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Biomarkers of Exposure in Environmental Epidemiology : the case of the effects of prenatal exposure to phenols and phthalates on pre- and post-natal growth.

Claire Philippat

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THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ DE GRENOBLE

Spécialité : Modèle, méthode et algorithmes en biologie

Arrêté ministériel : 7 août 2006

Présentée par: **Claire PHILIPPAT**

Thèse dirigée par Rémy Slama

Préparée au sein de l'Institut Albert Bonniot (CRI INSERM/UJF U823). Equipe d'Epidémiologie Environnementale appliquée à la Reproduction et la Santé Respiratoire dans l'école doctorale EDISCE

Utilisation des Biomarqueurs d'Exposition en Epidémiologie Environnementale ; application à l'étude des effets des expositions intra-utérines aux phénols et aux phtalates sur la croissance pré- et post-natale.

Biomarkers of Exposure in Environmental Epidemiology; the case of the effects of prenatal exposures to phenols and phthalates on pre- and post-natal growth.

Thèse soutenue publiquement le 26 août 2013
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¹ Conformément aux consignes de l'école doctorale Edisce concernant les thèses rédigées en anglais, chaque chapitre est précédé d'un résumé en français.

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List of abbreviations

AhR: Aryl hydrocarbon receptor
ARNT: Aryl hydrocarbon receptor nuclear translocator
BP: Butyl paraben
BP3: Benzophenone-3
BPA: Bisphenol A
CAR: Constitutive Androstane Receptor
CDC: Centers for Disease Control and Prevention
CI: Confidence interval
DEHP: Di(2-ethylhexyl) phthalate
EP: Ethyl paraben
ER: Estrogen receptors
GR: Glucocorticoid receptor
ICC: Intraclass correlation coefficient
IOTF: International Obesity Task Force
LOD: limit of detection
MBP: Mono-n-butyl phthalate
MBzP: Monobenzyl phthalate
MCNP: Monocarboxy-isononyl phthalate
MCOP: Monocarboxy-isoctyl phthalate
MCPP: Mono(3-carboxypropyl) phthalate
MECPP: Mono(2-ethyl-5-carboxypentyl) phthalate
MEHHP: Mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHP: Mono(2-ethylhexyl) phthalate
MEOHP: Mono(2-ethyl-5-oxohexyl) phthalate
MEP: Monoethyl phthalate
MiBP: Mono-isobutyl phthalate
MP: Methyl paraben
MW: Molecular weight
PP: Propyl paraben
PPAR: Peroxisome Proliferator–Activated Receptors
PXR: Xenosensors Pregnane X Receptor
RXR: Retinoid X receptor
TCS: Triclosan
TR: Thyroid hormone receptor
US: Ultrasound examination
WHO: World Health Organization
2,4-DCP: 2,4-dichlorophenol
2,5-DCP: 2,5-dichlorophenol
 \sum HMW: Molecular sum of high molecular weight phthalates
 \sum LMW: Molecular sum of low molecular weight phthalates
 \sum PB: Molecular sum of parabens

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CHAPTER I: INTRODUCTION

Introduction

La période prénatale ainsi que les premières années de vie sont considérées comme des périodes de sensibilité importante aux expositions à de nombreux facteurs environnementaux. Des modifications survenues durant cette période pourraient entraîner des effets à court mais aussi à long terme sur la santé, ainsi que formulé dans le contexte de l'hypothèse DOHaD ("*Developmental Origins of Health and Diseases*", (Hanson and Gluckman 2008)). Cette hypothèse considère que les effets chez l'adulte associés à des expositions survenues pendant la vie fœtale pourraient s'expliquer par une adaptation du développement en réponse à un environnement intra-utérin défavorable. Cette adaptation développementale est favorable pendant la période intra-utérine mais, à cause de modifications physiologiques et/ou métaboliques, pourrait entraîner des conséquences ultérieures néfastes (Barker 2004, 2007; Gluckman and Hanson 2004). C'est une des raisons pour lesquelles il est important de caractériser les événements et expositions survenant durant la grossesse et leur impact potentiel sur le développement du fœtus et de l'enfant dans les premières années de vie.

L'événement de santé étudié dans le cadre de ce travail de thèse est la croissance durant la vie fœtale et les premières années de vie. Une altération de la croissance fœtale est considérée comme un marqueur non spécifique des agressions subies pendant la vie fœtale. Un faible poids de naissance a été associé à un risque plus élevé de mortalité infantile, ainsi qu'à un risque plus élevé de développer des pathologies de type obésité, diabète, troubles cardiovasculaires à l'âge adulte (Barker 2007). Le poids à la naissance est un paramètre facilement mesurable et est couramment utilisé comme estimateur de la croissance fœtale dans les études épidémiologiques. Néanmoins, le poids est une mesure globale ne permettant pas d'identifier un effet sur une partie spécifique du corps et des mesures complémentaires telles que la taille et le périmètre crânien à la naissance sont souvent utilisées de concert. Une limitation à l'utilisation des mesures à la naissance est qu'elles ne fournissent pas d'information directe concernant des périodes spécifiques du développement du fœtus. L'utilisation de mesures échographiques répétées pendant la grossesse permet de mettre en évidence des périodes de sensibilité au cours de la vie fœtale. De telles mesures ont déjà été utilisées en épidémiologie environnementale pour évaluer les effets des polluants atmosphériques sur le développement fœtal (Aguilera et al. 2010; Hansen et al. 2008; Iniguez et al. 2012b; Slama et al. 2009; van den Hooven et al. 2012), mais encore très peu en relation avec des perturbateurs endocriniens à courte demi-vie (Snijder et al. 2013).

La croissance post-natale précoce a été identifiée comme un facteur de risque d'obésité infantile (Botton et al. 2008; Giles et al. 2013). L'obésité infantile est un problème de santé publique important. Cette pathologie, dont la prévalence augmente dans le monde, est associée à d'autres problèmes de santé graves dans la jeunesse, mais aussi plus tard dans la vie adulte, comme le diabète, l'hypertension artérielle et les maladies coronariennes (Barker 2007; Bibbins-Domingo et al. 2007). En plus des déterminants connus de la croissance pré- et post-natal (génétique, alimentation...), l'exposition à des polluants chimiques est de plus en plus suspectée d'affecter les trajectoires de croissance.

Les contaminants environnementaux étudiés dans ce travail appartiennent aux familles des phénols et des phtalates. Ils font partie des composés chimiques dont le niveau de production est très élevé (de l'ordre de 4 millions de tonnes dans le monde pour le Bisphénol A en 2006) et sont présents dans un vaste éventail de produits de consommation courante. Les phtalates de poids moléculaire inférieur à < 250 g / mol sont utilisés dans les produits de soins personnels (parfums, cosmétiques) et dans les industries pharmaceutiques (enrobage de certains médicaments) tandis que les phtalates de haut poids moléculaire (> 250 g / mol) sont plutôt retrouvés dans les revêtements de sols et de murs, les emballages alimentaires et des dispositifs médicaux (A. M. Calafat et al. 2006; Hauser and Calafat 2005).

Pour ce qui est des phénols, le bisphénol A est utilisé dans la fabrication des résines époxy couvrant l'intérieur des boîtes de conserve et dans certains plastiques de type polycarbonate. D'autres phénols sont utilisés pour leurs propriétés antibactériennes et de conservateurs dans les cosmétiques (parabènes) et les savons (triclosan). La benzophénone-3 est utilisée comme filtre anti rayons ultraviolets dans les crèmes solaires (Calafat et al. 2008). Ainsi l'exposition à ces composés est ubiquitaire. Ces substances et / ou leurs métabolites ont été mis en évidence dans la quasi totalité des urines testées en population générale, incluant des femmes enceintes (Casas et al. 2012; Woodruff et al. 2011). Le fœtus est lui aussi exposé et certains métabolites ont été détectés dans le sang du cordon ombilical, le liquide amniotique ou le méconium des nouveau-nés (Latini et al. 2003; Zhang et al. 2009).

Les phénols et les phtalates ont été identifiés comme étant des perturbateurs endocriniens. Ils peuvent interagir avec les récepteurs hormonaux, mais aussi la synthèse, la sécrétion, le transport, la fixation et l'élimination des hormones naturelles qui jouent un rôle important dans de nombreuses fonctions telles que le métabolisme, le comportement, la fertilité ou la croissance.

Une étude réalisée au sein d'une population de femmes enceintes new-yorkaise avait suggéré un effet de ces composés sur la croissance fœtale estimée à l'aide de mesures à la naissance; l'effet de certains phénols sur le poids de naissance était différent suivant le sexe de l'enfant (Wolff et al. 2008). Une seule étude s'était intéressée aux effets des expositions prénatales aux phénols sur la croissance postnatale (Harley et al. 2013), les autres études étant transversales (Teitelbaum et al. 2012; Trasande et al. 2012). Dans l'étude publiée par Harley et al (2013) une association négative entre les concentrations maternelles de bisphénol A et le risque d'être en surpoids à 9 ans a été mis en évidence chez les filles.

Les objectifs de ce doctorat étaient:

- 1) d'étudier les associations entre les concentrations urinaires maternelles de phénols et de phtalates et la croissance pré et post-natale de l'enfant (chapitre II);
- 2) de comparer les concentrations de phénols dosées dans l'urine maternelle à celle dosées dans le liquide amniotique, recueillis le même jour (Chapitre III);
- 3) d'étudier la variabilité des concentrations urinaires de polluants environnementaux non persistants (phénols) durant la grossesse (Chapitre III);
- 4) de caractériser le biais associé à l'utilisation d'un seul échantillon urinaire pour estimer l'exposition durant la grossesse, qui est l'approche utilisée dans la plupart des études épidémiologiques publiées à cette date (Chapitre III).

I. Pre- and post-natal growth in humans

1. Pre and post-natal growth as markers of well being

a) Prenatal growth

Fetal growth is the reflect of healthy development and is considered to be a non-specific marker of exposures and mechanisms taking place during fetal life (Gluckman and Hanson 2004; Hanson and Gluckman 2008). Birth weight is easy to collect and is often used as a surrogate measure for fetal growth in epidemiological studies. Term low birth weight (< 2500 g) has been linked with a higher risk of baby's mortality (Wilcox 2001), but also with occurrence of several chronic conditions including cardiovascular diseases, type 2 diabetes, obesity and hypertension (Barker 1995, 2004; Godfrey and Barker 2001). These results are in line with the Developmental Origins of Health and Diseases (DOHaD) hypothesis, which suggests that exposures during fetal life can result in long term effects on health. These long term effects may result from fetal developmental adaptations, which are beneficial during the intrauterine period, but may lead to permanent changes in body's structure, physiology and metabolism and thus may promote development of diseases later in life (Barker 2004, 2007; Gluckman and Hanson 2004).

Historically birth weight was analyzed independently of gestational duration, making it difficult to distinguish low birth weight due to restricted fetal growth from low birth weight due to preterm birth (Adams et al. 2010; Savitz et al. 2002). In studies on the effects of environmental factors on fetal growth, birth weight is now usually corrected for gestational age. In addition to low birth weight, metrics including reduction in birth weight (continuous variable), very low birth weight (< 1500 g) and small for gestational age (birth weight lower than the 10th percentile of a suitable sex- and gestational age-specific weight reference distribution) are commonly used in epidemiological studies. Weight at birth does not allow to identify an effect on a specific body part or organ and additional measurements of neonatal anthropometry at birth such as length and head circumference provide additional information.

A limitation to the use of birth outcomes in epidemiological studies is that they are measured at the end of intrauterine life. They therefore do not provide direct information on specific periods of fetal development (Hemachandra and Klebanoff 2006) and effects occurring at specific times of pregnancy could be missed. In addition, developmental

programming may occur independently of birth weight. Animal experiments have indeed shown that fetuses from mothers with nutrition restriction during early pregnancy may have normal weight at birth, but exhibited increased body weight and fat deposition later in life (Ford et al. 2007; Reynolds and Caton 2012). Repeated measurements of fetal anthropometry during pregnancy by ultrasound examinations may allow to highlight windows of heightened sensitivity to environmental contaminants during fetal life. Such measurements have already been used in environmental epidemiology to assess the effects of air pollutants on fetal development (Aguilera et al. 2010; Hansen et al. 2008; Iniguez et al. 2012b; Slama et al. 2009; van den Hooven et al. 2012) and very recently to characterize the effect of short half-life endocrine disruptors (Snijder et al. 2013).

b) Early postnatal growth and link with childhood obesity

The definition of overweight and obesity given by the World Health Organization (WHO) is an abnormal or excessive fat accumulation that presents a risk to health. A crude measure of overweight and obesity is given by the body mass index (BMI): an adult with a BMI above 25 kg/m² is defined as overweight; a BMI above 30 kg/m² is defined as obesity. During childhood, body composition strongly varies with age and sex. Child's weight status is therefore usually determined using an age- and sex-specific references rather than the BMI categories used for adults. Several definitions of child's obesity and overweight exist; the most common used are those from the WHO, the Centers for Disease Control and Prevention (CDC) and the International Obesity Task Force (IOTF) (Flegal and Ogden 2011).

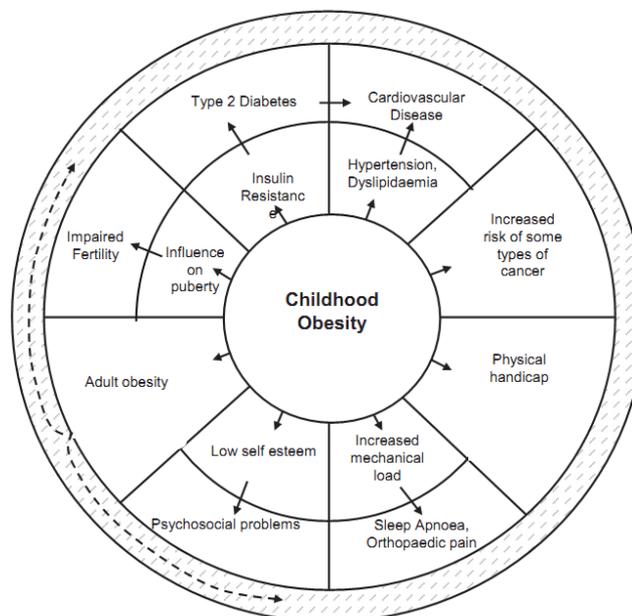
Table 1: Definitions of overweight and obesity commonly used in study of weight and adiposity in children.

Organization	Definition of Childhood Obesity and overweight
World Health Organization	Between birth to age 5: Obese: BMI > 3 standard deviations above the WHO growth standard median Overweight: BMI > 2 standard deviations above the WHO growth standard median
Centers for Disease Control and Prevention	Between 2 to 19 years: Obese: BMI ≥ 95 th percentile. Overweight: BMI between the 85 th and the 95 th percentiles
International Obesity Task Force	Age 2 to 18: Provides international BMI cut points by age and sex for overweight and obesity, the cut points correspond to an adult BMI of 25 (overweight) or 30 (obesity)

Abbreviations: BMI: Body Mass Index, WHO: World Health Organization

The prevalence of childhood overweight and obesity has strongly increased in developed countries. Surveys during the 1990s show that in Europe an additional 1 % of the entire child population became overweight each year and 15 % of the European school-aged children are estimated to be carrying excess body fat; among them a quarter are obese (IOTF definition (Lobstein et al. 2004)). Obesity is associated with other health troubles during youth and obese children and adolescents are likely to suffer from related comorbidities (Figure 1). These children are also more likely to be obese as adults (Bibbins-Domingo et al. 2007; Haslam and James 2005; Lakshman et al. 2012; Reilly et al. 2003; Singh et al. 2008).

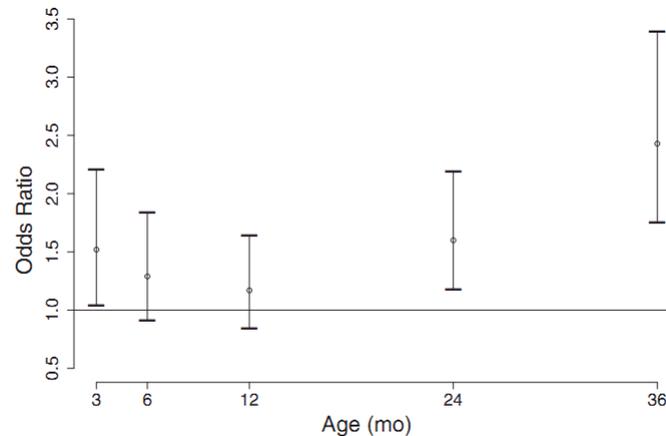
Figure 1: Summary of the complications related to childhood obesity (*Lakshman et al. 2012*).



Comorbidities of childhood obesity are depicted in the outer ring with their intermediate processes in the inner ring.

Several epidemiological studies have shown an association between rapid growth during the first years of life and the development of obesity later in the childhood (Botton et al. 2008; Giles et al. 2013; Ong et al. 2000; Taveras et al. 2009), suggesting that early post-natal growth is a risk factor for childhood obesity (Figure 2). Two periods in particular (up to 6 months and from 2 years onward) have been associated with a later risk of overweight (Botton et al. 2008). Such results highlight the importance of studying determinants of overweight in early life.

Figure 2: Odds ratios (ORs) and 95% CIs of being overweight (IOTF) in 325 adolescent boys and girls for a 1-SD increase in weight growth velocity at different ages between 3 and 36 months (*logistic model adjusted for sex, (Botton et al. 2008)*).



Early post-natal growth can be studied by collecting repeated measures of weight and height during childhood. Such measurements are easy to collect, especially in countries where they are regularly reported by the family pediatrician in the personal child health booklet (such as France). Skin fold thickness and abdominal circumference are not routinely measured. They can provide complementary information on the body fat composition and are also interesting to study early post-natal growth (Hauspie et al. 2004).

2. Determinants of pre- and early post-natal growth

a) Fetal growth

Fetal growth results from a complex interplay among factors including genetic, maternal, placental, hormonal and environmental factors (Kramer 1987; Sacks 2004; Williams 2005). A non-exhaustive overview of these determinants is given in this chapter.

Genetic influences

A study has reported that around 50% of the variation in weight, length and head circumference at birth may be explained by genetic factors (n = 101,748 parent-offspring pairs, data from Medical Birth Registry of Norway from 1967 to 2004). Among this share of

50%, the fetal genome might explain about 30% of the normal variation in birth weight while the maternal genome would explain the other 20% (Lunde et al. 2007). Infant sex is associated with fetal growth and at birth boys are on average 150 g heavier than girls (Valero De Bernabé et al. 2004). Several fetal chromosomal anomalies including trisomy 21, 18 and Turner's syndrome usually result in fetal growth retardation. Conversely, other genetic disorders can lead to pre- and/or post-natal over-growth. This is the case of the Beckwith-Wiedemann syndrome, caused by the disruption of imprinting of the short arm of chromosome 11, leading to over activity of the Insulin like Growth Factor-2 (IGF-2) gene (Gicquel and Le Bouc 2006).

Placental influences

The placenta is the main interface between mother and fetus during pregnancy and fetal growth is related to its capacity to transfer oxygen and nutrients to the fetus (Jansson and Powell 2007; Reece and Hobbons 2007). The placenta also produces a wide spectrum of hormones involved in fetal growth regulation (e.g., IGF-I, IGF-II, placental growth hormone, (Murphy et al. 2006; Newbern and Freemark 2011)) and modifications in placental functions are likely to lead to fetal growth alterations.

Endocrine regulation of fetal growth

A wide range of hormones influence growth and maturation, beginning in-utero and continuing throughout childhood. Hormones, such as IGFs, insulin, thyroid hormones and oestrogens control the rate of cell proliferation, apoptosis and differentiation in many tissues, they could either stimulate or inhibit growth in-utero.

Maternal (non genetic) influences

Maternal behavioral factors affecting fetal growth include alcohol consumption and active smoking during pregnancy, which has been associated with decrease in offspring birth weight (Mills et al. 1984; Rahmalia et al. 2012; Suzuki et al. 2008). Good maternal health is essential for normal fetal development. Pathologies limiting nutrients and oxygen transfer to the fetus, including preeclampsia and anemia, negatively affect fetal growth. Conversely infants born to mothers with gestational diabetes are more likely to be large for gestational age (Gillman et al. 2003). Other maternal factors associated with size at birth include maternal pre-pregnancy weight, maternal age and parity; the first child weights on average less than the

second and third children (Reece and Hobbons 2007; Thame et al. 2004; Valero De Bernabé et al. 2004)

Environmental factors

Environmental factors suspected to influence fetal growth include biological factors, microorganisms can cross the placenta and several agents including toxoplasma, rubella, cytomegalovirus and herpes simplex have been associated with fetal growth disorders, and chemical factors. We will focus here on the chemical environment.

There is growing concern that environmental contaminants might interact with developmental processes and fetal growth (Hanson and Gluckman 2008; Woodruff et al. 2010). Research in Humans is at a somewhat advanced stage for air pollutants, metals and persistent endocrine disruptors (DDT, PCBs)). Among them, lead (Bellinger 2005), PCBs (Govarts et al. 2012) and air pollutants, notably the fine particulate matter of diameter $\leq 2.5 \mu\text{m}$ (Dadvand et al. 2013; Shah and Balkhair 2011; Slama and Cordier In Press) have been identified as possibly inhibiting fetal growth. Exposure to environmental tobacco smoke has also been associated with an increased risk of having a baby with low birth weight (Salmasi et al. 2010). Data in Human are much more limited for the so-called "emerging chemicals", including brominated flame retardants, perfluorinated compounds, phthalates and phenols and do not allow to conclude yet about their potential effects on fetal growth (Slama and Cordier In Press). Specific review of the effects of phthalates and phenols, two families of non persistent endocrine disruptors, on fetal growth is available section III of this chapter (definition of endocrine disruptors is detailed page 18).

b) Post-natal growth, childhood obesity

Influences of prenatal growth on postnatal growth

Early postnatal growth is related to fetal growth. Smaller babies at birth are more likely to show an increased growth rates during early infancy compared to normal size newborns, what is commonly called catch up growth (Eleftheriades et al. 2006; Hindmarsh et al. 2008; Mook-Kanamori et al. 2011).

Factors influencing prenatal growth also suspected to influence postnatal growth

As for prenatal growth, postnatal growth is controlled by hormonal (e.g., growth hormone) and genetic factors (Hauspie et al. 2004; K Ong et al. 2002). Infant sex is associated

with postnatal growth (Regnault et al. 2010). Regarding childhood obesity it has been suggested that inheritance may account for around 25 to 40% of the BMI inter-individual variability (Maffeis 2000). Several other factors affecting fetal growth are suspected to affect postnatal growth. This is in particular the case for maternal smoking, maternal and paternal height and parity. As an example, a lower weight at 3 months (Regnault et al. 2010), but not later in childhood (Hindmarsh et al. 2008; Ong et al. 2000) has been reported for infants prenatally exposed to tobacco smoke. Regarding parity, infants of primiparous pregnancies, which are generally smaller and thinner at birth, exhibit an important postnatal catch-up in weight and length, which results in larger size during childhood (Ong et al. 2000). It is not well known in which extent these effects are mediated by effects on prenatal growth.

Postnatal factors

Postnatal factors, especially nutrition, play an important role in early post-natal growth. Marked differences in growth rates have been reported as early as at the age of 2-3 months between infants who were breast- or bottle-fed. Breastfed infants usually have lower weight growth velocity than formula-fed infants do. The difference in weights between these two groups is still observed at 31 months of age (KK Ong et al. 2002; Regnault et al. 2010).

Environmental chemicals

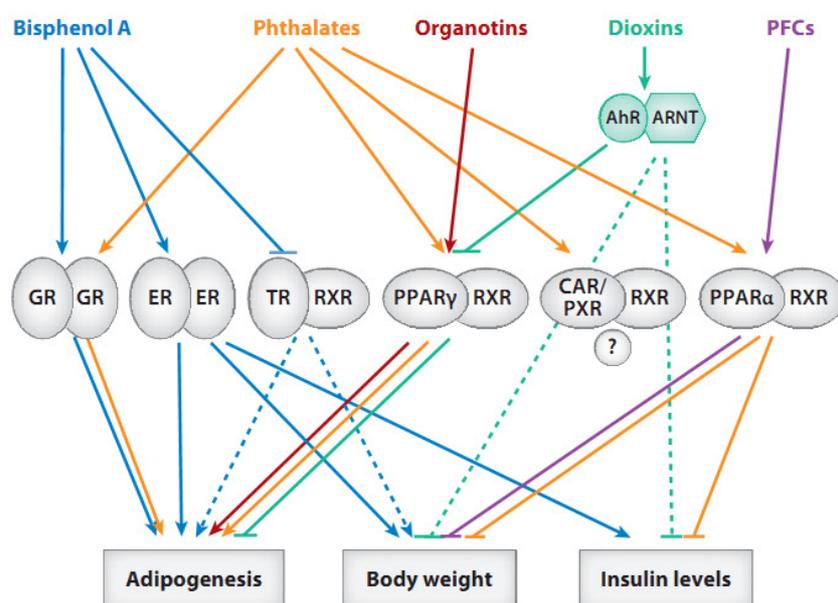
Exposure to industrial chemicals during critical periods of development has been recently mentioned as a potential risk factor for obesity development; this particularly concerns endocrine disruptors (Casals-Casas and Desvergne 2011; DiVall 2013; Newbold 2010; Newbold et al. 2008).

Endocrine disruptors have been defined as exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations (definition from the International Program on Chemicals (IPCS 2002)). Endocrine disruptors interfere with the normal functioning of the endocrine system by affecting the synthesis, secretion, transport, metabolism and elimination of the natural hormones; mimicking their action and / or interfering with their receptors. Both natural (e.g., phytoestrogens) and man-made substances have been identified to be endocrine disruptors.

Some endocrine disruptor chemicals are in particular able to interact with receptors implied in adipogenesis, lipid accumulation and metabolism (e.g., Peroxisome Proliferator-

Activated Receptors (PPARs), Estrogen Receptors, Figure 3) and may induce epigenetic modifications in obesity-related genes (Casals-Casas and Desvergne 2011; DiVall 2013). For these reasons, they are considered potential obesogens. Endocrine disruptor chemicals can be categorized according to their intended use, their structural properties and / or their persistence in the environment and organism. Persistent endocrine disruptors suspected to act as metabolic disruptors include dioxins, PCBs, organochlorine pesticides (Casals-Casas and Desvergne 2011). We will focus here on two families of short-lived endocrine disruptors: phthalates and phenols.

Figure 3: Nuclear receptors implied in the control of growth and adipogenesis, and endocrine disruptors likely to interact with them (Casals-Casas and Desvergne 2011).



PPAR: Peroxisome Proliferator–Activated Receptors; ER: estrogen receptors, TR: Thyroid hormone receptor, GR: Glucocorticoid receptor, PXR: xenosensors Pregnane X Receptor, CAR: Constitutive Androstane Receptor, AhR: Aryl hydrocarbon receptor, ARNT: Aryl hydrocarbon receptor nuclear translocator; RXR: Retinoid X receptor.

II. Phenols and phthalates

1. Exposure in the general population

Esters of phthalic acid, commonly named phthalates, are a group of chemicals with a wide spectrum of industrial applications (Table 2). Low molecular weight (MW) phthalates (MW < 250 g / mol) are primarily used in personal care products (perfumes, cosmetics) and as coating for pharmaceutical products while high MW phthalates (MW > 250 g / mol) are

mostly used in polyvinylchloride floor and wall covering, food packaging and medical devices (A. M. Calafat et al. 2006; Hauser and Calafat 2005). Bisphenol A, benzophenone-3, triclosan, dichlorophenols and parabens are phenolic compounds used in lot of common products: food packaging and polycarbonate plastics (bisphenol A), cosmetics (parabens), soap (triclosan) and sunscreen (benzophenone-3, (Calafat et al. 2008)). Precursors of dichlorophenols are used as intermediates in the production of several herbicides and insecticides (Agency for Toxic Substances and Disease 2006).

Exposure to these chemicals is prevalent in the general population, including pregnant women (Adibi et al. 2008a; Braun et al. 2011b; Cantonwine et al. 2010; Wolff et al. 2008; Ye et al. 2009; Ye et al. 2008).

Table 2: Non exhaustive list of sources of exposure to the phthalates and phenols studied in this thesis.

Chemicals	Sources of exposure, French and EU regulation
PHTHALATES	
Di-ethyl phthalate	Personal care products (fragrances, cosmetics), coatings (pharmaceutical products), dyes
Di-butyl phthalate	Personal care products, lacquers, vanishes, coating (pharmaceutical products), banned from toys and childcare products (2005/12/14) ^a
Di-isobutyl phthalate	Polish, lacquers
Di-n-octyl phthalate	Medical devices, cosmetics, food packaging
Butyl-benzyl phthalate	Vinyl flooring, adhesives and sealants, personal care products, food packaging, industrial solvents, banned from toys and childcare products (2005/12/14) ^a
Di(2-ethylhexyl) phthalate	PVC plastics used in household products (floor tiles, furniture), food packaging, medical devices, banned in toys and childcare products (2005/12/14) ^a
Di-isodecyl phthalate	Anticorrosive paints
Di-isononyl phthalate	Sealants, lacquers, paints
PHENOLS	
2,4-dichlorophenol	Metabolite of 1,3 dichlorobenzene: herbicides, pesticides, minor contaminant of 1,4-dichlorobenzène
2,5-dichlorophenol	Metabolite of 1,4 dichlorobenzene: pesticides, toilet-deodorizer blocks, banned from mothballs (2009/08/21) ^b
Bisphenol A	Polycarbonate plastics and epoxy resins: food packaging, toys, medical tubing, dental fillings, banned from baby bottles (2010/07/01) ^c and food containers for children below 3 years
Benzophenone-3	Sun cream, food packaging
Triclosan	Antibacterial and antifungal agent: soap, deodorant, toothpaste
Parabens	
Methyl paraben	
Ethyl paraben	Antibacterial agents, food and cosmetic preservatives
Propyl paraben	
Butyl paraben	

Phthalates were ranged in order of increasing molecular weight. Abbreviations: PVC : polyvinyl chloride

^a Directive 2005/84/CE

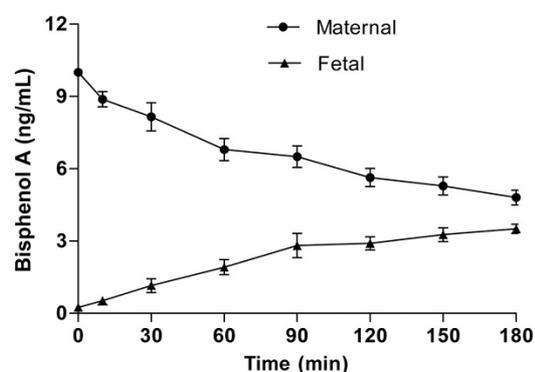
^b Directive 2007/565/CE

^c French Law 2010-729 and 2012-1442

2. Fetal exposure

Human studies have shown fetal exposure to phenols and phthalates. Metabolites of these chemicals have been detected in amniotic fluid (Engel et al. 2006; Huang et al. 2009; Jensen et al. 2012; Silva et al. 2004a; Yamada et al. 2002), cord blood (Latini et al. 2003) and meconium (Zhang et al. 2009). This was corroborated by experimental data showing that several phthalates and phenols are able to cross the placenta (Balakrishnan et al. 2010; Mose et al. 2007; Takahashi and Oishi 2000). In an ex-vivo experimental system, where 7 human placentas were in contact with a perfusate enriched in bisphenol A (10 µg/L), authors observed rapid transfer of this phenol across the placenta (Figure 4).

Figure 4: Bisphenol A concentration in maternal and fetal compartments during three hours of perfusion in an ex-vivo human placental perfusion system (Balakrishnan et al. 2010).



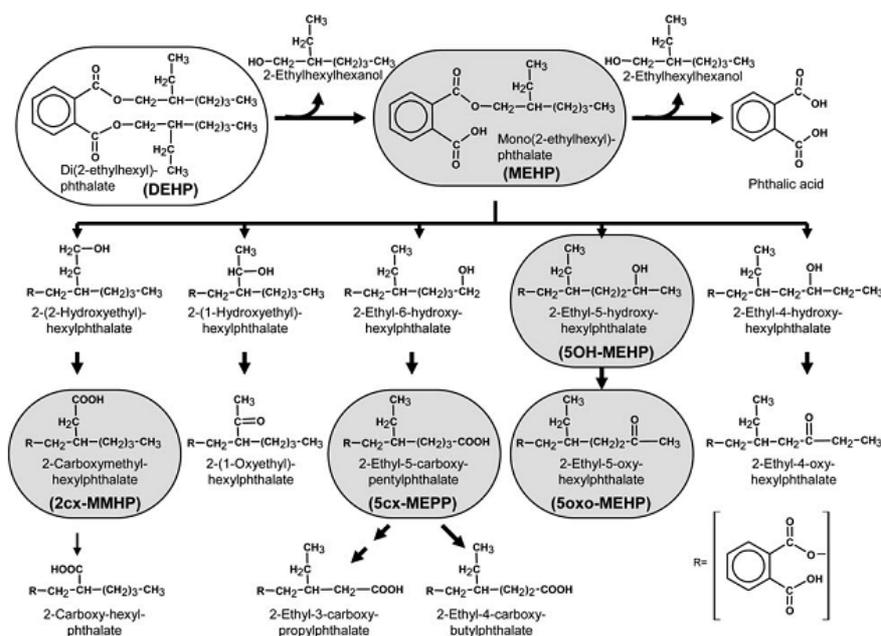
3. Metabolism

Phenols and phthalates have short half-lives in Human, generally estimated to be less than 24 hours (Janjua et al. 2008; Koch et al. 2004; Sandborgh-Englund et al. 2006; Volkel et al. 2002). To our knowledge none of these studies have specifically explored half-lives in pregnant women, which may differ from those observed in men and non-pregnant women because of the metabolic and physiologic changes occurring during pregnancy. The short half-life reported in Human is a priori a desirable feature in terms of reducing exposure levels, but creates strong challenges when it comes to assessing exposure levels, since these will vary very quickly throughout the day.

After exposure, phthalates and phenols are rapidly metabolized and mostly excreted in urine. Phenols are usually excreted unchanged or conjugated (conjugation increases water solubility and therefore increases urinary excretion). Phthalate biotransformation is related to their molecular weight: low molecular weight phthalates are changed into their hydrolytic monoesters, which can be excreted in urine or undergo conjugation. The metabolism of the

higher molecular weight phthalates is more complex and results in more metabolites (Figure 5). High molecular weight phthalates are also metabolized to hydrolytic monoesters, but then undergo enzymatic oxidation of the alkyl chain, to produce oxidative metabolites (Hauser and Calafat 2005).

Figure 5: Metabolism of di(2-ethylhexyl) phthalate (DEHP) in Human (*major metabolites are highlighted, (Koch et al. 2005)*)



4. Suspected effects of prenatal exposure to phthalates and phenols on health

Phthalates and phenols are known to be endocrine disruptors. They interact, among others with sex hormone receptors and therefore research first focused on their potential effects on reproductive development and sex differentiation. Experimental studies have suggested that phthalates may induce developmental abnormalities of the male genital tract (Foster 2006; Swan 2008). One study in 106 boys (Swan 2008) has suggested male reproductive toxicity (decreased anogenital distance) while another did not (cases-control study: n = 21 cases of hypospadias and 50 cases of undescended testis (Chevrier et al. 2012)).

