Exposure to nonpersistent endocrine disruptors during pregnancy using biomarkers of exposure : Within-subject variability and effects on respiratory health in the offspring

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Exposition à des perturbateurs endocriniens non-persistants pendant la grossesse : Variabilité intra-individuelle et effets sur la santé respiratoire de l'enfant

Exposure to nonpersistent endocrine disruptors during pregnancy using biomarkers of exposure: Within-subject variability and effects on respiratory health in the offspring

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Abstract

Background: Phenols and phthalates include chemicals widely used in daily-life products, resulting in ubiquitous exposure of the general population. There is growing concern regarding the effects on human health of these compounds, suspected to be endocrine disruptors, particularly during early life. Epidemiological research on the health effects of phenols and phthalates in offspring generally rely on a few biospecimens to assess exposure. These studies are limited by the possibly strong within-subject variability, which may result in exposure misclassification. The within-subject variability in the context of pregnancy and its possible impact on dose-response functions are poorly characterized.

Objective: The aim of this thesis was to study the exposure to several phenols and phthalates during pregnancy by: 1) investigating the possible associations between this exposure and respiratory outcomes in childhood; 2) characterizing the temporal within-subject variability of these compounds during pregnancy; and finally 3) studying the efficiency of a within-subject pooling approach using a small number of daily biospecimens for exposure assessment.

Methods: Associations between exposure to phenols and phthalates and respiratory health relied on n = 587 mother-child pairs from the French EDEN prospective cohort. Developments about the assessment of exposure during pregnancy relied on n = 16 pregnant participants of the SEPAGES-feasibility study who had collected all their urine samples for three weeks.

Results: Ethyl-paraben was associated with increased asthma rate in the first 5 years of life (Hazard Rate, HR per each unit increase in ln-transformed concentration: 1.10; 95% Confidence Interval, CI: 1.00, 1.21) and tended to be negatively associated with the forced expiratory volume in one second (FEV_1%) at 5 years of age (beta: -0.59; 95% CI: -1.24, 0.05). Bisphenol A tended to be associated with increased rates of asthma diagnosis (HR: 1.23; 95% CI: 0.97, 1.55) and bronchiolitis/bronchitis (HR: 1.13; 95% CI: 0.99, 1.30). Isolated trends for deleterious associations were also observed between 2,5-dichlorophenol and wheezing and between mono-(carboxynonyl) phthalate (MCNP), a metabolite of di-isodecyl phthalate (DIDP), and wheezing. Conversely, increases in methylparaben, propylparaben, benzophenone-3 and mono-(3-carboxypropyl) phthalate (MCPP, metabolite of di-n-butyl phthalate, DnBP; di-n-octyl phthalate, DNOP; and other high molecular weight phthalates) concentration tended to reduce rates of bronchiolitis/bronchitis and/or wheezing.

Most phenol biomarkers were highly variable over the course of a day (intraclass correlation coefficients, ICCs, below 0.3), while the between-day variability of their

daily averages over one week was much lower (ICCs above 0.6). This pattern was opposite for bisphenol S. The variability of the weekly averages considered several weeks apart was low for some compounds (2,5-dichlorophenol, butylparaben, methylparaben, propylparaben, ICCs above 0.8) and high for others (ethylparaben, bisphenol S, triclosan, ICCs below 0.4).

When estimating daily, weekly and pregnancy exposures, correlations between within-subject pools of all and of only three daily voids were above 0.8, except for benzophenone-3 and triclosan daily exposure (below 0.7). Relying on one biospecimen per subject for exposure assessment resulted in an attenuation bias in dose-response functions of 30% (methylparaben) and 68% (bisphenol A). Four and 18 samples, respectively for methylparaben and bisphenol A, were required to decrease bias in dose-response functions to 10%.

Conclusion: This work quantified the within-subject variability of phenol and phthalate biomarker concentrations during pregnancy over various time scales (day to months), and confirmed empirically that this variability is likely to strongly bias the dose-response functions in human-based epidemiological studies exploring the effects of gestational exposure to these chemicals. This thesis adds to the emerging literature on respiratory health impacts of early-life exposure to several phenols and phthalates. However, as for most studies on the human health effects of phenol and phthalate exposure, it is potentially challenged by this exposure assessment issue. Thus, this work emphasizes the relevance of more elaborate sampling strategies for exposure biomarkers in future epidemiological studies. These results have relevance for studies outside the context of pregnancy, and also for other nonpersistent compounds. New designs, such as the within-subject pooling of biospecimens validated in this study, are needed so as to efficiently characterize the health impact of nonpersistent chemicals.

