of canned tuna is very frequent in Spain, thus this finding may have important public health implications in this population but perhaps not in other populations where canned fish consumption is less frequent. The identification of predictors in pregnant women provide valuable input for the improvement of exposure assessment in future studies and importantly may contribute in providing effective recommendations for behaviour changes with the aim to reduce exposure levels during pregnancy.

6.4 Implications for Public Health

In the absence of a "magic pill" that may effectively treat the occurrence of obesity, disease prevention is very critical. Although further research is required to elucidate the role of early-life exposures to environmental pollutants on the Global Obesity Epidemic, the current state of evidence suggest that *in utero* exposures to POPs, BPA and phthalates even at the low-levels currently detected in the population may influence child postnatal growth. A growing number of studies suggests hazardous effects of early-life exposures to these environmental pollutants on other child health outcomes beyond the scope of this thesis including neurodevelopment, reproductive and respiratory health (Meeker et

al. 2012). Thus, studies evaluating these effects provide important insights into the development of new, more effective, strategies for the early prevention of obesity and other important diseases. Taking into account the existing evidence, the actual environmental regulations related to these pollutants should be reconsidered and/or behaviour changes should be encouraged to reduce the levels of exposure in the general population.

DDT has been the most controversial pesticide of the 20th century. Fifty years since the awareness raised by the book "Silent Spring" of Rachel Carson (1962) highlighting the toxic effects of DDT on wildlife and despite the significant efforts of public health professionals that have led to ban its use in many countries, today DDT is still used in developing countries for vector control. DDT is claimed to be "safe" and the most cost-effective method of saving lives from malaria by some organizations (Tren and Roberts 2010). However, the adverse health outcomes suggested by many studies, including the studies conducted in INMA, even at the low-levels of exposure that are nowadays detected in populations all over the world, indicate that the use of DDT should not be considered harmless. Although DDT has contributed in decreasing the number of cases and deaths from malaria in the past, in recent years the application of DDT is proven to be ineffective in many regions due to mosquito resistance and further, other effective chemical and nonchemical methods for malaria control are now available (van den Berg 2009). Given the current state of evidence, effective alternatives to DDT for vector control less harmful for environment and humans should be advocated. Recommendations aiming to decrease the levels of POP exposure in pregnant women and children through the reduction of the consumption of foods that are important sources of exposure, such as fish, have shown low effectiveness in recent simulation studies (Binnington et al. 2014). Thus, governmental regulations eliminating the use of POPs shown to be hazardous for humans may be the only effective way for reducing the levels exposure in the population.

Our findings suggest that changes in population behaviour may contribute in reducing the levels of exposure to BPA and phthalates, such as could be the reduction in the consumption of packaged and canned foods and in the particular case of phthalates the less frequent use of household cleaning products during pregnancy. This may have important public health implications, however because exposure to these widely currently used contaminants occur from multiple sources, pathways and routes and it is shown to depend on many individual factors (eg sociodemographics), the most effective way to reduce exposure would be at the level of environmental regulations. The actual regulations of the European commission that ban the use of BPA and phthalates in some child-care articles, although may contribute in reducing the levels of exposure in children, have limited value in reducing the levels of exposure in other groups of the population such as pregnant women. This is of particular concern as the most sensitive exposure period for the potentially health adverse effects of these pollutants is suspected to be fetal life and thus regulations aiming to reduce exposure levels in pregnant women are much needed. Advocating the precautionary principle, France is currently extending the EU regulations for the ban of BPA to food containers and other products which can be a good example to follow also in other places. Particularly attention should be paid though so that chemicals suspected to be hazardous, such as BPA and phthalates, are not replaced by new chemicals for which we know less about their health adverse effects and thus, may be more toxic for humans. This risk can be minimised by taking into account the scientific evidence on hazardous effects in the production of new chemicals. Protocols, such as the recently proposed “Tiered Protocol for Endocrine Disruption-TiPED” (www.tipedinfo.com), that can be applied in the chemical industry for the development of new chemicals that do not disrupt the endocrine systems and thus may be safer for both the environment and humans, are particularly usefull for this purpose.

Table 6.2 Main Characteristics and Findings of Prospective Studies on the Effects of Prenatal DDE Exposure on Childhood Growth and Obesity (studies ordered by levels of exposure)

Reference	Study population, year; Country	DDE exposure assessment	DDE concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Delvaux 2014	114 children from the Flemish Environment and Health Study, recruitment year: 2002-2006; Flanders, Belgium	Cord serum at birth	*Relatively low* Median: 40* IQ Range: 22-73*	**BMI-for-age-and-sex at 7-9 years** -Standardization using the British 1990 growth referent **Sum of 4 Skinfolds (biceps, triceps, subscapular, suprailiacal) at 7-9 years** **Waist-to-height ratio at 7-9 years**	Positive association with waist-to-height ratio in girls. Null associations with other outcomes in girls. Null associations with all outcomes in boys.	**Waist-to-height ratio at 7-9 years:** ns overall **+ girls** ns boys **BMI-for-age-and-sex/ Sum of 4 Skinfolds at 7-9 years:** ns overall ns girls ns boys
(Continued)						

Reference	Study population, year; Country	DDE exposure assessment	DDE concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Valvi 2014 – Paper II **(subcohort analysis in Mendez 2011)**	1285 infants from the INMA-birth cohort studies, recruitment year: 2003-2008; Spain	Pregnancy maternal serum (1st trimester)	*Relatively low* GM (GSD): 132 (2.4) Q1: ≤73.6 Q2: >73.6-118.8 Q3: 118.8-203.1 Q4: >203.1	**Rapid weight gain 0-6 months** (Weight-for-age-and-sex z-score difference 0-6 months >0.67 SDs)	Positive association with rapid weight gain. Associations mainly seen in boys, null associations in girls (P-interaction=0.04). No effect modification by maternal pre-pregnancy BMI and exclusive breastfeeding duration.	**Relatively low DDE and rapid weight gain 0-6 months:** **+ overall** **+ boys** ns girls
				Overweight at 14 months (BMI-for-age-and-sex z-score ≥85th percentile) -Standardization using the WHO referent	Positive association with overweight. No effect modification by child sex. Associations mainly seen in infants exclusively breastfed for a shorter period (≤4 months), null associations in infants breastfed for longer periods (P-interaction=0.04). No effect modification by maternal pre-pregnancy BMI.	**Relatively low DDE and overweight at 14 months:** **+ overall**

Valvi 2012 – Paper I	344 children from the INMA-Menorca birth cohort study, recruitment year: 1997-1998; Spain	Cord serum at birth	*Relatively low* Median: 173* IQ Range: 95-323* Mean (SD): 276 (340)* T1: <117* T2: 117-250* T3: >250*	**Overweight at 7 years** (BMI-for-age-and-sex z-score ≥85th percentile) -Standardization using the CDC growth charts	Positive non-linear association with overweight. Potentially enhanced effects shown in girls compared to boys (P-interaction=0.18). No effect modification by child high-fat intake. Associations not shown to be confounded by postnatal DDE exposure measured in child serum at 4 years (subgroup analysis).	**Relatively low DDE and overweight at 7 years:** **+ overall** **+ girls** ? boys
Verhulst 2009	138 infants, recruitment year: 2002-2004; Flanders, Belgium	Cord serum at birth	*Relatively low* Mean (SD): 212 (243) Range: 4.3-108.3	**BMI-for-age-and-sex z-scores from 1 to 3 years of age and at single time points** (1, 2 and 3 years of age) -Reported by the parents -Standardization using national references	Week positive association with BMI z-score from 1 to 3 years of age. -Enhanced effects in infants of mothers who smoked in pregnancy compared to infants of non-smokers (P-interaction=0.06).	**Relatively low DDE and BMI z-score at 1-3 years:** **+ overall**
(Continued)						

Reference	Study population, year; Country	DDE exposure assessment	DDE concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Høyer 2014	1109 children from the INUENDO birth cohort, recruitment year: 2002-2004; Greenland (n=525), Poland (n=92), Ukraine (n=492)	Pregnancy maternal serum	*Moderate* Greenland Median: 300 10-90 perc.: 75-954 Ponland Median: 385 10-90 perc.: 160-718 Ukraine Median: 639 10-90 perc.:329-1303	**BMI-for-age-and-sex at 5-9 years** -Self reported or measured -Standardization using the WHO referent	Null association with BMI in pooled analyses. (Negative association with BMI in Ukraine, null associations in other regions).	**Moderate DDE and BMI-for-age-and-sex at 5-9 years:** ns overall
Karmaus 2009	259 female adults from the Michigan fisheater cohort, time of pregnancy: 1950-1980; US	Maternal repeated serum post partum samples– Extrapolation of exposure levels back to time at birth	*Moderate* Median: 700* Q1: <250* Q2: 250-483* Q3: 483-1017* Q4: 1017-1567*	**Weight, Height BMI at 20-50 years** -Self-reported and measured	Positive associations with BMI and weight. Null associations with height.	**Moderate DDE and Weight/BMI at 20-50 years:** **+ women**

Garced 2012	253 infants, recruitment year: 2001-2005; Mexico	Pregnancy maternal serum (3 samples; in 1st, 2nd and 3rd trimesters of pregnancy)	*Moderate* 1st trimester GM (GSD): 1105 (2.7) 2nd trimester GM (GSD): 843 (2.9) 3rd trimester: GM (GSD): 711 (3.0)	**Weight/Weight-for-length/BMI-for-age-and-sex difference between birth and 1 year of age** -Standardization using the WHO referent	Null associations with weight, weight-for-length and BMI z-score difference between birth and 1 year of age. No effect modification by child sex and breastfeeding duration.	**Moderate DDE and Weight/Weight-for-length/BMI-for-age z-score difference between birth and 1 year of age:** ns overall ns boys, ns girls
Warner 2013 and 2014	261 children from the Center for the Health Assessment of Mothers and Children of Salinas study, recruitment year: 1999-2000; US (Mexicans)	Pregnancy maternal serum	*Moderate* Median: 1104 IQ Range: 613-2710	**BMI-for-age-and-sex z-score and waist circumference-for-age≥90th percentile at 2, 3.5, 5, 7 and 9 years of age** -Standardization using the CDC growth charts **Body fat % (foot-to-foot bioimpedance) at 9 years of age**	Positive associations with all anthropometric measures at 9 years in boys but null associations in girls (P-sex interaction=0.15 for BMI z-scores, 0.04 for overweight and 0.08 for waist circumference). Null associations with anthrometric measures at earlier ages. No effect modification by child sex, breastfeeding status or maternal pre-pregnancy BMI. Null associations with body fat %. No effect modification by child sex.	**Moderate DDE-and BMI z-score and overweight/elevated waist circumference at 9 years:** ns overall **+ boys** ns girls **Moderate DDE and body fat % at 9 years:** ns overall ns boys, ns girls
(Continued)						