The endocrine system is related to a wide range of organism functions and it has been recently shown that phenols and phthalates may impact other facets of health than reproduction. Experimental studies have reported effects on brain organization and behavior (Wolstenholme et al. 2011; Wolstenholme et al. 2012), respiratory health (Nakajima et al.

2012) and growth (Rubin and Soto 2009). In Human, epidemiological studies have reported associations between exposures to phthalates and phenols during perinatal periods and child neurodevelopment. For example, low molecular weight phthalates, measured in maternal urine collected during pregnancy, have been associated with a higher risk of developing behavioral, attention and depressive problems in children between 4 and 9 years ($n = 137$, (Engel et al. 2010; Miodovnik et al. 2011)). Prenatal exposure to bisphenol A has been associated with more anxious and depressed behaviors at 3 years (Braun et al. 2011a). Bisphenol A and phthalates have also been associated with respiratory health (Jaakkola and Knight 2008; Kwak et al. 2009; Spanier et al. 2012) and prenatal growth (Wolff et al. 2008). This thesis focuses on the effects of these chemicals on pre- and early post-natal growth.

III. Effects of prenatal exposures to phthalates and phenols on pre- and post-natal growth

1. In vitro studies suggesting that phthalates and phenols interact with mechanisms implied in growth

In vitro, phthalates have shown to be weak agonists of the estrogenic receptors ER_{α} and ER_{β} implied among others in growth and maintenance of a diverse range of tissues (mammary gland, uterus, bone, cardiovascular system...). These chemicals shown antagonist activities on androgen (National Institute of Health and Medical Research 2011) and thyroid receptors (Shen et al. 2009); they also interact with the triiodothyronine (T3) transportation (Boas et al. 2010; Ishihara et al. 2003). Fetal growth is regulated among other by thyroid hormones (Blazer et al. 2003; Shields et al. 2011). Phthalates are partial agonists of peroxisome proliferator-activated receptors ($PPAR_{\alpha}$ and $PPAR_{\gamma}$) involved in lipid metabolism, adipocyte differentiation and energy homeostasis. $PPAR_{\alpha}$ activation has been associated with the induction of fatty acid hydroxylase (CYP4A family) involved in several metabolic pathways (Desvergne et al. 2009; Hurst and Waxman 2003)).

Regarding phenols, affinity with estrogen receptors have been reported for benzophenone-3, triclosan and parabens (Kunz and Fent 2006; Witorsch and Thomas 2010); the estrogenic activity of parabens depends on the length of the alkyl group (ER affinity: butylparaben > propylparaben > ethylparaben > methylparaben (Routledge et al. 1998; Witorsch and Thomas 2010)). A recent in vitro study has reported that parabens promote adipocyte differentiation in mice cells by an activation of glucocorticoid receptors and / or

PPAR γ (Hu et al. 2012; Taxvig et al. 2012). Bisphenol A is one of the most studied phenols. Additionally to its effects on estrogen receptors, it can inhibit androgen (Sohoni and Sumpter 1998) and thyroid receptors (Moriyama et al. 2002; Pearce and Braverman 2009). For this phenol, in vitro studies have also suggested an agonist effect on glucocorticoid receptors, notably implied in adipocyte differentiation (Sargis et al. 2010).

Recent studies have suggested that effects of phthalates and phenols on obesity might be mediated by epigenetic modifications (DiVall 2013; Fleisch et al. 2012; Manikkam et al. 2013; Singh and Li 2012). As an example, prenatal exposure to bisphenol A has induced hypomethylation leading to an increased expression of the Agouti gene, inducing obesity and diabetes in mice (Dolinoy 2008).

2. Effects of prenatal exposures to phenols and phthalates on growth; evidence from animal studies

Originally, animal studies aiming at assessing phthalate effects on health did not specifically focus on growth. However authors usually reported pup's weight as secondary outcome.

a) Phthalates

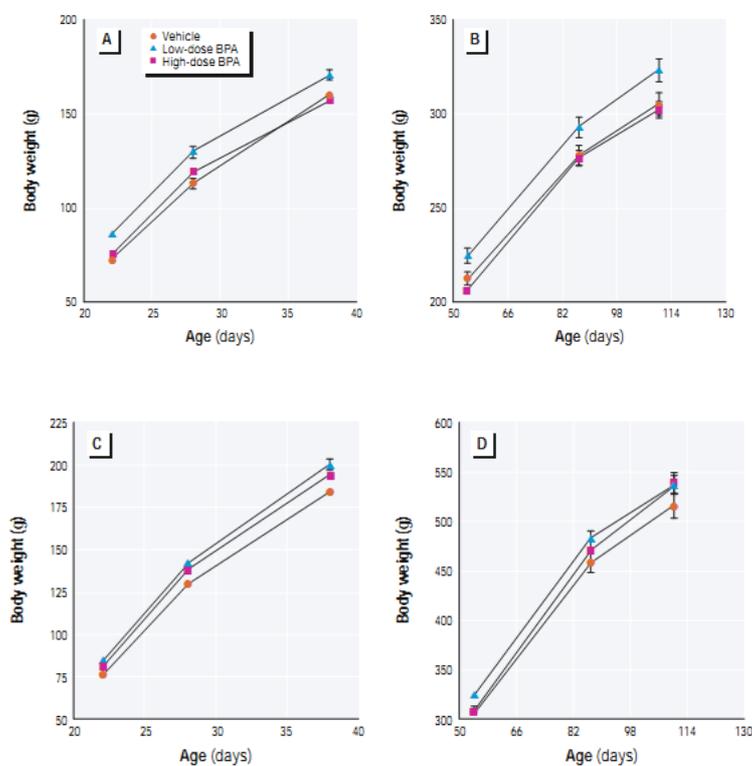
In rodents, exposure to several phthalates (di-iso-butyl phthalate, di-n-butyl phthalate and benzylbutyl phthalate) during the prenatal period has been inversely associated with fetal weight (Ema and Miyawaki 2002; Saillenfait et al. 2003, 2006, 2008). In another study, exposure to low dose of mono-(2-ethylhexyl) phthalate (MEHP, dose: 0.05 mg/kg of body weight/day), a metabolite of DEHP, has been associated with birth weight and fat mass increases in male offspring; no effect was observed with higher doses of MEHP (0.25 or 0.5 mg/kg of body weight/day, (Hao et al. 2013)). Two other studies, using high dose of DEHP (pregnant rats and mice were exposed to 50 mg/kg/day and 750 mg/kg/day) did not observe effect on weight at birth nor later in life (Gray et al. 2000; Tanaka 2005).

b) Phenols

Exposure to bisphenol A (0.1 and 1.2 mg/kg of body weight/day) during perinatal period (day 6 of pregnancy through the period of lactation) has been positively associated with weight at birth (Rubin and Soto 2009; Rubin et al. 2001). This association remained later

in life and tended to be non-monotonic, especially in female newborns: the effect of bisphenol A was more marked in the group exposed to 0.1 mg compared to the group exposed to 1.2 mg/kg of body weight/day (Figure 6). Birth and postnatal weight increase in association with exposure to bisphenol A during pregnancy has also been reported with lower doses of exposure (around 0.25 $\mu\text{g}/\text{kg}$ of body weight/day, (Ryan et al. 2010) and 70 $\mu\text{g}/\text{kg}/\text{day}$ (Somm et al. 2009)). Reports of body weight reduction at birth existed, however at high doses (300 and 1000 mg/kg) and the authors did not see any effect with the dose of 100 mg/kg (Kim et al. 2001). Data were sparse regarding effects of the other phenols. In rodents, weight at birth and in early life (postnatal day 7) decreased after perinatal (gestational and lactational periods) exposure to 2,4-dichlorophenol (Aoyama et al. 2005). 1,4-dichlorobenzene, a precursor of 2,5-dichlorophenol has been associated with birth weight decrease (Agency for Toxic Substances and Disease 2006).

Figure 6: Body weights of female (A, B) and male (C, D) offspring of Sprague-Dawley female rats exposed to 0.1 mg/kg of body weight/day ("low dose"), 1.2 mg/kg of body weight/day ("high dose ") of bisphenol A or to vehicle only (controls) during gestation and lactation (Rubin et al. 2001).



3. Effects of prenatal exposures to phenols and phthalates on growth, epidemiological studies

a) Phthalates

Findings of the studies aiming at studying the associations between prenatal exposure to phthalates and birth outcomes in the general population are summarized in Table 3. Several studies have reported positive associations between phthalates and birth outcomes (Brucker-Davis et al. 2010; Huang et al. 2009; Wolff et al. 2008). In an American cohort study of 404 mother–infant pairs, authors have reported a positive association between monobenzyl phthalate (MBzP), measured in maternal urine and birth length and between monoethyl phthalate (MEP) and head circumference at birth (Wolff et al. 2008). In another study with a small sample size ($n = 32$ female and 33 male newborns), a significant birth weight and birth length increase has been observed in female newborns with the highest concentrations of mono-butyl phthalate (MBP) in amniotic fluid (concentration $>$ median level); similar but not significant associations have been observed in male newborns (Huang et al. 2009). The concentration of MBP, measured in cord blood, has been positively correlated to weight, length and head circumference at birth in a study restricted to male newborns ($n = 86$, (Brucker-Davis et al. 2010)). Reports of inverse association also exist for the precursor of MBP measured in cord blood, which has been associated with an increase risk of low birth weight (< 2500 g) (Zhang et al. 2009). Suzuki et al. (2010) have studied the association between the molecular sum of phthalate metabolites measured in maternal urine and birth weight, and suggested a negative association ($n = 149$ boys and girls).

Comparisons across these studies are difficult because different matrices, including maternal urine, cord blood and amniotic fluid, have been used to measure biomarkers of exposure (Table 3). Sometimes the parent phthalate rather than its metabolites has been measured. This could lead to exposure misclassification. In the case of external contamination of the biological samples, levels of parent chemicals will be impacted but not those of their metabolites.

Associations between phthalate exposure and early postnatal growth have only been explored in a cross-sectional study. No data regarding exposure during fetal life was available (Teitelbaum et al. 2012). No clear association has been observed between any of the nine assessed phthalate metabolites (measured once in the child's urine collected between 6 and 8

years) and body mass index, waist circumference and height between 6 and 8 years ($n = 361$, (Teitelbaum et al. 2012)).

b) Phenols

The findings of the studies aiming at studying the association between prenatal exposure to phenols and pre- and post-natal growth in human are summarized in Table 4. Most of the studies used birth measurements as proxies of fetal growth and focused on bisphenol A. Only two studies had fetal biometry measured during pregnancy (Lee et al. 2010; Snijder et al. 2013).

The two studies with ultrasound measurements reported a negative association between prenatal exposure to bisphenol A and fetal head circumference (Lee et al. 2010; Snijder et al. 2013). Snijder et al. (2013) further reported a negative association with fetal weight. A negative association with fetal abdominal circumference has been observed in the study by Lee et al (2010). The negative associations with weight and head circumference reported in the study by Snijder et al. (2013) were only observed in the 80 subjects in whom three measurements of bisphenol A concentrations during pregnancy were used to assess exposure and no association was observed when only one ($n = 219$) or two ($n = 120$) samples were used. Inversely, in a study among 339 pregnant women from New-York city, maternal urinary concentrations of bisphenol A tended to be positively associated with birth weight ($p < 0.2$, (Wolff et al. 2008)). Dichlorophenols, benzophenone-3 and triclosan have been measured in this cohort. An inverse association between 2,5-dichlorophenol and birth weight, and a positive association between benzophenone-3 and birth weight in male, but not in female newborns, have been observed.

Only one study explored the potential effects of prenatal exposures to phenols on post-natal growth. Bisphenol A concentrations measured in maternal urine collected during pregnancy have been associated with a decrease risk of being overweight at 9 years. This association was observed in girls but not in boys (Harley et al. 2013). Bisphenol A concentrations were also measured in the urine of the children collected at 9 years. A positive association with overweight at age 9 have been reported in boys and girls. This positive associations as well as those observed in a cross-sectional study (Trasande et al. 2012) have to be interpreted cautiously because of reverse causality. Overweight children may consume more canned drink or processed foods, which are important sources of bisphenol A, than non-

overweight children. In addition, obesity and associated health disorders might affect bisphenol A metabolism, storage and elimination.

To summarize, only few human studies have explored the effects of prenatal exposures to phenols and phthalates on pre- and early post-natal growth, and most of them had a limited sample size (Tables 3, 4). Some of the reported associations might be due to false positive discovery induced by the high number of comparisons performed (high number of biomarkers assessed and high number of outcomes, especially for studies using repeated measurements of fetal biometry during pregnancy). Another limitation of these studies is the exposure assessment, usually based on a small numbers of maternal urine samples collected during pregnancy.

Table 3: Studies of associations between prenatal exposures to phthalates and fetal growth in the general population

Reference, area	Phthalates considered	Matrices for phthalate assay	Outcomes	Population	Main results
Brucker-Davis et al. 2010 France	MBP	Cord blood	Birth length Birth weight Head circumference at birth	Case-control study regarding cryptorchidism n = 86 controls (boys only)	Positive correlations with Weight: 0.27 (p = 0.09) Height: 0.29 (p = 0.007) Head circumference: 0.43 (p = 0.005)
Huang et al. 2009 Taiwan	MBP, MEHP, MEP, MBzP, MMP	Amniotic fluid Urine (1 st trimester)	Birth length Birth weight	Cohort of women with planned amniocentesis n=65 (girls and boys)	Girls: weight and height increase in the group heavily exposed to MBP Boys: no significant difference
Latini et al. 2003 Italy	DEHP, MEHP	Cord blood	Birth weight	Cohort n=84 (girls and boys)	No association with birth weight
Suzuki et al. 2010 Tokyo	MMP, MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MINP, MnOP	Urine (collected between 9 and 40 GW)	Birth length Birth weight Head circumference at birth	Cohort n = 149 (girls and boys)	No significant association but the molecular sum of the all phthalate metabolites tended to be negatively associated with birth weight ($\beta = -0.12$, p = 0.1)
Wolf et al. 2008 New York	MEHP, MEHHP, MEOHP, MECPP, MBzP, MCP, MiBP, MEP, MBP	Urine (collected between 25 and 40 GW)	Birth length Birth weight Head circumference at birth	Cohort n = 382 (girls and boys)	β (95% CI) for a 1 increase in ln-transformed concentration: MBzP / length : $\beta = 0.20$ (0.00; 0.40) MEP / head circumference: $\beta = 0.12$ (0.01; 0.23) Low-MWP / head circumference: $\beta = 0.13$ (0.01; 0.24) Low-MWP / gestational age: 0.14 (0.01; 0.27)
Zhang et al. 2009 China	DEP, DBP, DEHP MBP, MEHP	Cord blood Meconium	Low birth weight	Case-control n = 88 cases of low birth weight and 113 controls (girls and boys)	OR (95% CI) in the 4 th exposure quartile compared to the 1 st : MBP: 4,68 (2.1; 6.9) MEHP: 3,23 (1,3; 5.9)

Abbreviations : OR : odds ratio, GW: gestational week, MBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxisononyl phthalate, MCOP: monocarboxy-isoocetyl phthalate, MCP: mono(3-carboxypropyl) phthalate, MECPP: mono(2-ethyl-5-carboxypentyl) phthalate, MEHP: mono(2-ethylhexyl) phthalate, MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate, MEOHP: mono(2-ethyl-5-oxohexyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, Σ LMW: molecular sum of low molecular weight phthalates Σ HMW: molecular sum of high molecular weight phthalates

Table 4: Studies regarding the effects of prenatal exposures to phenols on pre- and post-natal growth in the general population

Reference, area	Phenols considered	Matrices for phenol assay	Outcomes	Population	Main results
Chou et al. 2011 Taiwan	BPA	Maternal and cord blood collected at birth	Birth weight Small for gestational age	Birth cohort n = 97 (girls and boys)	BPA in maternal blood was negatively correlated with weight U-shaped association between BPA (quartile) and the risk to be small for gestational age in males.
Harley et al. 2013 United States	BPA	Maternal and child urine	BMI, waist circumference, body fat, obesity	n = 311, 9 year-old children (boys and girls)	Prenatal exposure: negative association with BMI- Z-score, waist circumference and with the risk of being obese in girl but not in boys. Postnatal exposure (at age 9): positive association with BMI Z-score and waist circumference (not modified by sex).
Lee et al. 2010 Korea Abstract only	BPA	Maternal blood and urine	Biparietal and abdominal diameter, femur length measurements at 19 and 36 GW	n = 125 (girls and boys)	Negative association with: Head circumference in late pregnancy: $\beta = - 0.06$, $p = 0.02$ Abdominal circumference in late pregnancy: $\beta = - 0.32$, $p = 0.02$ Femur length in middle pregnancy: $\beta = - 0.04$, $p = 0.04$
Padmanabhan et al. 2008 Michigan (USA)	BPA	Maternal blood collected at birth	Birth weight	General population n = 40 (girls and boys)	No correlation between BPA concentrations and birth weight.
Wolff et al. 2008 New York	2,4-, 2,5-DCP BPA BP3 TCS	Maternal urines collected between 25 and 40 GW.	Birth length Birth weight Head circumference at birth	Cohort n = 339 (girls and boys)	Boys : weight and length decreased with exposure to 2,5-DCP, weight increased with exposure to BP3 Girls: weight decreased with exposure to BP3
Snijder et al. 2013 Netherlands	BPA	Maternal urines (1 to 3 samples).	Ultrasounds (T2 and T3) and birth measurements of weight and head circumference	n = 80, 120 or 219 depending of the analysis	When analyses were restricted to the 80 fetuses with 3 exposure assessments: Negative association with weight and HC ($p < 0.1$), which disappeared if using only 1 or 2 of the 3 samples were used.

Abbreviations: GW: gestational week, BPA: bisphenol A, BP3: benzophenone-3, DCP: dichlorophenol, TCS: Triclosan, T2: second trimester of pregnancy, T3: third trimester of pregnancy,

IV. Assessment of prenatal exposure to phenols and phthalates in Epidemiological studies

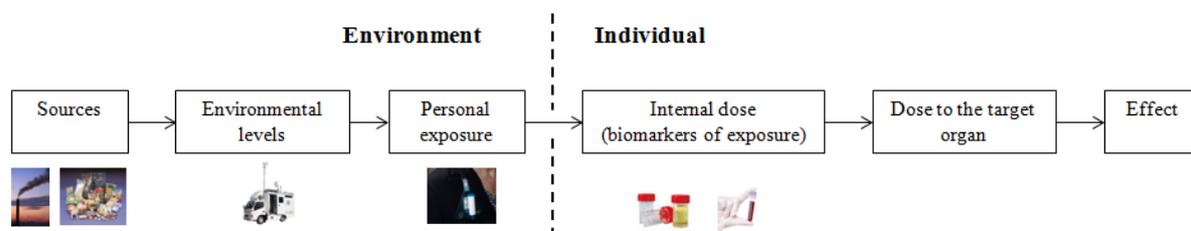
1. Exposure assessment to chemicals in epidemiological studies

Exposure assessment is a central issue in epidemiological studies aiming at exploring the effects of environmental contaminants on human health; exposure misclassification may indeed lead to bias in the health risk estimates and impacts statistical power (de Klerk et al. 1989; White et al. 2008). There are several methods to assess exposure to environmental contaminants and the choice of a particular one will depend on the characteristics, sources and routes of exposure to the studied chemical(s) (Figure 7). Studies focusing on atmospheric pollutants usually estimate chemical concentrations to which the participants may have been exposed (external dose) using environmental and/or personal measurements. This approach is not well adapted to chemicals with multiple sources and routes of exposure, such as phenols and phthalates. However, phthalate measurements in indoor air have been used in few previous studies exploring their effects on respiratory health (Bornehag et al. 2004; Kolarik et al. 2008). Other methods, such as questionnaires, could be used to estimate environmental exposure to pollutants. For chemicals with multiple sources of exposure, exposure assessment through questionnaires is not easy and is likely to suffer of important lack of precision. Job-exposure matrices are usually used in studies aiming at assessing professional exposure and allow to infer exposure on the basis of the job title, the industry and / or calendar times of employment (Nieuwenhuijsen 2003; Snijder et al. 2012).

Human biomonitoring consists in the assessment of the levels of chemicals or their metabolites in human biological samples such as blood or urine and provides an estimate of the dose that actually enters the body (internal dose) from all sources and routes of exposures. For these reasons, it is the most relevant option to assess exposure to phenols and phthalates (A.M. Calafat et al. 2006). Biomonitoring was primarily used in occupational medicine. Improvements in analytical techniques have allowed detection of relatively low chemical concentrations in human fluid and biomarkers of exposure are now widely used in epidemiological studies in the general population (Angerer et al. 2007; A. M Calafat et al. 2006; Schisterman and Albert 2012). The use of biomarkers does not guarantee an absence of exposure misclassification. Biomonitoring indeed only provides a snapshot in time of a

person's exposure and because of the short half-life of phenols and phthalates and of the episodic nature of the exposures, concentrations are likely to vary over time.

Figure 7: Schematic view of the different tools allowing to quantify exposure to the environmental chemicals in Environmental Epidemiology.



2. Specific issues related to the use of biomarkers to assess prenatal exposure to phenols and phthalates

Phthalates and phenols have been detected in indoor air and dust and biological sample contamination may occur during collection and processing specimens. Sampling materials might also contain these chemicals. In order to minimize the risk of exposure over-estimation due to external contamination of the biological samples, it is more desirable to measure metabolites, produced by transformation of the parent chemical in the organism, rather than the parent chemicals (Calafat and Needham 2009; A. M Calafat et al. 2006). The metabolism of high molecular weight phthalates leads to more than one metabolite and in addition to separately study each metabolite, their molecular sum is commonly considered (Hauser and Calafat 2005; Koch et al. 2005). This may be seen as an attempt to provide an estimate closer to the internal dose in the absence of toxicokinetic model.

a) Relevant biological matrices to measure biomarkers of exposure to phthalates and phenols

Urine and blood are the most commonly used matrices for biomonitoring. Phenols and phthalates have already been assessed in blood in epidemiological studies (Zhang et al. 2009). However, blood contains enzymes able to degrade phthalates and their metabolites; which could lead to exposure misclassification (Koch et al. 2004). In addition, collecting blood is invasive. Phthalates and phenols are mostly excreted in urine, which is easier and safer to collect than blood. Thus urine is the preferred matrix to assess exposure to these chemicals.

Many studies aiming at assessing exposure during fetal life used maternal urine samples collected during pregnancy (Braun et al. 2011a; Chevrier et al. 2012; Engel et al. 2010; Wolff et al. 2008). However it is unclear to which extent maternal urine reflects fetal exposure. Alternative matrices such as amniotic fluid and meconium have been proposed (Barr et al. 2005; A. M Calafat et al. 2006). During pregnancy, the fetus swallows amniotic fluid and fetal urine is one of the main source of amniotic fluid in the second half of pregnancy (Modena and Fieni 2004; Underwood et al. 2005). Thus, concentrations measured in this matrix might be a relevant dosimeter of fetal exposure. In humans, bisphenol A (Edlow et al. 2012; Engel et al. 2006; Ikezuki et al. 2002; Yamada et al. 2002) and 2,5-dichlorophenol (Bradman et al. 2003) have been detected in amniotic fluid collected from pregnant women receiving amniocentesis as part of their routine prenatal care. However, to our knowledge, no published study has evaluated human amniotic fluid concentration of other prevalent environmental phenols, nor has compared phenol biomarker concentrations in amniotic fluid with their concentrations in maternal urine.

Several studies have assessed phthalate metabolites in amniotic fluid and maternal urine sample from same woman and authors reported lower rates of detection in amniotic fluid compared to maternal urine (Huang et al. 2009; Wittassek et al. 2009). Huang et al. (2009) found a correlation of 0.16 ($p = 0.002$, $n = 64$) between urine and amniotic fluid concentration of monobutyl phthalate (MBP). A higher correlation coefficient has been reported in another study for mono-isobutyl phthalate (MiBP) ($\rho = 0.93$, $p < 0.001$, $n = 11$ (Wittassek et al. 2009)). Because of the difficulty encountered in the collection of amniotic fluid specimen, which requires an invasive procedure that can be harmful to the fetus and mother, this fluid is not commonly used in epidemiological studies.

Meconium corresponds to the earliest stools of the newborn. It starts accumulating in the fetal bowel at the beginning of the second trimester of pregnancy and has already been used to estimate fetal exposure to non persistent chemicals (Zhang et al. 2009), however collection standardization has to be improved to allow its routine use in epidemiological studies (Calafat and Needham 2009): contamination with the newborn urine is hardly avoidable; timing of collection after birth is also important because meconium composition changes rapidly.

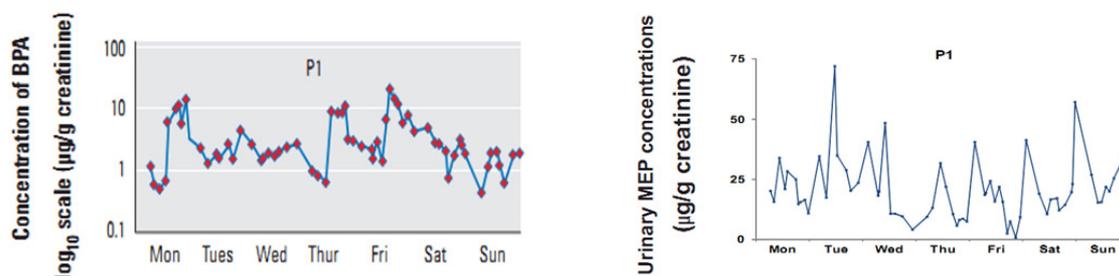
Cord blood, collected at birth, has also been used to assess phthalate exposures (Zhang et al. 2009). However due to the non persistence of the studied chemicals, concentrations of phenols and phthalates measured in this fluid may reflect exposure occurred at the hospital / maternity before and during delivery, which is likely to differ from exposure during the rest of the pregnancy. This is particularly true for di(2-ethylhexyl)phthalate and bisphenol A, which are found in medical tubing and medical devices potentially used during delivery (Calafat and Needham 2009). Such an issue has been highlighted in a study in which exposure biomarkers have been assayed in maternal urine collected at delivery (Vandentorren et al. 2011).

b) Temporal variability in phenol and phthalate urinary concentrations during pregnancy

Variability in phthalate and phenol urine concentrations has been studied in adults (Adibi et al. 2008a; Fromme et al. 2007; Hauser et al. 2004; Hoppin et al. 2002a; Peck et al. 2010; Preau et al. 2010; Ye et al. 2011a). Considerable within- and between-day variability in the bisphenol A concentrations measured in spot urine samples collected from the same participant (Ye et al. 2011a) has been reported. Similar variations have been observed for phthalates ((Preau et al. 2010) Figure 8). Data concerning pregnant women are limited (Adibi et al. 2008a; Braun et al. 2012; Braun et al. 2011b; Smith et al. 2012). Regarding phenols, reproducibility of the urinary concentrations of bisphenol A (Braun et al. 2012; Braun et al. 2011b) and parabens (Smith et al. 2012) in pregnant women has been evaluated. However, to date, no study has assessed temporal variability in urinary concentration of other phenols during pregnancy².

² Mecker et al have very recently published ICCs for benzophenone-3, triclosan and dichlorophenols among 105 pregnant women from Northern Puerto Rico (Mecker et al, 2013). This paper will be discussed in Chapter III of the thesis.

Figure 8: Concentrations of bisphenol A and monoethyl phthalate ($\mu\text{g/g}$ creatinine) for all of the spot urine samples collected from one participant over one week (*Preau et al. 2010; Ye et al. 2011a*)



A source of variability in phenol biomarker concentrations is heterogeneity in collection conditions. Based on the concentration variations shown in Figure 8, one can hypothesize that for two women with the same real exposure, variations in sampling conditions (e.g. hour and day of sampling) between these women could lead to high difference in the exposure estimates. Because it is rarely feasible to make collection conditions identical for all subjects in large-scale observational studies, a statistical method has been developed to standardize concentrations for collection conditions (this has been detailed in Chapter II, page 50, (Mortamais et al. 2012)). The aim of this approach was to increase the between-subjects comparability of biomarkers' levels estimated from urinary samples collected in varying conditions. While it has been used in epidemiological studies relating urinary biomarkers to health endpoints (Chevrier et al. 2012; Philippat et al. 2012), the impact of standardizing biomarker concentrations for collection conditions on reproducibility of urinary measures has never been studied.

c) Biomarker concentrations and urine dilution

Water contents usually differs across spot samples (e.g., urine tends to be more concentrated in the first morning-void compared to the void collected later in day) and this has to be taken into account when studying associations between biomarker concentrations and health outcomes. Adjustment for creatinine and specific gravity have been both proposed to solve this issue. Creatinine comes from creatine and creatine phosphate decomposition and is primarily cleared from the body through the kidney by glomerular filtration (Alessio et al. 1985). Specific gravity is the ratio of urine density to water density. These measurements have both limitations. Creatinine adjustment might be not appropriate for chemicals excreted by tubular secretion (active transport); specific gravity will be affected by medical conditions

leading to the presence of sugar and proteins in urine. There is no clear consensus about the superiority of one of them to account for urine dilution in epidemiological studies related to exposure biomarkers.

V. Objectives of the thesis

The objectives of this thesis were:

- 1) to study the effects of prenatal exposures to phenols and phthalates on pre- and early post-natal growth (Chapter II);
- 2) to assess the relationship between phenol concentrations measured in maternal urine and amniotic fluid collected on the same day (Chapter III);
- 3) to study the temporal variability in urinary phenol concentrations over the course of pregnancy (Chapter III) and to characterize the bias associated with the use of one urine sample to estimate exposure to non persistent chemicals (Chapter III).

**CHAPTER II: EFFECTS OF PRENATAL
EXPOSURES TO PHTHALATES AND PHENOLS
ON HUMAN GROWTH**

Effets de l'exposition prénatale aux phtalates et aux phénols sur la croissance

Introduction

Chez les rongeurs, l'exposition périnatale (gestation et / ou lactation) à certains phénols (notamment le bisphénol A) et phtalates, a été associée à une modification du poids à la naissance et durant les premiers jours de vie. Une étude chez l'homme a suggéré un effet du bisphénol A, mais aussi des dichlorophénols et de la benzophénone-3 sur le poids de naissance des garçons (Wolff et al. 2008). Plusieurs études s'étaient aussi intéressées aux effets des expositions prénatales aux phtalates sur le poids de naissance chez l'homme, néanmoins leurs résultats ne permettaient pas de conclure. Une seule de ces études avait utilisé des mesures échographiques pendant la grossesse pour estimer la croissance foetale, les autres études étant basées sur des mesures à la naissance (poids, taille, périmètre crânien). Les effets des expositions prénatales aux phénols et phtalates sur la croissance postnatale ont été peu étudiés. L'unique étude pour laquelle les expositions pendant la grossesse ont été estimées concernait le bisphénol A et suggérait une association négative avec l'indice de masse corporelle et la circonférence abdominale des filles à 9 ans (Harley et al. 2013).

Objectif: L'objectif de ce chapitre était d'étudier les associations entre l'exposition aux phénols et aux phtalates pendant la grossesse et la croissance durant la vie foetale et les trois premières années de vie.

Expositions prénatales aux phtalates et aux phénols et poids, taille et périmètre crânien à la naissance.

Méthodes: La population d'étude était issue d'une étude cas-témoins, concernant les malformations congénitales des organes génitaux masculins, nichée dans deux cohortes mère-enfant françaises, EDEN et PELAGIE, dont le recrutement a eu lieu entre 2002 et 2006 (n = 287). Pour évaluer les expositions prénatales, nous avons dosé 11 métabolites de phtalates (n = 287) et 9 phénols (n = 191) dans les urines maternelles prélevées entre 6 et 30 semaines de gestation. Le poids, la taille et le périmètre crânien des enfants à la naissance ont été recueillis à partir des dossiers de maternité. Les associations entre les concentrations urinaires des métabolites de phtalates et des phénols et les mesures à la naissance ont été étudiées à l'aide de modèles de régressions linéaires multiples, corrigées pour la surreprésentation des cas de malformation congénitales relative au design cas-témoins de l'étude.

Résultats : Le poids à la naissance ajusté diminuait de 77 g (intervalle de confiance 95 % (IC), -129 ; -25) et de 49 g (IC 95%, -86 ; -13) pour chaque augmentation unitaire de la concentration ln-transformée de 2,4- et 2,5-dichlorophénol, respectivement. La concentration de benzophénone-3 était positivement associée avec le poids ($\beta = 26$ g, IC 95%, -2 ; 54) et la circonférence crânienne à la naissance ($\beta = 0,1$ cm, IC 95%, 0,0 ; 0,2). Le périmètre crânien augmentait en moyenne de 0,3 cm (IC 95%, 0,0 ; 0,7) pour chaque augmentation unitaire de la concentration ln-transformée de bisphénol A. Aucun phtalate n'était associé de façon monotone au poids de naissance (p-valeur pour le test de monotonie > 0.14) mais des relations non monotones ont été observées entre le mono-(3-carboxypropyl) phtalate (MCPP), le mono(2-ethyl-5-carboxypentyl) phtalate (MECPP) et le poids de naissance.

Conclusion : Les résultats concernant les associations entre le 2,5-dichlorophénol et la benzophénone-3 et le poids à la naissance des garçons étaient cohérents avec ceux d'une étude antérieure menée aux États-Unis (Wolff et al. 2008). Des associations non-monotones avec le poids et la taille à la naissance ont été également suggérées dans notre analyse pour certains métabolites de phtalates.

Ces associations n'avaient, à notre connaissance, jamais été signalées auparavant chez les rongeurs ou chez l'homme, et ont donc été considérées avec prudence.

Effets des expositions prénatales aux phénols sur la croissance fœtale, estimée à l'aide de mesures échographiques pendant la grossesse, et sur la croissance post-natale précoce.

Les dosages urinaires de phénols et de métabolites de phtalates ont été étendus à d'autres femmes de la cohorte EDEN ayant accouchés d'un garçon (n = 410), dans le but 1) de répliquer les résultats obtenus précédemment, 2) d'étudier les associations avec l'anthropométrie fœtale mesurée à plusieurs reprises pendant la grossesse et la croissance de l'enfant durant les trois premières années de vie³.

Méthodes : La croissance fœtale a été évaluée à l'aide de données échographiques, réalisées en cours de grossesse ainsi que le poids, la taille et le périmètre crânien à la naissance. Les trajectoires individuelles de croissance postnatale ont été prédites en utilisant les mesures de poids et de taille réalisées de façon régulièrement durant les trois premières années de vie. La circonférence abdominale ainsi que l'épaisseur des plis cutanés et le périmètre crânien ont été mesurés à 3 ans.