Keywords: endocrine disruptors, phenols and phthalates, childhood respiratory health, prenatal exposure, within-subject temporal variability, exposure measurement error

Ph.D carried out at the Institute for Advances Biosciences: research center Inserm U 1209, CNRS UMR 5309, University Grenoble Alpes; F-38700 La Tronche, France.

Résumé

Contexte *: Les phénols et les phtalates incluent des composés très largement utilisés dans des produits de la vie quotidienne. Une grande partie de la population générale y est donc largement exposée. Ces composés sont suspectés d'être des perturbateurs endocriniens et des effets sur la santé chez l'Homme ont été rapportés, notamment après une exposition périnatale. Les études épidémiologiques sur les effets sur la santé humaine reposent généralement sur un faible nombre de biospécimens pour estimer l'exposition. Cependant, la variabilité intra-individuelle des phénols et des phtalates est potentiellement forte, ce qui peut entraîner une mauvaise classification de l'exposition dans les études sur les effets des phénols et des phtalates et limite leurs conclusions. La variabilité intra-individuelle des phénols et des phtalates au cours de la grossesse n'est pas très bien caractérisée à l'heure actuelle.*

Objectif *: L'objectif de cette thèse est d'explorer l'exposition aux phénols et aux phtalates et plus précisément : 1) d'étudier les associations entre une telle exposition pendant la grossesse et la santé respiratoire de l'enfant au cours de ses premières années de vie ; 2) de caractériser la variabilité temporelle intra-individuelle de ces composés au cours de la grossesse ; et 3) d'évaluer l'efficacité d'une approche basée sur le pooling intra-sujet d'un nombre réduit d'échantillons journaliers pour estimer l'exposition.*

Méthodes *: Les associations entre l'exposition aux phénols et phtalates et la santé respiratoire reposent sur $n = 587$ couples mères-enfants de la cohorte prospective française EDEN. Les développements sur l'estimation de l'exposition au cours de la grossesse s'appuient sur $n = 16$ femmes enceintes ayant participé à l'étude de faisabilité de la cohorte SEPAGES.*

Résultats *: L'exposition prénatale à l'éthylparabène est associée à une augmentation du ratio du taux d'incidence de l'asthme diagnostiqué par un médecin dans les 5 premières années de vie (rapport des risques instantanés ou Hazard Rate [HR] pour une augmentation de la concentration log-transformée d'une unité : 1.10; avec un intervalle de confiance [IC], à 95% : 1.00, 1.21) et a tendance à être associée avec une diminution du Volume Expiratoire Maximum par Seconde en pourcents prédits (VEMS%) à 5 ans (beta, -0.59 ; 95% IC: -1.24, 0.05). Nous avons aussi mis en évidence une tendance à l'augmentation du risque d'asthme (HR : 1.23 ; IC à 95% : 0.97, 1.55) et de bronchiolite ou bronchite (HR : 1.13 ; IC à 95% : 0.99, 1.30) avec l'exposition au bisphénol A. Des tendances d'associations délétères avec un unique paramètre respiratoire sont aussi observées entre le 2,5-dichlorophénol et le risque de sifflements dans la poitrine, et entre le mono-(carboxynonyl) phtalate (MCNP), un métabolite du di-isodécyl phtalate (DIDP), et le risque de sifflements. A l'inverse, nous avons observé une tendance à la*

diminution du risque de sifflements et/ou de bronchiolite/bronchite avec l'augmentation des concentrations de méthylparabène, propylparabène, benzophénone-3 et le mono-(3-carboxypropyl) phtalate (MCPP, métabolite du di-n-butyl phthalate, DnBP ; du di-n-octyl phtalate, DNOP ; et d'autres phtalates de haut poids moléculaire).

Pour la plupart des phénols, la variabilité intra-jour est forte, avec des coefficients de corrélation intra-classe (CCI) entre 0.03 et 0.5. La variabilité des moyennes journalières entre les jours d'une même semaine est plus faible, avec des CCI supérieurs à 0.6 sauf pour le bisphénol S (CCI, 0.14, IC à 95% : 0.00-0.39). La variabilité des moyennes hebdomadaires estimées à plusieurs semaines d'intervalle est faible pour certains composés (2,5-dichlorophénol, propylparabène, butylparabène et méthylparabène, CCI supérieur à 0.8) ; et forte pour d'autres (éthylparabène, bisphénol S, et triclosan, CCI inférieur à 0.4).