Reference	Study population, year; Country	DDE exposure assessment	DDE concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Gladen 2000	594 children from the North Carolina Infant Study, recruitment year: 1978-1982; US	Index of transplacental exposure based on breast milk, maternal blood, cord blood and placenta samples	*Moderate* Median: 2100* 95th percentile: 5800*	**Weight and height at 10-16 years** -Self-reported -Unstandardized for age and sex	Positive associations with weight and height in boys. Null associations with all outcomes in girls. (Associations overall not reported). Associations shown for prenatal but not for postnatal exposure through breastfeeding.	**Moderate DDE and weight and height at 10-16 years:** **+ boys** ns girls
Cupul-Uicab 2013	1809 children from the US Collaborative Perinatal Project, recruitment year: 1959-1965; US	Pregnancy maternal serum (3rd trimester)	*Relatively high* Median: 4098* IQ Range: 2822-6058*	**Overweight and obesity based on age-and-sex-specific percentiles at 7 years** -Standardization using the IOTF-Cole et al. criteria	Null associations with overweight and obesity. No effect modification by child sex, maternal smoking and pre-pregnancy BMI.	**Relatively high DDE and overweight/obesity at 7 years:** ns overall ns boys ns girls
Gladen 2004	304 boys from the Philadelphia Blood Pressure Project, recruitment year: 1959-1966; US	Pregnancy maternal serum	*Relatively high* Median: 5700 Range: 1000-25000 Q1:<3000 Q2: 3000-5.999	**Repeated BMI and skinfold (subscapular, triceps) measures between 10-20 years of age**	Null associations with BMI and skinfolds.	**Relatively high DDE and BMI/ skinfolds at 10-20 years:** ns boys

			Q3: 6000-8999 Q4: 9000-11.999 Q5: ≥12.000	-Unstandardized for age and sex		
Jusko 2006	399 children from the Child Health and Development Study, recruitment year: 1964-1967; US	Pregnancy maternal serum	*Relatively high* Mean: 6850 SD:4800 Median: 5880 IQ range: 3900-8560	**Weight and height for-sex-and-race z-scores at age 5 years** -Methods for standardization not specified	Null associations with weight and height. No effect modification by child sex.	**Relatively high DDE and weight/height z-scores at 5 years:** ns overall ns boys ns girls
Cupul-Uicab 2010	789 boys, recruitment year: 2002-2003; Mexico	Maternal serum at birth	*Relatively high* Q1: <=3000 Q2: 3001-6000 Q3: 6001-9000 Q4: >=9000	**Repeated BMI-for-age measures (n=2633) between (IQ age range) 14 and 22 months of age** -Internal standardization	Null associations with BMI-for age.	**Relatively high DDE and repeated BMI z-scores at 1-2 years:** ns boys

GM: geometric mean; GSD: geometric standard deviation; IQ: interquartile range; ns: non-significant

*If only not lipid adjusted concentrations are reported in the original paper, rough transformations of the concentrations into ng/g lipid have been performed using 0,006 g lipid per mL serum (i.e, the mean lipid concentration in the INMA-birth cohort studies and elsewhere [Tang-Péronard et al. 2011]) to facilitate comparisons.

[1]Studies may have evaluated other anthropometric-based obesity outcomes other than BMI (eg weight and height separately) that are not reported in this table. Outcome measures relied on medical registries or measurements conducted by the research team if not otherwise stated.

Table 6.3 Main Characteristics and Findings of Prospective Studies on the Effects of Prenatal PCB Exposure on Childhood Growth and Obesity (studies ordered by age at outcome assessment)

Reference	Study population, year; Country	PCB exposure assessment	PCB concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Valvi 2014 – Paper II **(subcohort analysis in Mendez 2011)**	1285 infants from the INMA-birth cohort studies, recruitment year: 2003-2008; Spain	Pregnancy maternal serum (1st trimester) -Congeners: 138, 153, 180	ΣPCB GM: 93.2 GSD: 2.0 Q1: ≤65.4 Q2: >65.4-101.3 Q3: 101.1-144.6 Q4: >144.6	**Rapid weight gain 0-6 months** (Weight-for-age-and-sex z-score difference 0-6 months >0.67 SDs) **Overweight at 14 months** (BMI-for-age-and-sex z-score ≥85th percentile) -Standardization using the WHO referent	Null association with rapid weight gain. No effect modification by child sex, maternal pre-pregnancy BMI and exclusive breastfeeding duration. Null association with overweight. No effect modification by child sex, maternal pre-pregnancy BMI and exclusive breastfeeding duration.	**PCBs and rapid weight gain 0-6 months:** ns overall ns boys ns girls **PCBs and overweight at 14 months:** ns overall ns boys ns girls
Jackson 2010	44 infants from the Prospective Pregnancy and Child Development	Maternal pregnancy serum (1st trimester)	ΣPCB Median: 1170 IQ Range: 792-1411	**Weight/Length/Weight-for-length -for-age z-scores at 2 years of age**	Null associations with all outcomes No effect modifications by child sex.	**PCBs and Weight/Length/Weight-for-length -for-age z-scores at 2 years of age:**

	Study of the New York State Angler Cohort Study, recruitment year: 1996-1999; US	-86 congeners, including: 28, 52, 101, 118, 138, 153, 170, 180		-Standardization using national references		ns overall ns boys ns girls
Verhulst 2009	138 infants, recruitment year: 2002-2004; Flanders, Belgium	Cord serum at birth -Congeners: 118, 138, 153, 170, 180	ΣPCB Mean (SD): 117 (76) Range: 9-442	**BMI-for-age-and-sex z-scores from 1 to 3 years of age and at single time points** (1, 2 and 3 years of age) -Reported by the parents -Standardization using national references	Week positive association with BMI z-score from 1 to 3 years of age. No effect modification by child sex and smoking status in pregnancy. No association between PCB-118 individually analysed and child growth outcomes.	**PCBs and BMI z-score at 1-3 years:** **+ overall** **+ boys** **+ girls**
Patandin 1998	418 infants from the Dutch PCB/Dioxin Project, recruitment year: 1990-1992; Netherlands	Maternal pregnancy serum (last month of gestation) and cord serum at birth -Congeners: 118, 138, 153, 180	ΣPCB Maternal serum Median : 300 Range:100-1200 Cord serum Median : 100 Range : 0-300	**Weight/Lenght-for-age-and-sex z-score differences between 0-3, 3-7, 7-18, 18-42 months** -Standardization using national references	Negative association with weight and length z-score difference between 0-3 months. Null associations with weight and length z-score differences at later ages. No association between postnatal PCB exposure and child growth outcomes.	**PCBs and weight/Lenght-for-age-and-sex z-score differences between 0-3 months:** - overall **between 3-7, 7-18, 18-42 months:** ns overall

(Continued)

Reference	Study population, year; Country	PCB exposure assessment	PCB concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Jacobson 1990	313 children from the Michigan cohort; recruitment year:?; US	Cord serum at birth -Congeners:?	ΣPCB Mean : 400 Range :0-2100	**Weight at 4 years of age** -Unstandardized for age and sex	Negative association in girls. Null association shown in boys.	**PCBs and weight at 4 years of age:** - girls ns boys
Hertz-Picciotto 2005	399 children from the Child Health and Development Study in San Francisco Bay Area, recruitment year: 1964-1967; US	Maternal pregnancy serum -Congeners: 105, 110, 118, 137, 138, 153, 170, 180, 187	ΣPCB Mean: 697 Median: 616 5th percentile: 378 95th percentile: 1115	**Weight/Height-for-age-and-sex at 5 years of age** -Internal Standardization	Positive associations with height and potentially weight in girls. Null associations shown in boys.	**PCBs and weight/height-for-age-and-sex at 5 years of age :** ns overall ns boys **+ girls**
Valvi 2012 – Paper I	344 children from the INMA-Menorca birth cohort study, recruitment year: 1997-1998; Spain	Cord serum at birth -Congeners 28, 52, 101, 118, 138, 153, 180	ΣPCB GM: 125* GSD: 2.0* T1: <100* T2: 100-150* T3: >150*	**Overweight at 7 years** (BMI-for-age-and-sex z-score ≥85th percentile) -Standardization using the CDC growth charts	Positive association with overweight. Stronger associations shown in girls compared to boys (P-interaction=0.06). No effect modification by child high-fat intake. Associations not confounded by postnatal PCB exposure measured in child serum at 4 years (subgroup analysis)	**PCBs and overweight at 7 years:** **+ overall** **+ girls** ns/- boys

Cupul-Uicab 2013	1809 children from the US Collaborative Perinatal Project, recruitment year: 1959-1965; US	Pregnancy maternal serum (3rd trimester) -Congeners: 28, 52, 74, 105, 118, 138, 153, 179, 180, 194, 203	ΣPCB Median: 456* IQ Range: 322-653*	**Overweight and obesity based on age-and-sex-specific percentiles at 7 years** -Standardization using the IOTF-Cole et al. criteria	Null associations with overweight and obesity. No effect modification by child sex, maternal smoking and pre-pregnancy BMI.	**PCBs and overweight/obesity at 7 years:** ns overall ns boys ns girls
Høyer 2014	1109 children from the INUENDO birth cohort, recruitment year: 2002-2004; Greenland (n=525), Poland (n=92), Ukraine (n=492)	Pregnancy maternal serum PCB-153	PCB-153 Greenland Median: 107 10-90 perc.: 30-369 Ponland Median: 11 10-90 perc.: 3-24 Ukraine Median: 27 10-90 perc.: 8-54	**BMI-for-age-and-sex at 5-9 years** -Self reported or measured -Standardization using the WHO referent	Null association with BMI in pooled analyses and separate analyses by region.	**PCBs and BMI-for-age-and-sex at 5-9 years:** ns overall