Résultats: La concentration urinaire de triclosan était négativement associée à tous les paramètres de croissance mesurés à la 3^{ème} échographie (p-valeurs comprises entre 0,01 pour la circonférence abdominale et 0,16 pour le périmètre crânien). Ce phénol était aussi associé de façon négative au périmètre crânien à la naissance ($\beta = - 1,4$ mm, IC 95%; -2,8 ; 0,0) et à 36 mois ($\beta = - 1,0$ mm, IC 95%; -2,8 ; 0,8). Les parabènes étaient positivement associés avec le poids de naissance (p-valeurs < 0,05). Le méthyle- et propyle-parabènes étaient aussi positivement associés avec le poids à 6, 12, 24 et 36 mois et avec la circonférence abdominale à 36 mois (p-valeurs comprises entre 0,02 et 0,14). Nous n'avons pas observé d'associations entre les dichlorophénols et le poids de naissance.

Conclusion : Nous disposions seulement d'un échantillon d'urine pour évaluer les expositions aux phénols et aux phtalates dans la cohorte EDEN. En raison de la courte demi-vie des composés étudiés et de la nature épisodique des expositions, nos résultats étaient probablement affectés par des erreurs de mesures concernant les expositions. Néanmoins, notre étude suggérait un effet potentiel de l'exposition prénatale à certains phénols sur la croissance pré- et post-natale. Les associations entre les dichlorophénols et le poids de naissance, suggérées dans notre 1^{ère} analyse, réalisée au sein d'un échantillon de 191 sujets de la cohorte EDEN, n'ont pas été confirmées dans un échantillon plus large de cette population (n = 520 sujets).

³ Les associations entre l'exposition aux phtalates et la croissance pré- et post-natale ont été étudiées par J. Botton (Inserm U1018, Université Paris-Sud) et ne sont pas présentées ici.

I. Effects of prenatal exposures to phthalates and phenols on birth outcomes

1. Aims of the chapter

Our aim was to study the relationships between prenatal exposures to phthalates and phenols and weight, length and head circumference at birth among male newborns⁴.

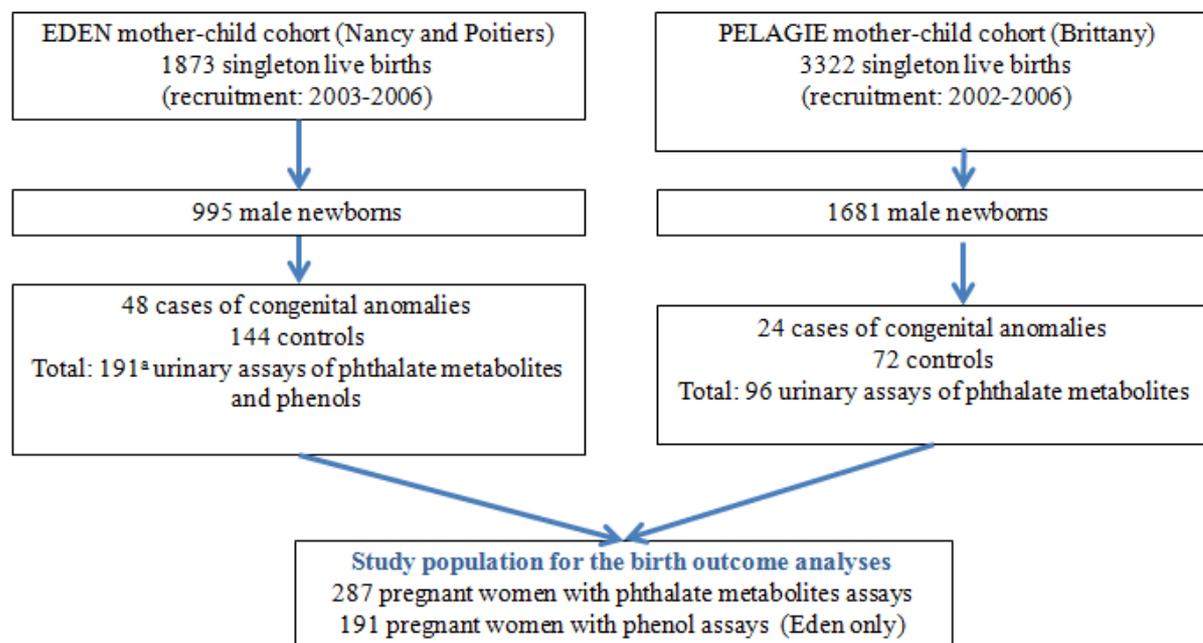
2. Population and Methods

a) Study population

The study population is a case-control population study of male malformations of the genitalia nested in the EDEN and PELAGIE mother-child cohorts. These cohorts aim to study the effects of the events happened during fetal life on development and health at birth and in childhood (Drouillet et al. 2009; Garlantezec et al. 2009). Briefly, the EDEN cohort consists of 2002 pregnant women recruited before the end of the 28th gestational week from April 2003 to March 2006 in the obstetrical departments of the University Hospitals of Nancy and Poitiers, France (Coordinator, M.A Charles, INSERM U1018). The PELAGIE cohort consists of 3421 pregnant women enrolled before 19 weeks of gestation from April 2002 to February 2006 in three districts of Brittany: Ille et Vilaine, Finistère and Côtes d'Armor, France (Coordinator: S. Cordier, Inserm U625). The present study includes all of the male newborns with undescended testis or hypospadias (identified at birth by pediatricians, n=48 in EDEN and 24 in PELAGIE, Figure 9). In addition, three male newborns without congenital malformation of the genitalia (controls) were matched to each case by recruitment center, date of recruitment (+ / - 6 months), day of week (week-end yes/no) and gestational week when the maternal urine sample was collected, for a total of 288 mother–newborn pairs (72 cases and 216 controls, Figure 9).

⁴ Our choice to focus on male newborns was that sex-specific associations have been reported, in particular between some phenols and birth weight.

Figure 9: Design of the study population for the birth outcome analyses (Gona_PE study, EDEN and PELAGIE cohorts, n = 287^a)



^a the vial of one control broke before assessments

Cases and controls were matched for center, date of recruitment and for gestational duration at the time of collection of the biological sample.

Participants provided informed consent for data and biological sample collection for themselves and their offspring. These cohorts received the approvals of the appropriate ethical committees. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was limited and determined not to constitute engagement in human subjects research

b) Outcomes

We extracted from hospital maternity records weight, length and head circumference assessed at birth.

c) Exposure assessment

Urine was collected between 6 and 19 gestational weeks in the PELAGIE cohort and between 24 and 30 gestational weeks in the EDEN cohort. Women were asked to provide a first morning urine void sample. Women from EDEN collected the urine at home before the hospital study visit. When forgotten, the urine sample was collected at the hospital during the

study visit. Urine samples were then stored at -80°C . For the PELAGIE cohort, each woman collected a urine sample at home and mailed it by standard 2-day mail to the research laboratory where, upon arrival, the samples were stored at -20°C .

Assessment of biomarker and creatinine concentrations in maternal urine was done by the National Center for Environmental Health laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA (Silva et al. 2007; Ye et al. 2005). We measured the urinary concentrations of eleven phthalate metabolites in samples from all mothers ($n = 287$, 72 cases and 215 controls because the vial of one control broke during the transport to the CDC laboratory). Nine phenols were measured in urine samples collected from mothers in the EDEN cohort only ($n = 191$, 48 cases and 143 controls). Phenols could not be measured in the PELAGIE cohort because a preservative added to the samples interferes with the assay used.

Table 5: Name of the metabolites (and of their parent compounds) assessed in urine of the pregnant women from the EDEN and PELAGIE (phthalates only) cohorts.

Phenols	Phthalates	
	Metabolites	Parents compounds
	Mono(3-carboxypropyl) phthalate (MCPP)	Di-n-octyl phthalate (DOP) et Di-n-butyl phthalate (DBP)
2,4-dichlorophenol (2,4-DCP)	Mono-n-butyl phthalate (MBP/MnBP)	Di-n-butyl phthalate (DBP/DnBP)
2,5-dichlorophenol (2,5-DCP)		
Bisphenol A (BPA)	Mono-isobutyl phthalate (MiBP)	Di-iso-butyl phthalate (DiBP)
Benzophenone-3 (BP3)	Monobenzyl phthalate (MBzP)	Benzylbutyl phthalate (BBzP)
Triclosan (TCS)	Monoethyl phthalate (MEP)	Diethyl phthalate (DEP)
	Monocarboxy-isononyl phthalate (MCNP)	Di-isodecyl phthalate (DiDP)
Parabens:	Monocarboxy-isoocetyl phthalate (MCOP)	Di-isononyl phthalate (DiNP)
Methyl Paraben (MPB)	Mono(2-ethyl-5-oxohexyl)phthalate (MEOHP)	
Ethyl Paraben (EPB)		
Propyl Paraben (PPB)	Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	Di (2-ethylhexyl) phthalate (DEHP)
Butyl Paraben (BPB)	Mono(2-ethylhexyl) phthalate (MEHP) Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	

Molar concentrations of four metabolites of DEHP (mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)) were summed as total DEHP; concentrations of phthalate metabolites of MW > 250 g/mol and of molecular weight < 250 g/mol were summed as total of high molecular weight phthalates (ΣHMW) and of low molecular weight phthalates (ΣLMW), respectively. Total paraben concentration

(\sum PB) was calculated by summing methyl paraben, ethyl paraben, propyl paraben and butyl paraben molar concentrations.

MEP and MBzP concentrations have been corrected using following factors: 0.66 for MEP and 0.72 for MBzP. These factors were provided by the CDC. MEP and MBzP concentrations have to be corrected because the analytical standards used by the CDC were of inadequate purity (Antonia Calafat, personal communication).

d) Statistical analyses

We replaced concentrations below the limit of detection (LOD) by $LOD/\sqrt{2}$. We corrected the over-representation of congenital abnormalities, induced by the case-control design, using a reweighting approach; we used center-specific weights corresponding to the inverse of the inclusion probability of controls, so as to give cases and controls the same relative weight as in the original cohorts (Richardson et al. 2007). Including cases and controls allows to maximize sample size and statistical power. Whereas excluding cases would possibly induce a selection bias and limits generalization as well as comparability (cases were not excluded from the previous studies). Reweighting approach is not common yet in environmental epidemiology and we also presented the analyses restricted to controls.

Phthalates and phenols are non persistent compounds in humans with a half life in non-pregnant subjects generally estimated to below 24 hours (Janjua et al. 2008; Koch et al. 2004; Volkel et al. 2002). A high day-to-day variability in their urinary concentrations has been documented among non pregnant (Hoppin et al. 2002b) and pregnant (Adibi et al. 2008b) women, as well as within-day variations for some phthalates metabolites (Preau et al. 2010; Silva et al. 2004b) and bisphenol A (Ye et al. 2011a). Another potential sources of variability in biomarkers levels may be variations in sampling conditions which constitute a possibly controllable source of exposure misclassification. To limit the impact of between-subject variations in urine sampling conditions, we standardized biomarker concentrations using a 2-step standardization method based on regression residuals (see Box 1 for more details). First, we estimated associations between ln-transformed biomarker concentrations and sampling conditions [hour of sampling (for EDEN only), gestational age at collection, duration of storage at room temperature before freezing, season and day of sampling] using separate linear regression models for each biomarker adjusted for maternal age, body mass index before pregnancy, parity, year of sampling, education, current occupation, active smoking and center. Using estimated parameters, we predicted the concentrations that would

have been observed if all samples had been collected under the same standard conditions. These standard conditions corresponded to the median values of sampling conditions observed in our population; for example, it corresponded to 7:30 AM for sampling hour, the sampling condition that had the largest influence on measured biomarkers levels and to 5 hours for the delay between urine sampling collection and freezing. Unless otherwise specified, all concentrations are the standardized values; we also report associations with biomarker concentrations not standardized for sampling conditions.

Associations between each standardized maternal urinary biomarker concentration and either birth weight, birth length or head circumference were estimated using separate weighted linear regressions. Biomarker concentrations (micrograms per liter) were ln-transformed or coded in tertiles. We performed tests of heterogeneity in outcome value across exposure tertiles; p-values of trend tests were estimated using categorical variables whose values corresponded to the tertile-specific median biomarker levels. Situations in which biomarkers exhibited heterogeneity in outcome across tertiles (low p-value, heterogeneity test) but with little support for a trend (high p-value, trend test) were considered as suggestive of a non-monotonic association (A monotonic trend does not reverse direction but may have flat segment (Rothman et al. 2008)). We also estimated adjusted relationships between standardized biomarker concentrations and birth weight using restricted cubic splines (Harrell 2001).

Adjustment factors were all variables possibly related to birth outcomes (based on *a priori* knowledge), including maternal pre-pregnancy weight (broken stick model with a knot at 60 kg, (Slama and Werwatz 2005)), maternal height (continuous), maternal smoking (never, 1-5, 6 cigarettes per day and more), parity (0, 1, ≥ 2), education level (high school or less, up to 2 years after high school, ≥ 3 years after high school), gestational duration (linear and quadratic terms) and recruitment center. In addition, we adjusted for urinary creatinine concentration (continuous, non-transformed), as a marker of urine dilution. Gestational duration was estimated using the date of last menstrual period (LMP, (Slama et al. 2008)) or gestational duration assessed by the obstetrician if it differed from the LMP-based estimate by more than two weeks. Adjustment for the obstetrician-estimated gestational age instead of the LMP-based gestational age did not modify results (not shown). Models for head circumference at birth were also adjusted for the mode of delivery (because passage through the birth canal may influence head circumference at birth).

We performed sensitivity analyses excluding women with pregnancy-induced hypertension (n=17) or gestational diabetes (n=15).

All analyses were performed using STATA/SE version 11 (College station, TX 77845).

In the result interpretation, we gave more weight to results in agreement with our a priori hypotheses, namely an effect of benzophenone-3 and 2,5-dichlorophenol on male birth weight (Wolff et al. 2008). Other associations (highlighted on the basis of their p-values, without relying on threshold p-value to define statistical significance) were considered as hypothesis-generating.

Box 1: Standardization of urinary concentration for sampling conditions

This method was developed by Marion Mortamais (Mortamais et al. 2012).

For each biomarker, we first fit a linear regression model of log-transformed concentration $\ln([\text{Conc}]) = Y$, including as covariates all sampling conditions and potential confounders for the relation between sampling conditions and biomarkers concentration:

$$(Y^i)^{\text{measured}} = \alpha + \sum_j [\beta_{\text{samp cond } j} \times X_j^i] + \sum_k [\gamma_k \times Z_k^i] + \varepsilon^i \quad (\text{A1})$$

$(Y^i)^{\text{measured}}$: measured concentration in each subject i ,

$\beta_{\text{samp cond } j}$: regression parameter quantifying the effect of sampling condition X_j on the biomarker's concentration,

Z_k correspond to the potential confounders (age, socio-economic status, smoking, etc.),

ε^i : variability in Y^i not explained by sampling characteristics and potential confounders.

In a second step, the standardized concentration $(Y^i)^{\text{standardized}}$ is estimated as:

$$(Y^i)^{\text{standardized}} = (Y^i)^{\text{measured}} - \sum_j [\beta_{\text{samp cond } j} \times (X_j^i - X_j^{\text{std}})] \quad (\text{A2})$$

X_j^{std} : *standard* value for sampling condition j (e.g., 7:30 AM, in the case of sampling hour).

Formula (A2) can be justified the following way: We start by writing that the expected log-transformed biomarker concentration for subject i if sampling conditions correspond to the standard ones (and if potential confounders Z have the values corresponding to those observed for each subject i) is:

$$(Y^i)^{\text{standardized}} = \sum_j [\beta_{\text{samp cond } j} \times X_j^{\text{std}}] + \sum_k [\gamma_k \times Z_k^i] + \varepsilon^i \quad (\text{A3})$$

Equivalently to (A1), one can write:

$$\varepsilon^i = (Y^i)^{\text{measured}} - \sum_j [\beta_{\text{samp cond } j} \times X_j^i] - \sum_k [\gamma_k \times Z_k^i] \quad (\text{A4})$$

We now assume that the residuals ε of formulas (A1) and (A3) are identical. This will be the case if this residual is uncorrelated to the covariates X and potential confounders Z , and if the effect measure of X on Y is not modified by Z . With these assumptions, we now replace ε^i in (A3) by its expression (A4), which gives:

$$\begin{aligned} (Y^i)^{\text{standardized}} &= \sum_j [\beta_{\text{samp cond } j} \times X_j^{\text{std}}] + \sum_k [\gamma_k \times Z_k^i] + (Y^i)^{\text{measured}} - \sum_j [\beta_{\text{samp cond } j} \times X_j^i] - \sum_k [\gamma_k \times Z_k^i] \\ &= (Y^i)^{\text{measured}} - \sum_j [\beta_{\text{samp cond } j} \times (X_j^i - X_j^{\text{std}})] \end{aligned}$$

which corresponds to formula (A2).

3. Results

a) Study population

The 287 women with available phthalate urinary concentrations were 29 years old on average; 17 % smoked during the first trimester of pregnancy (Table 1). Average gestational age at delivery was 39.8 weeks, average birth weight was 3393 g (5th-95th percentiles, 2640 - 4130) and 7 newborns (2 %) weighed less than 2500 g. Average birth length was 50 cm (5th - 95th percentiles, 47 - 54) and average head circumference at birth was 35 cm (5th - 95th percentiles, 32 - 37, Table 6).

Table 6: Characteristics of French pregnant women and of their offspring (Gona_PE study, n = 287 mother-child pairs from EDEN and PELAGIE cohorts, 2002-2006).

Characteristic	n or mean ^a	(%)	Percentiles		
			5 th	50 th	95 th
Maternal age (years) (mean)	29.3		22	29	38
Duration of gestation^b (mean)	39.7		37	40	42
Birth weight (g) (mean)	3393		2640	3390	4130
Birth length (cm) (mean)	50		47	50	54
Head circumference at birth (cm) (mean)	34.7		32	35	37
Gestational age at sampling^b (weeks) (mean)	21.7		9	26	28
Creatinine concentration (g/L) (mean)	1.2		0.4	1.1	2.2
Parity					
0	115	(40)			
1 previous child	114	(40)			
≥ 2 previous children	58	(20)			
Maternal Education					
≤ high school	133	(47)			
high school + 2 years	62	(22)			
≥ high school + 3 years	86	(31)			
missing value	6				
Active smoking					
0	238	(83)			
1-5 cigarettes/day	30	(11)			
≥ 6 cigarettes/day	17	(6)			
missing value	2				
Pre-pregnancy BMI (kg/m²)					
< 18.5	29	(10)			
18.5 to 25.0	181	(64)			
> 25.0	73	(26)			
missing value	4				
Hour of urinary sampling					
before 8 AM	108	(65)			
8 to 10 AM	40	(24)			
after 10 AM	18	(11)			
missing value	121				

^a Sample size, unless otherwise specified.

^b Weeks of amenorrhea assessed by the date of the last menstrual period.

BMI: Body Mass Index

b) Exposure

We detected 8 of the 11 phthalate metabolites and 5 of the 9 phenols in at least 95 % of the samples. Among phthalates, the highest medians were observed for the low MW phthalates: MEP, MiBP, MBP (Table 7).

Table 7: Urinary phenol (n = 191) and phthalate (n = 287) biomarker concentrations after correction for case-control sampling (Gona_PE study, n = 287 mother-child pairs from EDEN and PELAGIE cohorts, 2002-2006).

Analytes	LOD (µg/L)	%>LOD	Standardized concentrations ^a			Measured concentrations		
			Percentiles (µg/L)			Percentiles (µg/L)		
			5 th	50 th	95 th	5 th	50 th	95 th
Phenols								
2,4-DCP	0.2	95.9	LOD	0.8	8.6	LOD	0.9	10.2
2,5-DCP	0.2	99.5	1.4	6.4	316.0	1.8	10.2	442.0
BPA	0.4	98.5	0.8	3.1	10.1	0.6	2.7	9.8
BP3	0.4	80.5	<LOD	1.3	74.5	<LOD	1.7	143.0
TCS	<LOD	84.1	1.0	17.5	464.6	<LOD	24.1	634.0
MP	1.0	100.0	9.0	104.3	2689.7	9.1	97.8	3520
EP	1.0	67.7	<LOD	1.5	38.2	<LOD	4.1	62.3
PP	0.2	96.9	0.3	10.4	267.7	0.5	12.5	402.0
BP	0.2	79.5	<LOD	2.2	63.6	<LOD	1.7	53.8
Phthalates								
MEP	0.5	100.0	28.1	105.3	727.0	24.9	110.22	983.4
MBP	0.6	100.0	10.2	58.1	487.5	7.6	48.1	398.0
MiBP	0.3	100.0	15.6	64.7	365.3	10.9	45.9	219.0
MBzP	0.2	100.0	2.7	21.7	209.2	2.0	17.7	116.6
MCPP	0.2	98.3	0.6	3.2	13.8	0.4	2.2	10.0
MEHP	1.2	91.8	1.8	10.5	62.3	<LOD	7.1	40.7
MEHHP	0.7	100	8.0	48.3	246.2	4.6	32.3	147.0
MEOHP	0.7	99.7	6.3	36.0	169.6	3.6	25.0	112.0
MECPP	0.6	100.0	18.9	67.2	303.0	11.6	43.8	183.0
MCOP	0.7	92.1	0.9	3.9	25.8	<LOD	2.7	17.2
MCNP	0.6	91.8	0.9	3.1	22.8	LOD	1.7	11.7

Abréviations : BBzP : benzylbutyl phthalate , DBP : di-n-butyl phthalate, DCP: dichlorophenol, DEP : diethyl phthalate, DiBP : di-iso-butyl phthalate, DiDP : di-isodecyl phthalate, DiNP : di-isononyl phthalate, DOP: n-octyl phthalate, LOD : limite de détection, MBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isooctyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MECPP: mono(2-ethyl-5-carboxypentyl) phthalate, MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate, MEHP: mono(2-ethylhexyl) phthalate, MEOHP: mono(2-ethyl-5-oxohexyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, MP: methyl paraben, PP: propyl paraben, TCS: Triclosan.

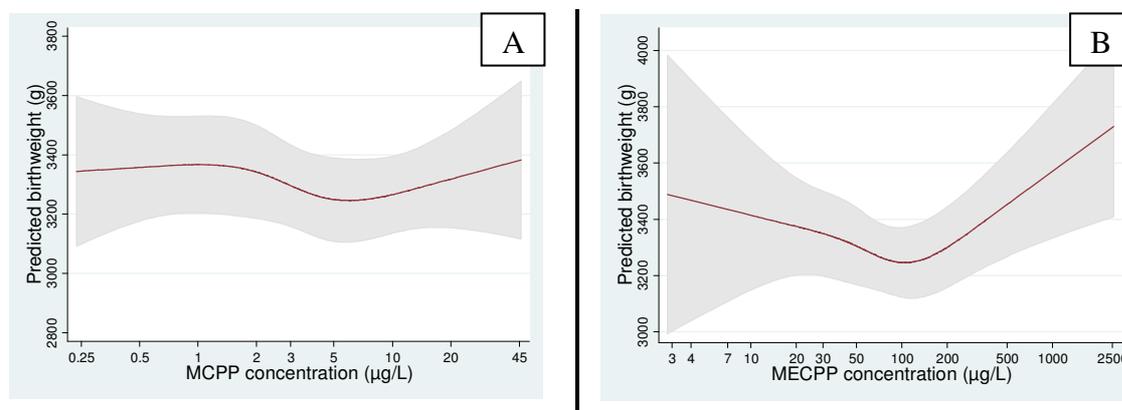
^a Concentrations were standardized for conditions of sampling such as hour of sampling (EDEN only), time elapsed between sample collections and freezing, season and day of sampling and gestational age at collection.

c) Association between phthalate concentrations and birth outcomes

For phthalate metabolite concentrations and birth weight, all p for trend were above 0.14 (Table 8). There was some evidence of heterogeneity in mean birth weight across concentration tertiles for some phthalate metabolites: the lowest p-values for heterogeneity

were observed for MCPPE (p-values = 0.03) and MECPP (p-values = 0.08), given the high values of p for trend (0.73 and 0.59 for MCPPE and MECPP, respectively), results were suggestive of non-monotonic associations (Table 8). For MECPP, but less so for MCPPE, this non-monotonic association was also to some extent supported by the restricted cubic spline analysis (Figure 10).

Figure 10: Birth weight as a function of MCPPE (A) and MECPP concentrations (B) standardized for sampling conditions and coded as restricted cubic splines (log scale) (Gona_PE study, n = 287 mother-child pairs from EDEN and PELAGIE cohorts, 2002-2006).



The predicted curves are adjusted for gestational duration, maternal pre-pregnancy weight and height, maternal smoking, maternal education level, parity, recruitment center and creatinine concentration.

Shading indicates 95% confidence interval.

Abbreviations: MCPPE: mono(3-carboxypropyl) phthalate, MECPP: mono(2-ethyl-5-carboxypentyl) phthalate

Regarding other birth outcomes, the lowest p-values for heterogeneity were observed with MCPPE, MCOP, MCNP, MEHP and the sum of the high MW phthalates for birth length and with the sum of the low MW phthalates for head circumference at birth (Table 8).

Table 8: Adjusted associations between maternal urinary concentrations of phthalate biomarkers standardized for sampling conditions and birth outcomes (Gona_PE study, n = 287 mother-child pairs from EDEN and PELAGIE cohorts, 2002-2006).

Analytes ($\mu\text{g/L}$) ^a	Change ^a in birth weight n = 287				Change ^a in birth length n = 286				Change ^a in head circumference n = 285			
	β (g)	95% CI	p_{het} ^b	p_{trend} ^c	β (cm)	95% CI	p_{het} ^b	p_{trend} ^c	β (cm)	95% CI	p_{het} ^b	p_{trend} ^c
MEP												
< 113.8	0	0	0.61	0.60	0	0	0.22	0.58	0	0	0.32	0.14
113.8 – 275.7	46	[-102; 194]			0.5	[-0.2; 0.1]			0.2	[-0.3; 0.7]		
≥ 275.7	-14	[-162; 133]			0.0	[-0.6; 0.7]			0.4	[-0.1; 1.0]		
Ln(MEP)	3	[-51; 57]			0.0	[-0.3; 0.2]			0.1	[-0.2; 0.3]		
MBP												
< 45.6	0	0	0.44	0.42	0	0	0.69	0.91	0	0	0.89	0.63
45.6 – 85.5	52	[-101; 206]			0.3	[-0.4; 0.9]			0.1	[-0.5; 0.6]		
≥ 85.5	-30	[-174; 114]			0.1	[-0.6; 0.7]			0.1	[-0.4; 0.7]		
Ln(MBP)	-13	[-61; 35]			0.1	[-0.2; 0.3]			0.0	[-0.2; 0.2]		
MiBP												
< 48.2	0	0	0.41	0.48	0	0	0.46	0.54	0	0	0.50	0.40
48.2 – 97.9	61	[-77; 200]			0.4	[-0.3; 1.1]			-0.1	[-0.6; 0.4]		
≥ 97.9	-31	[-190; 129]			0.3	[-0.4; 1]			0.2	[-0.5; 0.9]		
Ln(MiBP)	-44	[-110; 23]			0.0	[-0.3; 0.3]			-0.1	[-0.4; 0.1]		
MCPP												
< 2.1	0	0	0.03	0.73	0	0	0.03	0.55	0	0	0.20	0.77
2.1 – 4.4	-198	[-343; -52]			-0.7	[-1.4; -0.1]			-0.5	[-0.1; 0.1]		
≥ 4.4	-95	[-243; 52]			-0.1	[-0.8; 0.7]			-0.3	[-0.9; 0.4]		
Ln(MCPP)	-34	[-91; 22]			0.1	[-0.2; 0.4]			-0.2	[-0.4; 0.1]		
MBzP												
< 17.6	0	0	0.67	0.43	0	0	0.99	0.88	0	0	0.55	0.32
17.6 – 57.2	14	[-141; 170]			0.0	[-0.7; 0.7]			-0.2	[-0.8; 0.4]		
≥ 57.2	-50	[-223; 123]			0.1	[-0.9; 0.7]			-0.3	[-0.9; 0.3]		
Ln(MBzP)	-23	[-71; 24]			0.1	[-0.3; 0.2]			0.0	[-0.2; 0.2]		
MEHP												
< 6.8	0	0	0.20	0.93	0	0	0.12	0.69	0	0	0.55	0.56
6.8 – 17.1	-122	[-261; 17]			-0.6	[-1.2; 0.0]			0.2	[-0.7; 0.3]		
≥ 17.1	-37	[-184; 110]			-0.3	[-0.9; 0.3]			0.1	[-0.5; 0.6]		
Ln(MEHP)	1	[-60; 62]			0.0	[-0.3; 0.2]			0.0	[-0.2; 0.2]		
MEOHP												
< 25.2	0	0	0.42	0.28	0	0	0.33	0.27	0	0	0.94	0.75
25.2 – 56.8	-37	[-179; 105]			-0.2	[-0.8; 0.4]			0.0	[-0.5; 0.6]		
≥ 56.8	60	[-89; 209]			0.3	[-0.4; 1.0]			-0.1	[-0.6; 0.5]		
Ln(MEOHP)	5	[-56; 66]			0.1	[-0.2; 0.3]			0.0	[-0.3; 0.2]		
MEHHP												
< 32.2	0	0	0.58	0.65	0	0	0.61	0.80	0	0	0.88	0.76
32.2 – 77.9	-60	[-202; 81]			-0.3	[-0.9; 0.4]			-1.0	[-0.6; 0.4]		
≥ 77.9	7	[-139; 154]			0.0	[-0.7; 0.7]			-0.1	[-0.7; 0.4]		
Ln(MEHHP)	4	[-54; 62]			0.1	[-0.2; 0.3]			0.0	[-0.2; 0.2]		

Table 8: Continued, Adjusted associations between maternal urinary concentrations of phthalate biomarkers standardized for sampling conditions and birth outcomes (Gona_PE study, n = 287 mother-child pairs from EDEN and PELAGIE cohorts, 2002-2006).

Analytes ($\mu\text{g/L}$) ^a	Change ^a in birth weight n=287				Change ^a in birth length n=286				Change ^a in head circumference n=285			
	β (g)	95% CI	P_{het} ^b	P_{trend} ^c	β (cm)	95% CI	P_{het} ^b	P_{trend} ^c	β (cm)	95% CI	P_{het} ^b	P_{trend} ^c
MECPP												
< 45.8	0	0	0.08	0.59	0	0	0.40	0.43	0	0	0.90	0.65
45.8 – 105.4	-141	[-277; -5]			-0.3	[-1.0; 0.4]			0.0	[-0.5; 0.6]		
≥ 105.4	-20	[-162; 121]			0.2	[-0.6; 0.9]			0.1	[-0.4; 0.6]		
Ln(MECP)P	5	[-64; 73]			0.1	[-0.2; 0.4]			0.0	[-0.2; 0.3]		
MCOP												
< 2.4	0	0	0.87	0.87	0	0	0.11	0.19	0	0	0.77	0.79
2.4 – 5.9	-40	[-192; 110]			-0.2	[-0.9; 0.4]			-0.1	[-0.7; 0.4]		
≥ 5.9	-27	[-200; 147]			0.4	[-0.5; 1.2]			0.0	[-0.6; 0.6]		
Ln(MCOP)	-8	[-72; 55]			0.1	[-0.2; 0.4]			0.0	[-0.2; 0.3]		
MCNP												
< 2.3	0	0	0.85	0.99	0	0	0.11	0.08	0	0	0.89	0.72
2.3 – 4.6	-40	[-186; 107]			0.5	[-0.1; 1.2]			-0.1	[-0.7; 0.4]		
≥ 4.6	-15	[-189; 158]			0.9	[0.0; 1.7]			-0.1	[-0.8; 0.5]		
Ln(MCNP)	-3	[-67; 61]			0.3	[-0.1; 0.6]			-0.1	[-0.3; 0.1]		
DEHP ($\mu\text{mol/L}$)												
< 0.4	0	0	0.43	0.29	0	0	0.18	0.39	0	0	0.80	0.68
0.4 – 0.9	-54	[-197; 88]			-0.4	[-1.0; 0.2]			0.1	[-0.3; 0.6]		
≥ 0.9	36	[-112; 186]			0.1	[-0.6; 0.9]			0.2	[-0.4; 0.7]		
Ln(DEHP)	5	[-60; 70]			0.1	[-0.2; 0.4]			0.0	[-0.2; 0.2]		
ΣLMW ($\mu\text{mol/L}$)												
< 1.2	0	0	0.32	0.14	0	0	0.29	0.53	0	0	0.12	0.52
1.2 – 2.7	-3	[-141; 135]			0.4	[-0.2; 1.1]			0.5	[0.0; 0.9]		
≥ 2.7	-100	[-248; 47]			0.0	[-0.7; 0.7]			0.4	[-0.3; 1.0]		
Ln(Σ LMW)	-38	[-109; 34]			-0.1	[-0.4; 0.2]			-0.1	[-0.4; 0.2]		
ΣHMW ($\mu\text{mol/L}$)												
< 0.5	0	0	0.17	0.37	0	0	0.06	0.26	0	0	0.82	0.54
0.5 – 1.3	-99	[-243; 44]			-0.5	[-1.2; 0.1]			0.0	[-0.5; 0.5]		
≥ 1.3	19	[-161; 198]			0.2	[-0.6; 1.0]			0.2	[-0.5; 0.8]		
Ln(Σ HMW)	-2	[-70; 65]			0.1	[-0.2; 0.4]			0.0	[-0.2; 0.2]		

Regression models were corrected for the over-representation of cases of malformations of the genitalia by a weighting approach, Abbreviations: MBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxyisononyl phthalate, MCOP: monocarboxy-isooctyl phthalate, MCP: mono(3-carboxypropyl) phthalate, MECPP: mono(2-ethyl-5-carboxypentyl) phthalate, MEHP: mono(2-ethylhexyl) phthalate, MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate, MEOHP: mono(2-ethyl-5-oxohexyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate. DEHP: molecular sum of 4 metabolites of di(2-ethylhexyl) phthalate (MEHP, MEHHP, MEOHP,MECPP), Σ LMW: molecular sum of low molecular weight phthalates (MEP, MBP, and MiBP), Σ HMW: molecular sum of high molecular weight phthalates (MBzP, MCP, MEHP, MECPP, MEHHP, MEOHP, MCOP and MCNP).

^a Adjusted for gestational duration, maternal pre-pregnancy weight and height, maternal smoking, maternal education level, parity, recruitment center and creatinine level. Models for head circumference were also adjusted for mode of delivery (cesarean section yes/no).

^b p-values of heterogeneity test.