Les estimations d'exposition moyenne de la journée, de la semaine et de la grossesse, obtenues par la méthode du pooling intra-sujet "allégée" (reposant sur le pooling répété de 3 échantillons journaliers) ou la méthode de pooling intra-sujet "idéale" (pooling de tous les échantillons journaliers) sont très corrélées (coefficient de corrélation de Pearson supérieurs à 0.8), excepté pour l'estimation des moyennes d'exposition journalières de benzophénone-3 et triclosan (coefficients de corrélation inférieurs à 0.7). L'utilisation d'un biospécimen unique pour estimer l'exposition au méthylparabène entraîne un biais d'atténuation de 30% dans les estimations des relations doses-réponses. Ce biais est encore plus important pour le bisphénol A (68%). L'utilisation d'au moins 4 et 18 biospécimens, respectivement pour le méthylparabène et le bisphénol A, est nécessaire pour ramener ce biais dans les estimations des relations doses-réponses sous le seuil des 10%.

***Conclusion** : Les travaux de cette thèse quantifient la variabilité intra-individuelle des concentrations urinaires des biomarqueurs d'exposition aux phénols et des phtalates au cours de la grossesse pour des échelles de temps variées (du jour à plusieurs mois). Ils confirment empiriquement que cette variabilité peut biaiser fortement les fonctions doses-réponses dans les études épidémiologiques explorant les effets de l'exposition fœtale à ces composés chez l'Homme. Les résultats de cette thèse enrichissent la littérature émergente sur les effets des expositions précoces aux phénols et phtalates sur la santé respiratoire de l'Homme. Cependant, notre étude ainsi que la plupart des recherches précédentes sont potentiellement limitées par les problématiques liées à la mesure de l'exposition. Ce travail souligne l'importance de stratégies d'échantillonnage des biomarqueurs d'exposition plus élaborées pour l'étude de ces composés dans de futures études épidémiologiques. Ces résultats sont aussi pertinents en dehors du contexte de la grossesse et pour d'autres composés non-persistants. De nouvelles approches, telles que le pooling répété pour chaque sujet d'un petit nombre de biospécimens journaliers, validé dans cette thèse, sont nécessaires pour caractériser efficacement l'impact des composés non-persistants sur la santé de l'Homme.*

***Mots-clés**: perturbateurs endocriniens, phénols et phtalates, santé respiratoire de l'enfant, exposition prénatale, variabilité temporelle intra-individuelle, erreur de mesure de l'exposition*

Thèse réalisée au sein du l'Institut pour l'Avancée des Biosciences: centre de recherche Inserm U 1209, CNRS UMR 5309, Université Grenoble Alpes; F-38700 La Tronche, France.

Contents

Index of acronyms

ANOVA Analysis of variance. 101, 102, 105, 108, 199

ATS American Thoracic Society. 51

BBzP Butylbenzyl phthalate. 9–12, 33, 34, 37, 38, 48, 65

BMI Body Mass Index. 191

CDC Centers for Disease Control and Prevention. 50, 92, 98, 99, 132, 139, 189

CIR Cumulative Indicence Rate. 54

CI Confidence Interval. iii, 38, 47, 54, 56–64, 86, 88, 89, 101, 104, 106, 108, 110, 111, 113, 114, 153–155

COPD Chronic Obstructive Pulmonary Disease. 30, 32

DBP Di-butyl phthalate. 11, 12, 240, 243, 244

DDT Dichlorodiphenyl-trichloroethane. 1–3

DEHP Di(2-ethylhexyl) phthalate. xv, 9–12, 22, 32–34, 37, 38, 48–50, 54, 59, 65–67, 86, 87, 89, 185, 188, 207, 240, 243

DEP Diethyl phthalate. 9, 10, 12, 14, 22, 37, 240, 243, 244

DiBP Di-isobutyl phthalate. 9, 10, 33, 240, 244

DIDP Di-isodecyl phthalate. iii, v, 9–11, 46–48, 64, 65, 67, 184, 240

DINP Di-isononyl phthalate. 9–11, 32, 38, 48, 49, 65, 240, 243, 244

DnBP Di-n-butyl phthalate. iii, vi, 9, 10, 32, 33, 37, 38, 64, 65, 184, 243, 244

DNOP Di-*n*-octylphthalate. iii, vi, 9, 10, 64, 184

DOHaD Developmental Origins of Health and Diseases hypothesis. 31

EDEN Etude des Déterminants pré et post natals du développement et de la santé de l'ENfant (cohort study). i–iii, v, xv, 43–45, 47, 50, 54, 60, 66, 72, 73, 86, 88, 89, 167, 168, 187, 190–194, 199