(Continued)

Reference	Study population, year; Country	PCB exposure assessment	PCB concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Delvaux 2014	114 children from the Flemish Environment and Health Study, recruitment year: 2002-2006; Flanders, Belgium	Cord serum at birth -Congeners: 138, 153, 180	ΣPCB Median: 23* IQ Range: 15-42*	**BMI-for-age-and-sex at 7-9 years** -Standardization using the British 1990 growth referent **Sum of 4 Skinfolds (biceps, triceps, subscapular, suprailiacal) at 7-9 years** **Waist-to-height ratio at 7-9 years**	Null associations with all outcomes. No effect modification by child sex.	**BMI-for-age-and-sex/ Sum of 4 Skinfolds /Waist-to-height ratio at 7-9 years:** ns overall ns girls ns boys
Lamb 2006	150 children from the Columbia – Presbyterian cohort of the National Collaborative Perinatal Project, recruitemnet year: 1959-1962; US (African	Pregnancy maternal serum -Congeners, non-ortho-substituted: 15 mono-ortho-substituted: 28, 56, 66,	ΣPCB-all Median: 1400* IQ Range: 1133-1733* PCB-15 Median: 167* IQ Range: 83-283*	**Repeated weight and height measures at 4, 7 and 17 years** -Unstandardized for age and sex	ΣPCBmono, ΣPCBdi, ΣPCBtri: Negative associations in girls. Null association shown in boys. PCB-15 (non-orthosubstituted) Null association with all outcomes in girls and boys.	**PCBs and weight at 4, 7 and 17 years of age:** - girls ns boys

	Americans)	74, 105, 118, 156, 167 di-ortho-substituted: 99, 101, 138, 146, 153, 170, 180 tri-ortho-substituted: 174, 183, 187, 199, 203	ΣPCBmono Median: 450* IQ Range: 383-583* ΣPCBdi Median: 600* IQ Range: 500-833* ΣPCBtri Median: 117* IQ Range: 71-167*			
Blanck 2002	308 girls from the Michigan cohort, recruitment years: 1976-1979; US	Extrapolation of postnatal maternal serum concentrations to maternal PCB exposure at time of pregnancy -Aroclor 1254	Aroclor 1254 Median: 800* Range: up to 13000*	**Weight-adjusted-for-height at 5-24 years** -Self-reported	Negative associations with weight-adjusted for height. Associations not confounded by postnatal exposure through breast-milk.	**PCBs and weight-adjusted-for-height at 5-24 years of age:** - girls

(Continued)

Reference	Study population, year; Country	PCB exposure assessment	PCB concentrations in ng/g lipid*	Outcome[1]	Results	Summary
Gladen 2000	594 children from the North Carolina Infant Study, recruitment year: 1978-1982; US	Index of transplacental exposure based on breast milk, maternal blood, cord blood and placenta samples	ΣPCB Median: 1500* 95th percentile: 3300*	**Weight and height at 10-16 years** -Self-reported -Unstandardized for age and sex	Positive associations with weight adjusted for height in white girls. Null associations with all outcomes in boys. (Associations overall not reported). Associations shown for prenatal but not for postnatal exposure through breastfeeding.	**PCBs and weight and height at 10-16 years:** **+ girls** ns boys
Karmaus 2009	259 female adults from the Michigan fisheater cohort, time of pregnancy: 1950-1980; US	Maternal repeated serum post partum samples– Extrapolation of exposure levels back to time at birth. Aroclor 1260	Aroclor 1260 Median: 400* Q1: <0.83* Q2: 0.83-323* Q3: 323-575* Q4: 575-1180*	**Weight, Height BMI at 20-50 years** -Self-reported and measured	Null associations with all outcomes.	**PCBs and Weight, Height, BMI at 20-50 years:** ns women

GM: geometric mean; GSD: geometric standard deviation; IQ: interquartile range; ns: non-significant.
*If only not lipid adjusted concentrations are reported in the original paper, rough transformations of the concentrations into ng/g lipid have been performed using 0,006 g lipid per mL serum (i.e, the mean lipid concentration in the INMA-birth cohort studies and elsewhere [Tang-Péronard et al. 2011]) to facilitate comparisons.
[1]Studies may have evaluated other anthropometric-based obesity outcomes other than BMI (eg weight and height separately) that are not reported in this table. Outcome measures relied on medical registries or measurements conducted by the research team if not otherwise stated.

7

Conclusions

- At the levels of exposure assessed, prenatal exposures to DDE and HCB and less clearly to PCBs and DDT, may influence child growth and increase obesity risk up to 7 years of life. The effects on childhood growth and obesity of at least some of the these POPs may vary according to child sex. *In vivo* and *in vitro* studies are requited to better understard the associations between POP exposures and obesity risk supported by a growing number of epidemiologic studies.

- Prenatal exposure to BPA may increase child BMI and waist circumference in early childhood but perhaps not earlier in infancy.

- Prenatal exposure to high molecular weight phthalates (DEHP metabolites and MBzP) may influence postnatal growth and obesity risk differently in boys and girls. Prenatal exposure to low molecular weight phthalates (MEP, MiBP and MnBP) did not influence postnatal growth in either boys or girls. Further, prenatal exposures to high and low molecular weight phthalates may decrease systolic blood pressure in childhood. Inconsistencies in findings with those previous shown in few cross-sectional studies highlight the necessity to study these effects in prospective studies.

- The consumption of canned fish may increase the levels of BPA exposure and the frequent use of cleaning products may increase the levels of phthalate exposure in pregnant women. Other sociodemographic and lifestyle characteristics may further determine the levels of phthalate exposure in pregnant women residing in Spain.

- Given the rising number of studies suggesting adverse effects of early-life exposure to POPs, BPA and phthalates on growth and other health outcomes such as neurodevelopment, reproductive and respiratory health, the actual environmental regulations should be reconsidered and behaviour changes should be encouraged to reduce the levels of exposure in the general population.

8

Future Research

The influence of developmental exposures to chemicals with endocrine disrupting properties on growth and obesity risk throughout the life course is a challenging and a very wide field for future research. Although, sufficient evidence supporting the Environmental Obesogen Hypothesis and the implications of the DOHaD concept in the development of obesity is already available, some of the great challenges in the years to come will be: 1) to identify the environmental pollutants that influence growth and increase the risks of obesity and other chronic metabolic diseases, 2) to detect the most critical windows of exposure susceptibility to these pollutants, 3) to elucidate the potential interrelationships between environmental pollutants and other putative causes, such as genetic predisposition and diet, in the development of obesity, and 4) to better understand the mechanisms that underlie these effects.

Important future aims for the INMA birth cohort studies include the continuous follow-up of children and the evaluation of the persistency of the associations shown in the studies that form part of this thesis at later ages. We aim to evaluate the effects of prenatal exposure to POPs on obesity risk at later ages in the INMA-new birth cohort studies integrating data also from the INMA-Asturias birth cohort where POP determinations in maternal serum are now available. Potential effect modifications by child sex, genetic and dietary factors that are hypothesised to influence these effects will be considered in this new analysis conducted in a larger population. Further, the INMA-Menorca cohort where children have now reached adolescence offers the opportunity not only to evaluate the persistency of the associations at later ages but further to evaluate the influence of puberty status on these associations. The evaluation of the persistency of the associations shown between prenatal

exposure to BPA and phthalates with obesity outcomes at later ages is also planned. Future studies conducted in INMA will integrate data on longitudinal growth trajectories, direct measures of fat mass and biomarkers of metabolic dysfunction which are important considerations also for future studies conducted in other settings as it has been discussed more above (*see Chapter 6*).

More prospective studies are currently needed to elucidate the influence of early-life exposures to POPs, BPA and phthalates on growth and obesity. Future studies should aim to evaluate the influence of both prenatal and postnatal exposures and further to evaluate the suggested obesogenic effects of other environmental pollutants for which, despite the growing body of experimental evidence, human evidence is still scarce or inexistent. These environmental pollutants include traffic-related pollutants and other new emerging pesticides and plasticizers that are currently widely used. New multidisciplinary collaborative projects in the field of environmental obesogens integrating both experimental and epidemiologic data, such as the recent FP7/2009 European Project OBELIX (Legler et al. 2013), can provide new insights into the current state of evidence and help to bridge the existing gaps in evidence from experimental and human studies. Further, collaborative projects between the environmental birth cohort studies that are currently evaluating the influence of early-life exposure to environmental pollutants on childhood obesity as well as on other health outcomes are required with the aim to improve causal inference in this field (Vrijheid et al. 2012).

Little efforts have been made so far to evaluate any potential synergies in the effects of multiple chemical exposures on health. However, humans are simultaneously exposed to many environmental pollutants throughout their life course that may interact at different degrees on the development of diseases such as obesity. The concept of the "exposome" was recently suggested to capture the totality of environmental exposures from conception

and onwards throughout the life course that can interact with individuals genetic predisposition on the development of human diseases (Wild 2005 and 2012). More holistic exposure approaches are required in epidemiologic studies to elucidate the influences of the "exposome" on human health. CREAL is currently leading one of the first multidisciplinary projects in this field, the EF7 European project "HELIX" that aims to exploit novel tools and methods to address the health effects of the "early-life exposome" (Vrijheid et al. 2014). HELIX and other similar research projects aiming to improve exposure assessment, to integrate data on biological mechanisms using new-techniques, such as "omics", and to advance the statistical tools available for evaluating these associations will importantly contribute in elucidating in the future the role of the "exposome" on the development of human diseases such as obesity.