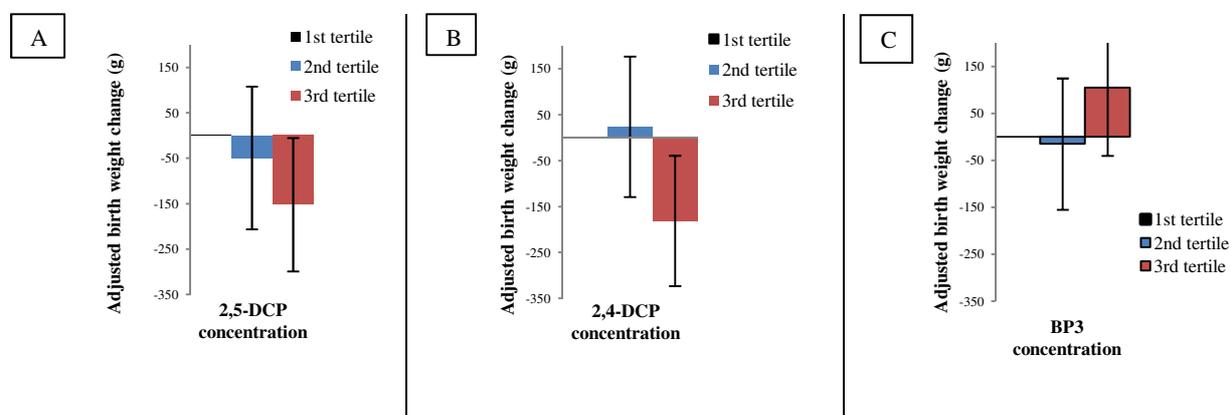
^c p-values of monotonic trend test.

d) Association between phenol concentrations and birth outcomes

Birth weight decreased by 49 g (95% Confidence Interval (CI), -86 ; -13) in association with a 1-unit increase in ln-transformed 2,5-dichlorophenol concentration; after categorizing exposures in tertiles, boys in the highest exposure tertile were 152 g lighter on average, compared with boys in the lowest tertile (95% CI, -299 ; -5) (Figure 11). We observed a similar association between 2,4- dichlorophenol and birth weight (Figure 11, Table 9), consistent with the high correlation between 2,4- dichlorophenol and 2,5- dichlorophenol ($r = 0.95$). 2,5- dichlorophenol ln-transformed concentration was also inversely associated with head circumference at birth (-0.1 cm, 95% CI, -0.2 ; 0.0).

Each 1-unit increase in ln-transformed benzophenone-3 concentration was associated with an increase of 26 g in birth weight (95% CI, -2; 54) and of 0.1 cm in head circumference at birth (95% CI, 0.0; 0.2).

Figure 11: Adjusted^a associations between 2,5-DCP (A), 2,4-DCP (B) and BP3 (C) maternal urinary concentrations (tertiles) standardized for sampling conditions and birth weight (Gona_PE study, n = 191 mother-child pairs from EDEN cohort, 2003-2006).



Abbreviations: 2,4-DCP: 2,4-dichlorophenol, 2,5-DCP: 2,5-dichlorophenol, BP3: benzophenone-3.

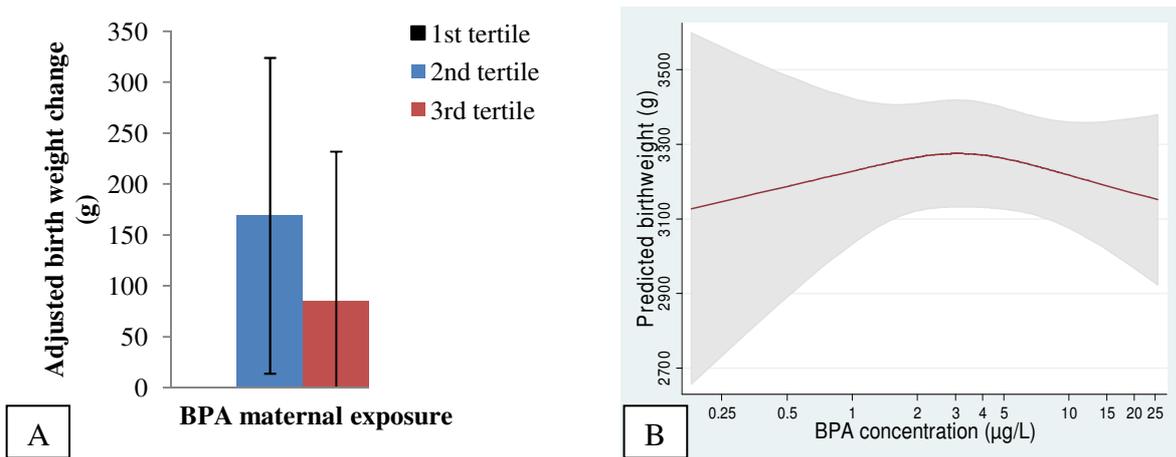
^a Adjusted for gestational duration, maternal pre-pregnancy weight and height, maternal smoking, maternal education level, parity, recruitment center and urine dilution (creatinine level).

Vertical bars indicate 95% confidence intervals.

For bisphenol A, estimates suggested an inverse U-shape association: birth weight increased by 169 g (95% CI, 14 ; 324) in the second bisphenol A concentration tertile and by 85 g (95% CI, -62 ; 233) in the third concentration tertile, compared to the first (Figure 12). BPA concentrations were positively associated with head circumference, which increased by

0.8 cm in the highest bisphenol A concentration tertile, compared to the lowest tertile (95% CI 0.2 ; 1.3).

Figure 12: Birth weight as a function of bisphenol A concentrations standardized for sampling conditions, categorized in tertile (A) and coded as restricted cubic splines (log scale, B) (Gona_PE study, n = 191 mother-child pairs from EDEN cohort, 2003-2006).



Abbreviations: BPA : bisphenol A.
Shading indicates 95% confidence interval.

For all other phenols, there was no evidence of associations with offspring measures at birth [all p for heterogeneity were above 0.24 (Table 9) and curves obtained using restricted cubic splines did not clearly support an association (data not shown)].

Table 9: Adjusted associations between maternal urinary concentrations of phenol biomarkers standardized for sampling conditions and birth outcomes (Gona_PE study, n = 191 mother-child pairs from EDEN cohort, 2003-2006).

Analytes ($\mu\text{g/L}$) ^a	Change ^a in birth weight n = 191				Change ^a in birth length n = 190				Change ^a in head circumference n = 189			
	β (g)	95% CI	p_{het} ^b	p_{trend} ^c	β (cm)	95% CI	p_{het} ^b	p_{trend} ^c	β (cm)	95% CI	p_{het} ^b	p_{trend} ^c
2,4-DCP												
< 0.6	0	0	<0.0	<0.01	0	0	0.09	0.77	0	0	<0.01	0.01
0.6 - 1.3	24	[-129; 177]	1		0.7	[0.1; 1.3]			0.8	[0.2; 1.4]		
≥ 1.3	-181	[-323; -40]			0.2	[-0.5; 0.9]			-0.3	[-0.8; 0.3]		
Ln(2,4-DCP)	-77	[-129; -25]			-0.1	[-0.4; 0.2]			-0.1	[-0.3; 0.1]		
2,5-DCP												
< 3.9	0	0		0.03	0	0	0.53	0.70	0	0	0.14	0.10
3.9 – 13.9	-49	[-206; 108]	0.10		0.4	[-0.3; 1.0]			-0.3	[-0.8; 0.3]		
≥ 13.9	-152	[-299; -5]			0.3	[-0.4; 1.0]			-0.5	[-1.1; 0.0]		
Ln (2,5-DCP)	-49	[-86; -13]			0.0	[-0.2; 0.2]			-0.1	[-0.2; 0.0]		
BPA												
< 2.2	0	0		0.70	0	0	0.98	0.83	0	0	0.04	0.01
2.2 - 4.7	169	[14; 324]	0.10		0.0	[-0.7; 0.8]			0.3	[-0.3; 0.9]		
≥ 4.7	85	[-62; 233]			0.1	[-0.7; 0.9]			0.8	[0.2; 1.3]		
Ln(BPA)	-9	[-98; 80]			0.0	[-0.4; 0.4]			0.3	[0.0; 0.7]		
BP3												
< 0.7	0	0		0.09	0	0	0.17	0.14	0	0	0.12	0.04
0.7 - 2.7	-15	[-155; 124]	0.20		-0.3	[-0.1; 0.3]			0.2	[-0.5; 0.8]		
≥ 2.7	105	[-40; 250]			0.4	[-0.3; 1.0]			0.5	[-0.0; 1.0]		
Ln(BP3)	26	[-2; 54]			0.1	[-0.1; 0.2]			0.1	[0.0; 0.2]		
TCS												
< 4.5	0	0		0.79	0	0	0.68	0.59	0	0	0.31	0.18
4.5 - 51.3	-58	[-194; 78]	0.68		-0.2	[-0.9; 0.4]			0.2	[-0.3; 0.7]		
≥ 51.3	-40	[-171; 90]			0.1	[-0.6; 0.7]			-0.2	[-0.7; 0.3]		
Ln(TCS)	-6	[-31; 19]			0.0	[-0.1; 0.1]			-0.1	[-0.2; 0.0]		
MPB												
< 62.8	0	0		0.58	0	0	0.47	0.23	0	0	0.79	0.58
62.8 – 213.0	-32	[-168; 105]	0.75		0.2	[-0.5; 0.8]			0.2	[-0.4; 0.7]		
≥ 213.0	24	[-121; 168]			0.4	[-0.3; 1.2]			0.2	[-0.4; 0.8]		
Ln(MPB)	-2	[-37; 34]			0.1	[-0.1; 0.3]			0.0	[-0.1; 0.2]		
EPB												
< 0.6	0	0	0.98	0.86	0	0	0.24	0.97	0	0	0.47	0.94
0.6 - 3.7	10	[-129; 148]			0.6	[-0.1; 1.3]			0.4	[-0.2; 1.0]		
≥ 3.7	17	[-145; 178]			0.3	[-0.4; 0.9]			0.2	[-0.4; 0.8]		
Ln(EPB)	-1	[-43; 41]			0.1	[-0.1; 0.3]			0.1	[-0.1; 0.3]		

Table 9: Continued, Adjusted associations between maternal urinary concentrations of phenol biomarkers standardized for sampling conditions and birth outcomes (Gona_PE study, n = 191 mother-child pairs from EDEN cohort, 2003-2006).

Analytes ($\mu\text{g/L}$) ^a	Change ^a in birth weight n = 191				Change ^a in birth length n = 190				Change ^a in head circumference n = 189			
	β (g)	95% CI	p_{het} ^b	p_{trend} ^c	β (g)	95% CI	p_{het} ^b	p_{trend} ^c	β (g)	95% CI	p_{het} ^b	p_{trend} ^c
PPB												
< 4.7	0	0	1.00	0.95	0	0	0.98	0.93	0	0	0.52	0.62
4.7 – 24.8	-2	[-137; 132]			-0.1	[-0.7; 0.6]			-0.3	[-0.8; 0.2]		
\geq 24.8	-5	[-151; 140]			0.2	[-0.8; 0.7]			-0.2	[-0.8; 0.3]		
Ln(PPB)	-10	[-40; 19]			0.0	[-0.2; 0.2]			-0.1	[-0.2; 0.1]		
BPB												
< 0.6	0	0	0.97	0.95	0	0	0.58	0.49	0	0	0.61	0.45
0.6 – 6.8	-15	[-156; 126]			0.2	[-0.5; 1.0]			0.2	[-0.3; 0.7]		
\geq 6.8	-2	[-155; 150]			0.1	[-0.9; 0.7]			0.3	[-0.3; 0.8]		
Ln(BPB)	-1	[-32; 29]			0.0	[-0.2; 0.2]			0.1	[-0.1; 0.2]		
ΣPB ($\mu\text{mol/L}$)												
< 0.5	0	0	0.95	0.86	0	0	0.61	0.38	0	0	0.80	0.66
0.5 – 1.6	-16	[-150; 118]			0.2	[-0.4; 0.9]			0.2	[-0.4; 0.7]		
\geq 1.6	6	[-143; 156]			0.4	[-0.4; 0.1]			0.2	[-0.4; 0.7]		
Ln(Σ PB)	-3	[-39; 33]			0.1	[-0.1; 0.3]			0.0	[-0.2; 0.1]		

Abbreviations: BP: butyl paraben BPA: bisphenol A, BP3: benzophenone-3, DCP: 2,5-dichlorophenol, MP: methyl paraben, EP: ethyl paraben, PP: propyl paraben, TCS: Triclosan, Σ PB: molecular sum of parabens.

Regression models were corrected for the over-representation of cases of malformations of the genitalia by a weighting approach.

^a Adjusted for gestational duration, maternal pre-pregnancy weight and height, maternal smoking, maternal education level, parity, recruitment center and creatinine level. Models for head circumference at birth were also adjusted for mode of delivery (cesarean section yes/no).

^b p-values of heterogeneity test.

^c p-values of monotonic trend test.

e) Sensitivity analyses

For phenols, associations with birth outcomes remained similar after exclusion of 48 cases of male malformations of the genitalia (not shown) Using biomarker concentrations not standardized for sampling conditions instead of concentrations standardized for sampling conditions did not markedly change associations between either 2,4-, 2,5-dichlorophenol, benzophenone-3 or bisphenol A and birth weight. Similarly, associations between either benzophenone-3 or bisphenol A and head circumference at birth remained similar (not shown).

In analyses restricted to controls only or using concentrations not standardized for sampling conditions, findings concerning phthalates were consistent with those obtained in the main analysis, excepted for MECPP: The birth weight change observed in the second MECPP concentration tertile was -141 g (95% CI, -277; -5) in the whole study population, - 79 g (95% CI, -220; 61) after exclusion of cases and -51 g (95% CI, -187; 84) using non-standardized biomarker concentrations. These sensitivity analyses are difficult to interpret because the restriction to controls decreased population size, and because standardization sometimes induced strong variations in the distribution of biomarker concentrations and hence in the cut-off values of tertiles.

Excluding women with pregnancy-induced hypertension or gestational diabetes did not modify our main results (data not shown).

4. Discussion

Within our study population of 191 male newborns, maternal urinary concentrations of 2,4- and 2,5-dichlorophenol were associated with a birth weight decrease, while urinary concentrations of benzophenone-3 were positively associated with weight and head circumference at birth. Bisphenol A urinary concentrations were positively associated with head circumference; there was no evidence of monotonic associations between phthalate metabolite concentrations and birth weight.

a) Phenols and birth outcomes

In our study population, birth weight decreased by 152 g in the highest 2,5-dichlorophenol concentration tertile compared to the lowest tertile (95% CI, -299 ; -5); the only other human study addressing this issue reported a decrease by 210 g in the third 2,5-dichlorophenol concentration tertile compared to the first (95% CI, -348, -71, ((Wolff et al. 2008)). Concentrations of 2,5-dichlorophenol (and hence tertiles) were much higher in the study by Wolff et al. (median: 53 µg/L) than in ours (median standardized concentration: 6.4 µg/L). Wolff et al. also reported that boys were 0.3 cm shorter at birth per increase by one in ln(2,5-DCP) (95% CI, -0.6; -0.4); our results did not clearly support such an association (0.0 cm per increase by one in ln(2,5-DCP), 95% CI; -0.2; 0.2), but birth length is not very accurately assessed, implying potentially strong measurement error. 2,5-dichlorophenol is a metabolite of 1,4-dichlorobenzene, which is used as chemical intermediate in the production of dyes and organic chemicals and which is found in mothballs and toilet-deodorizer blocks (Agency for Toxic Substances and Disease 2006; Yoshida et al. 2002). Dichlorophenols may also be released from water treatments (Abrahamsson and Xie 1983).

2,4-dichlorophenol was also associated with a birth weight decrease. It is a metabolite of 1,3-dichlorobenzene, a minor contaminant of 1,4-dichlorobenzene (Agency for Toxic Substances and Disease 2006; Yoshida et al. 2002), which may explain the high correlation reported between concentrations of both dichlorophenols. 2,4-dichlorophenol is also an environmental transformation intermediate of the antiseptic agent triclosan and of some herbicides such as 2,4-dichlorophenoxyacetic acid and 2-(2,4-dichlorophenoxy) propionic acid (Yang et al. 2010; Zona et al. 2002). Our results concerning 2,4-dichlorophenol are difficult to compare with those of Wolff et al (2008), who studied male and female newborns altogether for this compound.

Taken together, these studies suggest an effect of dichlorophenols, or one of their precursors, on birth weight.

Benzophenone-3 urinary concentrations were positively associated with weight and head circumference at birth. Boys were 105 g heavier (95% CI, -40 ; 250) in the highest benzophenone-3 concentration tertile compared with the lowest. Similarly, Wolff et al (2008) noted a birth weight increase in male infants in the highest benzophenone-3 concentration tertile compared to the lowest (betas not reported); they did not report effect estimates for benzophenone-3 and head circumference in males. Exposure to benzophenone-3 likely results from use of consumer products as sunscreens or cosmetics (Calafat et al. 2008).

Bisphenol A urinary concentrations were positively associated with head circumference at birth. After categorizing exposures in tertiles, we observed an inverse U-shape association between bisphenol A concentrations and birth weight. Such non-monotonic dose-response curves between perinatal exposures to bisphenol A and weight in early life have been reported in rodents (Rubin and Soto 2009; Rubin et al. 2001). However, urinary bisphenol A concentrations were relatively low in our study population, enhancing the analytical uncertainties and hence the potential for exposure misclassification, which may limit our ability to distinguish monotonic from non-monotonic associations.

b) Phthalates and birth outcomes

There was no strong evidence of monotonic association between phthalate metabolite concentrations and birth outcomes, excepted for the possible positive association between MCNP and birth length. Our analyses suggested non-monotonic associations with birth weight and birth length for some phthalate metabolites. Such associations between phthalate metabolites and birth weight have, to our knowledge, never been reported previously in rodents or in humans and therefore should be considered cautiously.

c) Study population

To correct the over-representation of cases induced by our case-control design, we weighted the observations in regression models (Richardson et al. 2007). We also performed non-weighted analyses, restricted to controls, and our main results regarding birth weight remained similar to those based on the whole weighted study population.

d) Exposure assessment

Sampling conditions, such as hour of urine sampling or storage duration before freezing, may influence the concentrations of several biomarkers (Mahalingaiah et al. 2008; Samandar et al. 2009). We used a 2-step standardization method based on regression residuals to reduce undesirable variability in biomarker urinary concentrations due to sampling conditions (Mortamais et al. 2012). To our knowledge it is the first time that such an approach has been applied to study associations between phthalate or phenol prenatal exposures and birth outcomes. We repeated our analyses using concentrations not standardized for sampling conditions and associations between dichlorophenols or benzophenone-3 and birth weight or between benzophenone-3 and bisphenol A and head circumference were similar.

e) Limitations

The assayed phenols and phthalates and their metabolites have relatively short half-lives in humans (typically, one day or less), but accurate information on half-lives in pregnant women, in whom metabolism may differ compared to non-pregnant women, is not available. We assessed exposures from the urinary concentrations at a single point during pregnancy; increasing the number of urine samples collected would have provided a more accurate estimate of the average exposure during the whole gestation. Adibi et al. reported that for phthalates, the probability of correctly classifying a woman into a low exposure group based on a single urine sample, if she truly had low exposure based on multiple measurements, was between 0.43 for monoethyl phthalate (MEP) and 0.95 for mono-isobutyl phthalate (MiBP) (Adibi et al. 2008a). Concerning phenols, bisphenol A concentrations also vary during pregnancy (Braun et al. 2011b). Therefore, there clearly is exposure misclassification, whose amplitude differs according to the biologically relevant exposure window (if any) and to the chemical considered.

We adjusted for many potential confounders, but residual confounding cannot be discarded. For example, specific metabolic disorders associated with both fetal growth and xenobiotic metabolism would constitute potential confounders; in our study, excluding women with pregnancy-induced hypertension or gestational diabetes did not alter associations with bisphenol A and benzophenone-3 (not shown). Multiple comparisons are generally an issue in studies relying on several biomarkers; although we did not formally correct for multiple comparisons, our choice to focus our conclusions on previously reported associations limits the risk of chance being a likely explanation for our main findings.

5. Conclusion

In our study, there was no strong evidence of monotonic association between phthalate metabolite concentrations and birth outcomes. Urinary concentrations of 2,4- and 2,5-dichlorophenols were negatively associated with birth weight while benzophenone-3 concentrations were associated with a birth weight increase. Results concerning 2,5-dichlorophenol and benzophenone-3 were in agreement with another publication concerning male newborns from New-York (Wolff et al. 2008) and we aimed at replicating our findings in a larger sub-population of pregnant women from the EDEN cohort.

II. Effects of prenatal exposures to phenols on fetal growth, estimated using repeated ultrasound measurements and early postnatal growth

1. Aims of the chapter

The objectives of this study were 1) to expand phenol measurements to 410 other women from the EDEN mother child-cohort delivering boys, in order to replicate results on birth outcomes published in 2012 (Philippat et al. 2012) and reported above (see I of this chapter); 2) to explore the associations between phenol exposures during pregnancy and repeated ultrasound measurements; 3) to study the associations between phenol exposures during pregnancy and growth during the first three years of life.

2. Population and methods

a) Population and exposure assessments

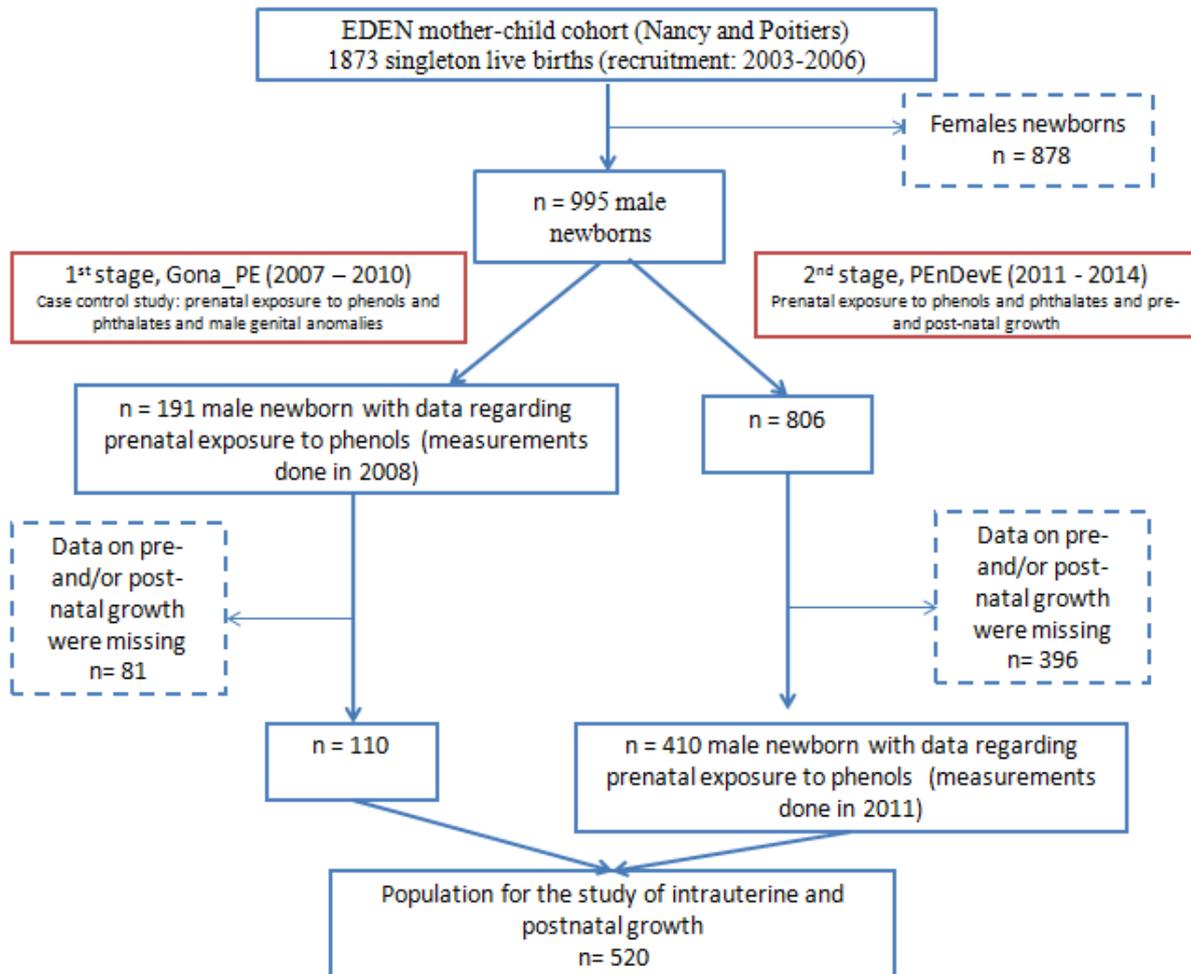
The study population relies on a subgroup of the EDEN mother-child cohort. Inclusion criteria for the present study was delivering boy with complete data on prenatal (three ultrasound measurements and biometry at birth) and postnatal growth (≥ 4 measurements of weight and height within the first three years of life), corresponding to a total of 520 mother-child pairs. Among those, 110 were included in the Gona_PE analysis aiming at studying associations between prenatal exposure to phthalates and phenols on birth outcomes described previously in this Chapter (See II, page 44) and had their urine samples analyzed for phenols in 2008 (Chevrier et al. 2012; Philippat et al. 2012). We extended these measurements to all women matching the inclusion criteria and 410 other urine samples were analyzed in 2011 (Figure 13).

Urine samples were collected between 22 and 29 gestational weeks. Women were asked to collect the first morning urine void at home before the hospital study visit. If forgotten, the urine sample was collected at the hospital. Urine samples were aliquoted and stored at -80°C . Measurements of nine phenol biomarkers⁵ and creatinine were done by the National Center for Environmental Health laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA (Ye et al. 2005). Total paraben concentration

⁵ Phthalates metabolites were also measured. J. Botton (Inserm U1018, Université Paris-Sud), in collaboration with our team, is in charge of the analyses regarding the associations between phthalates and pre- and post-natal growth, which are not presented in this manuscript.

(Σ PB) was calculated by summing molar concentrations of methyl-, ethyl-, propyl- and butyl-parabens.

Figure 13: Study population for fetal and postnatal growth analyses (PEndDevE study, n = 520 mother-child pairs from the EDEN cohort).



Abbreviations: EDEN: Etude sur les Déterminants pré- et post-nataux précoces du développement psychomoteur et de la santé de l'ENfant, Gona_PE: Gonades et Perturbateurs Endocriniens, PEndDevE: Perturbateurs Endocriniens et Développement de l'ENfant

b) Growth assessment

We assessed biparietal diameter (BPD) by ultrasound (US) at three occasions during pregnancy (around 12.6, 22.5 and 32.6 gestational weeks (GW)) while the other measures of fetal size (head circumference (HC), abdominal circumference (AC) and femur length (FL))

were assessed only during the two last ultrasound examinations (Albouy-Llaty et al. 2011). We estimated fetal weight (EFW) using the formula by (Hadlock et al. 1985):

$$\log(\text{EFW}) = 1.3596 - 0.00386 \cdot \text{AC} \cdot \text{FL} + 0.0064 \cdot \text{HC} + 0.00061 \cdot \text{BPD} \cdot \text{AC} + 0.0424 \cdot \text{AC} + 0.174 \cdot \text{FL}$$

Weight and length at birth were extracted from hospital maternity records.

Infants were weighed and measured at 1 and 3 years during study specific examinations. Additionally, at 4, 8, 12, 24 and 36 months, mothers reported by self-questionnaires the weight and height measures contained in the child health booklet which is filled in by the family pediatrician in routine follow-up. We used these repeated measurements to model child growth individually using an adaptation of the Jenss growth curve model (see Box 2 for more detail on this model). This model was used to predict weight and height at 6, 12, 24 and 36 months. Head circumference was assessed in duplicate within 4 days after birth and at 3 years; in main analyses we used the average of the two measures at each age. At 3 years, children tricipital and subscapular skinfold thicknesses were measured in triplicate and abdominal circumference in duplicate. We used the average of these measures.

For growth parameters measured three times or more, we additionally computed an average growth rate between two successive measurements:

$$\text{growth rate} = (\text{measurement at } t_2 - \text{measurement at } t_1) / (t_2 - t_1).$$

Box 2: Prediction of individual postnatal growth curves in the EDEN cohort

Postnatal growth modeling was done by J. Botton (Inserm, U1018 - Univ Paris-Sud).

A neonatal weight loss is usually observed the first few days after birth. After this few days, growth velocity is very high but steadily declines over time in order to reach a constant value around 2 years. Growth velocity is then constant until puberty where growth accelerates again. Several mathematical equations have been used to describe this shape of growth; Jenss, Reed and Count models were shown to be efficient for studying growth between birth and 3 years (Hauspie et al. 2004). The Jenss model (see equation below) was the one with the highest fit in the EDEN cohort.

$$Y_i(t) = e^{A_i} + e^{-B_i} \cdot t + e^{C_i} \cdot (1 - e^{-D_i \cdot t}) + \varepsilon_i$$

t: age in months

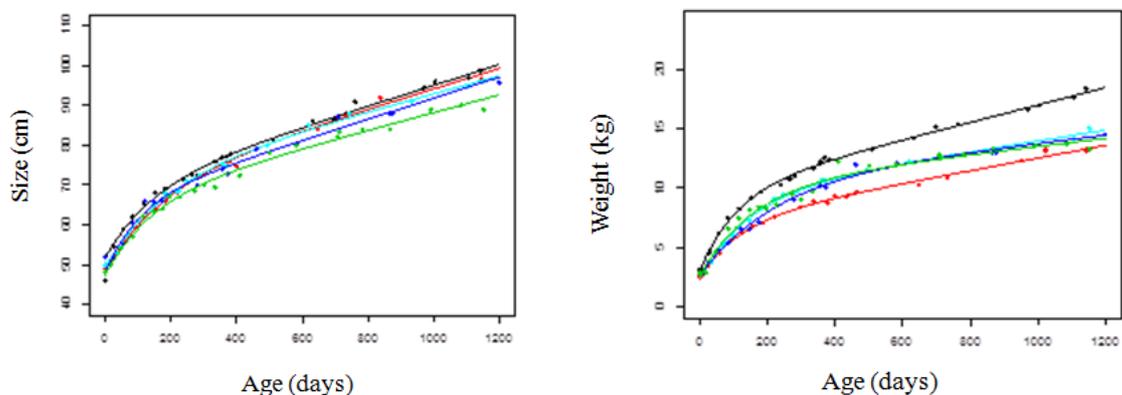
Y_i : weight (kg) or height (cm) of the child at age t

ε_i : residual error at age t

e^{A_i} : birth weight

The first part of the model ($e^{A_i} + e^{-B_i} \cdot t$) is a straight line allowing to describe the straight trend of growth between 2 years and puberty. The other part is an exponential term that fits the increase observed in the first months of life and the following weight growth deceleration (Botton et al. 2008). This model allows to take into account the nonlinearity of weight and height growth during the first years of life and to investigate different periods of growth between birth and 3 years.

Figure 14: Fitted weight and height growth curve between birth and 3 years for 5 randomly selected children of the EDEN cohort (*points correspond to observations, lines to modeled curves*).



c) Statistical methods

We replaced phenol biomarker concentrations below the limit of detection (LOD) by instrumental reading values. Concentrations were standardized for collection conditions using a previously defined approach (See method section of the chapter II, II.2, page 50, (Mortamais et al. 2012)). In contrast to Mortamais et al. (2012), we only standardized for sampling conditions associated with the phenol concentrations (p -value < 0.2). Phenol concentrations were coded either as continuous variables (ln-transformed concentration) or in tertiles. Unless otherwise specified, β estimates are given for an increase by one interquartile range (IQR) of phenol concentration.

To study the associations between phenol concentrations and fetal growth measurements (ultrasound and birth measurements), we used linear regression models with a random effect variable corresponding to the mother–child pair. To allow effect modifications along pregnancy we included interaction terms between phenol concentration and gestational age (longitudinal analyses, see Box 3 page 71). Models were adjusted for factors possibly related to prenatal growth (based on a priori knowledge). This included maternal and paternal height (continuous) and weight (continuous), maternal weight gain during pregnancy (continuous), maternal active (continuous) and passive (yes / no) smoking before the ultrasound measurement, maternal education level (3 categories: high school or less, up to 2 years after high school, ≥ 3 years after high school), parity, gestational age at measurement (third order polynomial) and recruitment center. Gestational age was estimated using the date of last menstrual period (LMP) or gestational duration assessed by the obstetrician if it differed from the LMP-based estimate by > 2 weeks.

In order to study the associations between phenol concentrations and measures of postnatal growth (predicted weight and height at 6, 12, 24 and 36 months, skin fold thickness abdominal and head circumference at 36 months), we used linear regression models adjusted for maternal and paternal height (continuous) and weight (continuous), maternal active (continuous) and passive (yes/no) smoking during pregnancy, maternal education level (high school or less, up to 2 years after high school, ≥ 3 years after high school), recruitment center, parity and breastfeeding (never, ≤ 3 months, > 3 months).

Sensitivity analyses

In sensitivity analyses, models for postnatal weight were additionally adjusted for 1) birth weight, 2) height predicted at the same age, 3) average caloric intake at 4 months for growth parameters measured at 6 months and average caloric intake at 8 months for growth parameters measured at 12 months and more. Average caloric intake was computed based on three days dietary inquiry occurring at 4 and 8 months). In order to test for effect modification by breastfeeding (postnatal growth only), we performed additional models including an interaction term between these variables and exposure.

Head circumference of the newborn was also measured at birth. We tested in what extend using this measure rather than the measure performed few days after birth, affect our results.

In order to allow comparisons with the results published in 2012 among 191 pregnant women from the EDEN cohort (see Chapter II, II.2, page 44), we performed cross sectional analyses to study the associations between phenol exposures and birth measurements (weight, height, head circumference).

Box 3: Description of two different approaches commonly used to estimate the effects of environmental exposures on pre-natal growth (Slama et al, submitted)

We consider Y_{it} a measure of fetal size assessed by ultrasonography at various time points throughout pregnancy; Y can for example be estimated fetal weight or biparietal diameter (assessed on a continuous scale).

The first approach corresponds to a linear regression model with a random effect variable corresponding to the mother–child pair and interaction terms of gestational age with exposure and covariates (Slama et al. 2009). The model can be written:

$$Y_{it} = \beta_0 + \beta_1 \times E_i + \beta_2 \times f(GA_{it}) + \beta_3 E_i \times f(GA_{it}) + \beta_4 X_i + \beta_5 X_i \times f(GA_{it}) + R_i + \varepsilon_{it} \quad (1)$$

Y_{it} : ultrasound measurement for woman i , at time j (for example, biparietal diameter).

E : biomarker concentrations (standardized for sampling conditions).

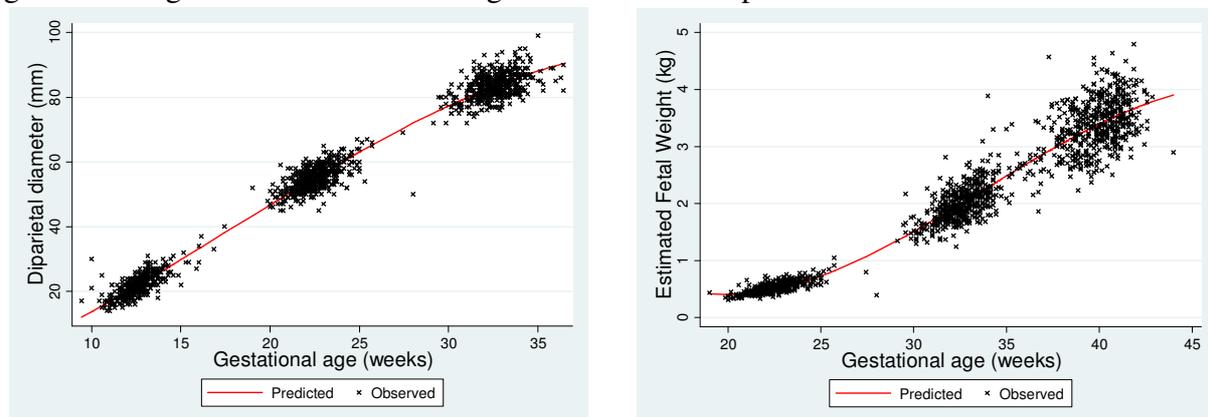
$f(GA)$: gestational age, where f is a third order polynomial (other functional forms could be used).