The ongoing and future research conducted in the wide and multidisciplinary field of environmental exposures and health expands our knowledge about health risks linked to the low levels of environmental chemical exposures that are currently detected in the general population. From a public health point of view, this has great implications for public health as it can contribute in developing more efficient strategies for the early prevention of severe chronic disorders and diseases, such as obesity, that are leading causes of the global morbidity and mortality. Thus, future research in this field can have important long-term benefits on human health and the financial costs that are currently linked to the treatment of chronic diseases. These long-term benefits should be carefully considered in the short-sighted perspectives currently applied with the aim to reduce research funding as an "effective" austerity measure against the current financial crisis.

"All our science, measured against reality,
is primitive and childlike – and yet it is
the most precious thing we have."

ALBERT EINSTEIN
(1879-1955)

Annex

About the Author

Damaskini Valvi received her graduate degree in Medicine at the University of Crete (Greece) in 2007 and her Master of Public Health at the Pompeu Fabra University and Autonoma University of Barcelona (Spain) in 2010. She joined the Centre for Research in Environmental Epidemiology (CREAL) in 2008 where the present thesis has been executed from 2010 to present. As part of her PhD training, she did a 4-month stay as a research scholar at the Gillings School of Public Health in the University of North Carolina-Chapell Hill, US (November 2012-March 2013). A summary of the research activity of the author during the thesis is provided below.

List of Publications

Govarts E, Casas M, Schoeters G, Eggesbø M, Valvi D, Nieuwenhuijsen M, Bonde JP, on behalf of the CHICOS Consortium. 2014. Prenatal exposure to PCB-153 and Decreased Birth Weight: the role of Gestational Weight Gain. Environ Health Perspect 122(4):A89. (*Letter*)

Valvi D, Méndez MA, García-Esteban R, Ballester F, Ibarluzea J, Goñi F, Grimalt JO, Llop S, Santa Marina L, Vizcaino E, Sunyer J, Vrijheid M. 2014. Prenatal exposure to persistent organic pollutants and rapid weight gain and overweight in infancy. Obesity 22: 488–496.

Valvi D, Casas M, Mendez MA, Ballesteros-Gómez A, Luque N, Rubio S, Sunyer J, Vrijheid M. 2013. Prenatal Bisphenol A Urine Concentrations and Early Rapid Growth and Overweight Risk in the Offspring. Epidemiology 24(6):791-799.

Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin A, Fernandez M, Koch HM, Mendez MA, Sunyer J, Rubio S, Vrijheid M. 2013. Dietary and Sociodemographic Determinants of Bisphenol A Urine Concentrations in Pregnant Women and Children. Environ Int 56:10-18.

Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M. 2012. Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: A prospective Birth Cohort Study. Environ Health Perspect 120(3):451-457.

Other papers submitted

Valvi D, Casas M, Romaguera D, Monfort N, Ventura R, Martinez D, Sunyer J, Vrijheid M. Prospective Evidence on the Effects of Phthalate Exposure on Childhood Growth and Blood Pressure. Submitted to Environ Health Perspect, June 2014.

Villar-Vidal M, Amiano P, Rodriguez Bernal C, Santa Marina L, Mozo I, Vioque J, Navarrete-Muñoz EM, Romaguera D, Valvi D, Fernández-Somoano A, Tardón A, Ibarluzea J. Compliance of nutritional recommendations of Spanish pregnant women according to sociodemographic and lifestyle characteristics. Submitted to Maternal and Child Nutrition, March 2014.

Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, Vrijheid M. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Submitted to Int J Hyg Environ Health, February 2014.

Morales E, Rodriguez A, Valvi D, Iñiguez C, Esplugues A , Vioque J, Santa Marina L, Jiménez A, Espada M, Rodriguez Dehli C, Fernández-Somoano A, Vrijheid M, Sunyer J. Deficit of vitamin D

in pregnancy and growth and overweight in the offspring. Submitted to JAMA, January 2014.

Casas M, Nieuwenhuijsen M, Martínez D, Ballester F, Basagaña X, Basterrechea M, Chatzi L, Chevrier C, Eggesbø M, Fernandez MF, Govarts E, Guxens M, Grimalt JO, Iszatt N, Kasper-Sonnenberg M, Kiviranta H, Kogevinas M, Palkovicova L, Ranft U, Schoeters G, Patelarou E, Petersen MS, Torrent M, Trnovec T, Valvi D, Toft GV, Weihe P, Weisglas-Kuperus N, Wilhelm M, Wittsiepe J, Vrijheid M, Bonde JP. Prenatal exposure to PCB-153, p,p'-DDE and birth outcomes in 11 European birth cohorts: dose-response relationship and effect modifiers. Submitted to Environ Int, February 2014.

Summary of PhD Training and Teaching

Oral Presentations

Invited Speaker

"Early-life exposure to persistent organic pollutants and childhood growth and obesity", PPTOX IV (Prenatal Programming and Toxicity) Conference, 26-29th October 2014, Boston, MA, US.

"Early-life exposure to environmental hazards and childhood obesity", Scientific meeting on Early-life Exposures and Prevention Strategies, 24th March 2014, Heraklion, Greece.

Others

"Prenatal exposure to persistent organic pollutants and cardiometabolic function in children of preschool age", INMA 2013 annual scientific meeting, 14-15th November 2013, Donostia-San Sebastian, Spain.

"Prenatal bisphenol A urine concentrations and rapid growth and overweight in the first years of life", Environment and Health – ISEE, ISES, ISIAQ, 23rd August 2013, Basel, Switzerland.

"Prenatal Bisphenol A exposure and rapid growth and overweight in infancy", EB2013 - Experimental Biology conference, 21st April 2013, Boston, MA, US.

"Endocrine-disrupting chemicals and childhood obesity – Evidence from the Spanish INMA birth cohorts", Reproductive Perinatal Pediatric Epidemiology (RPPE) Seminar, 6th February 2013, UNC-Chapel Hill, NC, US.

"The BPA debate – first INMA results on exposure, predictors, and child health effects", CREAL scientific seminar, 29th June 2012, PRBB, Barcelona, Spain.

"INMA birth cohort study – Review on the advances of the Obesity Group", CREAL Scientific Retreat, 25th November 2011, UAB – Casa de Convalescència, Barcelona, Spain.

"Overweight and obesity classification in childhood; Which cut-offs to use?", INMA 2011 annual scientific meeting, 16-17th June 2011, Barcelona, Spain.

"Obesogenic effects of prenatal concentrations of organochlorine compounds; Does sex or a high-fat intake of the child play a role?", CREAL scientific seminar, 22nd November 2010, PRBB, Barcelona, Spain.

Poster Communications

Valvi D, Romaguera D, Horton BJ, Pritchard D, Herring AH, Vrijheid M, Sunyer J, Mendez MA. "Plasma levels of cardiometabolic biomarkers in overweight and centrally obese normal weight Spanish children of preschool age", 2nd International

Conference on Nutrition and Growth, 30th January-2nd February 2014, Barcelona, Spain.

Valvi D, Romaguera D, Horton BJ, Pritchard D, Herring AH, Vrijheid M, Sunyer J, Mendez MA. "Prenatal exposure to persistent organic pollutants and plasma levels of cardiometabolic biomarkers in early childhood", 2nd International Conference on Nutrition and Growth, 30th January-2nd February 2014, Barcelona, Spain.

Valvi D, Romaguera D, Horton BJ, Herring AH, Vrijheid M, Sunyer J, Mendez MA. "Elevated cardiometabolic risk in overweight and centrally obese normal weight Spanish children of preschool age", IUNS 20th International Congress of Nutrition, 15th-20th September 2013, Granada, Spain.

Valvi D, Mendez MA, Ballester F, Ibarluzea J, Goñi F, Grimalt JO, Vizcaino E, Sunyer J, Vrijheid M. "Prenatal exposure to persistent organic pollutants and early postnatal growth: Prospective evidence from the Spanish INMA birth cohorts", Environment and Health – ISEE, ISES, ISIAQ, 20th-23rd August 2013, Basel, Switzerland.

Casas M, Valvi D, Garcia R, Ballesteros-Gómez A, Iñiguez C, Luque N, Rubio S, Fernandez MF, Sunyer J, Vrijheid M. "Exposure to Bisphenol A during pregnancy and fetal growth in a Spanish birth cohort", Environment and Health – ISEE, ISES, ISIAQ, 20th-23rd August 2013, Basel, Switzerland.

Monfort N, Ventura R, Valvi D, Balcells G, Vrijheid M, Segura J. "Phthalates in urine as markers of blood transfusion in sports: Population concentrations of five DEHP metabolites and reference limits". 30th Cologne Workshop on Dope Analysis (Manfred Donike Workshop), February 2013, Cologne, Germany.

Valvi D, Mendez MA, Garcia R, Ibarluzea J, Santa Marina L, Goñi F, Llop S, Ballester F, Vizcaino E, Grimalt JO, Sunyer J, Vrijheid M. "Prenatal exposure to organochlorine compounds and early

postnatal growth in the Spanish INMA Cohort ", PPTOX III Conference (Prenatal Programming and Toxicity), 14-16th May 2012, Paris, France.

Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M "Persistent organic pollutants and overweight at age 6.5 years; Do sex or high-fat intakes of the child matter?", 23rd International ISEE Conference-International Society for Environmental Edidemiology, 13-16th September 2011, Barcelona, Spain.

Valvi D, Mendez MA, Martinez D, Grimalt JO, Torrent M, Sunyer J, Vrijheid M "Obesogenic effects of early exposure to persistent organic pollutants: does sex or a high-fat intake of the child play a role?", 18th European Congress on Obesity – ECO2011, 25-28th May 2011, Instambul, Turkey.

Training Seminars and Courses (Offered by the Author)

"An Introduction to Directed Acyclic Graphs (DAGs) - As a method in Epidemiology and how to deal with them", seminar for researchers, statisticians and students of the RHEA birth cohort study (duration: 2 h), 27th March 2014, University of Crete, Heraklion, Greece.

"Methods for assessing nutritional status in children and adolescents", course for students of the Master in Public Health (duration: 6 hrs), Pompeu Fabra University and Autonoma University of Barcelona, 17th April 2012, Pompeu Fabra University, Barcelona, Spain.

"Prenatal environmental chemical exposures and childhood obesity – an example of environmental epidemiology", seminar for students of environmental sciences (duration: 1 h), Autonoma University of Barcelona, 29th November 2011, PRBB, Barcelona, Spain.

Supervision of Master Thesis

2013-2014. Ashley Michel Oberndorfer. "Prenatal exposures to metals and rapid weight gain and risk of overweight in the first 4 years of life". Master in Public Health (MPH), Pompeu Fabra University and Autonoma University of Barcelona, Barcelona, Spain. [with Dr. Martine Vrijheid]

Participation in Research Grant Proposals and Projects

"Childhood obesity and the risk of metabolic syndrome in adulthood: epigenetic mechanisms". Eugenio Rodriguez Pascual Foundation research grants 2013, PI: Dr. Josep C Jimenez Chillaron (San Juan de Dios Foundation - University of Barcelona, San Juan de Dios Hospital, Barcelona, Spain). *Participant of the grant proposal*. [Declined].

"Obesogenic lifestyle in pregnancy and early life and development of different growth trajectories and metabolic syndrome in children and adolescents". FIS-Institute Carlos III 2013, PI: Dr. Dora Romaguera (Centre for Research in Environmental Epidemiology- CREAL, Barcelona Spain). *Participant of the grant proposal*. [Declined].

"Exposure to emerging contaminants and childhood obesity and metabolic disruption". FIS-Institute Carlos III (Nº: PI12/01890), January 2013 - January 2015, PI: Dr. Martine Vrijheid (Centre for Research in Environmental Epidemiology- CREAL, Barcelona Spain). *Participant of the grant proposal*. [Ongoing].

"HELIX-The Human Early-Life Exposome - novel tools for integrating early-life environmental exposures and child health across Europe" (http://www.projecthelix.eu/). European Commission 7th Framework Programme (Nº: FP7-ENV-2012-308333), January 2013-June 2017, PI: Dr. Martine Vrijheid (Centre for Research in Environmental Epidemiology- CREAL, Barcelona

Spain). *Project collaborator and coordinator of the field work on obesity outcomes*. [Ongoing].

"PICOS- Nutrition, Environment, Epigenetics: Programming of Childhood Obesity". "ARISTEIA 2012" confunded by European Union and Greek State, PI: Dr. Leda Chatzi (University of Crete, Heraklion,Greece). *Participant of the grant proposal*. [Declined].

"Determinants of Childhood Obesity: The role of prenatal and early-life environmental and social factors". RecerCaixa (2010ACUP 00349), January 2011- April 2013, PI: Dr. Martine Vrijheid (Centre for Research in Environmental Epidemiology- CREAL, Barcelona Spain). *Participant of the grant proposal*. [Completed].

"CHICOS-Developing a Child Cohort research strategy for Europe" (http://www.chicosproject.eu/cohorts/), January 2010- December 2012, PI: Dr. Martine Vrijheid (Centre for Research in Environmental Epidemiology- CREAL, Barcelona Spain). *Collaborator in the working group of fetal and postnatal growth.* [Completed].

Reviews for Peer-Reviewed Scientific Journals

Environmental Health Perspectives (1 review), Environment International (3 reviews), American Journal of Epidemiology (1 review), International Journal of Obesity (2 reviews), Environmental Science and Technology (1 review), Food and Chemical Toxicology (1 review).

Awards and Fellowships

2012 Student Travel Award Winner - Society of Toxicology - PPTOX III Conference for the abstract presentation: Valvi D, Mendez MA, Garcia R, Ibarluzea J, Santa Marina L, Goñi F, Llop S, Ballester F, Vizcaino E, Grimalt JO, Sunyer J, Vrijheid M. "Prenatal exposure to organochlorine compounds and early postnatal growth in the Spanish INMA Cohort ", PPTOX III (Prenatal Programming and Toxicity) Conference, 14-16th May 2012, Paris, France.

2010 Institute Carlos III predoctoral fellowship – Spanish Ministry of Innovation Science, Register number: FI10/00399 (Duration: 4 years).

2009 FPU predoctoral fellowship – Spanish Ministry of Education, Register number: Nº: 550206789 (Duration: 4 years). [Rejected by the applicant].

References

Adetona O, Horton K, Sjodin A, Jones R, Hall DB, Aguillar-Villalobos M, Cassidy BE, Vena JE, Needham LL, Naeher LP. 2013. Concentrations of select persistent organic pollutants across pregnancy trimesters in maternal and in cord serum in Trujillo, Peru. Chemosphere 91:1426-1433.

Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, Nelson H, Bhat HK, Perera FP, Silva MJ, Hauser R. 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ Health Perspect 116:467-473.

Armstrong BG. 1998. Effect of measurement error on epidemiological studies of environmental and occupational exposures. Occup Environ Med 55:651-656.

Arsenescu V, Arsenescu RI, King V, Swanson H, Cassis LA. 2008. Polychlorinated biphenyl-77 induces adipocyte differentiation and proinflammatory adipokines and promotes obesity and atherosclerosis. Environ Health Perspect 116:761-768.

Baillie-Hamilton PF. 2002. Chemical toxins: a hypothesis to explain the global obesity epidemic. J Altern Complement Med 8:185-192.

Baker D, Nieuwenhuijsen. 2008. Environmental epidemiology: study methods and application. Oxford, New York, Oxford University Press. (Book)

Barker DJ, Osmond C. 1986. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1:1077-1081.

Barker DJ. 2007. The origins of the developmental origins theory. J Intern Med 261:412-417.

Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. 2012. Developmental origins of non-communicable disease: Implications for research and public health. Environ Health 11:42.

Basterrechea M, Lertxundi A, Iniguez C, Mendez M, Murcia M, Mozo I, Goni F, Grimalt J, Fernandez M, Guxens M. 2014. Prenatal exposure to hexachlorobenzene (HCB) and reproductive effects in a multicentre birth cohort in Spain. Sci Total Environ 466-467:770-776.

Bastien M, Poirier P, Lemieux I, Despres JP. 2014. Overview of epidemiology and contribution of obesity to cardiovascular disease. Prog Cardiovasc Dis 56:369-381.

Bastos SL, Kamstra JH, Cenijn PH, van Rijt LS, Hamers T, Legler J. 2013. Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicol In Vitro 27:1634-1643.

Behl M, Rao D, Aagaard K, Davidson TL, Levin ED, Slotkin TA, Srinivasan S, Wallinga D, White MF, Walker VR, Thayer KA, Holloway AC. 2013. Evaluation of the association between maternal smoking, childhood obesity, and metabolic disorders: a national toxicology program workshop review. Environ Health Perspect 121:170-180.

Berthoud HR. 2012. The neurobiology of food intake in an obesogenic environment. Proc Nutr Soc 71:478-487.

Bhandari R, Xiao J, Shankar A. 2013. Urinary bisphenol A and obesity in U.S. children. Am J Epidemiol 177:1263-1270.

Binnington MJ, Quinn CL, McLachlan MS, Wania F. 2014. Evaluating the effectiveness of fish consumption advisories: modeling prenatal, postnatal, and childhood exposures to persistent organic pollutants. Environ Health Perspect 122:178-186.

Blanck HM, Marcus M, Rubin C, Tolbert PE, Hertzberg VS, Henderson AK, Zhang RH. 2002. Growth in girls exposed in utero and postnatally to polybrominated biphenyls and polychlorinated biphenyls. Epidemiology 13:205-210.

Blouin K, Veilleux A, Luu-The V, Tchernof A. 2009. Androgen metabolism in adipose tissue: recent advances. Mol Cell Endocrinol 301:97-103.

Boas M, Feldt-Rasmussen U, Skakkebaek NE, Main KM. 2006. Environmental chemicals and thyroid function. Eur J Endocrinol 154:599-611.

Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, Juul A, Main KM. 2010. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect 118:1458-1464.

Boberg J, Metzdorff S, Wortziger R, Axelstad M, Brokken L, Vinggaard AM, Dalgaard M, Nellemann C. 2008. Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. Toxicology 250:75-81.

Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TH, Vinggaard AM. 2001. Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 158:141-153.

Branchi I, Alleva E, Costa LG. 2002. Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. Neurotoxicology 23:375-384.

Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, Hauser R. 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ Health Perspect 120:739-745.

Brisbois TD, Farmer AP, McCargar LJ. 2012. Early markers of adult obesity: a review. Obes Rev 13:347-367.

Browning LM, Hsieh SD, Ashwell M. 2010. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0.5 could be a suitable global boundary value. Nutr Res Rev 23:247-269.

Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. 2001. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 98:2396-2402.

Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, Calafat AM, Crespo N, Jimenez-Velez B, Padilla IY, Alshawabkeh AN, Meeker JD. 2014. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. Environ Int 62:1-11.

Carson R. 1962. Silent Spring. Houghton Mifflin. (Book)

Casals-Casas C, Desvergne B. 2011. Endocrine disruptors: from endocrine to metabolic disruption. Annu Rev Physiol 73:135-162.

Casas M, Nieuwenhuijsen M, Martínez D, Ballester F, Basagaña X, Basterrechea M, Chatzi L, Chevrier C, Eggesbø M, Fernandez MF, Govarts E, Guxens M, Grimalt JO, Iszatt N, Kasper-Sonnenberg M, Kiviranta H, Kogevinas M, Palkovicova L, Ranft U, Schoeters G, Patelarou E, Petersen MS, Torrent M, Trnovec T, Valvi D, Toft GV, Weihe P, Weisglas-Kuperus N, Wilhelm M, Wittsiepe J, Vrijheid M, Bonde JP. 2014. Prenatal exposure to PCB-153, p,p'-DDE and birth outcomes in 11 European birth cohorts: dose-response relationship and effect modifiers. (Submitted to Environ Int)

CDC 2000. Growth charts for United States: Methods and development. Available at: http://www.cdc.gov/growthcharts/background.htm [accessed May 2014].

Chivers P, Hands B, Parker H, Beilin L, Kendall G, Bulsara M 2009. Longitudinal modelling of body mass index from birth to 14 years. Obes Facts 2:302-310.