X_i : adjustment variables.

R_i : random effect variable corresponding to the mother–child pair.

The model can be used to test an effect measure modification of exposure by gestational duration. The main results correspond to the parameters associated with exposure at a given gestational age. This approach was used in the work presented in the thesis. Examples of the growth curves obtained using this model are presented in Figure 15

Figure 15: Biparietal diameter (mm) and estimated fetal weight (kg) as a function of gestational age at measurement among 520 mother-child pairs from EDEN cohort.



The predicted curves are adjusted for gestational age, maternal age, maternal height and weight before pregnancy, maternal weight gain during pregnancy, paternal height and weight, maternal active and passive smoking during pregnancy, maternal education level, parity, and recruitment center.

The second approach (2-step approach based on Z-Scores, (Aguilera et al. 2010; Iniguez et al. 2012a)) is an extension of what has been proposed by Royston and Altman (Royston and Altman 1995). The main steps are:

1) Build an adjusted regression model that does not include the exposure variable:

$$Y_{it} = \beta_0 + \beta_1 \times f(GA_{it}) + \beta_2 X_i + \beta_3 X_i \times f(GA_{it}) + R_i + \varepsilon_{it} \quad (2)$$

Y_{it} : ultrasound measurement for woman i , at time t (for example, biparietal diameter).

$f(GA)$: gestational age, where f is a third order polynomial (other functional forms could be used).

X_i : adjustment variables including maternal weight, height and age, maternal education level, active and passive smoking, parity, center.

R_i : random effect variable corresponding to the mother–child pair.

The values of Y according to gestational age predicted by this model for a given set of values of the adjustment variables correspond to what can be seen as a standardized growth curve.

2) Z-score computation

If ultrasound measurements occurred at the same gestational age for all subjects, then one could compute a Z-score at time t using the following formula:

$$Z_{it} = \frac{Y_{it(obs)} - Y_{it(sdz)}}{SD_{t(sdz)}} \quad (3)$$

$Y_{it(obs)}$: Ultrasound measurement observed at t.

$Y_{it(sdz)}$: Ultrasound measurement predicted at t using standardized growth curve.

$SD_{t(sdz)}$: Standard deviation of predicted biparietal diameter (estimated using standardized growth curve).

However ultrasound measurements may not all have taken place exactly at the same time t but at some time $t \pm \Delta t_i$. The ultrasound parameter observed at t will be approximated using Y observed at $t \pm \Delta t_i$ and the growth curve defined in (2):

$$\hat{Y}_{it} = Y_{i,t \pm \Delta t_i} \times \frac{Y_{i,t(sdz)}}{Y_{i,t \pm \Delta t_i(sdz)}} \quad (4)$$

\hat{Y}_{it} : Approximated ultrasound measurement at t.

$Y_{i,t \pm \Delta t_i}$: Ultrasound measurement observed at $t \pm \Delta t_i$

$Y_{i,t(sdz)}$: Ultrasound measurement predicted at t using standardized growth curve.

$Y_{i,t \pm \Delta t_i(sdz)}$: Ultrasound measurement predicted at $t \pm \Delta t_i$ using standardized growth curve.

With this approximation, one can then perform the Z-score computation as if all ultrasound measures had occurred at time t.

$$Z_{it} = \frac{\hat{Y}_{it} - Y_{it(sdz)}}{SD_{t(sdz)}} \quad (5)$$

3) Regress the Z-score at time t on the exposure variable E:

$$Z_{it} = \beta_0 + \beta_1 \times E_i + \beta_2 \times X_i + \varepsilon_{it} \quad (6)$$

E_i : Exposure variable.

X_i : adjustment variables (may include the adjustment variables of model 2 and possibly additional ones).

The parameter associated with E provides an estimate of the effect of exposure on the fetal size measure at time t. In a previous paper related on air pollution and fetal growth this approach was slightly modified; author did not standardized the difference between $Y_{it(obs)}$ and $Y_{it(sdz)}$ (step 3, (Hansen et al. 2008)).

3. Results

a) Population and growth characteristics

Average maternal age was 29.7 years. Women were mostly non-smokers during pregnancy (87%), 26% were overweight (BMI > 25 kg/m²). Regarding their babies, 23 (4%) were born before 37 gestational weeks and 11 (2%) had a weight at birth below 2500 g (Table 10).

Compared to all women from EDEN cohort delivering boys and to the women studied in the framework of the Gona_PE study (chapter II, paragraph 2.1), women of the PEnDevE study population were less exposed to active and passive smoking (Table 10). The percentage of women recruited in Poitiers was higher in the PEnDevE population than in the two sub-mentioned populations (60% versus to 53% in the whole EDEN cohort and 48% among women included in the Gona_PE project, Table 10).

Table 10: Characteristics of the whole EDEN cohort delivering boys and comparison with PEnDevE^b and Gona_PE^c population.

Characteristic	All male newborns, EDEN cohort (n = 995) ^a	Women of EDEN cohort included in PEnDevE study (n = 520) ^b	Women of EDEN cohort included in Gona_PE study (n = 191) ^c
	n (%)	n %	n %
Maternal age (years)			
< 25	200 (20)	85 (16)	35 (18)
25-29	376 (38)	200 (38)	71 (37)
30-34	285 (29)	159 (31)	57 (30)
≥ 35	134 (13)	76 (15)	28 (15)
Parity			
0	434 (44)	248 (48)	71 (38)
1	373 (37)	192 (37)	75 (39)
≥ 2	187 (19)	79 (15)	44 (23)
Missing	1 (0)	1 (0)	
BMI (kg/m²)			
<18.5	91 (9)	45 (9)	19 (10)
≥ 18.5 to <25	634 (64)	331 (64)	115 (60)
≥ 25	249 (25)	137 (26)	54 (28)
Missing	21 (2)	7 (1)	3 (2)
Maternal Education			
Less than 2y after high school	457 (46)	211 (41)	95 (50)
High school + 2 years	218 (22)	124 (24)	39 (20)
≥ High school + 3 years	297 (30)	178 (34)	51 (27)
Missing	23 (2)	7 (1)	6 (3)
Active smoking (cig/day)^d			
0	825 (83)	453 (87)	159 (83)
1 to 5	81 (8)	39 (8)	15 (8)
> 5	86 (9)	27 (5)	17 (9)
Missing	3 (0)	1 (0)	
Passive smoking			
No	710 (71)	411 (79)	131 (69)
Yes	277 (28)	108 (21)	59 (31)
Missing	8 (1)	1 (0)	1 (1)
Center			
Poitiers	530 (53)	312 (60)	91 (48)
Nancy	465 (47)	208 (40)	100 (52)
Gestational duration			
< 37 gestational week	63 (6)	23 (4)	5 (3)
≥ 37 gestational week	932 (94)	497 (96)	186 (97)
Birth weight			
< 2500 g	47 (5)	11 (2)	4 (2)
≥ 2500 g	948 (95)	509 (98)	187 (98)
Year of birth			
2003-2004	560 (56)	286 (55)	89 (47)
2005-2006	440 (44)	234 (45)	102 (53)
Breastfeeding			
Never	268 (27)	135 (26)	51 (27)
≤ 3months	422 (42)	209 (40)	84 (44)
> 3months	298 (30)	176 (34)	56 (29)
Missing	7 (1)	0 (0)	0 (0)

^a Singleton live births.^b Study population for the intrauterine and postnatal growth analyses (inclusion criteria: deliver boys with complete data on pre and post natal growth).^c Study population for the birth outcome analyses (See Chapter II; paragraph II.2).^d Second trimester of pregnancy.

The first, second and third ultrasound examination were performed on average at 12.6 (5th-95th centiles, 11.1-14.0), 22.5 (5th-95th centiles, 20.7-24.4) and 32.6 (5th-95th centiles, 30.6-34.2) gestational weeks. Fetal and child biometry are described in details in Table 11. On average fetal weight and head circumference were multiplied by 6 and 2 between the second ultrasound examination and birth, respectively. On average, weight doubled between birth and 6 months of life (Table 11, Figure 14).

Table 11: Descriptive statistics of growth measurements in the PEnDevE population (n = 520, EDEN cohort, 2003-2006).

Measurements	Percentiles			
	mean	5 th	50 th	95 th
Biparietal diameter (mm)				
US 1	22	17	22	27
US 2	55	49	55	61
US 3	83	77	83	90
Head circumference (mm)				
US 2	202	181	201	223
US 3	300	280	300	323
Birth	349	330	349	370
36 months	506	483	506	529
Abdominal circumference (mm)				
US 2	180	160	179	202
US 3	284	256	283	314
36 months	499	457	500	546
Femur length (mm)				
US 2	39	34	38	44
US 3	62	57	62	67
Height (mm)				
Birth	501	460	500	530
6 months ^a	675	643	675	710
12 months ^a	762	727	762	802
24 months ^a	874	827	874	924
36 months ^a	966	912	966	1023
Weight (g)				
US 2 (estimated)	527	388	522	699
US 3 (estimated)	2003	1530	1977	2556
Birth	3371	2620	3370	4105
6 months ^a	7877	6752	7819.6	9204.8
12 months ^a	9975	8559	9911	11648
24 months ^a	12543	10685	12519	14551
36 months ^a	14723	12453	14551	17226
Skinfold thickness at 36 months (mm)				
Tricipital	8.3	5.3	8	11.7
Subscapular	5.2	3.9	5.1	7.1

^a Predicted by the Jenness growth curve model, based on postnatal measures, US: ultrasound measurement.

Only biparietal diameter has been measured at US 1.

Correlations observed between repeated measures of the same fetal growth parameter were lower than those observed between different fetal parameters measured at the same ultrasound examinations. Biparietal diameter measured at the second ultrasound examination was correlated at 0.3 with biparietal diameter measured in the other ultrasound examinations while the correlation observed between this measurement and the other parameters measured at the second ultrasound examination were greater than 0.7 (Table 12). Head circumference measured in duplicate within 4 days after birth was correlated at 0.8 with head circumference at birth and these measurements were correlated at 0.3 (head circumference at birth) and 0.4 (measurement performed within 4 days after birth) with head circumference measured in third ultrasound examination.

Correlations between measurements of child weight between 6 and 36 months were high ($\rho > 0.67$). These measurements were moderately correlated with birth weight ($\rho < 0.5$). Similar pattern was observed for child height (Table 12). We observed relatively high correlations between child weight and size predicted at the same age (correlation coefficients ranged between 0.65 and 0.73, Table 12).

Table 12: Correlations between pre- and post-natal growth measurements in the PEnDevE population (n = 520 male newborns from the EDEN cohort, 2003-2006).

	Biparietal diameter			Head circumference			Femur length		Size					Abdominal circumference			Weight					
	US 1	US 2	US 3	US 2	US 3	Birth ^a	US 2	US 3	Birth	6 mo	12 mo	24 mo	36 mo	US 2	US 3	36 mo	US 2	US 3	Birth	6 mo	12 mo	24 mo
Biparietal diameter																						
US 2	0.26	1																				
US 3	0.09	0.31	1																			
Head circumference																						
US 2	0.23	0.88	0.26	1																		
US 3	0.12	0.23	0.79	0.24	1																	
Birth ^a	0.01	0.18	0.32	0.21	0.41	1																
Femur length																						
US 2	0.20	0.74	0.15	0.79	0.19	0.18	1															
US 3	0.05	0.16	0.55	0.18	0.59	0.26	0.27	1														
Size																						
Birth	-0.08	0.03	0.14	0.07	0.16	0.55	0.10	0.22	1													
6 months	-0.02	0.06	0.21	0.10	0.23	0.36	0.18	0.38	0.48	1												
12 months	-0.02	0.02	0.17	0.06	0.17	0.25	0.14	0.34	0.35	0.90	1											
24 months	-0.02	0.02	0.15	0.06	0.14	0.19	0.13	0.32	0.28	0.73	0.94	1										
36 months	-0.02	0.03	0.15	0.07	0.15	0.19	0.15	0.34	0.29	0.74	0.89	0.97	1									
Abdominal circumference																						
US 2	0.24	0.72	0.23	0.76	0.22	0.16	0.73	0.21	0.10	0.15	0.11	0.09	0.10	1								
US 3	0.09	0.20	0.60	0.22	0.60	0.29	0.18	0.61	0.20	0.26	0.21	0.18	0.18	0.34	1							
3 years	-0.03	0.06	0.11	0.04	0.10	0.12	0.03	0.16	0.17	0.31	0.35	0.38	0.40	0.08	0.18	1						
Weight																						
US 2 (est)	0.25	0.82	0.22	0.87	0.23	0.19	0.90	0.24	0.10	0.16	0.12	0.11	0.12	0.94	0.28	0.06	1					
US 3 (est)	0.10	0.23	0.74	0.25	0.74	0.33	0.22	0.79	0.21	0.32	0.27	0.24	0.24	0.32	0.94	0.19	0.30	1				
Birth	0.02	0.12	0.26	0.18	0.28	0.70	0.18	0.30	0.73	0.49	0.34	0.26	0.26	0.21	0.38	0.16	0.20	0.37	1			
6 months	-0.04	0.02	0.18	0.03	0.17	0.33	0.06	0.24	0.39	0.66	0.61	0.52	0.51	0.10	0.26	0.49	0.08	0.27	0.44	1		
12 months	-0.04	-0.01	0.14	0.00	0.14	0.25	0.03	0.21	0.29	0.59	0.65	0.63	0.61	0.07	0.23	0.58	0.05	0.24	0.33	0.92	1	
24 months	-0.02	0.04	0.15	0.04	0.15	0.21	0.06	0.23	0.23	0.55	0.66	0.70	0.70	0.08	0.22	0.69	0.07	0.24	0.28	0.77	0.93	1
36 months	0.00	0.08	0.18	0.08	0.18	0.21	0.10	0.25	0.22	0.54	0.63	0.69	0.72	0.10	0.22	0.73	0.10	0.26	0.27	0.67	0.80	0.95

^a assessed in duplicate within 4 days after birth

Abbreviation: mo: months, US: Ultrasound measurement

b) Exposure

We detected 6 of the 9 assayed phenols in at least 93% of the samples. Highest medians were observed for methyl paraben (122 µg/l), followed by triclosan (30 µg/l) and propyl paraben (17 µg/l, Table 13). Pearson correlation coefficients between 2,4- and 2,5-dichlorophenol was 0.83 and they ranged from 0.52 to 0.81 for the four parabens.

Table 13: Concentrations of phenol biomarkers in maternal urine in the PEnDevE population (EDEN cohort, 2003-2006, n = 520).

Phenols	LOD (µg/L)	%> LOD	Standardized concentrations ^a (1)			Measured concentrations (2)			Spearman correlation between (1) and (2)
			Percentiles (µg/L)			Percentiles (µg/L)			
			5 th	50 th	95 th	5 th	50 th	95 th	
2,4-dichlorophenol	0.2	98	0.3	1.0	9.8	0.3	1.0	10.1	0.92
2,5-dichlorophenol	0.2	100	1.7	9.2	305	1.6	9.8	291	0.96
Bisphenol A	0.4	99	0.8	2.3	9.5	0.7	2.5	11	0.85
Benzophenone-3	0.4	93	<LOD	2.1	72	<LOD	2.2	85	0.95
Triclosan	2.3	81	<LOD	31	763	<LOD	30	755	0.99
Methyl paraben	1	100	8.3	106	1148	8.1	122	2020	0.94
Ethyl paraben	1	73	<LOD	3.6	66	<LOD	4.7	72	0.93
Propyl paraben	0.2	99	0.5	14	250	0.6	17	317	0.96
Butyl paraben	0.2	84	<LOD	2.1	61	<LOD	2.0	59	0.96
∑Parabens (µmol/L)			0.1	0.9	9.8	0.1	1.1	17	0.94

^aAll concentrations were standardized for creatinine concentrations. Bisphenol A concentration was additionally standardized for gestational age at sampling and time elapsed between urine collection and freezing; benzophenone-3 was standardized for gestational age at sampling and hour of sampling. All of the parabens were standardized for hour of sampling ; butyl paraben was additionally standardized for day of sampling and gestational age at sampling
LOD: limit of detection.

We did not observe strong differences in exposure pattern between the subpopulation with phenol assessments in 2008 and the subpopulation with assessments in 2011: p-values for Student tests were above 0.4 for all phenols, except 2,4-dichlorophenol, which tended to increase from 2008 to 2011 (p, 0.11).

c) Association between phenols and growth parameters

We observed negative associations between triclosan concentrations and all of the growth parameters measured at the third ultrasound examinations (p-values ranged between 0.01 for abdominal circumference and 0.16 for head circumference), but not earlier in

pregnancy (p-values > 0.30, Table 14). At birth, this phenol was also negatively associated with head circumference ($\beta = -1.4$ mm, 95% CI; -2.8; 0.0) but not with weight ($\beta = 8.7$ g, 95% CI; -27; 44), nor height ($\beta = 0.1$ mm, 95% CI; -2.3; 2.5). The negative association with head circumference observed at birth was less marked at 36 months ($\beta = -1.0$ mm, 95% CI; -2.8; 0.8). Triclosan concentration was not associated with postnatal heights and weights nor as with abdominal circumference and skinfold thicknesses at 36 months (p-values ≥ 0.2 , Table 14).

Regarding prenatal growth rate, triclosan was negatively associated with growth in biparietal diameter between US examination one and two ($\beta = -0.03$ mm/week; 95% CI; -0.07; -0.02). This phenol was positively associated with weight growth between US examination two and three ($\beta = -3.1$ g/week; 95% CI; -5.9; -0.3) and US examination three and birth ($\beta = 7.6$ g/week; 95% CI; 1.1; 14, Table 15).

The strongest association between parabens and ultrasound measurements was observed between butylparaben and head circumference at the third ultrasound ($\beta = -1.3$ mm, 95% CI; -0.2; 2.7); This association did not remain at birth ($\beta = 0.1$ mm, 95% CI; -1.4; 1.5, Table 14) and butylparaben tended to be negatively associated with head circumference growth between both measurements ($\beta = -0.3$ mm/week; 95% CI; -0.7; 0.1). At birth, all parabens, as well as their molecular sum, were positively associated with weight (p-values < 0.05). This might be explained by an effect on growth in late pregnancy since all of these phenols are also positively associated with weight growth between the third ultrasound examination and birth (Table 15). Positive associations were observed between methyl- and propyl-paraben and weight at 6, 12, 24 and 36 months and abdominal circumference at 36 months (p-values ranged between 0.02 and 0.14, Table 14). These phenols tended to be positively associated with subscapula skinfold thickness at 36 months ($\beta = 0.08$ mm, 95% CI; -0.04; 0.21 and $\beta = 0.11$ mm, 95% CI; -0.03; 0.24 for methyl- and propyl-paraben respectively). Similar but weaker patterns of association were observed for butyl paraben (Table 14). Additional adjustment for birth weight weakened the positive associations observed between parabens and postnatal weight, and only the positive association between methylparaben and weight at 36 months was maintained ($\beta = 167$ g; 95% CI; 1; 332). Regarding postnatal growth rate, parabens tended to be positively associated with average weight growth between 12 and 24 months and between 24 and 36 months but not earlier in the child life (intervals birth - 6 months and 6 - 12 months, Table 15). Only

methylparaben tended to be positively associated with size at birth and during early childhood (p-values ranged between 0.05 and 0.14, Table 15).

Bisphenol A tended to be positively associated with abdominal circumference ($\beta = 1.4$ mm; 95% CI -0.2; 2.9) but negatively with femur length ($\beta = -0.2$ mm; 95% CI -0.5; 0.04) at the third ultrasound examination. This phenol was not clearly associated with the other pre- and post-natal measurements (p-values ≥ 0.18 , Table 14). After adjustment for height, bisphenol A was not associated either with weight at birth and 6 months, but tended to be associated with weight at 12, 24 and 36 months which respectively increased by 59 g (95% CI; -22; 140), 100 g (95% CI; 0.3; 200) and 102 g (95% CI; -18; 221) for each IQR increase in bisphenol A concentration.

2,4 dichlorophenol was negatively associated with abdominal circumference at the third ultrasound examination ($\beta = -1.2$ mm, 95% CI; -2.3; 0.0), but positively with this outcome recorded at 36 months ($\beta = 2.4$ mm, 95% CI; -0.2; -4.9, Table 15). This phenol was also positively associated with weight growth between 24 and 36 months which increased by 0.9 g/weeks for each IQR increase in 2,4-dichlorophenol concentration (Table 15). It was not clearly associated with weight measured at the endpoint of this period ($\beta = 31$ g; 95% CI, -94; 155). We observed isolated associations between 2,5-dichlorophenol and head circumference ($\beta = 1.2$ mm, 95% CI; 0.1; 2.3) at the third ultrasound examination.

For benzophenone-3, the strongest association was observed with weight at birth ($\beta = 17$ g; 95% CI, -6.1; 40, Table 14).

Table 14: Adjusted associations between phenol maternal urinary concentrations and pre- and post-natal size in the PEnDevE population (EDEN cohort, 2003-2006, n = 520 male newborns).

Growth parameter	Phenolic compounds																			
	2,4-DCP		2,5-DCP		BPA		BP3		TCS		MP		EP		PP		BP		Σ PB	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Biparietal diameter (mm)																				
1 st US	-0.09	0.49	-0.11	0.49	-0.03	0.87	-0.02	0.87	0.19	0.33	0.09	0.61	-0.01	0.94	0.04	0.85	-0.05	0.81	0.09	0.62
2 nd US	-0.06	0.64	-0.03	0.85	-0.05	0.79	-0.13	0.30	-0.16	0.42	0.07	0.69	0.12	0.55	0.02	0.90	0.28	0.16	0.06	0.73
3 rd US	-0.04	0.74	0.26	0.10	-0.23	0.18	0.06	0.62	-0.41	0.04	-0.11	0.53	0.02	0.90	-0.13	0.51	0.34	0.10	-0.12	0.52
Head circumference (mm)																				
2 nd US	-0.37	0.44	0.02	0.98	0.26	0.67	-0.28	0.55	-0.06	0.93	0.57	0.38	1.04	0.14	0.05	0.94	1.01	0.17	0.56	0.40
3 rd US	0.11	0.81	1.19	0.03	-0.49	0.42	0.33	0.46	-0.97	0.16	-0.11	0.86	1.00	0.15	0.14	0.84	1.25	0.08	0.04	0.96
Birth	-0.23	0.63	0.03	0.96	0.08	0.90	0.35	0.46	-1.39	0.05	-0.09	0.90	0.03	0.97	0.20	0.77	0.05	0.95	-0.02	0.98
36 months	-0.18	0.77	0.10	0.90	0.16	0.84	-0.38	0.51	-0.98	0.27	0.28	0.73	-0.75	0.40	0.19	0.82	-0.58	0.54	0.19	0.82
Femur length (mm)																				
2 nd US	-0.03	0.81	0.08	0.54	0.16	0.24	-0.02	0.85	0.01	0.94	0.06	0.66	0.08	0.63	0.01	0.94	0.11	0.50	0.07	0.65
3 rd US	-0.12	0.24	0.00	0.98	-0.23	0.09	0.04	0.70	-0.30	0.05	-0.04	0.80	0.15	0.34	-0.20	0.19	0.10	0.54	-0.03	0.83
Length (mm)																				
Birth	-0.53	0.50	-0.53	0.58	1.08	0.30	0.45	0.56	0.09	0.94	2.21	0.05	0.84	0.49	1.52	0.20	1.73	0.17	2.24	0.05
6 months ^a	-0.07	0.94	0.22	0.84	-0.09	0.94	0.59	0.48	-0.11	0.93	2.19	0.07	0.04	0.97	1.28	0.31	0.77	0.57	2.21	0.07
12 months ^a	-0.70	0.45	-0.55	0.63	-1.03	0.40	0.53	0.55	-0.47	0.73	1.89	0.14	-0.38	0.79	0.77	0.57	0.69	0.64	1.91	0.14
24 months ^a	-1.05	0.35	-0.91	0.51	-1.63	0.27	0.52	0.63	-0.85	0.61	2.25	0.14	-0.11	0.95	0.84	0.61	1.39	0.43	2.26	0.15
36 months ^a	-0.57	0.65	-0.59	0.70	-1.53	0.36	0.48	0.70	-0.71	0.71	3.21	0.07	0.45	0.81	1.29	0.49	1.96	0.33	3.16	0.08
Abdominal circumference (mm)																				
2 nd US	-0.07	0.90	0.22	0.76	0.58	0.45	0.17	0.76	0.33	0.71	0.22	0.79	0.29	0.74	0.04	0.96	0.73	0.44	0.19	0.82
3 rd US	-1.16	0.05	-0.05	0.95	1.35	0.08	0.33	0.58	-2.24	0.01	0.30	0.72	0.98	0.27	0.03	0.97	1.44	0.12	0.29	0.73
36 months	2.36	0.07	2.19	0.17	0.61	0.72	1.14	0.36	2.49	0.20	4.15	0.02	1.88	0.34	3.37	0.07	3.52	0.08	4.15	0.02
Weight (g)																				
2 nd US	4.68	0.70	6.07	0.68	7.62	0.63	-1.53	0.90	6.57	0.72	18.86	0.25	25.3	0.16	12.8	0.46	17.9	0.34	18.8	0.27
3 rd US	-18.1	0.12	-0.91	0.95	3.37	0.83	7.09	0.53	-37.3	0.03	-8.06	0.61	6.69	0.70	-10.1	0.55	21.7	0.23	-6.86	0.67
Birth	-3.82	0.76	6.96	0.64	0.52	0.97	17.4	0.15	8.68	0.63	33.1	0.05	47.4	0.01	38.9	0.03	52.4	0.01	38.9	0.02
6 months ^a	-12.1	0.71	-15.5	0.70	-1.93	0.96	23.3	0.46	3.72	0.94	84.9	0.06	6.00	0.90	80.6	0.09	45.0	0.38	84.7	0.06
12 months ^a	-33.8	0.40	-32.4	0.51	30.8	0.56	39.4	0.31	6.23	0.92	86.2	0.12	-4.88	0.94	86.9	0.14	38.5	0.54	85.1	0.13
24 months ^a	-17.8	0.73	-13.2	0.83	50.8	0.45	28.8	0.56	33.9	0.66	137	0.05	35.9	0.64	134	0.07	88.3	0.27	138	0.06
36 months ^a	30.5	0.63	34.4	0.66	50.1	0.55	-1.13	0.99	69.8	0.46	208	0.02	102	0.28	188	0.04	153	0.12	210	0.02
Skinfold at 36 months (mm)																				
Triceps	-0.03	0.73	-0.08	0.40	0.03	0.79	-0.01	0.86	0.07	0.55	0.01	0.96	-0.02	0.90	-0.02	0.87	-0.07	0.58	-0.01	0.94
Subscapula	0.05	0.34	0.02	0.75	0.03	0.59	0.00	0.95	0.12	0.10	0.08	0.20	0.08	0.28	0.11	0.12	0.03	0.66	0.08	0.22

^a Parameter individually estimated by the Jenss growth curve model, based on postnatal measures. Abbreviations: BP: butylparaben, BPA: bisphenol A, BP3: benzophenone-3, DCP: dichlorophenol, EP: ethylparaben, MP: methylparaben, PP, propylparaben, TCS: triclosan, Σ PB: molecular sum of paraben concentrations.

Table 15: Adjusted associations between phenol maternal urinary concentrations and growth rate in the PEnDevE population (EDEN cohort, 2003-2006, n = 520 male newborns).

Growth rate	Phenolic compounds																			
	2,4-DCP		2,5-DCP		BPA		BP3		TCS		MP		EP		PP		BP		ΣPB	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Biparietal diameter^a																				
1 st to 2 nd US	0.00	0.98	0.01	0.61	0.00	0.96	-0.01	0.43	-0.03	0.06	-0.01	0.51	0.00	0.81	-0.01	0.58	0.03	0.18	-0.01	0.48
2 nd to 3 rd US	0.01	0.49	0.02	0.21	-0.02	0.34	0.01	0.70	-0.03	0.25	0.00	0.95	0.01	0.53	0.00	0.97	0.01	0.65	0.00	0.95
Head circumference^a																				
2 nd to 3 rd US	0.06	0.25	0.07	0.26	-0.04	0.54	0.03	0.56	-0.07	0.37	0.01	0.93	0.05	0.52	0.10	0.18	0.06	0.51	0.02	0.76
3 rd US to birth	-0.12	0.32	-0.23	0.12	0.15	0.35	0.05	0.66	-0.09	0.64	-0.07	0.70	-0.27	0.14	-0.21	0.24	-0.31	0.11	-0.09	0.58
Weight^b																				
2 nd to 3 rd US	-1.29	0.18	-0.15	0.90	0.97	0.44	0.22	0.81	-3.12	0.03	0.07	0.96	1.68	0.24	0.04	0.98	2.10	0.17	0.16	0.91
3 rd US to birth	2.71	0.22	0.68	0.80	-1.86	0.53	1.77	0.41	7.64	0.02	7.02	0.02	6.98	0.04	7.83	0.02	5.67	0.11	8.00	0.01
0 to 6 months	-0.15	0.89	-0.81	0.57	-0.71	0.64	0.58	0.61	-0.83	0.63	1.19	0.45	-1.37	0.43	-0.23	0.89	-0.04	0.98	0.89	0.58
6 to 12 months	-0.83	0.21	-0.65	0.42	1.26	0.15	0.62	0.34	0.10	0.92	0.05	0.96	-0.42	0.67	0.24	0.80	-0.25	0.81	0.02	0.99
12 to 24 months	0.31	0.44	0.37	0.45	0.39	0.46	-0.20	0.60	0.53	0.38	0.98	0.08	0.78	0.19	0.91	0.12	0.96	0.13	1.01	0.07
24 to 36 months	0.93	0.03	0.92	0.07	-0.01	0.98	-0.57	0.16	0.69	0.28	1.36	0.02	1.27	0.04	1.03	0.09	1.25	0.06	1.39	0.02
Size^a																				
0 to 6 months	0.03	0.44	0.03	0.51	-0.07	0.12	0.02	0.47	-0.04	0.42	-0.02	0.74	0.00	0.95	-0.07	0.15	-0.02	0.78	-0.02	0.71
6 to 12 months	-0.02	0.16	-0.03	0.16	-0.04	0.11	0.00	0.89	-0.01	0.59	-0.01	0.63	-0.02	0.53	-0.02	0.44	0.00	0.90	-0.01	0.64
12 to 24 months	-0.01	0.44	-0.01	0.51	-0.01	0.31	0.00	0.98	-0.01	0.58	0.01	0.56	0.01	0.69	0.00	0.92	0.01	0.32	0.01	0.58
24 to 36 months	0.01	0.21	0.01	0.50	0.00	0.84	0.00	0.91	0.00	0.81	0.02	0.07	0.01	0.33	0.01	0.42	0.01	0.35	0.02	0.09

^a mm/week^b g/week

Abbreviations: BP: butylparaben, BPA: bisphenol A, BP3: benzophenone-3, DCP: dichlorophenol, EP: ethylparaben, MP: methylparaben, PP, propylparaben, TCS: triclosan, ΣPB: molecular sum of paraben concentrations.

d) Sensitivity analyses

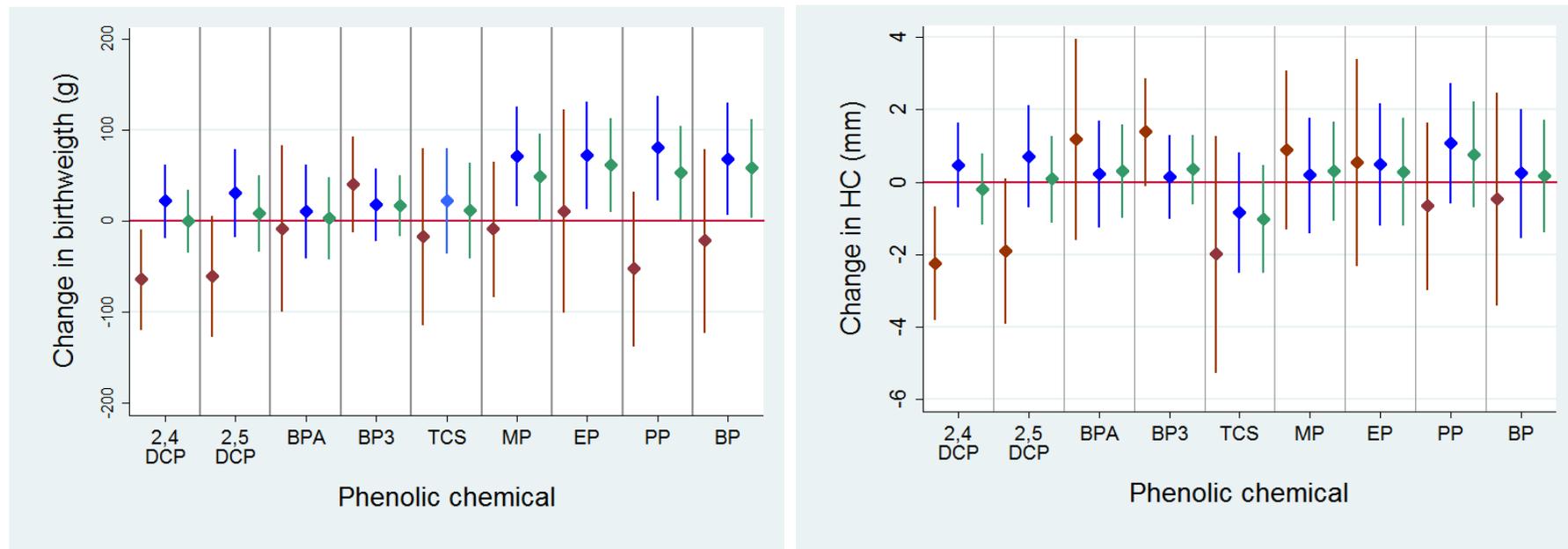
When we used head circumference at birth rather than the duplicate assessment performed few days after birth, the negative association observed with triclosan was weakened ($\beta_{HC \text{ at birth}} = -1.1 \text{ mm}$; 95% CI -3.0; 0.5 compared to $\beta_{HC \text{ duplicate assessment}} = 1.4 \text{ mm}$, 95% CI, -2.8; 0.0, Table 14), while a positive association with benzophenone-3 ($\beta = 1.0 \text{ mm}$; 95% CI 0.0; 2.0), not observed with the duplicate assessment ($\beta = 0.4 \text{ mm}$; 95% CI -0.6; 1.3), appeared.

Adjustment for the child caloric intake at 4 and 8 months did not strongly affect our results and the positive associations between parabens and postnatal weight were maintained. Similarly, after taking into account child caloric intake at 8 months (child caloric intake was not available later in child life) and child height, bisphenol A was still positively associated with weight at 24 and 36 months. The association between bisphenol A and weight at 12 months was weakened after adjustment for caloric intake at 8 months ($\beta = 36 \text{ g}$; 95% CI, -52, 124) compared to in the analysis not adjusted for caloric intake ($\beta = 61 \text{ g}$; 95% CI; -27; 149; $n = 403$, analysis restricted to children with data on caloric intake).

We did not find clear evidence of effect modification by breastfeeding duration for any of the phenolic chemicals (p-values for interaction > 0.1).

In order to compare our results with those previously observed among a smaller subsample of the EDEN cohort (Gona_PE study, see Chapter II, paragraph II.1) we performed transversal analyses for weight and head circumference at birth. The negative associations observed between dichlorophenols, birth weight and head circumference were only observed in the subsample of 191 women (Gona_PE study). The positive associations between parabens and weight at birth were not observed in women of the Gona_PE study, however the confidence interval of the estimated β tended to overlap between both studies (Figure 16).

Figure 16: Adjusted^a associations between phenols and weight and head circumference^b at birth in different subpopulations of the EDEN cohort (transversal analysis, β given for an IQR increase in phenol concentration).



- ◆ n = 191, population of the case-control study with phenol measurements in 2008 (Gona_PE study, models corrected for case-control sampling)
- ◆ n = 410, PEnDevE population not in Gona_PE
- ◆ n = 520, all women delivering boys with complete data on pre- and post-natal growth (PEnDevE study)

Abbreviations: BP: butylparaben, BPA: bisphenol A, BP3: benzophenone-3, DCP: dichlorophenol, EP: ethylparaben, MP: methylparaben, PP, propylparaben, TCS: triclosan

^a Adjustment factors were: maternal and paternal height and weight, maternal weight gain during pregnancy, maternal active and passive smoking during pregnancy, maternal education level, parity, gestational duration (3rd order polynomial) and recruitment center. Model for head circumference was additionally adjusted for the time elapsed between birth and measurements.

^b Head circumference was assessed in duplicate within 4 days after birth; results in Gona_PE slightly differed to those presented in Table 9 (page 58) because in this table, we used head circumference measured at birth rather than the duplicate measurement performed within 4 days after birth.

4. Discussion

Within our study population of male newborns, triclosan concentration was negatively associated with fetal growth measurements in late pregnancy, head circumference at birth and less strongly with head circumference at 36 months. The parabens were not associated with fetal weight during pregnancy but were positively associated with weight at birth. This positive association with weight remained until 36 months for methyl- and propyl-paraben; which were also positively associated with abdominal circumference and subscapula skinfold thickness at 36 months.

The negative association observed between triclosan and head circumference at birth is consistent with the results obtained in the subsample of 191 male newborns from the EDEN cohort (Gona_PE study, $\beta = -2.0$ mm; 95% CI; -5.3; 1.3, β given for an IQR increase in log-transformed TCS concentrations). A negative association has been previously suggested in one study among boys and girls ($\beta = -0.4$ mm; 95% CI; -1.3; 0.04, β given for an increase by one in log-transformed TCS concentrations, results for boys only were not reported (Wolff et al. 2008)). Head circumference has been shown to be a predictor of the brain volume (Bartholomeusz et al. 2002) and both brain size and head circumference have been correlated with intelligence quotient and cognitive ability (Castellanos et al. 2002; Gale et al. 2006; Ounsted et al. 1988). To our knowledge no study has evaluated the association between triclosan and neurodevelopment. Animal studies have shown that perinatal exposure to triclosan may disturb the homeostasis of thyroid hormones, which are required for the fetus and infant normal growth and brain development (Paul et al. 2010; Paul et al. 2012). In Human, no study has specifically focused on triclosan exposure and thyroid hormone levels in pregnant women. Among a subset of the NHANES population, a positive association between this phenol, measured in spot urine samples, and the total triiodothyronine concentration in blood has been observed in adolescents (ages 12–19), but not in adults; no association was observed with the other thyroid hormones as thyroxin (Koeppel et al. 2013).

Methylparaben is one of the most commonly used parabens in food and cosmetics (Soni et al. 2005) and in our population, methylparaben urine concentration was around 10 fold higher than those observed for the other parabens. The positive associations observed with postnatal growth measurements tended to be stronger for this paraben than for the other ones. Our findings suggested that the associations observed between parabens and weight at 6 and 12 months may result of an accelerated weight growth at the end of pregnancy. These

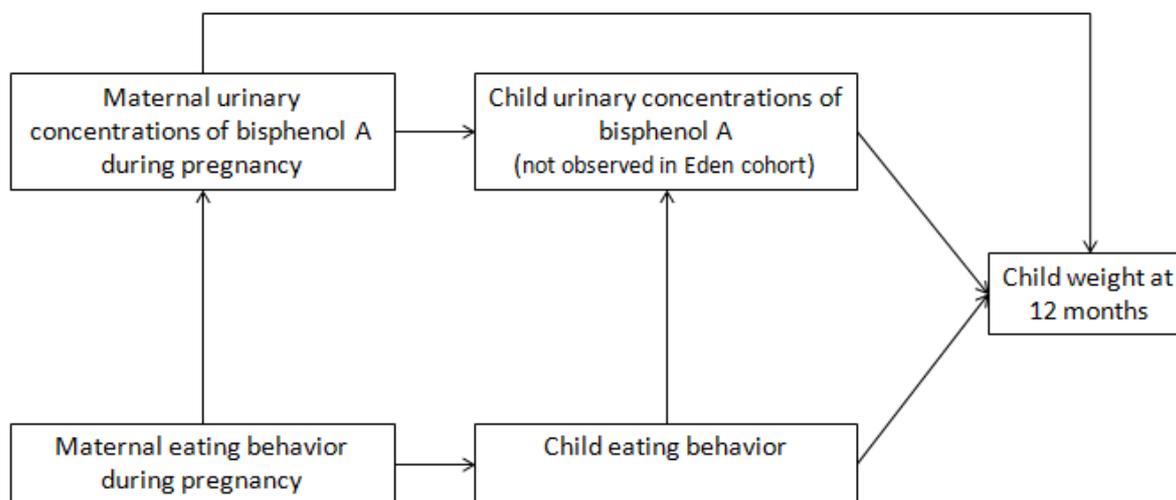
associations were indeed weakened by birth weight adjustment and parabens were positively associated with weight growth between the third ultrasound examination and birth, but not between birth and 6 months nor as between 6 and 12 months. Inversely, the positive association between parabens and weight at 36 months was not affected by birth weight adjustment and may result of an accelerated weight growth between 12 and 36 months. A recent *in vitro* study has reported that parabens may promote adipocyte differentiation in murine cells by an activation of the glucocorticoid receptor (GR) and / or the peroxisome proliferator activated receptor gamma (PPAR γ , (Hu et al. 2012; Taxvig et al. 2012)). These mechanisms may play a role in the positive associations observed with postnatal weight and skinfold thickness. Paraben estrogenic activities (Chen et al. 2007; Golden et al. 2005; van Meeuwen et al. 2008) may also be implied in the positive association observed with child growth since the estrogenic receptors ER α and ER β are implied in the growth of diverse tissues. In Human, a positive association between the placental total effective xenoestrogen burden and birth weight has been observed in boys (Vilahur et al. 2013). To our knowledge, if we except our previous study among a subsample of 191 male newborns from EDEN cohort (Philippat et al. 2012) and in which we did not observe any association between parabens and weight at birth ($\beta = - 3$ g; 95% CI, - 39, 33) no other study has explored associations between these chemicals and pre- and early post-natal growth in Human.

In our population, bisphenol A was negatively associated with femur length and positively associated with abdominal circumference measured at the third ultrasound examination. This chemical was not associated with weight, height or length at birth. A few studies have already assessed the effect of bisphenol A on fetal growth in Human; some have reported positive associations with weight and head circumference at birth (Philippat et al. 2012; Wolff et al. 2008) while others, with repeated ultrasound measurements during pregnancy, have reported negative associations with fetal femur length, head and abdominal circumference (Lee et al. 2010), and with fetal weight (Snijder et al. 2013). Urinary concentrations of bisphenol A have been shown to be highly variable during pregnancy with an Intraclass Correlation Coefficient (ICC) around 0.10 (Braun et al. 2011b). All of the sub-mentioned studies used a small numbers of urine samples to assess exposure (one sample; except for Snijder et al. who performed three measurements) and their findings are likely to be affected by exposure misclassification. This may explain the lack of consistency observed across human studies for bisphenol A. This issue is further discussed and studied in chapter III.

In our population, bisphenol A was positively associated with weight at 12, 24 and 36 months, after height adjustment. In vitro studies have reported an adipogenic activity for bisphenol A (Masuno et al. 2005; Sargis et al. 2010) and in animal, obesogenic effects have been reported (doses: 0.1 to 1.2 mg/kg of body weight/day of bisphenol A, (Rubin et al. 2001; Ryan et al. 2010; Somm et al. 2009)). Previous studies exploring the effects of bisphenol A on growth during childhood focused on older children than those of our study (6 to 19 years, Harley et al. 2013; Trasande et al. 2012). Only the study published by Harley et al. relies on measurements in maternal urine collected during pregnancy and no association between prenatal exposure to bisphenol A and BMI or waist circumference at 9 years was observed in boys.

The main source of bisphenol A is probably diet and eating behaviors may affect urinary concentrations of bisphenol A (Agence nationale de sécurité sanitaire de l'alimentation de l'environnement et du travail 2013; Rudel et al. 2011). It is likely that women and children who regularly consume heavily-packaged, processed and canned foods have higher levels of bisphenol A. In our study, we cannot exclude that higher urinary concentration of bisphenol A is a surrogate for factors predictive of child overweight such as maternal and child (when food diversification began) eating behaviors, rather than a direct cause of overweight (Figure 17). We were limited in studying confounding of the observed associations between bisphenol A and child postnatal weight by eating behaviors because we only had an estimation of the mother and child caloric intake rather than data on the kind of food (canned, processed or fresh food) they usually eat. In addition, data on the child and mother alimentation were collected by questionnaires and our estimation are likely to suffer from lack of precision. Nevertheless, in our study the association of maternal pregnancy bisphenol A level with child weight at 12 months was weakened by adjustment for the child caloric intake at 8 months, which is suggestive of confounding by child diet. Adjustment for maternal caloric intake did not affect the association.

Figure 17: Assumed associations of maternal pregnancy bisphenol A level with child weight at 12 months and potential confounding by maternal and child eating behaviors.



Some of our findings, including the negative association between triclosan and head circumference at birth and the positive association between benzophenone-3 and weight at birth were consistent with those previously reported in a smaller subsample of the EDEN cohort (Gona_PE study, chapter II, paragraph II.2). However, the negative associations between dichlorophenols and weight at birth observed among the 191 mother-child pairs from the EDEN cohort (Gona_PE study) were not replicated in the largest PEnDevE group ($n = 520$); a few associations not observed in the Gona_PE population also appeared (e.g., positive association between parabens and weight at birth). Differences in findings between our two studies might be explained by 1) random variations, we explored a high number of associations and we cannot exclude that a part of our findings are due to chance; 2) exposure measurement errors; our results might be biased because of exposure misclassification resulting from the use of one urine sample to assess exposure to short half-life chemicals (this issue is further discussed and studied in chapter III). Less likely sources of finding differences across our studies are 3) confounding; we indeed cannot discard that unmeasured factors which confound the associations between phenols and birth outcomes differed across subpopulations. However both subpopulations are from the EDEN cohort and we did not observed strong differences between them; 4) difference in urine processing; phenol measurements were performed in 2008 for the Gona_PE study and in 2011 for the PEnDevE study. Differences in urine processing, which may differently impact the phenol measured

concentrations in both subpopulations, might have occurred. However, the fact that all measurements were performed by the same laboratory (CDC, Atlanta, USA) and that we did not observe strong differences in concentration distributions between samples analyzed in 2008 and 2011 were not in favor of that explanation.

Errors in outcome measurements will also impact effect estimates. We indeed observed different results using head circumference measured at birth rather than head circumference measured in duplicate few days after birth. Because head circumference could be distorted by the labor of giving birth (Bronfin 2001), we recommend to use a measure of head circumference performed few days after birth rather than at birth.

Strengths of the study are the prospective design, allowing exposure assessment during one of the biologically relevant windows (fetal period); the use of repeated measurements of growth during fetal and early postnatal life and the sample size, larger than those of the previous studies (Philippat et al. 2012; Snijder et al. 2013; Wolff et al. 2008). One of the main limitations is the availability of only one urine sample during pregnancy in the EDEN cohort to assess phenol concentrations.

5. Conclusion

Our study lends support to a potential effect of prenatal exposure to some phenols on pre- and early post-natal growth. However, because of the short half-life of the studied phenols and the likely episodic nature of the exposures, our findings may be affected by exposure misclassification. Replications are needed in other populations with repeated urine samples during pregnancy.

**CHAPTER III: ASSESSMENT OF PRENATAL
EXPOSURE TO PHENOLS AND PHTHALATES:
METHODOLOGICAL ISSUES**

Estimation des expositions prénatales aux phénols et aux phtalates : questions méthodologiques

Contexte : Les phénols et les phtalates sont rapidement métabolisés et éliminés dans les urines qui sont de ce fait, couramment utilisées dans les études épidémiologiques pour estimer l'exposition à ces composés (dosage de biomarqueurs). Dans le cadre des études s'intéressant aux expositions prénatales c'est l'urine maternelle, recueillie en général un faible nombre de fois pendant la grossesse (1 à 3), qui est utilisée. L'utilisation de biomarqueurs d'exposition permet de prendre en compte l'ensemble des sources d'exposition mais est, dans certain cas, associée à de l'erreur de mesure. Du fait de la courte demi-vie dans l'organisme des composés étudiés et de la nature épisodique des expositions, les concentrations urinaires de phénols et de phtalates sont susceptibles de varier au cours du temps et il est probable qu'un faible nombre de mesures ne reflète qu'imparfaitement l'exposition durant la grossesse entière. Lorsque que ce travail de thèse a été initié, une variabilité importante avait été reportée pour les concentrations de bisphénol A pendant la grossesse mais aucune étude concernant les autres phénols n'avait été publiée.

D'autres matrices tel que le méconium ou le liquide amniotique pourraient être plus proche de l'exposition fœtale que l'urine maternelle, néanmoins elles n'ont été que très peu utilisées en Epidémiologie Environnementale. Le bisphénol A et le 2,5-dichlorophénol ont déjà été détectés dans des échantillons de liquide amniotique recueillis auprès de femmes enceintes recevant une amniocentèse dans le cadre de leurs soins prénataux. Cependant, à notre connaissance, les autres phénols n'ont jamais été dosés dans ce liquide et aucune étude n'a comparé les concentrations de phénols dosées dans le liquide amniotique et l'urine maternelle recueillie en cours de grossesse.

Objectifs : Nous avons étudié la variabilité des concentrations urinaires de phénols pendant la grossesse et la relation entre les niveaux dosés dans les urines et le liquide amniotique recueillis le même jour, chez des femmes enceintes de la ville de New York.

Méthodes : Soixante et onze femmes se présentant pour une amniocentèse ont été incluses. Le liquide amniotique restant à la fin des tests cliniques a été utilisé pour cette étude. Un échantillon d'urine maternelle a été recueilli le jour de l'amniocentèse, puis à deux reprises durant la grossesse. Les phénols suivants : 2,4 et 2,5-dichlorophénol, bisphénol A, triclosan, benzophénone-3, méthyle, éthyle, propyle et butyle parabènes ont été dosés dans les urines et le liquide amniotique par le Center for Disease Control and Prevention (Atlanta, USA).

Résultats : Dans l'ensemble, le pourcentage de détection des phénols dans le liquide amniotique était faible, seuls la benzophénone-3 et le propylparabène ont été détectés dans plus de la moitié des échantillons. Le bisphénol A a été détecté dans seulement deux échantillons de liquide amniotique. Les concentrations de benzophénone-3 et de propyle parabène dosées dans le liquide amniotique et l'urine de la mère recueillie le jour de l'amniocentèse, étaient positivement associées. En ce qui concerne la variabilité des concentrations urinaires, le Coefficient de Corrélation Intra-classe (CCI) variait entre 0,48 et 0,61 pour la plupart des phénols, sauf pour le bisphénol A (CCI = 0,11).

Discussion : Les taux de détection des phénols dans le liquide amniotique étaient faibles et seuls la benzophénone-3 et le propyle parabène ont été détectés dans plus de 50 % des échantillons de liquide amniotique. Pour ces deux phénols, une corrélation positive a été observée entre les concentrations dosées dans le liquide amniotique et l'urine maternelle. La variabilité temporelle observée dans notre étude pour les concentrations urinaires de phénols suggère qu'il est nécessaire de recueillir plusieurs échantillons d'urine pour évaluer de façon correcte l'exposition aux phénols pendant toute la grossesse.

I. Prenatal exposure to short half-life chemicals: concentrations in amniotic fluid and variability in phenol urinary concentrations during pregnancy⁶.

1. Aims of the chapter

Our objectives were to assess the relationship between phenol biomarker concentrations measured in maternal urine and amniotic fluid collected on the same day, and to study the temporal variability in urinary phenol concentrations over the course of pregnancy. A secondary aim was to evaluate if concentration standardization for collection conditions could improve reliability in phenol concentrations during pregnancy.

2. Population and methods

a) Study population

The Study of Advanced Reproductive Age and Environmental Health (SARAEH) recruited 97 women who were referred for amniocentesis screening at the Mount Sinai Medical Center in New York, NY. Recruitment occurred between 2005 and 2008. Study eligibility requirements were fluency in English or Spanish, maternal age between 18 and 40 years, singleton pregnancy and intent to deliver at Mount Sinai Hospital. Women were approached for participation in the waiting area prior to their amniocentesis and provided their first spot urine sample immediately following their procedure. Two additional spot urine samples were collected from the participant later in their pregnancy, with collections planned at least two weeks apart and the final sample collected after the 27th week of gestation. This current study includes participants providing all three urine samples (n=71). Of these, an amniotic fluid sample was available for 69 participants (97%). Following delivery, medical records were abstracted for information pertaining to fetal health.

This study was approved by the Mount Sinai Program for the Protection of Human Subjects and women gave informed consent to be part of the study. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

⁶ This part of the thesis has been done in collaboration with the Pr Stephanie Engel during my stay at the University of North Carolina, Chapel Hill, USA (January to June 2012).

b) Exposure assessment

Following collection, amniotic fluid was delivered to the Mount Sinai Medical Center clinical cytogenetics laboratory for standard clinical care processing, which includes centrifugation and cell separation. Before being released for research purposes amniotic fluid supernatant was stored in polypropylene containers at -20 °C (time range: 0 to 38 weeks). Once released, SARAHEH research personnel thawed the amniotic fluid overnight at 4°C, and then aliquoted the supernatant into 2 mL cryovials for storage at -80 °C. Following collection, urine was immediately aliquoted into 2 mL cryovials and stored at -80 °C. Urine and amniotic fluid samples were shipped overnight on dry ice to the CDC for measurements of environmental phenols (free + conjugated forms): 2,4- and 2,5-dichlorophenols, bisphenol A, benzophenone-3, triclosan, and methyl-, ethyl-, propyl- and butyl-parabens⁷. Phenols were quantified using online solid phase extraction–high performance liquid chromatography–isotope dilution tandem mass spectrometry (Ye et al. 2005). Specific gravity of urine was measured using a handheld refractometer in each thawed aliquot prior to shipment to the CDC, with the exception of 11 urine samples which had specific gravity measured at the CDC. Creatinine concentration in urine was measured using an enzymatic reaction at the CDC.

c) Statistical analyses

Analyses were conducted on natural-log-transformed concentrations (ln). For phenols detected in at least 50% of the samples we replaced amniotic fluid concentrations below the limit of detection (LOD) by the instrumental reading values. We replaced the undetectable urine concentrations by $LOD/\sqrt{2}$ because the instrumental reading values were not available. The following formula was used to correct urinary concentrations for specific gravity: $C_{SG} = C \times [(SG_{mean} - 1)/(SG - 1)]$, where C_{SG} is the specific gravity corrected biomarker concentration, SG_{mean} is the specific gravity arithmetic mean in our population and C is the measured biomarker concentration. Creatinine corrected concentrations were computed by dividing the phenol concentrations by the creatinine concentration.

⁷ Biomarkers of exposure to phthalates have also been measured in maternal urine and amniotic fluid. K. Hoffman (University of North Carolina, Chapel Hill, USA) is working on the data. Analyses are not finalized yet and so are not presented in this thesis.

d) Relation between maternal urine and amniotic fluid biomarker concentrations

We used graphical techniques to compare amniotic fluid and urinary biomarker concentrations collected on the same day, and computed ratios of urinary (not corrected for urine dilution) to amniotic fluid concentrations.

For benzophenone-3 and propylparaben, the two phenols detected in more than 50% of the amniotic fluid samples, we computed Spearman correlation coefficients between urinary (uncorrected and specific gravity corrected) and amniotic fluid concentrations, collected on the same day. To explore the possible predictors of amniotic fluid benzophenone-3 and propylparaben concentrations, we performed Tobit regression models for a left-censored dependent variable (Lubin et al. 2004). We regressed amniotic fluid biomarker concentrations on maternal urinary concentrations taken on the same day, fetal sex, gestational age at amniocentesis (<17, 17 to 18.9, ≥ 19 gestational weeks), maternal age (<31, 31 to 35.9, ≥ 36), maternal pre-pregnancy body mass index (BMI, <25, ≥ 25), race (white versus non-white), pregnancy complications related to placental function (including preeclampsia, placenta previa, small placenta, oligohydroamnios and chorioamnionitis), and time that elapsed between amniotic fluid collection and processing (≤ 4 , 4 to 16, 16 to 20, > 20 weeks). Models were simultaneously adjusted for all of these factors. We also performed sensitivity analyses excluding four women with abnormal amniotic fluid conditions, namely oligohydramnios and polyhydramnios.

e) Variability in urinary concentrations

We evaluated variability in measures of dilution in spot urine samples across pregnancy by plotting creatinine urinary concentrations and urine specific gravity as a function of gestational age. To assess variability in phenol concentrations across pregnancy, we computed Intraclass Correlation Coefficients (ICCs) between phenol concentrations, measured in the three spot urine samples, using random intercept linear mixed models (Rabe-Hesketh 2008). The ICC is the ratio of the between-woman variability to the total variability (between- plus within- woman variability). We also computed Spearman correlations between pairs of phenol concentrations from the first, second and third spot urine collections. Our interpretation for ICCs and Spearman correlation coefficients was based on the following general guidelines: < 0.4: weak association, 0.4 to 0.6: moderate, > 0.6: good. Analyses of urinary concentration variability were performed using uncorrected, specific gravity-corrected

and creatinine-corrected concentrations. We were missing creatinine concentrations for 11 of the first spot urine samples. Thus, analyses based on creatinine-corrected concentrations were restricted to 60 women with complete creatinine and environmental phenol biomarker data.

In a secondary analysis, we computed Spearman correlations among pairs of urine samples collected in specific time intervals: fewer than 4 weeks apart, 4-6 weeks apart, 6-10 weeks apart, 10-12 weeks apart, 12-14 weeks apart, 14-16 weeks apart, and greater than 16 weeks apart. Samples were not independent within a given category (e.g., women can be included twice in the same category if they have equally spaced intervals between their three collections).

f) Influence of sampling conditions on phenol urinary concentrations

Finally, we estimated associations between urinary phenol biomarker concentrations and collection conditions (gestational age at collection, hour, day and season of sampling, urine specific gravity) using a random intercept linear mixed model simultaneously adjusted for each collection condition along with BMI, maternal age, year of collection, maternal education and maternal race (Mortamais et al. 2012). We used the measured urinary biomarker concentrations and the estimated effects of collection conditions on the measured urine concentrations to predict concentrations that would have been observed if all samples had been collected under the same conditions (concentrations standardized for collection conditions (Mortamais et al. 2012)). We only considered collection conditions associated with the measured concentration ($p < 0.2$) for the standardization. We evaluated whether standardization of urinary concentrations improved the calculated reliability across repeated spot urine samples by computing ICCs using concentrations standardized for collection conditions.

All analyses were performed using STATA/SE, version 12 (StataCorp, College Station, TX, USA).

3. Results

a) Study population and exposure

Women were 35.5 years old on average; mostly white (72%) and 55% went to graduate school (Table 16). The first, second and third urine samples were collected on average (\pm standard deviation (SD)), at 17.6 (\pm 1.6), 22.8 (\pm 3.5) and 32.8 (\pm 3.1) gestational weeks. The average (\pm SD) time elapsed between urine sampling was 5 (\pm 2.8) weeks between the first and second samples and 10 (\pm 3.9) weeks between the second and third samples.

Table 16: Characteristics of the 71 women with three repeated urine measurements during pregnancy (SARAEH study, 2005 to 2008).

Characteristic	N	%
Maternal age (years)		
< 30	5	7
30 to 34.9	21	30
\geq 35	45	63
Race		
White	51	72
Black	5	7
Hispanic	11	15
Asian	3	4
American Indian	1	1
Pre-pregnancy BMI (kg/m²)		
< 18.5	3	4
18.5 to 24.9	53	75
\geq 25	15	21
Maternal education		
High school	9	13
Any college	23	32
Graduate school, graduate degree	39	55
Conception methods		
Reproductive Techniques	3	4
Natural	68	96
Amniotic fluid sampling reason		
Advanced Maternal Age	49	69
Abnormal Genetic Screen	10	14
Elective	11	15
Other	1	1

Overall, the first sample was collected later in the day than the others (the number of women with urine collections before 10 a.m. was 1, 15 and 16 for the first, second and third samples, respectively). With the exception of ethylparaben, the medians of the uncorrected phenol concentrations, as well as creatinine concentrations and specific gravity, were lower in the first urine samples as compared to samples collected later in pregnancy (Table 17). Detection frequencies of environmental phenols in urine were high; 2,5-dichlorophenol,

benzophenone-3, methyl- and propyl- parabens were detected in at least 99% of the samples (Table 17). Detection frequencies in amniotic fluid were much lower; only benzophenone-3 and propylparaben were detectable in at least half of the samples. Bisphenol A was detected only in two amniotic fluid samples at a concentration equal to the LOD (0.4 µg/L); it was detected in 62% of the urine samples collected on the same day (Table 17).

Table 17: Distribution of phenol concentrations ($\mu\text{g/L}$) measured in amniotic fluid and in urine collected at three time periods during pregnancy among women of the SARA EH study, 2005 to 2008.

Analyte	Urine (n=71) ^a													Amniotic fluid (n=69) ^b (2)				Ratio (1)/(2) ^c		
	1 st sample (1)					2 nd sample				3 rd sample										
	LOD	% >LOD	5 th	50 th	95 th	% > LOD	5 th	50 th	95 th	% > LOD	5 th	50 th	95 th	% > LOD	5 th	50 th	95 th	5 th	50 th	95 th
Phenols ($\mu\text{g/L}$)																				
2,4-DCP	0.2	68	<LOD	0.6	5.7	94	<LOD	1.1	8.3	93	<LOD	1.0	14	1 ^c	<LOD	<LOD	LOD	1.4	1.4	9
2,5-DCP	0.2	100	0.8	7.5	178	100	1.8	12.8	364	100	1.7	14.2	466	16	<LOD	LOD	5.2	3.5	297	1756
BPA	0.4	62	<LOD	0.6	7.0	92	<LOD	1.8	9.5	82	<LOD	1.2	5.0	3	<LOD	<LOD	LOD	1.4	12	85
BP3	0.4	100	4.1	53.5	2220	100	5.7	77	6740	100	8.9	76.1	3180	61	<LOD	0.8	15.7	8	110	1648
TCS	2.3	79	<LOD	6.5	789	75	<LOD	15.4	734	80	<LOD	16.2	514	6	<LOD	<LOD	19.4	1.4	16	354
MP	1.0	100	9.6	100	985	100	19	272	1670	100	9.7	156	1830	42	<LOD	<LOD	3.3	23	196	827
EP	1.0	59	<LOD	2.8	58	65	<LOD	4.5	347	59	<LOD	1.7	144	0	<LOD	<LOD	<LOD	3.5	30	304
PP	0.2	100	0.5	28.7	424	100	1.6	45.6	531	99	0.8	36.5	589	58	<LOD	0.3	1.4	7	116	2130
BP	0.2	70	<LOD	1.5	26	85	<LOD	1.9	56.3	73	<LOD	1.7	58.2	6	<LOD	<LOD	0.3	1.4	22	179
Markers of urine dilution																				
Creat (mg/dL) ^d	3.5	100	13.7	44.1	256	100	15.3	86.8	221	100	16	90.9	175							
Specific gravity			1.003	1.008	1.026		1.003	1.02	1.03		1.004	1.015	1.026							

^a Concentrations not corrected for urine dilution, concentrations below LOD were replaced by $\text{LOD}/\sqrt{2}$.

^b Concentrations below LOD were replaced by instrumental reading values.

^c 2,4-DCP was only assessed in 11 amniotic fluid samples.

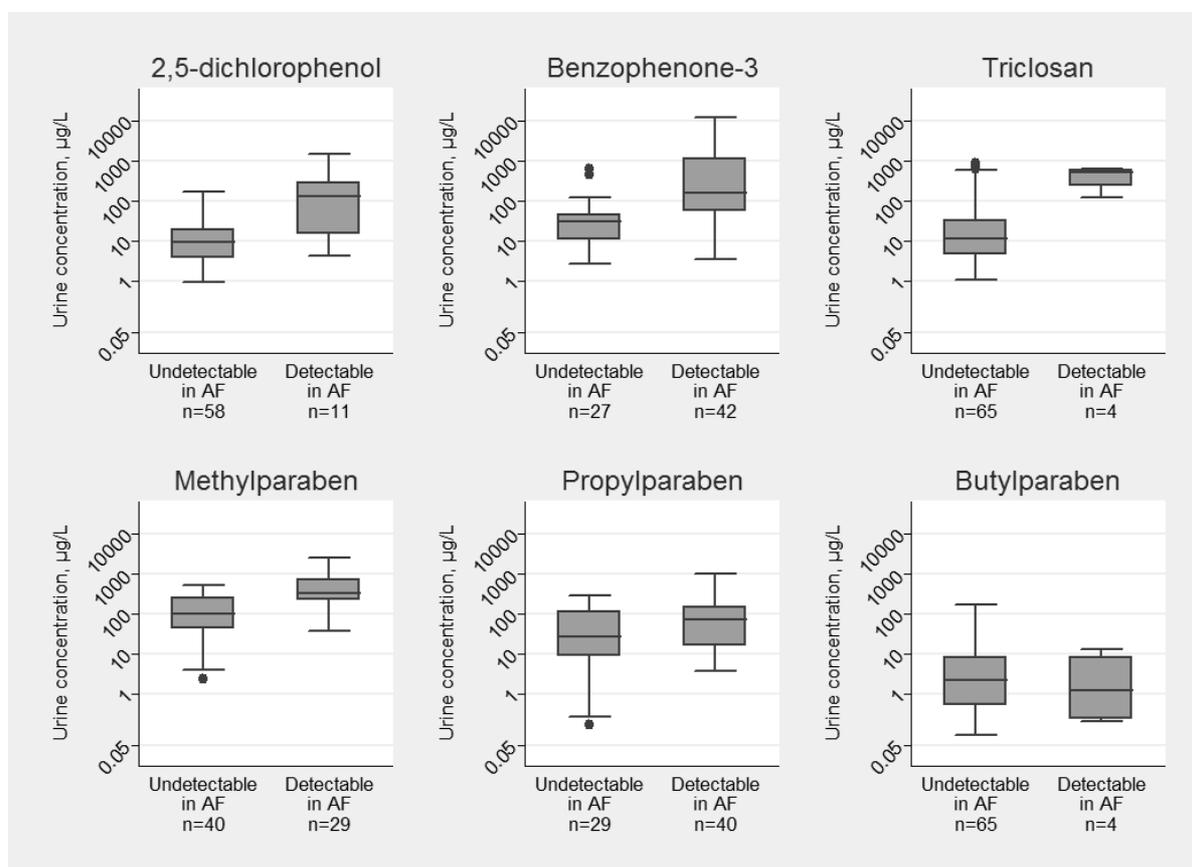
^d n = 60, creatinine concentration was missing for 11 urine samples from the first sampling.

Abbreviations: DCP: dichlorophenol, BP: butylparaben, BPA: bisphenol A, BP3: benzophenone-3, creat: creatinine, EP: ethylparaben, MP: methylparaben, PP, propylparaben, TCS: triclosan.

b) Relation between maternal urine and amniotic fluid biomarker concentrations

Among women with no abnormal amniotic fluid condition (i.e. excluding our four subjects with oligohydramnios and polyhydramnios), ratios of urine concentration in the first sample (not corrected for urine dilution) to amniotic fluid concentration were greater than one (Table 17). However, we observed higher benzophenone-3 and butylparaben concentrations in amniotic fluid than in the first urine sample for one women with oligohydramnios (urine - amniotic fluid ratio = 0.2 for benzophenone-3 and 0.7 for butylparaben). Median urinary concentrations of phenol biomarkers, whether corrected or not for urinary dilution, were higher in women with detectable amniotic fluid concentrations, with the exception of butylparaben, which was detected in only four amniotic fluid samples (Figure 18).

Figure 18: Box plots of the distribution of specific-gravity corrected urinary phenol concentrations are presented according to whether detectable levels of phenol metabolites were measured in amniotic fluid taken at the same time. *Bisphenol A, 2,4-dichlorophenol and ethyl paraben were not displayed in the graphic form because they were detected in too few amniotic fluid samples. Lower whiskers represent the lowest value within 1.5 IQR of the 25th percentile. The bottom of box is the 25th percentile, the line is the median, and the top of box is the 75th percentile. Upper whisker is the highest value within 1.5 IQR of the 75th percentile. Dots represents outlier values.*



The correlation between amniotic fluid and urinary concentration (uncorrected for urine dilution) was moderate for benzophenone-3 ($\rho = 0.53$) and weak for propylparaben ($\rho = 0.32$). Correction for urinary dilution did not change these correlation. After adjustment, for each one-unit increase in ln-transformed, specific gravity corrected urinary biomarker concentration, ln-amniotic fluid concentration increased by 0.52 (95% CI: 0.3; 0.7) for benzophenone-3, and by 0.36 (95% CI: 0.2; 0.5) for propylparaben (Table 18). Including urine specific gravity as a covariate instead of using phenol concentrations corrected for specific gravity did not change the results (data not shown). There was no clear association between gestational age at urine collection and amniotic fluid concentrations, although there was a limited range of gestational ages. After adjustment for the urinary concentration of the considered phenol, maternal age was negatively associated with amniotic concentrations of benzophenone-3 (p for trend = 0.09). Propylparaben amniotic fluid concentration varied according to fetal sex (Table 18) and we observed lower amniotic fluid concentrations among girl fetuses as compared to boy fetuses ($\beta = -0.56$; 95% CI: -1.2; 0.1). Compared to a storage time shorter than 4 weeks, we observed higher propylparaben concentration in amniotic fluid samples stored at -20°C for 4 to 16 weeks before being processed ($\beta = 0.93$; 95% CI: 0.0; 1.9, $n = 12$ samples); no change was observed for longer storage times (Table 18). Excluding four women with abnormal amniotic fluid conditions (oligohydramnios and polyhydramnios) did not change the positive associations observed between urine and amniotic fluid biomarker concentrations.