Colborn T, Clement C. (Eds). 1992. Chemically-induced Alterations in Sexual and Functional Development. The Wildlife/Human Connection. Princeton Scientific Publishing Co. (Book)

Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 320:1240-1243.

Cole TJ, Lobstein T. 2012. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes 7:284-294.

Crinnion WJ. 2010. The CDC fourth national report on human exposure to environmental chemicals: what it tells us about our toxic burden and how it assist environmental medicine physicians. Altern Med Rev 15:101-109.

Cupul-Uicab LA, Hernandez-Avila M, Terrazas-Medina EA, Pennell ML, Longnecker MP. 2010. Prenatal exposure to the major DDT metabolite 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) and growth in boys from Mexico. Environ Res 110:595-603.

Cupul-Uicab LA, Klebanoff MA, Brock JW, Longnecker MP. 2013. Prenatal exposure to persistent organochlorines and childhood obesity in the US collaborative perinatal project. Environ Health Perspect 121:1103-1109.

de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ 85:660-667.

de Onis M, Garza C, Onyango AW, Rolland-Cachera MF. 2009. [WHO growth standards for infants and young children]. Arch Pediatr 16:47-53.

Dee A, Kearns K, Neill O, Sharp L, Staines A, Dwyer O, Fitzgerald S, Perry IJ. 2014. The direct and indirect costs of both overweight and obesity: a systematic review. BMC Res Notes 7:242.

Delvaux I, Van Cauwenberghe J, Den Hond E, Schoeters G, Govarts E, Nelen V, Baeyens W, Van Larebeke N, Sioen I. 2014. Prenatal exposure to environmental contaminants and body composition at age 7-9 years. Environ Res 132C:24-32.

Diamanti-Kandarakis E, Palioura E, Kandarakis SA, Koutsilieris M. 2010. The impact of endocrine disruptors on endocrine targets. Horm Metab Res 42:543-552.

Dieudonne MN, Pecquery R, Leneveu MC, Giudicelli Y. 2000. Opposite effects of androgens and estrogens on adipogenesis in rat preadipocytes: evidence for sex and site-related specificities and possible involvement of insulin-like growth factor 1 receptor and peroxisome proliferator-activated receptor gamma2. Endocrinology 141:649-656.

Druet C, Stettler N, Sharp S, Simmons RK, Cooper C, Smith GD, Ekelund U, Levy-Marchal C, Jarvelin MR, Kuh D, Ong KK. 2012. Prediction of childhood obesity by infancy weight gain: an individual-level meta-analysis. Paediatr Perinat Epidemiol 26:19-26.

Dubois L, Girad M. 2007. Accuracy of maternal reports of pre-schoolers' weights and heights as estimates of BMI values. Int J Epidemiol 36:132-138.

Eggesbo M, Stigum H, Longnecker MP, Polder A, Aldrin M, Basso O, Thomsen C, Skaare JU, Becher G, Magnus P. 2009. Levels of hexachlorobenzene (HCB) in breast milk in relation to birth weight in a Norwegian cohort. Environ Res 109:559-566.

Feige JN, Gerber A, Casals-Casas C, Yang Q, Winkler C, Bedu E, Bueno M, Gelman L, Auwerx J, Gonzalez FJ, Desvergne B. 2010. The pollutant diethylhexyl phthalate regulates hepatic energy metabolism via species-specific PPARalpha-dependent mechanisms. Environ Health Perspect 118:234-241.

Frederiksen H, Nielsen JK, Morck TA, Hansen PW, Jensen JF, Nielsen O, Andersson AM, Knudsen LE. 2013. Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs. Int J Hyg Environ Health 216:772-783.

Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. 2009. Risk factors and adult body mass index among overweight children: the Bogalusa Heart Study. Pediatrics 123:750-757.

Fry J, Finley W. 2005. The prevalence and costs of obesity in the EU. Proc Nutr Soc 64:359-362.

Garced S, Torres-Sanchez L, Cebrian ME, Claudio L, Lopez-Carrillo L. 2012. Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and child growth during the first year of life. Environ Res 113:58-62.

Gladen BC, Ragan NB, Rogan WJ. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr 136:490-496.

Gladen BC, Klebanoff MA, Hediger ML, Katz SH, Barr DB, Davis MD, Longnecker MP. 2004. Prenatal DDT exposure in relation to anthropometric and pubertal measures in adolescent males. Environ Health Perspect 112:1761-1767.

Gluckman PD, Hanson MA. 2006a. The Developmental Origins of Health and Disease, 2006a. Advances in Experimental Medicine and Biology. Springer US. (Book Chapter)

Gluckman PD, Hanson MA. 2006b. Mismatch: Why our World no
Longer fits our Bodies. Oxford University Press: Oxford. (Book)

Goodman M, Lakind JS, Mattison DR. 2014. Do phthalates act as obesogens in humans? A systematic review of the epidemiological literature. Crit Rev Toxicol 44:151-175.

Govarts E, Nieuwenhuijsen M, Schoeters G, Ballester F, Bloemen K, de Boer M, Chevrier C, Eggesbo M, Guxens M, Kramer U, Legler J, Martinez D, Palkovicova L, Patelarou E, Ranft U, Rautio A, Petersen MS, Slama R, Stigum H, Toft G, Trnovec T, Vandentorren S, Weihe P, Kuperus NW, Wilhelm M, Wittsiepe J, Bonde JP. 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect 120:162-170.

Graversen L, Sorensen TI, Petersen L, Sovio U, Kaakinen M, Sandbaek A, Laitinen J, Taanila A, Pouta A, Jarvelin MR, Obel C. 2014. Preschool weight and body mass index in relation to central obesity and metabolic syndrome in adulthood. PLoS One 9:e89986.

Gregoire FM, Smas CM, Sul HS. 1998. Understanding adipocyte differentiation. Physiol Rev 78:783-809.

Grun F, Blumberg B. 2006. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology 147:S50-S55.

Grun F, Blumberg B. 2007. Perturbed nuclear receptor signaling by environmental obesogens as emerging factors in the obesity crisis. Rev Endocr Metab Disord 8:161-171.

Grun F, Blumberg B. 2009. Endocrine disrupters as obesogens. Mol Cell Endocrinol 304:19-29.

Guo SS, Wu W, Chumlea WC, Roche AF. 2002. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. Am J Clin Nutr 76:653-658.

Guxens M, Ballester F, Espada M, Fernandez MF, Grimalt JO, Ibarluzea J, Olea N, Rebagliato M, Tardon A, Torrent M, Vioque J, Vrijheid M, Sunyer J. 2012. Cohort Profile: The INMA--INfancia y Medio Ambiente--(Environment and Childhood) Project. Int J Epidemiol 41(4):930-40.

Han JC, Lawlor DA, Kimm SY. 2010. Childhood obesity. Lancet 375:1737-1748.

Hao C, Cheng X, Xia H, Ma X. 2012. The endocrine disruptor mono-(2-ethylhexyl) phthalate promotes adipocyte differentiation and induces obesity in mice. Biosci Rep 32:619-629.

Harley KG, Aguilar SR, Chevrier J, Tyler K, Aguirre H, Bradman A, Holland NT, Lustig RH, Calafat AM, Eskenazi B. 2013. Prenatal and postnatal bisphenol A exposure and body mass index in childhood in the CHAMACOS cohort. Environ Health Perspect 121:514-516.

Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, Webster TF. 2008. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environ Health 7:27.

Heindel JJ, vom Saal FS. 2009. Role of nutrition and environmental endocrine disrupting chemicals during the perinatal period on the aetiology of obesity. Mol Cell Endocrinol 304:90-96.

Hennig B, Reiterer G, Toborek M, Matveev SV, Daugherty A, Smart E, Robertson LW. 2005. Dietary fat interacts with PCBs to induce changes in lipid metabolism in mice deficient in low-density lipoprotein receptor. Environ Health Perspect 113:83-87.

Hernan MA, Hernandez-Diaz S, Robins JM. 2004. A structural approach to selection bias. Epidemiology 15:615-625.

Hertz-Picciotto I, Charles MJ, James RA, Keller JA, Willman E, Teplin S. 2005. In utero polychlorinated biphenyl exposures in relation to fetal and early childhood growth. Epidemiology 16:648-656.

Høyer BB, Ramlau-Hansen CH, Henriksen TB, Pedersen HS, Góralczyk K, Zviezdai V, Jönsson BA, Heederik D, Lenters V, Vermeulen R, Bonde JP, Toft G. 2014.Body mass index in young school-age children in relation to organochlorine compounds in early life: a prospective study. Int J Obes (Lond). doi: 10.1038/ijo.2014.58.

Hollingworth W, Hawkins J, Lawlor DA, Brown M, Marsh T, Kipping RR. 2012. Economic evaluation of lifestyle interventions to treat overweight or obesity in children. Int J Obes (Lond) 36:559-566.

Holtcamp W. 2012. Obesogens: an environmental link to obesity. Environ Health Perspect 120:a62-a68.

Howe LD, Tilling K, Matijasevich A, Petherick ES, Santos AC, Fairley L, Wright J, Santos IS, Barros AJ, Martin RM, Kramer MS, Bogdanovich N, Matush L, Barros H, Lawlor DA. 2014. Linear spline multilevel models for summarising childhood growth trajectories: A guide to their application using examples from five birth cohorts. Stat Methods Med Res (Epub Ahead of Print)

Howell G, III, Mangum L. 2011. Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. Toxicol In Vitro 25:394-402.

Hugo ER, Brandebourg TD, Woo JG, Loftus J, Alexander JW, Ben Jonathan N. 2008. Bisphenol A at environmentally relevant doses inhibits adiponectin release from human adipose tissue explants and adipocytes. Environ Health Perspect 116:1642-1647.

IASO International Association for the Study of Obesity. Obesity Facts 2012. Available at: http://www.worldobesity.org/aboutobesity/ [accessed May, 2014].

Jackson LW, Lynch CD, Kostyniak PJ, McGuinness BM, Louis GM. 2010. Prenatal and postnatal exposure to polychlorinated biphenyls and child size at 24 months of age. Reprod Toxicol 29:25-31.

Jacobson JL, Jacobson SW, Humphrey HE. 1990. Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol 12:319-326.

Janesick A, Blumberg B. 2011. Endocrine disrupting chemicals and the developmental programming of adipogenesis and obesity. Birth Defects Res C Embryo Today 93:34-50.

Janesick A, Blumberg B. 2012. Obesogens, stem cells and the developmental programming of obesity. Int J Androl 35:437-448.

Jusko TA, Koepsell TD, Baker RJ, Greenfield TA, Willman EJ, Charles MJ, Teplin SW, Checkoway H, Hertz-Picciotto I. 2006. Maternal DDT exposures in relation to fetal and 5-year growth. Epidemiology 17:692-700.

Karmaus W, Osuch JR, Eneli I, Mudd LM, Zhang J, Mikucki D, Haan P, Davis S. 2009. Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring. Occup Environ Med 66:143-149.

Kasper-Sonnenberg M, Koch HM, Wittsiepe J, Wilhelm M. 2012. Levels of phthalate metabolites in urine among mother-child-pairs - results from the Duisburg birth cohort study, Germany. Int J Hyg Environ Health 215:373-382.

Keyes KM, Utz RL, Robinson W, Li G. 2010. What is a cohort effect? Comparison of three statistical methods for modeling cohort effects in obesity prevalence in the United States, 1971-2006. Soc Sci Med 70:1100-1108.

Kiyosawa N, Kwekel JC, Burgoon LD, Williams KJ, Tashiro C, Chittim B, Zacharewski TR. 2008. o,p'-DDT elicits PXR/CAR-, not ER-, mediated responses in the immature ovariectomized rat liver. Toxicol Sci 101:350-363.

Koch HM, Lorber M, Christensen KL, Palmke C, Koslitz S, Bruning T. 2013. Identifying sources of phthalate exposure with human biomonitoring: results of a 48h fasting study with urine collection and personal activity patterns. Int J Hyg Environ Health 216:672-681.

Koo WW, Walters JC, Hockman EM. 2000. Body composition in human infants at birth and postnatally. J Nutr 130:2188-2194.

Kramer MS, Martin RM, Bogdanovich N, Vilchuk K, Dahhou M, Oken E. 2014. Is restricted fetal growth associated with later adiposity? Observational analysis of a randomized trial. Am J Clin Nutr (Epub Ahead of Print)

La Merrill M, Birnbaum LS. 2011. Childhood obesity and environmental chemicals. Mt Sinai J Med 78:22-48.

La Merrill M, Emond C, Kim MJ, Antignac JP, Le Bizec B, Clement K, Birnbaum LS, Barouki R. 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ Health Perspect 121:162-169.

Lakind JS, Naiman DQ. 2011. Daily intake of bisphenol A and potential sources of exposure: 2005-2006 National Health and Nutrition Examination Survey. J Expo Sci Environ Epidemiol 21:272-279.

Lakind JS, Goodman M, Mattison DR. 2014. Bisphenol A and indicators of obesity, glucose metabolism/type 2 diabetes and cardiovascular disease: a systematic review of epidemiologic research. Crit Rev Toxicol 44:121-150.

Lamb MR, Taylor S, Liu X, Wolff MS, Borrell L, Matte TD, Susser ES, Factor-Litvak P. 2006. Prenatal exposure to polychlorinated biphenyls and postnatal growth: a structural analysis. Environ Health Perspect 114:779-785.

Landrigan PJ, Carlson JE, Bearer CF, Cranmer JS, Bullard RD, Etzel RA, Groopman J, McLachlan JA, Perera FP, Reigart JR, Robison L, Schell L, Suk WA. 1998. Children's health and the environment: a new agenda for prevention research. Environ Health Perspect 106 Suppl 3:787-794.

Lee DH, Lee IK, Porta M, Steffes M, Jacobs DR, Jr. 2007. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia 50:1841-1851.

Lee DH, Steffes MW, Sjodin A, Jones RS, Needham LL, Jacobs DR, Jr. 2011. Low dose organochlorine pesticides and polychlorinated biphenyls predict obesity, dyslipidemia, and insulin resistance among people free of diabetes. PLoS One 6:e15977.

Legler J. 2013. An integrated approach to assess the role of chemical exposure in obesity. Obesity (Silver Spring) 21:1084-1085.

Li DK, Miao M, Zhou Z, Wu C, Shi H, Liu X, Wang S, Yuan W. 2013. Urine bisphenol-A level in relation to obesity and overweight in school-age children. PLoS One 8:e65399.

Li J, Li N, Ma M, Giesy JP, Wang Z. 2008. In vitro profiling of the endocrine disrupting potency of organochlorine pesticides. Toxicol Lett 183:65-71.

Longnecker MP, Klebanoff MA, Gladen BC, Berendes HW. 1999. Serial levels of serum organochlorines during pregnancy and postpartum. Arch Environ Health 54:110-114.

Lopez-Espinosa MJ, Murcia M, Iniguez C, Vizcaino E, Llop S, Vioque J, Grimalt JO, Rebagliato M, Ballester F. 2011. Prenatal exposure to organochlorine compounds and birth size. Pediatrics 128:e127-e134.

Low FM, Gluckman PD, Hanson MA. 2011. Developmental plasticity and epigenetic mechanisms underpinning metabolic and cardiovascular diseases. Epigenomics 3:279-294.

MacLehose RF, Dunson DB, Herring AH, Hoppin JA. 2007. Bayesian methods for highly correlated exposure data. Epidemiology 18:199-207.

Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. 2013. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One 8:e55387.

Meaney MJ, Szyf M, Seckl JR. 2007. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. Trends Mol Med 13:269-277.

Meeker JD, Sathyanarayana S, Swan SH. 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos Trans R Soc Lond B Biol Sci 364:2097-2113.

Meeker JD. 2012. Exposure to environmental endocrine disruptors and child development. Arch Pediatr Adolesc Med 166:952-958.

Mei Z, Grummer-Strawn LM, Pietrobelli A, Goulding A, Goran MI, Dietz WH. 2002. Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. Am J Clin Nutr 75:978-985.

Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goni F, Fochs S, Sunyer J. 2011. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect 119:272-278.

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.

Monteiro PO, Victora CG. 2005. Rapid growth in infancy and childhood and obesity in later life--a systematic review. Obes Rev 6:143-154.

Moreno-Aliaga MJ, Matsumura F. 2002. Effects of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (p,p'-DDT) on 3T3-L1 and 3T3-F442A adipocyte differentiation. Biochem Pharmacol 63:997-1007.

Muthen B, Muthen LK. 2000. Integrating person-centered and variable-centered analyses: growth mixture modeling with latent trajectory classes. Alcohol Clin Exp Res 24:882-891.

Neel BA, Sargis RM. 2011. The paradox of progress: environmental disruption of metabolism and the diabetes epidemic. Diabetes 60:1838-1848.

Newbold RR. 2010. Impact of environmental endocrine disrupting chemicals on the development of obesity. Hormones (Athens) 9:206-217.

Oken E, Levitan EB, Gillman MW. 2008. Maternal smoking during pregnancy and child overweight: systematic review and meta-analysis. Int J Obes (Lond) 32:201-210.

Painter RC, Roseboom TJ, Bleker OP. 2005. Prenatal exposure to the Dutch famine and disease in later life: an overview. Reprod Toxicol 20:345-352.

Patandin S, Koopman-Esseboom C, de Ridder MA, Weisglas-Kuperus N, Sauer PJ. 1998. Effects of environmental exposure to polychlorinated biphenyls and dioxins on birth size and growth in Dutch children. Pediatr Res 44:538-545.

Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TL, Jorens PG, Blust R, Vanparys C. 2013. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PLoS One 8:e77481.

Pereira-Fernandes A, Dirinck E, Dirtu AC, Malarvannan G, Covaci A, Van Gaal L, Vanparys C, Jorens PG, Blust R. 2014. Expression of obesity markers and Persistent Organic Pollutants levels in adipose tissue of obese patients: reinforcing the obesogen hypothesis? PLoS One 9:e84816.

Pollack AZ, Perkins NJ, Mumford SL, Ye A, Schisterman EF. 2013. Correlated biomarker measurement error: an important threat to inference in environmental epidemiology. Am J Epidemiol 177:84-92.

Porta M, Jariod M, Lopez T, Pumarega J, Puigdomenech E, Marco E, Malats N, Grimalt JO, Real FX. 2009. Correcting serum concentrations of organochlorine compounds by lipids: alternatives to the organochlorine/total lipids ratio. Environ Int 35:1080-1085.

Robinson S, Yardy K, Carter V. 2012. A narrative literature review of the development of obesity in infancy and childhood. J Child Health Care 16:339-354.

Ross MG, Desai M. 2013. Developmental programming of offspring obesity, adipogenesis, and appetite. Clin Obstet Gynecol 56:529-536.

Rothman K.J, Greenland S, Lash TL. 2008. Modern Epidemiology, 3rd Edition. Philadelphia, PA: Lippincott, Williams & Wilkins. (Book)

Rubin BS, Soto AM. 2009. Bisphenol A: Perinatal exposure and body weight. Mol Cell Endocrinol 304:55-62.

Savitz DA. 2014. Invited commentary: interpreting associations between exposure biomarkers and pregnancy outcome. Am J Epidemiol 179:545-547.

Schisterman EF, Cole SR, Platt RW. 2009. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 20:488-495.

Silva DR, Ribeiro AS, Pavao FH, Ronque ER, Avelar A, Silva AM, Cyrino ES. 2013. Validity of the methods to assess body fat in children and adolescents using multi-compartment models as the reference method: a systematic review. Rev Assoc Med Bras 59:475-486.

Skinner MK, Manikkam M, Tracey R, Guerrero-Bosagna C, Haque M, Nilsson EE. 2013. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. BMC Med 11:228.

Slining MM, Herring AH, Popkin BM, Mayer-Davis EJ, Adair LS. 2013. Infant BMI trajectories are associated with young adult body composition. J Dev Orig Health Dis 4:56-68.