Table 18: Adjusted associations between ln-transformed amniotic fluid concentrations of selected phenols^a and their potential predictors among 68 pregnant women from the SARAHEH study, 2005 to 2008.

Characteristics	n	Change in BP3 AF concentration (ln-unit, (µg/L))		Adjusted BP3 AF concentration (GM, µg/L)	p-trend ^b	Change in PP AF concentration (ln-unit, (µg/L))		Adjusted PP AF concentration (GM, µg/L)	p-trend ^b
		β	95% CI			β	95% CI		
Urine concentration (ln-unit, µg/L)^c		0.5	[0.3;0.7]			0.4	[0.2; 0.5]		
Time of amniotic fluid storage at -20 C (weeks)					0.35				0.35
≤ 4	17	Reference		0.71		Reference		0.20	
4 to 16	12	-0.3	[-1.6; 1.0]	0.54		0.9	[0.0; 1.9]	0.50	
16 to 20	14	-0.4	[-1.7; 0.9]	0.48		0.0	[-0.9; 1.0]	0.20	
> 20	27	-0.6	[-1.9; 0.7]	0.39		-0.3	[-1.3; 0.6]	0.14	
Gestational age at sampling (weeks)					0.71				0.49
15 to 16.9	31	Reference		0.51		Reference		0.20	
17 to 18.9	26	0.1	[-0.7;0.9]	0.57		-0.2	[-0.8; 0.4]	0.17	
19 to 23	11	-0.4	[-1.8; 1.1]	0.36		0.6	[-0.4; 1.5]	0.35	
Maternal age (year)					0.09				0.56
<31	10	Reference		1.03		Reference		0.18	
31 to 35.9	24	-0.6	[-1.8; 0.7]	0.58		0.1	[-0.9; 1.0]	0.20	
36 to 40	34	-1.0	[-2.3; 0.2]	0.37		0.2	[-0.7; 1.1]	0.23	
BMI					0.67				0.34
< 25	53	Reference		0.53		Reference		0.23	
≥ 25	15	-0.2	[-1.3; 0.9]	0.42		-0.4	[-1.2; 0.4]	0.15	
Race									
White	48	Reference		0.63		Reference		0.20	
Non-white	20	-0.8	[-1.9; 0.3]	0.29		0.2	[-0.6; 0.9]	0.23	
Pregnancy complications related to placenta^d									
No	58	Reference		0.50		Reference		0.23	
Yes	10	-0.1	[-1.3; 1.2]	0.48		-0.8	[-1.7; 0.1]	0.11	
Baby sex									
Boy	36	Reference		0.59		Reference		0.27	
Girl	32	-0.4	[-1.2; 0.4]	0.41		-0.6	[-1.2; 0.1]	0.15	

^a Phenols detected in at least 50% of the amniotic fluid samples.

^b p-trend was estimated using categorical variables whose values corresponded to the category-specific median value (Rothman et al. 2008).

^c Corrected for specific gravity.

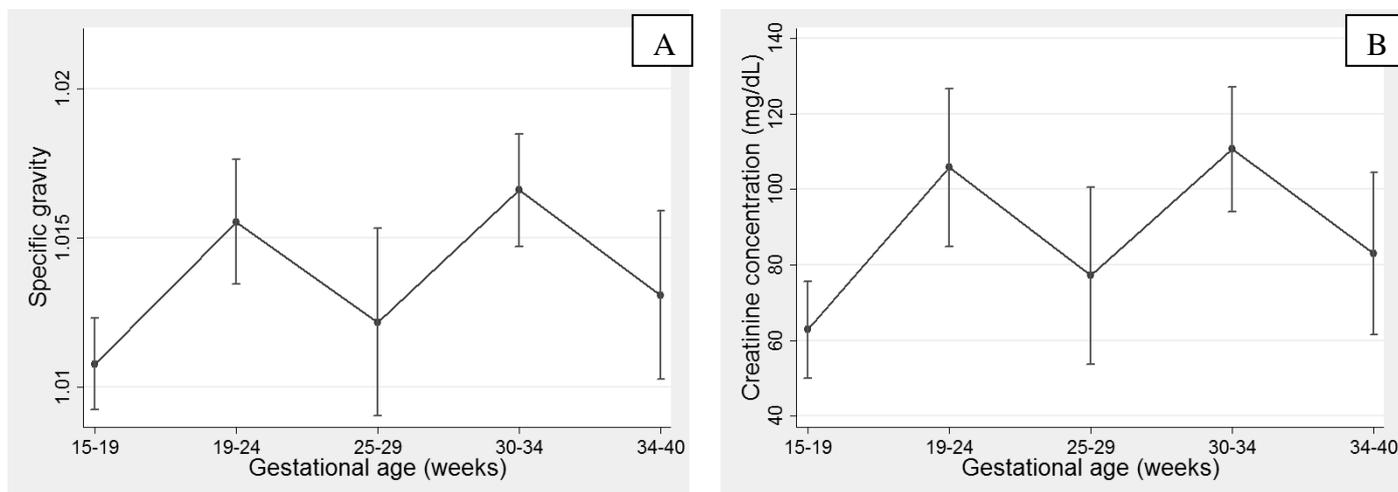
^d Including oligohydroamnios, choriamnionitis, small placenta, placenta praevia and pre-eclampsia.

Abbreviations: BP3: benzophenone-3, GM: geometric mean, PP: propyl paraben.

c) Variability in urinary concentrations

We observed similar variations in urinary specific gravity and creatinine concentrations throughout pregnancy (Figure 19), and both measures of urinary dilution were highly correlated (spearman correlation coefficient = 0.93).

Figure 19: Means of urine specific gravity (A) and urine creatinine concentrations (B) by gestational age (SARAEH study, 2005 to 2008).



I are confidence intervals.

n = 213 urine samples for specific gravity and 202 for creatinine.

ICCs and Spearman correlations using phenol concentrations corrected for creatinine or specific-gravity were very similar to one another, and with the exception of ethylparaben and bisphenol A, higher than those based on concentrations that were not corrected for urine dilution (Table 19, Table 23). ICCs were moderate to good, and of comparable magnitude for 2,4-, 2,5-dichlorophenol, benzophenone-3, triclosan, methyl-,ethyl- propyl- and butyl-parabens (Table 19). For these phenols, we observed similar trends with the Spearman correlation coefficients, which ranged from 0.41 to 0.77 (specific-gravity corrected concentrations), 0.40 to 0.86 (creatinine corrected concentrations) and 0.35 to 0.70 (concentrations not corrected for urine dilution, Table 19). ICCs for bisphenol A ranged between 0.11 (specific gravity corrected) to 0.23 (uncorrected). Although collections of spot urines were planned to occur at least two weeks apart, four women had less than a two week interval between successive collections. Excluding these women did not change our results (data not shown).

Table 19: Intraclass Correlation Coefficients (ICC) for phenol biomarker concentrations measured in urine samples collected at several times during pregnancy (n = 71 women from the SARAHEH study, 2005 to 2008).

Analyte	Specific gravity corrected concentrations ^a	Creatinine corrected concentrations ^b	Uncorrected concentrations ^a	Concentrations standardized for collection conditions ^{a,c}
2,4-dichlorophenol	0.60	0.48	0.47	0.60
2,5-dichlorophenol	0.61	0.62	0.52	0.60
Bisphenol A	0.11	0.14	0.23	0.15
Benzophenone-3	0.62	0.70	0.57	0.61
Triclosan	0.58	0.61	0.56	0.60
Methylparaben	0.61	0.61	0.52	0.62
Ethylparaben	0.48	0.44	0.48	0.48
Propylparaben	0.55	0.54	0.51	0.54
Butylparaben	0.56	0.64	0.54	0.61

^a n = 213 samples^b n = 180 samples^c All concentrations were standardized for specific gravity. Bisphenol A concentration was additionally standardized for gestational age at sampling and sampling hour. Butylparaben, triclosan and 2,4-dichlorophenol were additionally standardized for hour of sampling. Benzophenone-3 and methylparaben were additionally standardized for hour and season of sampling, propylparaben for season of sampling and ethylparaben for day of sampling.

Correlations between urinary concentrations measured within different time intervals are shown in Table 20. Our expectation was that correlations would be higher for samples collected with shorter intervening time intervals, and longer for samples collected with longer intervening time intervals. As expected, for 2,4-, 2,5-dichlorophenol and benzophenone-3, we overall observed lower coefficients (i.e., less reliability) for samples with longer time intervals between collections. However, correlations for bisphenol A continued to be weak, regardless of the length of the intervening time between sample collections.

Table 20: Spearman correlation coefficients between urine samples collected at specific time intervals using specific gravity corrected (A), creatinine corrected (B) and uncorrected (C) phenol concentrations (SARAEH study, 2005 to 2008).

Analytes	Interval between samples (weeks)						
	<4	4 to 6	6 to 10	10 to 12	12 to 14	14 to 16	≥ 16
A) Specific gravity corrected concentrations							
Nb of sample pairs	32	32	30	36	24	29	30
2,4-dichlorophenol	0.79	0.71	0.70	0.67	0.69	0.49	0.29
2,5-dichlorophenol	0.83	0.71	0.70	0.61	0.59	0.71	0.40
Bisphenol A	0.29	-0.10	0.34	0.25	0.43	0.38	-0.32
Benzophenone-3	0.80	0.74	0.59	0.7	0.75	0.58	0.29
Triclosan	0.57	0.70	0.71	0.46	0.70	0.28	0.35
Methyl paraben	0.59	0.56	0.61	0.58	0.70	0.61	0.63
Ethyl paraben	0.58	0.45	0.44	0.49	0.48	0.48	0.26
Propyl paraben	0.51	0.43	0.64	0.37	0.38	0.62	0.56
Butyl paraben	0.75	0.55	0.58	0.4	0.61	0.69	0.29
B) Creatinine standardized concentrations ^a							
Nb of sample pairs	28	25	29	29	21	22	26
2,4-dichlorophenol	0.88	0.65	0.63	0.69	0.41	0.41	0.22
2,5-dichlorophenol	0.77	0.89	0.71	0.66	0.44	0.81	0.38
Bisphenol A	0.22	0.05	0.07	0.19	0.38	0.53	-0.08
Benzophenone-3	0.85	0.85	0.71	0.73	0.82	0.65	0.47
Triclosan	0.64	0.68	0.54	0.48	0.74	0.56	0.49
Methyl paraben	0.60	0.61	0.57	0.51	0.58	0.65	0.58
Ethyl paraben	0.48	0.46	0.40	0.30	0.27	0.58	0.42
Propyl paraben	0.52	0.45	0.52	0.36	0.37	0.78	0.58
Butyl paraben	0.75	0.60	0.64	0.41	0.62	0.76	0.41
C) Crude concentrations							
Nb of sample pairs	32	32	30	36	24	29	30
2,4-dichlorophenol	0.67	0.75	0.40	0.58	0.43	0.29	0.16
2,5-dichlorophenol	0.84	0.64	0.52	0.57	0.49	0.40	0.32
Bisphenol A	0.43	0.14	0.48	0.41	0.36	0.22	0.06
Benzophenone-3	0.71	0.76	0.53	0.64	0.56	0.58	0.32
Triclosan	0.67	0.67	0.51	0.51	0.69	0.31	0.49
Methyl paraben	0.50	0.50	0.53	0.56	0.6	0.44	0.63
Ethyl paraben	0.46	0.54	0.41	0.44	0.57	0.51	0.35
Propyl paraben	0.57	0.32	0.64	0.45	0.55	0.49	0.51
Butyl paraben	0.72	0.63	0.63	0.49	0.49	0.64	0.28

^a Creatinine concentrations were missing for 11 samples

Table 21: Spearman correlation coefficients between pairs of ln-transformed phenol concentrations from the first, second and third samplings (specific gravity corrected (A), creatinine corrected (B) and uncorrected for urine dilution (C)), SARAHEH study, 2005 to 2008.

Sampling	2,4-dichlorophenol		2,5-dichlorophenol		Bisphenol A		Benzophenone-3		Triclosan		Methylparaben		Ethylparaben		Propylparaben		Butylparaben	
	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd	1 st	2 nd
A) Specific gravity corrected concentrations (n = 71 women, 213 samples)																		
2 nd	0.70		0.75		0.10		0.77		0.57		0.53		0.51		0.48		0.59	
3 rd	0.54	0.62	0.56	0.63	0.26	0.18	0.53	0.64	0.49	0.61	0.63	0.73	0.41	0.53	0.53	0.54	0.49	0.61
B) Creatinine corrected concentrations (n = 60 women, 180 samples) ^a																		
2 nd	0.74		0.84		0.15		0.86		0.6		0.55		0.42		0.47		0.61	
3 rd	0.41	0.56	0.58	0.63	0.39	0.07	0.65	0.70	0.57	0.57	0.64	0.68	0.40	0.44	0.57	0.55	0.61	0.71
C) Crude concentrations (n = 71 women, 213 samples)																		
2 nd	0.59		0.70		0.25		0.68		0.59		0.47		0.48		0.45		0.59	
3 rd	0.35	0.56	0.43	0.59	0.42	0.28	0.47	0.60	0.53	0.58	0.58	0.58	0.48	0.47	0.56	0.50	0.52	0.62

^aCreatinine concentrations were missing for 11 samples.

d) Influence of collection conditions on phenol urinary concentrations

Regarding the associations between collection conditions and phenol urinary concentrations; specific gravity was positively associated with all urinary phenol biomarker concentrations; association was weaker for ethylparaben (p-value of 0.1) compared to the other phenols (p-values < 0.001, Table 22). Similar associations were observed using creatinine concentration (data not shown). We observed heterogeneity in 2,4-dichlorophenol, benzophenone-3, triclosan and butylparaben concentrations according to hour of sampling (Table 22). Benzophenone-3 concentrations were higher in samples collected in spring and summer (adjusted geometric mean: 110 µg/L) than in those collected in fall and winter (adjusted geometric mean: 69 µg /L).

Correlation coefficients between urinary concentrations standardized for collection conditions (Mortamais et al. 2012) and urinary concentrations corrected for specific gravity were high (Spearman correlation coefficients > 0.96). The use of concentrations standardized for collection conditions did not notably improve ICCs between spot urine collections (Table 19).

Table 22: Adjusted^a geometric mean of urinary phenol concentrations according to collection conditions among 213 urine samples from the 71 women of Study of Advanced Reproductive Age and Environmental Health, 2005 to 2008.

Collection conditions	n	2,4-DCP		2,5-DCP		BPA		BP3		TCS		MP		EP		PP		BP	
		GM	p ^b	GM	p ^b	GM	p ^b	GM	p ^b	GM	p ^b	GM	p ^b	GM	p ^b	GM	p ^b	GM	p ^b
Gestational age (weeks)			0.39		0.49		0.01		0.49		0.3		0.44		0.87		0.25		0.64
≤ 18 ^c	54	0.7		12.2		1.0		105		15.8		159		4.1		38.3		1.8	
18.01 to 22	54	0.8		11.6		1.4		85.4		17.0		162		4.8		31.1		1.8	
22.01 to 32	58	0.9		13.8		1.3		71.5		15.6		123		3.9		22.8		1.4	
> 32	47	0.9		15.5		1.0		85.3		25.5		143		4.0		31.1		2.1	
Sampling season			0.87		0.33		0.3		0.02		0.98		0.17		0.30		0.1		0.31
Oct-March ^c	112	0.8		12.2		1.1		68.7		17.8		161		4.7		35.6		1.9	
April-Sep	101	0.8		14.2		1.3		110		17.9		130		3.7		25.1		1.5	
Hour of sampling			0.04		0.6		0.12		0.02		0.01		0.17		0.91		0.47		0.01
8.00 to 11.00 ^c	67	1.0		14.0		1.2		116		20.6		182		4.1		33.4		2.6	
11.01 to 13.00	56	0.9		13.2		1.0		56.8		25.0		136		4.7		35.1		1.9	
13.01 to 15.00	56	0.8		13.7		1.4		100		16.4		126		3.8		24.6		1.2	
15.01 to 18.00	34	0.6		10.5		1.2		71.8		8.8		132		4.2		26.8		1.2	
Day of sampling			0.69		0.45		0.28		0.81		0.95		0.56		0.20		0.75		0.38
Tuesday to Friday ^c	176	0.8		13.5		1.2		86.6		17.9		148		4.4		29.7		1.8	
Monday	37	0.8		11.6		1.0		81.8		17.6		133		3.1		32.2		1.4	
Specific gravity			<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		0.11		<0.001		<0.001
1.002 to 1.009 ^c	74	0.4		5.4		0.6		54.5		10.1		69.7		3.3		13.5		1.0	
1.01 to 1.017	75	1.0		15.3		1.4		86.5		23.1		166		3.8		38.3		1.8	
1.018 to 1.036	64	1.8		30.5		2.2		143		25.3		293		6.0		57.8		3.1	

^a Linear mixed models were simultaneously adjusted for the collection conditions and also for BMI, maternal age, year of sampling, maternal education and maternal race.

^b p-values of heterogeneity test.

^c Reference category.

Abbreviations: BP: butylparaben, BPA: bisphenol A, BP3: benzophenone-3, DCP: dichlorophenol, EP: ethylparaben, MP: methylparaben, PP, propylparaben, TCS: triclosan

4. Discussion

In our population of pregnant women referred for amniocentesis, the detection frequencies of phenol biomarkers in amniotic fluid were low, with only two phenols detected in 50% or more of the samples. With the exception of bisphenol A, the reproducibility of urine phenol biomarker concentrations in urine collected at three times during pregnancy was fairly good (ICCs of 0.48 – 0.62).

a) Phenol concentrations in maternal urine

Urine collected on the day of the amniocentesis tended to be more dilute than those collected later; however no specific instructions (with respect to hydration) or procedures (e.g., use of intravenous therapy) which could impact urinary dilution was given before or during the amniocentesis. Methyl- and propyl- parabens are the most commonly used parabens in food and cosmetics (Soni et al. 2005), and as expected, urine concentrations of these phenols were higher than those observed for ethyl- and butyl- parabens. Triclosan and bisphenol A concentrations were similar; 2,5-dichlorophenol concentrations were lower than those reported in a previous mother-child cohort study conducted earlier at the Mount Sinai Medical Center (1998 to 2002), and which included predominantly Hispanic and African American participants (medians were 11 µg/L for triclosan, 1.3 µg/L for bisphenol A and 53 µg/L for 2,5-dichlorophenol (Wolff et al. 2008)). Urinary concentrations of parabens were not reported in the study published by Wolff et al. Benzophenone-3 urine concentrations observed in our study population (medians ranged between 53 and 77 µg/L) were considerably higher than those reported in the aforementioned study (median of 7.5 µg/L). It is likely that this is at least in part reflective of the different racial/ethnic composition of these two Mount Sinai studies, since benzophenone-3 is found in sunscreens.

b) Phenol concentrations in amniotic fluid

While the presence of phenol biomarkers in amniotic fluid highlights the potential for direct fetal exposure, the low detection frequency observed for the majority of these compounds may be suggestive of low transfer and/or rapid clearance of the phenols in amniotic fluid. That being said, we cannot exclude the possibility that the low detection of phenol biomarkers in amniotic fluid was the result of processing and/or storage of samples in the clinical laboratory prior to being released for research purposes. Ye et al. reported good stability of total phenol concentrations in urine stored at -70°C (Ye et al. 2007), but no

published data regarding the impact of storage at -20°C , nor data examining the stability of biomarker concentrations in amniotic fluid, exist.

We detected bisphenol A in only one amniotic fluid sample. Low concentrations of bisphenol A in amniotic fluid collected during pregnancy and at birth have been previously reported (medians ranged from 0.26 to 1.1 $\mu\text{g/L}$ (Edlow et al. 2012; Engel et al. 2006; Ikezuki et al. 2002; Yamada et al. 2002)). In an ex-vivo experimental system, where 7 human placentas were in contact with a perfusate enriched in bisphenol A (10 $\mu\text{g/L}$), authors observed rapid transfer of this phenol across the placenta (Balakrishnan et al. 2010); however, this concentration was higher than those typically observed in the urine of pregnant women from the general population (Woodruff et al. 2011). Regarding the other phenols, in a study of 20 second trimester amniotic fluid samples from women referred for amniocentesis at a Children's Hospital Central California in Madera (California), Bradman et al. (2003) reported lower geometric mean of 2,5-dichlorophenol concentrations (0.39 $\mu\text{g/L}$) than in our population (geometric mean = 0.29 $\mu\text{g/L}$). To our knowledge, no previous study has examined amniotic fluid concentrations of benzophenone-3, triclosan or parabens in humans.

c) Predictors of amniotic fluid concentrations

Women with the highest concentrations of phenols in urine were more likely to have detectable concentrations of environmental phenols in amniotic fluid. Because of the high percentage of undetectable concentrations, we were only able to evaluate predictors of amniotic fluid concentrations for benzophenone-3 and propylparaben, and for these substances, maternal urine concentration was found to be a strong predictor of amniotic fluid concentration. There have been few comparisons of maternal and fetal exposure for phenolic compounds (Ikezuki et al. 2002; Yamada et al. 2002). Ikezuki et al. (2002) compared bisphenol A concentration in maternal serum and amniotic fluid, and reported amniotic fluid biomarker concentrations (arithmetic mean 8.3 $\mu\text{g/L}$) about 5 times higher than in maternal serum (arithmetic mean 1.5 $\mu\text{g/L}$). However, a bisphenol A amniotic fluid mean concentration of 8.3 $\mu\text{g/L}$ is extraordinarily high relative to values observed in other studies (medians in amniotic fluid ranged from 0.26 to 1.1 $\mu\text{g/L}$ (Edlow et al. 2012; Engel et al. 2006; Yamada et al. 2002)), raising concerns regarding potential external contamination from environmental sources of free bisphenol A.

We found no association between gestational age at collection and benzophenone-3 or propylparaben amniotic fluid concentrations. However, we were limited in evaluating this

relationship by a lack of substantial variability in gestational age at amniocentesis (range: 15 to 23 gestational weeks). Two previous studies have reported higher bisphenol A concentrations in amniotic fluid collected in the second trimester as compared to later pregnancy. However, in these studies, the samples collected in the second and third trimesters were from different women who may have had different levels of exposure (Edlow et al. 2012; Ikezuki et al. 2002). We observed that amniotic fluid concentration of propylparaben was lower for female fetuses compared to male fetuses. This difference may have been due to chance, or might suggest sex differences in fetal metabolism or excretion. For example, a study of 55 fetuses showed sex-differences in the levels of several mRNA transcripts encoding hepatic enzymes (O'Shaughnessy et al. 2011) suggesting that fetal hepatic function may differ by sex.

d) Variability in urinary concentrations

The poor reproducibility in bisphenol A concentration measured in serial spot urine samples collected during pregnancy is consistent with previous reports (Braun et al. 2012; Braun et al. 2011b) and taken together, these findings suggest that substantial exposure misclassification might occur in epidemiological studies assessing prenatal bisphenol A exposure using a single, or even a few spot urine samples. Exposures to the eight other phenols or their precursors appear to be more consistent throughout pregnancy (e.g., use of sunscreen, cosmetics, indoor deodorizers); for these we indeed observed a higher degree of temporal reproducibility in urinary concentrations, with ICC ranging between 0.5 and 0.6. A correlation of 0.6 between several measurements indicates that on average the correlation between the concentration from a spot sample and the biologically relevant exposure will be moderate. Although a correlation of this magnitude is superior to many rapidly metabolized compounds, it may still result in bias due to exposure misclassification. In order to limit the effects of this, studies aiming to assess the effects of phenol prenatal exposure on health should try to collect repeated urine samples during pregnancy (White et al. 2008).

Two previous studies among pregnant women have shown lower ICCs for methyl-, propyl- and butyl-parabens (ICCs < 0.5) relative to our population; the authors did not report results for ethylparaben (Meeker et al. 2013; Smith et al. 2012). In the study published by Meeker et al. (2013), ICCs for the other phenols were also lower than we reported here (0.47, 0.38 and 0.49 for triclosan, 2,4- and 2,5-dichlorophenol), except for benzophenone-3 for which an ICC of 0.62 has been reported. The difference may be attributable to differences in

the exposure patterns between study populations or to differences in time elapsed between consecutive urinary collections. For example, the first, second and third urine samples were collected at gestational weeks 5.8, 20.6 and 33.5 in average in the Smith et al. study compared to weeks 17.6, 22.8 and 32.8 in our study.

Correction for urine dilution improved the temporal reproducibility in urinary concentration for most phenols evaluated in this study. We observed similar patterns of urinary creatinine and specific gravity measures throughout pregnancy, and the estimates of urinary concentration reliability (correlations, ICCs) were similar using both methods. These findings suggested that either could be used to correct for urine dilution in pregnant women.

e) Influence of collection conditions on phenol urinary concentrations

Because benzophenone-3 is commonly used in sunscreen for its sun-blocking properties (Calafat et al. 2008), exposure is more likely to be high during the summer months as compared to the rest of the year, and we indeed observed this seasonal variability in benzophenone-3 concentrations in our population. The associations observed between hour of collection and benzophenone-3, triclosan, butylparaben and 2,4-dichlorophenol urinary concentrations suggested that exposures changed considerably throughout the day. We sought to remove the variability in phenol urinary concentrations due to heterogeneity in collection conditions by implementing the 2-step standardization method proposed by Mortamais et al. (2012). Our results suggest that study related variability in sample collection conditions did not strongly influence intra-subject comparability of biomarkers' levels, because overall the ICCs obtained with the standardized concentrations were close to those obtained from the unstandardized concentrations.

f) Study strengths and limitations

Our study is the first to report phenol concentrations in amniotic fluid and maternal urine collected on the same day. In addition, we examined variability in phenol biomarker concentrations over time during the prenatal period, for a diverse set of environmental phenols including some that have not been widely studied (triclosan, benzophenone-3 and dichlorophenols). Limitations include the relatively small sample size, and the low detection frequency of phenols in amniotic fluid. Additionally, although we made no exclusions on the basis of race/ethnicity, most of our study population was white. Since there is some

racial/ethnic variability in exposure patterns, our results may not fully represent any underlying variability in the general population.

To better understand the link between maternal and fetal exposures further studies with more diverse populations, having time-matched amniotic fluid and maternal samples may be required. Collecting more urine samples or having larger time intervals between sample collection may improve the characterization of the urine concentration variability during pregnancy.

5. Conclusion

The degree of reproducibility in benzophenone-3, triclosan, 2,4- and 2,5-dichlorophenols, methyl-, ethyl-, propyl- and butyl-parabens urinary concentrations over the course of the pregnancy suggest that a small number of urine samples (more than one) may reasonably represent the individual exposure across pregnancy to these compounds or their precursors. As previously reported, we observed low reproducibility in bisphenol A urinary concentration among pregnant women with little evidence of secondary fetal exposure to bisphenol A via amniotic fluid. Given the infrequent detection and much lower concentrations of most biomarkers in amniotic fluid than in urine, our results suggest that amniotic fluid may not be a suitable matrix for assessing fetal exposure to non-persistent phenols using the currently available detection techniques.

II. Impact of exposure misclassification and of number of urine samples collected on bias and power in biomarkers based epidemiological studies; a simulation study applied to short half-life endocrine disruptors

1. Background

Measurement error occurs whenever we cannot exactly observe one or more of the variables that enter into a model of interest (Buonaccorsi 2010). In this we focus on measurement error in exposure. There are several ways to describe and classify measurement error. Measurement error can be differential or not (a concept that will not be detailed here); it can have a Berkson or classical component and be of additive or multiplicative structure (Armstrong 1998; Heid et al. 2004; Rothman et al. 2008). Classical measurement error arises when the estimator of the true exposure (e.g., phenol concentrations measured in one urine sample) varies around the true value (e.g., the average pregnancy exposure) in such way that the average of many replicate measurements, conditional on the true value, will equal the true individual exposure. Classical error is statistically independent of the true value (Armstrong 1998; Carroll et al. 1995; Heid et al. 2004). This type of error usually results in effect estimates that are smaller than the true effect, a phenomenon commonly called attenuation bias (Bateson and Wright 2010). Berkson error is independent of the surrogate measure of exposure and typically occurs when the same surrogate is used for many subjects (e.g., use of a central monitoring site to estimate the individual exposures of people living around this site) (Armstrong 1998; Bateson and Wright 2010; Zeger et al. 2000). Berkson error usually does not induce notable bias on the effect estimates but rather increases standard errors of the effect estimate and so weakens their precisions (Bateson and Wright 2010; Heid et al. 2004).

A recognized limitation of the studies aiming at exploring effects of prenatal exposure to short half-life endocrine disruptors is exposure estimation, generally based on biomarker measurements in maternal urine samples collected one to three times during pregnancy (Braun et al. 2011a; Philippat et al. 2012; Wolff et al. 2008). Because of the short half-life of these chemicals and the episodic nature of the exposures, concentrations are likely to be variable during pregnancy. Depending of the chemicals, the ICCs described in the previous chapter and in other studies ranged between 0.1 and 0.6 (Adibi et al. 2008a; Braun et al. 2011b; Meeker et al. 2013; Philippat et al. In revision), suggesting that using a small number of measures during pregnancy may imperfectly reflect the global exposure throughout the

whole fetal life or during a specific time window and lead to measurement error in exposure estimation, which may in turn bias dose-response relationship.

Increasing the number of urine samples during pregnancy and pooling them could be a solution to improve exposure assessment to short half-life chemicals without increasing analytical cost (White et al. 2008). Conversely, increasing study sample size will lead to increased analytical cost without decreasing bias if error is of classical nature .

In this chapter, using simulated data, we characterized the bias associated with the use of one urine sample to estimate exposure to non persistent chemicals, and the impact of increasing the number of measurements on bias and statistical power of epidemiological studies aiming at describing associations between pregnancy exposure to non persistent chemicals and a birth outcome. We here assume that one considers birth weight, analyzed as a continuous variable as an outcome.

2. Methods

We considered two chemicals A and B with intraclass correlation coefficients (ICC) of 0.6 (A) and 0.15 (B), corresponding to the ICCs observed between ln transformed urine concentrations of benzophenone-3 and bisphenol A measured in three urine samples collected during pregnancy (see section I of this chapter, (Philippat et al. In revision)). For 500 subjects $i (i = 1 - 500)$, we generated $T = 294$ variables $E_{it} (t = 1 - 294)$ with standard normal distribution and a coefficient of correlation between any two of the within subject $E_{it(t = 1 - 294)}$ variables of 0.6; these variables represent the daily concentrations of A measured in spot urine samples collected in subject i during each day of pregnancy. E_{it} was standardized by the standard deviation of (\bar{E}_i) , which represents the average pregnancy concentration of A in subject i . We supposed that the real effect of A on birth weight (Y_i) corresponded to a decrease of 80 g in mean birth weight for each increase by one in ln-transformed concentration of A. That is:

$$Y_i = \alpha_0 - 80x\bar{E}_i + \varepsilon_i$$

α_0 : constant (3300g)

\bar{E}_i : Prenatal exposure to A for subject i (pregnancy average); $\bar{E}_i = \sum_{t=1}^{294} E_{it} / 294$

ε_i : Random error: variable normally distributed, centered on 0, with standard deviation (SD) of 456, corresponding to the observed SD of birth weight in EDEN cohort.

We randomly selected j days during pregnancy and assumed that urine samples were collected on each of these days, so that exposure was assessed as the mean concentration of A during these j days. We tested values of $j = 1, 2, 3, 4, 5, 10, 25, 50, 75$ and 100. We estimated the association between the mean of the j concentrations and birth weight using linear regression.

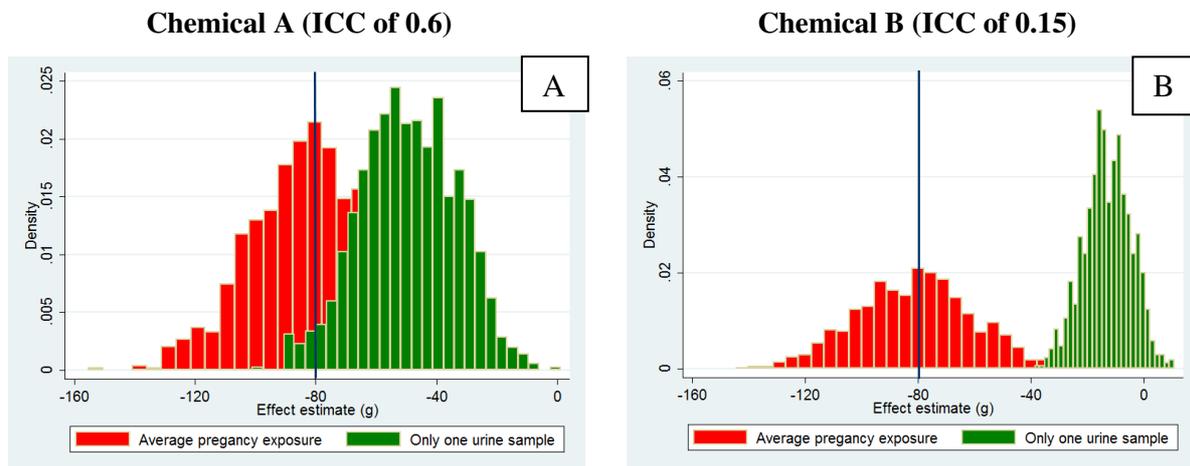
For each value of j we performed 1000 simulations and computed the mean of the 1000 estimated β parameters. In order to estimate the impact on statistical power we counted how many times the association was significant ($p < 0.05$ and $\beta < 0$).

A similar approach was used for chemical B, assuming that correlation between any two concentrations on two different days was 0.15 instead of 0.6.

3. Results

For chemical A, as expected the empirical average correlation between two random samples from a given woman was 0.6. When only one urine sample was used, the average effect estimate was $\beta_{1\text{sample}} = -49$ g compared to $\beta_{\text{ref}}, -80$ g (an attenuation by 40%). The statistical power was 83 %, compared to 97 % if all daily samples are used to estimate exposure. Using 5 samples led to an effect estimate of -72 g (attenuation by around 10%) and a statistical power of 95% (Table 23, Figure 20).

Figure 20: Distribution of the β parameter characterizing the association between exposure and birth weight using the average pregnancy exposure \bar{E}_i or only one urine sample to assess exposure to chemicals with ICC of 0.6 (A) and to chemicals with ICC of 0.15 (B) (1000 simulations were performed).