Smink A, Ribas-Fito N, Garcia R, Torrent M, Mendez MA, Grimalt JO, Sunyer J. 2008. Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr 97:1465-1469.

Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, Huppi PS. 2009. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect 117:1549-1555.

Sonnenschein C, Soto AM. 1998. An updated review of environmental estrogen and androgen mimics and antagonists. J Steroid Biochem Mol Biol 65:143-150.

Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, Concha H, Hassan M, Ryden M, Frisen J, Arner P. 2008. Dynamics of fat cell turnover in humans. Nature 453:783-787.

Stephens SK, Cobiac LJ, Veerman JL. 2014. Improving diet and physical activity to reduce population prevalence of overweight and obesity: an overview of current evidence. Prev Med 62:167-178.

Stolkhom Convention on Persistent Organic Pollutants. 2004. Homepage Available at: http://chm.pops.int [accessed May 2014].

Symonds ME, Mendez MA, Meltzer HM, Koletzko B, Godfrey K, Forsyth S, van der Beek EM. 2013. Early life nutritional programming of obesity: mother-child cohort studies. Ann Nutr Metab 62:137-145.

Tang-Péronard JL, Andersen HR, Jensen TK, Heitmann BL. 2011. Endocrine-disrupting chemicals and obesity development in humans: A review. Obes Rev 12(8):622-636.

Taxvig C, Dreisig K, Boberg J, Nellemann C, Schelde AB, Pedersen D, Boergesen M, Mandrup S, Vinggaard AM. 2012. Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARgamma activation. Mol Cell Endocrinol 361:106-115.

Teitelbaum SL, Mervish N, Moshier EL, Vangeepuram N, Galvez MP, Calafat AM, Silva MJ, Brenner BL, Wolff MS. 2012. Associations between phthalate metabolite urinary concentrations and body size measures in New York City children. Environ Res 112:186-193.

Thayer KA, Heindel JJ, Bucher JR, Gallo MA. 2012. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. Environ Health Perspect 120:779-789.

Trasande L, Cronk C, Durkin M, Weiss M, Schoeller DA, Gall EA, Hewitt JB, Carrel AL, Landrigan PJ, Gillman MW. 2009 Environment and obesity in the National Children's Study. Environ Health Perspect 117:159-166.

Trasande L, Attina TM, Blustein J. 2012. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA 308:1113-1121.

Trasande L, Sathyanarayana S, Spanier AJ, Trachtman H, Attina TM, Urbina EM. 2013a. Urinary phthalates are associated with higher blood pressure in childhood. J Pediatr 163:747-753.

Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. 2013b. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect 121:501-506.

Tren R, Roberts D. 2010. DDT and malaria prevention. Environ Health Perspect 118:A14-A15.

Vafeiadi M, Vrijheid M, Fthenou E, Chalkiadaki G, Rantakokko P, Kiviranta H, Kyrtopoulos SA, Chatzi L, Kogevinas M. 2014. Persistent organic pollutants exposure during pregnancy, maternal gestational weight gain, and birth outcomes in the mother-child cohort in Crete, Greece (RHEA study). Environ Int 64:116-123.

van den Berg H. 2009. Global status of DDT and its alternatives for use in vector control to prevent disease. Environ Health Perspect 117:1656-1663.

Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. 2007. Human exposure to bisphenol A (BPA). Reprod Toxicol 24:139-177.

Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR, Jr., Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr Rev 33:378-455.

Vandenberg LN, Hunt PA, Myers JP, vom Saal FS. 2013. Human exposures to bisphenol A: mismatches between data and assumptions. Rev Environ Health 28:37-58.

Vandenberg LN. 2014. Low-dose effects of hormones and endocrine disruptors. Vitam Horm 94:129-165.

VanderWeele TJ, Hernan MA. 2012. Results on differential and dependent measurement error of the exposure and the outcome using signed directed acyclic graphs. Am J Epidemiol 175:1303-1310.

Verhulst SL, Nelen V, Hond ED, Koppen G, Beunckens C, Vael C, Schoeters G, Desager K. 2009. Intrauterine exposure to environmental pollutants and body mass index during the first 3 years of life. Environ Health Perspect 117:122-126.

Vizcaino E, Grimalt JO, Fernandez-Somoano A, Tardon A. 2014. Transport of persistent organic pollutants across the human placenta. Environ Int 65:107-115.

vom Saal FS, Nagel SC, Coe BL, Angle BM, Taylor JA. 2012. The estrogenic endocrine disrupting chemical bisphenol A (BPA) and obesity. Mol Cell Endocrinol 354:74-84.

Vrijheid M, Casas M, Bergstrom A, Carmichael A, Cordier S, Eggesbo M, Eller E, Fantini MP, Fernandez MF, Fernandez-Somoano A, Gehring U, Grazuleviciene R, Hohmann C, Karvonen AM, Keil T, Kogevinas M, Koppen G, Kramer U, Kuehni CE, Magnus P, Majewska R, Andersen AM, Patelarou E, Petersen MS, Pierik FH, Polanska K, Porta D, Richiardi L, Santos AC, Slama R, Sram RJ, Thijs C, Tischer C, Toft G, Trnovec T, Vandentorren S, Vrijkotte TG, Wilhelm M, Wright J, Nieuwenhuijsen M. 2012. European birth cohorts for environmental health research. Environ Health Perspect 120:29-37.

Vrijheid M, Slama R, Robinson O, Chatzi L, Coen M, van den HP, Thomsen C, Wright J, Athersuch TJ, Avellana N, Basagana X, Brochot C, Bucchini L, Bustamante M, Carracedo A, Casas M, Estivill X, Fairley L, van Gent D, Gonzalez JR, Granum B, Grazuleviciene R, Gutzkow KB, Julvez J, Keun HC, Kogevinas M, McEachan RR, Meltzer HM, Sabido E, Schwarze PE, Siroux V, Sunyer J, Want EJ, Zeman F, Nieuwenhuijsen MJ. 2014. The Human Early-Life

Exposome (HELIX): Project Rationale and Design. Environ Health Perspect (Epub Ahead of Print)

Walker DM, Goetz BM, Gore AC. 2014. Dynamic postnatal developmental and sex-specific neuroendocrine effects of prenatal polychlorinated biphenyls in rats. Mol Endocrinol 28:99-115.

Wang H, Zhou Y, Tang C, He Y, Wu J, Chen Y, Jiang Q. 2013. Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PLoS One 8:e56800.

Wang HX, Zhou Y, Tang CX, Wu JG, Chen Y, Jiang QW. 2012. Association between bisphenol A exposure and body mass index in Chinese school children: a cross-sectional study. Environ Health 11:79.

Warner M, Aguilar Schall R, Harley KG, Bradman A, Barr D, Eskenazi B. 2013. In utero DDT and DDE exposure and obesity status of 7-year-old Mexican-American children in the CHAMACOS cohort. Environ Health Perpect 121(5):631-636.

Warner M, Wesselink A, Harley KG, Bradman A, Kogut K, Eskenazi B. 2014. Prenatal Exposure to Dichlorodiphenyl-trichloroethane and Obesity at 9 Years of Age in the CHAMACOS Study Cohort. Am J Epidemiol.

Wei J, Lin Y, Li Y, Ying C, Chen J, Song L, Zhou Z, Lv Z, Xia W, Chen X, Xu S. 2011. Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology 152:3049-3061.

Weng SF, Redsell SA, Swift JA, Yang M, Glazebrook CP. 2012. Systematic review and meta-analyses of risk factors for childhood overweight identifiable during infancy. Arch Dis Child 97:1019-1026.

Wild CP. 2005. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev 14:1847-1850.

Wild CP. 2012. The exposome: from concept to utility. Int J Epidemiol 41:24-32.

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

Wohlfahrt-Veje C, Tinggaard J, Winther K, Mouritsen A, Hagen CP, Mieritz MG, Renzy-Martin KT, Boas M, Petersen JH, Main KM. 2014. Body fat throughout childhood in 2647 healthy Danish children: agreement of BMI, waist circumference, skinfolds with dual X-ray absorptiometry. Eur J Clin Nutr (Epub Ahead of Print)

Wolff MS, Teitelbaum SL, Windham G, Pinney SM, Britton JA, Chelimo C, Godbold J, Biro F, Kushi LH, Pfeiffer CM, Calafat AM. 2007. Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls. Environ Health Perspect 115:116-121.

Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, Kushi LH, Erdmann C, Hiatt RA, Rybak ME, Calafat AM. 2010. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. Environ Health Perspect 118:1039-1046 .

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.

Zhang HY, Xue WY, Li YY, Ma Y, Zhu YS, Huo WQ, Xu B, Xia W, Xu SQ. 2014. Perinatal exposure to 4-nonylphenol affects adipogenesis in first and second generation rats offspring. Toxicol Lett 225:325-332.

Zheng H, Lenard NR, Shin AC, Berthoud HR. 2009. Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. Int J Obes (Lond) 33 Suppl 2:S8-13.

Abbreviations

In alphabetical order

BBzP	Benzyl-butyl
BMI	Body Mass Index
BPA	Bisphenol A
CI	Confidence Interval
DAGs	Directed Acyclic Graphs
DOHaD	Developmental Origins of Health and Disease
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethylene
DEHP	Di-2-ethylhexyl
DES	Diethylstilbestrol
DINP	Di-isononyl
DnBP	Di-n-butyl
DXA	Dual-energy X-ray Absorptiometry
EDCs	Endocrine Disrupting Chemicals
GM	Geometric Mean
GSD	Geometric Standard Deviation
HCB	Hexachlorobenzene
HPA	Hypothalamic-Pituitary-Adrenocortical
ICC	Intraclass Correlation Coefficient
IQ	Interquartile
LOD	Limit Of Detection
MBzP	Mono-benzyl Phthalate
MEHP	Mono-(2-ethylhexyl) Phthalate
MEP	Mono-ethyl Phthalate
MiBP	Mono-iso-butyl Phthalate
MnBP	Mono-n-butyl Phthalate
PCBs	Polychlorinated Biphenyls
PPARs	Peroxisome Proliferator-Activated Receptors
POPs	Persistent Organic Pollutants
WAT	White Adipose Tissue