For chemical B, the empirical average correlation between two random samples from a given woman was 0.15. Using only one sample to estimate exposure led to an effect estimate biased toward the null ($\beta_{1\text{sample}}, -13$ g compared to $\beta_{\text{ref}}, -80$ g) and to low statistical power (33%). In addition, we observed a positive but not significant association (p-values > 0.2) for 61 of the 1000 simulations. Our results suggest that more than 25 urine samples would be needed to correctly estimate the pregnancy average exposure and to obtain an effect estimate close to the expected β (less than 10% variation compared to β_{ref}) for chemicals B (Table 23). A statistical power of 87% is reached with 10 samples per subject.

Table 23: Statistical power and effect estimate (β) characterizing the association between birth weight and exposure assessed during pregnancy (simulation study, $n = 500$ women, 1000 simulations)

Number of urine samples used	Chemical A (ICC, 0.6)			Chemical B (ICC, 0.15)		
	B		Statistical power (%)	B		Statistical power (%)
	mean	(SD)		mean	(SD)	
Reference ^a	-81	20	97	-81	21	98
1	-49	16	83	-13	8	33
2	-61	18	91	-22	11	52
3	-66	19	95	-28	12	62
4	-70	19	95	-34	14	71
5	-72	19	95	-39	15	76
10	-76	20	96	-53	17	87
25	-79	20	97	-67	19	94
50	-80	20	97	-75	20	96
75	-81	20	97	-77	20	97
100	-81	20	97	-78	20	98

^a corresponds to using the 294 pregnancy urine samples to assess exposure

4. Discussion

Our results suggest that studies relying on one urine sample to assess exposure to short half-life endocrine disruptors in human are likely to be biased; the bias is very high for chemicals showing high intra-individual variability such as bisphenol A. The type of bias observed in our study is commonly referred to as attenuation bias. Attenuation bias has been previously described in situations of classical measurement errors and is not expected if error is only of Berkson nature (Bateson and Wright 2010; Heid et al. 2004; Whitehead et al. 2012). Such results was expected with regards to the way we simulated our data (the true exposure (pregnancy average) was considered to be the average of the values of the surrogate measures, which is typical of classical error).

One of the limitation of our study was the hypothesis regarding ICCs. The dataset simulation was based on correlation coefficients observed in the SARA EH study (Philippat et al. In revision) in which only three measurements per women during pregnancy have been collected. In addition, correlation was assumed to be the same between any two samples at whatever day of pregnancy, which may not be realistic. Having more precise information on phenol concentration variability within-day, -week and between-trimesters would be useful to make more realistic simulations. Our research team aims at collecting such information in the framework of a feasibility study (SEPAGES-Feasibility cohort) taking place at Grenoble and

including about 30 women with intensive urine collection (around 180 samples per woman). Also, we assumed that the biologically relevant exposure windows was the whole pregnancy, although for specific outcomes narrower time windows may be relevant. Finally, we did not take into account additional sources of error such as sampling and storage conditions; nor we have considered potential confounders, which might also be measured with error.

Our results showed that increasing the number of samples per participant and pooling them could be an efficient method to decrease measurement error in epidemiological studies. For chemicals with ICC of 0.6, using 4 or 5 samples gave an estimate close to the expected β (attenuation < 10 %). For chemicals with ICC of 0.15, around 25 samples were needed to reach an estimate close to the expected β . Collecting 25 samples per woman during pregnancy in the framework of an epidemiological study would imply a high observance of the participants and a complex organization for the research team in charge of the collection and processing of the urine samples. This would also be cumbersome for the participants and therefore might induce selection bias and low participation rate. In addition, decreasing measurement error by increasing the number of urine samples per participant is doable for further cohort studies but is not applicable to existing cohorts. For these reasons, statistical methods aiming at estimating an effect estimate (β) and 95% CI "corrected" for the measurement error have been developed (e.g., regression calibration, simulation extrapolation, (Buonaccorsi 2010; Carroll et al. 1995; White et al. 2008)). To our knowledge, these methods have not been used in studies aiming at assessing the effects of phenols and phthalates on human health. They have been used in other settings (Bateson and Wright 2010; Spiegelman et al. 1997). We recently began working on the application of these methods in the framework of the study of the effects of short half-life chemicals. I am currently supervising a master 2 internship aiming at testing different measurement error models (based on simulated data and on the ICCs observed in the SARA EH study, chapter III, paragraph II.2) in order to identify a measurement error model that could be used to correct the β estimated in the previous studies using a small number of urine samples to assess exposure to phenols and phthalates.

CHAPTER IV: OVERALL DISCUSSION

I. Prenatal exposure to phthalates and phenols and pre- and post-natal growth

1. Associations with birth outcomes

When this PhD was initiated in 2010, only two studies in Humans had explored the associations between prenatal exposure to phenols and prenatal growth (Padmanabhan et al. 2008; Wolff et al. 2008); none had considered postnatal growth. The study by Wolff et al. (2008) had suggested possible sex-specific effects of some phenols on birth weight. Effects of prenatal exposure to phthalates on fetal growth were a little bit more documented (n = 4 studies published before 2010 (Huang et al. 2009; Latini et al. 2003; Wolff et al. 2008; Zhang et al. 2009)), but that was not enough to allow to conclude about their effects on fetal growth. Limitation of these previous studies were the use of birth outcomes (weight, length and head circumference) as proxies of fetal growth (none of these had repeated measurements of fetal growth during pregnancy) and their relatively low sample size; only the study published by Wolff et al. (2008) had more than 150 subjects of each sex. In addition, because of the high number of comparisons performed, a part of their findings might have been due to chance and had to be replicated in another population before drawing any conclusion. For this reason, in the first part of my PhD, we aimed at reproducing previous published results, regarding the associations between these chemicals and birth outcomes.

Our study population consisted in a case–control study of male malformations of the genitalia nested in the EDEN and PELAGIE cohorts (Gona_PE study). This particular design has been taken into account in our analyses by using a weighting approach specifically developed to re-use of case–control data for analysis of new outcome variables (Reilly et al. 2005; Richardson et al. 2007). A part of our findings on phenols and birth weight was in relatively good agreement with those reported by Wolff et al. (2008). An inverse association of 2,5-dichlorophenol and a positive association of benzophenone-3 with male birth weight were indeed observed in both studies, even if urine concentrations were lower in our population. Regarding phthalates, we observed non-monotonic associations with birth weight which had never been reported previously in rodents or in Humans and that we therefore considered cautiously.

2. Associations with fetal biometry and early postnatal growth

In 2011, a new funding allowed us to extend the measurements of phenol and phthalate urinary concentrations to all women of the EDEN cohort delivering boys with complete data on fetal biometry assessed during pregnancy by repeated ultrasound measurements and postnatal growth until 3 years of age (n=410); 110 women previously included in the case-control study matched these inclusion criteria and were also considered in our analyses (PEnDevE study). The objectives of this study were to replicate our previous findings on birth outcomes, to study the associations with the repeated ultrasound measurements of fetal biometry (which could allow highlighting potential susceptibility windows) and to study the association with child growth until 3 years of age. These three points contributed to the project originality since, when it was initiated, none of the authors of the previous studies had to our knowledge tried to reproduce their published findings and no study using ultrasound measurements or early postnatal growth measurements in relation of exposure to short half-life endocrine disruptors had been published. Because of limited funding and of our choice to try to replicate our previous findings rather than generating new ones, we did not assess phenols and phthalates in urine from mother delivering girls. Women of the PELAGIE cohort were not included because data from ultrasound examinations had not been collected in this cohort. The phthalates were studied by Jérémie Botton (Inserm U1018 - Villejuif) and only the results regarding phenols have been reported in this thesis.

We were not able to replicate the negative associations previously observed between dichlorophenols and weight and head circumference at birth among the subsample of 191 women from the EDEN cohort. Several possible explanations can be considered. These include chance findings and bias induced by exposure misclassification related to the short half-life of the studied chemicals; our simulation study has indeed shown that results of studies using one urine sample to assess exposure could be highly biased, even for chemicals showing relatively good ICC, such as dichlorophenols. We observed consistency across our studies regarding the positive association between benzophenone-3 and birth weight (ICC = 0.6).

Within the larger subpopulation of 520 male newborns, we observed a negative association between triclosan concentration and all of the third ultrasound measurements,

head circumference at birth and at 36 months. Parabens, especially the methyl, were positively associated with postnatal weight and weight at birth.

3. Methodological issues

a) Confounding

A confounding factor can be defined as being associated with both the exposure and the studied outcome without being affected by them (Rothman et al. 2008). In our study, we adjusted for many potential confounders, but residual confounding cannot be discarded. In our study, the association of maternal pregnancy bisphenol A level with child weight at 12 months was weakened by adjustment for the child caloric intake at 8 months, which is suggestive of confounding by child diet (this was detailed Chapter II, page 88).

A consideration when associating urinary biomarkers with health effects is that a part of the between-subject differences in the measured concentrations are due to variations in metabolism and xenobiotic excretion across participants. Specific metabolic disorders associated with both fetal growth and xenobiotic metabolism thus constitute potential confounders in our study. We considered this issue in Gona_PE study and excluding women with pregnancy-induced hypertension or gestational diabetes did not alter our findings.

We did not adjust our analyses for exposures which may co-exist with phenols and phthalates. This leaves room for residual confounding by unmeasured co-exposures.

b) Selection bias and finding generalization

Selection bias arises when the relationship between exposure and disease is different for those who participate and for all of those who are eligible for the study, including those who do not participate (Rothman et al. 2008). The fact that we did not observe strong differences between the characteristics of the women selected to study the associations between phenols and pre- and post-natal growth and those of the whole EDEN women delivering boys, is not in favor of such bias in our study.

Comparison between the EDEN cohort population and a representative sample of women who delivered in 2003 throughout France (Blondel et al. 2006) has shown no difference in term of length of gestation and weight at birth of the newborns. Women included in EDEN were more highly educated and more often worked during pregnancy than women from the general population (Slama et al. 2009). These characteristics might be associated

with exposure to several of the studied chemicals; as an example, in the EDEN cohort we observed an inverse association between maternal education and the urinary bisphenol A concentrations during pregnancy (data not shown); this was also reported in a population of American pregnant women (Braun et al. 2011b). We therefore cannot discard that the urinary concentration of phthalates and phenols differ between women included in EDEN and the whole pregnant women of the general population.

Studying male newborns only prevents the generalization of our results to female newborns, but is not a source of bias. In term of public health, observing an effect of phenols on fetal growth in male newborns may be enough to call for more stringent regulations of the substances. Our choice to focus on males was that sex-specific associations had been reported, in particular between some phenol concentrations and male birth weight (Wolff et al. 2008); in this context warranting for analyses stratified on sex, we opted for a study of male newborns only, which had in expectancy a higher statistical power than two sex-specific analyses on populations samples with half our sample size.

II. Variability in urine concentration and correspondence between urine and amniotic fluid concentration

A few papers have reported high between and within day variability of bisphenol A concentrations (Ye et al. 2011a) and in a study where three spot urine samples have been collected during pregnancy (n = 389 pregnant women from Cincinnati, Ohio), low intraclass correlation coefficient (0.11) had been reported for this phenol (Braun et al. 2011b). This suggests that single spot urine sample, as we had in our study, will not be representative of the whole pregnancy exposure and will strongly misclassify bisphenol A exposure. Even if this seems essential for the understanding of published epidemiological studies relating their urinary biomarkers to health endpoints, no data on urinary concentration variability during pregnancy was published for the other phenols. We aimed at filling this gap, by publishing the first study presenting data on urinary concentration reproducibility during pregnancy of triclosan, benzophenone-3 and dichlorophenol. Bisphenol A and parabens were also reported in our study and our results for these chemicals were generally in agreement with the few papers published before ours (Braun et al. 2012; Smith et al. 2012). For bisphenol A, our results were consistent with previous reports (Braun et al. 2012; Braun et al. 2011b) and we observed poor reproducibility along pregnancy (Philippat et al. In revision). For the other

phenols we observed a higher degree of reproducibility in urinary concentrations (ICCs ~ 0.5-0.6), suggesting that fewer samples would be needed to efficiently estimate pregnancy exposure for these phenols compared to bisphenol A. However, even in this range there might be exposure misclassification leading to bias in epidemiological studies using a single spot urine sample. In order to estimate the bias associated with such ICCs, we performed a simulation study and illustrated that using one urine sample to assess exposure to short half-life phenols will lead to bias in the estimated relationship between exposure and a health outcome and that; bias will be stronger for phenols showing high variability during pregnancy (e.g., ICC < 0.2). We provide the first estimate of the optimal number of samples required to characterize average pregnancy exposure; around 5 for chemicals such as benzophenone-3 with an ICC of 0.6, and above 20 for chemicals with an ICC of 0.15. These estimations could be made more accurate by using more precise data on phenols urine concentrations variability during pregnancy (e.g., variability within-day, -week and between-trimesters). In the framework of the SARAHEH study we indeed had only three measurements per woman, which had been collected in a narrow time window (the first, second and third urine samples were collected in average at gestational weeks 18, 23 and 33) .

Another originality of this work is that an amniotic fluid sample collected the same day of one of the urine sample was available for 69 women of the SARAHEH study. Although previous studies have measured environmental phenols in this fluid, none of them had simultaneously collected urine and amniotic fluid samples (Edlow et al. 2012; Engel et al. 2006; Yamada et al. 2002). This was particularly interesting since most of the epidemiological studies used maternal urine samples to assess fetal exposure while it is not well known to what extent maternal urine reflects it. We hypothesized that, because the fetus swallows amniotic fluid and because fetal urine is one of the main sources of this fluid in the second half of pregnancy (Modena and Fieni 2004; Underwood et al. 2005), concentrations measured in this matrix might be a relevant dosimeter of fetal exposure. Only two of the nine phenols assessed in amniotic fluid were detected in more than 50 % of the samples, and for these chemicals we observed a positive correlation with their concentrations measured in urine collected the same day. The low frequencies of detection suggest that amniotic fluid may not be a suitable matrix for assessing fetal exposure to non-persistent phenols using the currently available detection techniques.

III. Conclusion and research perspectives

The work performed in the EDEN and PELAGIE cohorts lends support to a potential effect of prenatal exposures to some phenols and phthalates on pre- and early post-natal growth. However our study had several limitations, especially regarding exposure assessment. Our findings need to be replicated in other Human studies with improved exposure assessment. This is also true for the other published studies, since repeated urine measurements of biomarkers in maternal urine have only been used in one study of fetal growth, with a sample size of 80 male and female newborns (Snijder et al. 2013).

1. Improving exposure assessment during fetal life

Increasing the number of urine samples collected per participants during pregnancy is an obvious way to improve exposure assessment in cohort of pregnant women. This seems feasible in practice for phenols with relatively good reproducibility during pregnancy (ICCs \approx 0.6), for which our simulation study showed that about 5 random samples could reasonably well represent the whole pregnancy exposure (although it must be noted that most previous studies collected first morning voids and that collecting urine samples at random hours during the day may be cumbersome for some study participants). However for chemicals showing high variability during pregnancy, such as bisphenol A, we estimated that around 25 urine samples during pregnancy would be needed. Such a sampling scheme implies a strong implication of the enrolled women and results in an important workload for the research team to collect and process all the samples. Our ongoing experience from SEPAGES-feasibility cohort with 30 women from Grenoble urban area shows that this is feasible. An efficient alternative or complement could be the use of statistical methods specifically aiming at correcting the effect estimates for measurement errors. This includes approaches such as regression calibration and simulation extrapolation (Buonaccorsi 2010; Carroll et al. 1995).

Maternal urine concentrations are commonly used in epidemiological studies as a proxy of fetal exposure. This can be discussed in terms of relevance of using urinary metabolites as a marker of exposure as well as in terms of matrix used. Alternative matrices might be indeed useful in estimating direct fetal exposure. We showed low frequencies of detection for most phenols in amniotic fluid. In addition, with regards to the risks for the mother and fetus (e.g. amniotic fluid leakage, chorioamnionitis...), it would be unethical to subject women to amniocentesis purely for research purposes. Other matrices allowing to

avoid invasive collection exit (e.g., meconium). Meconium starts accumulating in the fetal bowel as early as the 13th week of gestation and measuring chemicals in this matrix might reflect fetal exposure during the second and third trimesters of pregnancy. Meconium has been already used to evaluate fetal exposure to alcohol, tobacco and cocaine (Joya et al. 2012; Ryan et al. 1994; Spanier et al. 2011); analytical techniques to assess non persistent chemicals also exist (Zhang et al. 2009) but have not been widely used yet.

In our study, we focused on the effects of prenatal exposure to phenols and phthalates on prenatal growth. Exposure during childhood might also have an effect on postnatal growth. Use of the child urine to assess his exposure to phenols and phthalates suffers of the same limitations than maternal urine; high variability has been reported in concentrations of these chemicals measured in repeated urine samples collected during childhood (Teitelbaum et al. 2008).

2. Link between the toxicological and epidemiological studies

In Human studies exposure to phthalates and phenols during pregnancy is usually estimated by measuring their biomarker concentrations in urine. These concentrations are wrongly called "exposure" and basically differ from the exposure available in toxicological studies (e.g. when animals are exposed through gavage). Urine concentrations indeed depend on exposure but are also determined by the metabolism and excretion capacity of each subject (Longnecker 2006). Physiologically Based Pharmacokinetic (PBPK) models are mathematical modeling techniques allowing prediction of the absorption, distribution, metabolism and excretion of chemicals in the organism and could be used to estimate exposure on the basis of urinary levels of metabolites (reverse dosimetry). Such models have been developed for several of the chemicals studied in the thesis (Fisher et al. 2011; Kawamoto et al. 2007), but they have been very little used in epidemiological studies and generally outside of the context of pregnancy. Using these models would make easier comparisons between exposures encountered in human populations with exposures used in animal experimentation.

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ANNEXES

I. Curriculum vitae

CLAIRE PHILIPPAT, Pharm D

Education

From Oct. 2010	PhD student, Environmental Health, National Institute of Health and Medical Research (Inserm, U823) and University Joseph Fourier, Grenoble, France
June 2010	Summer school in Epidemiology and Public Health, University Paris-Sud 11, Paris, France
2009-2010	M.S., Environment and Health, University Joseph Fourier, Grenoble, France
2007-2008:	International exchange program: University of Montreal, Montréal, Canada
2004-2010	Pharmacy studies, University Joseph Fourier, Grenoble, France
2004	French Baccalaureate - major in Sciences, Pravaz High School, Le Pont de Beauvoisin, France

Professional Experience, Internships

From Oct. 2010	PhD student, Environmental Health, Inserm (U823) and University Joseph Fourier, Grenoble, France
Jan-May 2012	Research mobility, University of North Carolina, Chapel Hill, North Carolina, USA
Jan-Sep 2010	Internship, Inserm U823, entitled: "Prenatal exposure to phthalates and phenols and fetal development"
Mar-Aug 2009	Internship, Grenoble University Hospital, Department of Occupational Medicine, entitled: "Occupational exposure to Polycyclic Aromatic Hydrocarbons"

Publications

- Philippat C**, Wolff M.S, Calafat A.M, Ye X, Bausell R, Meadows M, et al. Prenatal Exposure to Environmental Phenols: Concentrations in Amniotic Fluid and Temporal Variability in Phenol Urinary Concentrations During Pregnancy. *Environ Health Perspect*. In revision.
- Rahmalia A, Giorgis-Allemand L, Lepeule J, **Philippat C**, Galineau J, Hulin A, et al. 2012. Pregnancy exposure to atmospheric pollutants and placental weight: an approach relying on a dispersion model. *Environ Int* 48: 47-55.
- Casas M, Chevrier C, Hond ED, Fernandez MF, Pierik F, **Philippat C**, et al. 2012. Exposure to brominated flame retardants, perfluorinated compounds, phthalates and phenols in European birth cohorts: ENRIECO evaluation, first human biomonitoring results, and recommendations. *Int J Hyg Environ Health*.
- Mortamais M, Chevrier C, **Philippat C**, Petit C, Calafat AM, Ye X, et al. Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates: a 2-step standardization method based on regression residuals. *Environ Health*. 2012 Apr 26;11(1):29.
- Chevrier C, Petit C, **Philippat C**, Mortamais M, Slama R, Rouget F, et al. Maternal urinary phthalates and phenols and male genital anomalies. *Epidemiology*. 2012 Mar;23(2):353-6.
- Philippat C**, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. Exposure to phthalates and phenols during pregnancy and offspring size at birth. *Environ Health Perspect*. 2012 Mar;120(3):464-70. Epub 2011 Sep 7.
- Philippat C**, Chevrier C, Giorgis-Allemand L, Cordier S, Charles MA, Jégou B, et al. Use of analgesics during pregnancy and undescended testis in the offspring within Eden mother-child cohort. *Epidemiology*. 2011 Sep;22(5):747-9.

International presentations

Philippat C, Botton J, Calafat A.M, Ye X, Charles M.A, Slama R. Prenatal exposures to phenols and postnatal growth in boys during the three first years of life. Submitted at the International Society of Environmental Epidemiology, Basel, Switzerland, August, 2013.

Philippat C, Wolff M.S, Calafat A.M, Ye X, Bausell R, Meadows M, et al. Amniotic Fluid Transfer and Temporal Stability in Phenol Exposure During Pregnancy. Presented at the International Society of Environmental Epidemiology, Columbia, South Carolina, August, 2012.

Philippat C, Botton J, Giorgis-Allemand L, Calafat A.M, Ye X, Manori J. S et al. Prenatal exposures to phenols and fetal growth estimated using repeated ultrasound measurements. Presented at the International Society of Environmental Epidemiology, Columbia, South Carolina, August, 2012.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. Maternal exposures to phthalates and phenols and birth weight. Presented at the International Society of Environmental Epidemiology, Seoul, Korea, August, 2010.

Contracts & Grants

2010-2013	Pre-doctoral fellow, competitive grant from French ministry of research.
2011-2012	Mobility grant from Région Rhône-Alpes
2011-2014	PEndEvE - Influence of Endocrine Disruptors on Child Development, grant from ANSES (French Agency for Food, Environmental and Occupational Health & Safety), total, 150 k€, PI: R. Slama

Contact Information

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II. PhD training and teaching

1. Catégorie : Insertion professionnelle

	Année	Crédits
Cours donnés par le doctorant :		
Master méthode de recherche en Environnement et Santé, Grenoble	2011	0.25
UE Environnement et Santé, Lyon	2011	0.5
DU d'Epidémiologie, Lyon	2011	0.5
Master méthode de recherche en Environnement et Santé, Grenoble	2012	0.25
Encadrement :		
Etudiant de master 1 "Recherche bibliographique sur les récepteurs cibles des perturbateurs endocriniens et leurs effets potentiels sur la santé" (O. Cea)	2011	
Etudiant de master 2 "A comparison study of urinary biomarkers of benzene exposure among non-smoking pregnant women" (N. Miljkovic)	2012	
Etudiant Ingénieur " Erreurs de mesures de l'exposition aux polluants non persistants : impact sur l'estimation des effets sanitaires" (F. Perrier)	2013	
Autres :		
Mobilité internationale : University of North Carolina, Chapel Hill, USA (6months)	2012	4.5
Valorisation des résultats de la recherche scientifique pour l'Inserm	2011-2013	6
Revue d'articles pour Science of the total Environment et Environmental Health Perspectives	2012-2013	
Représentants des doctorants	2010-2012	3

2. Formations

Ecole d'été de santé publique et d'épidémiologie de Bicêtre, Paris	2010	10
Anglais communication, Grenoble	2010-2011	1.5
Anglais rédaction scientifique, Grenoble	2010-2011	4

III. Publications

1. Related to the thesis

Philippat C, Wolff M.S, Calafat A.M, Ye X., Bausell R., Meadows M., et al. Prenatal Exposure to Environmental Phenols: Concentrations in Amniotic Fluid and Variability in Phenol Urinary Concentrations during Pregnancy. *Environ Health Perspect.* In revision.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. Exposure to phthalates and phenols during pregnancy and offspring size at birth. *Environ Health Perspect.* 2012 Mar;120(3):464-70. Epub 2011 Sep 7.

Philippat C, Botton J, Calafat A.M, Ye X, Charles M.A, Slama R. Prenatal exposures to phenols and pre- and early postnatal growth in boys. *In preparation*

2. Other publications

Rahmalia A, Giorgis-Allemand L, Lepeule J, **Philippat C**, Galineau J, Hulin A, et al. 2012. Pregnancy exposure to atmospheric pollutants and placental weight: an approach relying on a dispersion model. *Environ Int* 48: 47-55.

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Mortamais M, Chevrier C, **Philippat C**, Petit C, Calafat AM, Ye X, et al. Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates: a 2-step standardization method based on regression residuals. *Environ Health.* 2012 Apr 26;11(1):29.

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RESUME DE LA THESE

Contexte : Les phtalates et les phénols sont des perturbateurs endocriniens à courte demi-vie. L'exposition à ces composés chimiques est ubiquitaire en population générale, y compris pour les femmes enceintes. Certains sont capables de traverser le placenta et le fœtus est également exposé. Les données concernant les effets de l'exposition prénatale à ces composés sur la croissance fœtale et durant l'enfance sont limitées et suggèrent un effet dépendant du sexe de certains phénols sur le poids de naissance.

Une des limites des études visant à évaluer les effets des expositions prénatales aux phénols et phtalates chez l'Homme est l'estimation de l'exposition, généralement basée sur la mesure de leurs concentrations dans un petit nombre d'échantillons d'urine maternelle. En raison de la faible persistance de ces composés chimiques dans l'organisme et de la nature épisodique probable de l'exposition, les concentrations urinaires varient dans le temps. Un petit nombre d'échantillons urinaires prélevés pendant la grossesse sont susceptibles de refléter de façon imparfaite l'exposition globale sur l'ensemble de la vie fœtale. Les concentrations dosées dans d'autres matrices, comme le liquide amniotique, pourraient être pertinentes pour estimer l'exposition fœtale.

Objectifs : Les objectifs de la thèse étaient : 1) d'étudier les impacts potentiels de l'exposition prénatale aux phénols et aux phtalates sur la croissance du fœtus et de l'enfant ; 2) de caractériser la variabilité des concentrations urinaires de composés non persistants (phénols) au long de la grossesse et de comparer les concentrations de phénols dosées dans le liquide amniotique et l'urine maternelle recueillis le même jour ; 3) de caractériser le biais et l'impact sur la puissance statistique de l'utilisation d'un faible nombre d'échantillons urinaires pour estimer les expositions.

Méthodes : Les associations entre les phénols, les phtalates et la croissance pré- et post-natale précoce ont été étudiées parmi les femmes de la cohorte mère-enfant EDEN ayant accouché de garçons. La croissance fœtale a été estimée à l'aide d'échographies, réalisées à trois reprises pendant la grossesse et de mesures à la naissance. La croissance postnatale a été modélisée à partir de mesures répétées normalisées, réalisées entre la naissance et 3 ans. Les biomarqueurs d'exposition aux phtalates et phénols ont été dosés dans les urines maternelles collectées une fois pendant la grossesse. Ces dosages ont été réalisés en 2008 pour 191 femmes et en 2012 pour 410 autres femmes ($n_{\text{tot}} = 601$). Parmi elles, 520 avaient des enfants ayant des données complètes sur la croissance pré- et post-natale.

La variabilité des concentrations urinaires de phénols et la correspondance avec les concentrations mesurées dans le liquide amniotique collecté le même jour ont été étudiées chez 71 femmes enceintes recevant une amniocentèse au centre médical Mount Sinai (étude SARAHEH, NY, États-Unis). Un échantillon d'urine maternelle a été recueilli le jour de l'amniocentèse, et à deux autres reprises pendant la grossesse. Neuf phénols ont été dosés dans l'urine et le liquide amniotique. L'étude concernant les biais est basée sur des données simulées.

Résultats : Parmi le sous-échantillon de 191 femmes de la cohorte EDEN ayant eu des dosages en 2008, nous avons mis en évidence une association négative entre les dichlorophénols et le poids de naissance ainsi qu'une association positive entre la benzophénone-3 et le poids à la naissance (Philippat et al. 2012). Les associations avec les dichlorophénols n'ont pas été observées lorsque la taille de l'échantillon de population a été augmentée ($n = 520$). Les concentrations de triclosan étaient négativement associées à tout les paramètres de croissance mesurés au troisième examen échographique (p-valeurs comprises entre 0,01 et 0,16) et avec la périmètre crânien mesuré à la naissance ($\beta = -1,4$ mm, IC 95%: -2,8; 0,0) et à 36 mois ($\beta = -1,0$ mm, IC 95%: -2,8; 0,8). Les parabènes étaient associés positivement avec le poids à la naissance ($p < 0,05$). Ces associations se maintenaient dans l'enfance pour le méthyle et propyle parabènes, qui étaient positivement associés au poids à 6, 12, 24 et 36 mois et la circonférence abdominale à 36 mois (p-valeurs comprises entre 0,02 et 0,14).

En ce qui concerne la variabilité des concentrations urinaires de phénols pendant la grossesse, les coefficients de corrélation intra-classe (ICC) variaient entre 0,48 et 0,62 pour l'ensemble des phénols sauf le bisphénol A (ICC = 0,11). Seuls la benzophénone-3 et le propyle parabène ont été détectés dans au moins 50 % des échantillons de liquide amniotique ; les autres phénols n'étant détectés que très rarement (par exemple, le bisphénol A a été détecté dans seulement deux échantillons). Les concentrations de benzophénone-3 et de propyle parabène dosées dans l'urine maternelle et le liquide amniotique, recueillis le même jour, étaient positivement associés (Philippat et al. en révision).

Dans le cadre d'une simulation, nous avons estimé que pour les produits chimiques avec un ICC de 0,6, utiliser 5 échantillons donnait un β proche du β obtenu lorsque que tout les échantillons d'urine ($n = 294$ par femme) étaient utilisés (atténuation $< 10\%$), mais que pour des produits chimiques avec un ICC de 0,15, environ 25 échantillons étaient nécessaires pour atteindre une estimation proche de ce β .

Discussion / Conclusion : La variabilité importante des concentrations de bisphénol A suggère que des erreurs de classification de l'exposition sont très susceptibles de se produire dans les études épidémiologiques basées sur un faible nombre d'échantillons urinaires pour estimer l'exposition. Une assez bonne reproductibilité a été observée pour les autres phénols (ICC $\sim 0,5-0,6$); néanmoins, même dans cette gamme d'ICCs, une mauvaise classification de l'exposition pouvant entraîner des biais est susceptible de se produire dans les études épidémiologiques utilisant un seul échantillon d'urine pour estimer l'exposition.

Un seul échantillon d'urine était disponible pour évaluer les expositions des femmes de la cohorte EDEN et nos résultats peuvent être affectés d'un biais résultant d'erreurs de classification des expositions. Néanmoins, notre étude suggérait un effet de l'exposition prénatale à certains phénols sur la croissance pré- et post-natale.

THESIS ABSTRACT

Background: Phthalates and phenols belong to the family of short half-life endocrine disruptors. Exposure to these chemicals is ubiquitous in the general population including pregnant women. Some of these can cross the placental barrier and fetuses are also exposed. Data concerning the effects of prenatal exposure on fetal and early post-natal growth in Human are limited and suggest a sex-specific effect of some phenols on birth weight.

One of the limitations of the studies aiming at assessing effects of prenatal exposure to phenols and phthalates in Human is exposure assessment usually based on the measurement of their concentrations in a small number of maternal urine samples. Because of their low persistence in the organisms and the likely episodic nature of the exposures, urinary concentrations of these chemicals are likely to vary. A small number of urine samples collected during pregnancy may imperfectly reflect the global exposure throughout the whole fetal life. Chemical concentrations measured in alternative matrix, such as amniotic fluid, might be a relevant dosimeter of fetal exposure.

Objectives: Objectives of the thesis were 1) to study the potential effects of prenatal exposures to phenols and phthalates on pre- and early post-natal growth; 2) to characterize variability in maternal urine concentrations of non persistent chemicals (phenols) throughout pregnancy and to compare phenol concentrations in amniotic fluid to those measured in maternal urine collected same day; 3) to characterize the impact of increasing the number of measurements to estimate exposure on bias and statistical power of epidemiological studies.

Methods: Associations between phenols, phthalates and pre- and early post-natal growth were studied among pregnant women of the French EDEN cohort, delivered boys. Pregnant women have been recruited before 24th gestational week and followed up until the child is 5 years old. We measured fetal growth with ultrasound (three times during pregnancy) and birth measurements. We used standardized measures acquired between birth and 3 years of age to model postnatal growth. We measured biomarkers of phthalates and phenols in maternal urines collected once during pregnancy: 191 women were assessed in 2008 and 410 other women in 2012 ($n_{\text{tot}} = 601$). Among them, 520 had complete data on pre- and post-natal growth of the offspring.

Variability in phenol urine concentrations and relationship between concentrations measured in amniotic fluid and maternal urine collected on the same day were studied among 71 pregnant women presenting for an amniocentesis at the Mount Sinai Medical Center (SARAEH study, NY, USA). Maternal urine was collected at the time of the amniocentesis appointment, and on two subsequent occasions. Urine and amniotic fluid were analyzed for nine phenols.

The study aiming at characterizing bias was based on simulated data.

Results: Among the subsamples of 191 pregnant women from the EDEN cohort, we observed a negative association between dichlorophenols and birth weight and a positive association between benzophenone-3 and birth weight (Philippat et al. 2012). The associations with dichlorophenols were not replicated in the larger subsample of the EDEN cohort ($n = 520$). Triclosan concentration was negatively associated with all of the growth parameters measured at the third ultrasound examination (p-values ranged between 0.01 and 0.16) and with head circumference measured at birth ($\beta = - 1.4$ mm, 95% CI; -2.8; 0.0) and at 36 months ($\beta = - 1.0$ mm, 95% CI; -2.8; 0.8). All of the parabens were positively associated with weight at birth ($p < 0.05$). These associations remained in childhood for methyl- and propyl-parabens.

Regarding the variability in phenol urinary concentrations during pregnancy, the intraclass correlation coefficients (ICC) ranged between 0.48 and 0.62 for all phenols except bisphenol A (ICC = 0.11). Only benzophenone-3 and propylparaben were detectable in at least half of the amniotic fluid samples; other phenols were detected infrequently (e.g., bisphenol A was detected in only two samples). Concentrations of benzophenone-3 and propylparaben in maternal urine and amniotic fluid, collected on the same day, were positively associated (Philippat et al. In revision).

In a simulation study, we estimated that for chemicals with ICC of 0.6, using 4 or 5 samples gave a β close to the β obtained when all of the urine samples collected during pregnancy ($n = 294$ per women) were used to estimate exposure (attenuation < 10 %); however for chemicals with ICC of 0.15 around 25 samples would be needed to reach an estimate close to this β .

Discussion / Conclusion: The poor reproducibility in bisphenol A concentration suggests that substantial exposure misclassification occurs in epidemiological studies using a single spot urine sample to assess prenatal exposure to this phenol. Higher degree of reproducibility in urinary concentrations was observed for the other phenols (ICCs ~ 0.5-0.6). However, even in this range there might be exposure misclassification leading to substantial bias in epidemiological studies using a single spot urine sample.

We only had spot urine sample to assess exposure in the EDEN cohort and findings may be affected by exposure misclassification. Nevertheless, our study lends support to a potential effect of prenatal exposure to some phenols on pre- and early post-natal growth.