ED Endocrine Disruptors. 2–4, 7, 9, 28, 32, 38, 43, 184, 195, 201, 204, 206, 207

EFSA European Food Safety Authority. 3, 11

EPA United States Environmental Protection Agency. 2, 11

ERS European Respiratory Society. 51, 196

EWAS Environment-Wide Association Studies. 206

$FEV_{0.5}$ Forced Expiratory Volume in 0.5 second. 66, 205

$FEV_{0.75}$ Forced Expiratory Volume in 0.75 second. 66, 205

$FEV_1\%$ Forced Expiratory Volume in 1 second in percent predicted. xv, xvii, 37, 47, 51, 53, 54, 56–66, 72, 77–84, 87–89, 191, 196

FEV_1 Forced Expiratory Volume in 1 second. 37, 47, 51, 65, 66, 184, 187, 188

FVC Forced Vital Capacity. 37, 65, 66

GLI Global Lung Initiative. 51, 191

HELIX Human Early-Life Exposome. 194, 205–207

HMW High Molecular Weight. xv, 9, 10, 12, 14, 21, 22, 51, 64, 184, 240, 243, 244

HR Hazard Rate. iii, v, xv, 46, 47, 56–63, 86–88

ICC Intraclass Correlation Coefficient. iii, iv, xv–xviii, 22, 24–27, 29, 86, 87, 89, 95, 101, 102, 104–106, 108, 110–116, 121, 123–127, 136, 142, 153–157, 159–161, 166–168, 178, 184–186, 188, 189, 194, 195, 199, 202, 204

IgE Immunoglobulin E. 33, 34, 37, 38, 64

IgG1 Immunoglobulin G1. 33

IL4 Interleukin 4. 33, 34, 64

IL5 Interleukin 5. 33

IPCS International Program for Chemical Safety. 2, 3

ISAAC International Study of Allergy and Asthma in Children. 51, 187

LMW Low Molecular Weight. xv, 9, 10, 12, 14, 21, 51, 240, 244

LOAEL Lowest Observed Adverse Effect Levels. 207

LOD Limit of Detection. 52, 99, 101, 104, 106, 108, 110, 139, 140, 145, 148–151, 189, 199

MAR Missing At Random. 193

MBP Mono-butyl phthalate. 37, 185, 191, 243, 244

MBzP Monobenzyl phthalate. 10, 25, 34, 37, 52, 62, 65, 86, 185

MCAR Missing Completely At Random. 193

MCNP Mono-(carboxynonyl) phthalate. iii, v, 10, 25, 46, 47, 52, 59, 62, 65, 86, 87, 184, 188

MCOP Monocarboxy-isooctyl phthalate. 10, 25, 52, 59, 62, 65, 86, 87, 188

MCPP Mono (3-carboxypropyl) phthalate. iii, vi, 10, 25, 52, 59, 61, 64, 65, 86, 87, 184, 185

MECPP Mono(2-ethyl-5-carboxypentyl) phthalate. 10, 25, 52, 54, 63

MEHHP Mono(2-ethyl-5-hydroxyhexyl) phthalate. xv, 10, 22, 23, 25, 34, 52, 62

MEHP Mono(2-ethylhexyl) phthalate. 10, 25, 37, 52, 63, 243, 244

MEOHP Mono(2-ethyl-5-oxohexyl) phthalate. 10, 25, 52, 62

MEP Monoethyl phthalate. 10, 22, 25, 37, 52, 54, 61, 86, 185, 244

MiBP Mono-isobutyl phthalate. 10, 25, 52, 59, 61, 65, 86, 185, 188, 191, 244

MICE Multiple Imputation by Chained Equations. 45

MINP Mono-isononyl phthalate. 243

MnBP Mono-n-butyl phthalate. 10, 25, 37, 52, 61, 65, 86

NMAR Not Missing At Random. 193

NHANES National Health and Nutrition Examination Survey. 38

NOAEL No Observed Adverse Effect Levels. 207

OECD Organisation for Economic Cooperation and Development. 3

PBPK Physiological Based Pharmacokinetic. 207

PCB Polychlorinated biphenyls. 2, 3

PHBA *p*-hydroxybenzoic acid. 15, 244, 245

PHHA *p*-hydroxyhippuric acid. 244, 245

POP Persistent Organic Pollutants. 2

PPAR-α Peroxisome proliferator-activated receptor α. 33

PPAR-γ Peroxisome proliferator-activated receptor γ. 34

PPAR Peroxisome proliferator-activated receptor. 8, 33, 34, 64, 66

PVC polyvinyl chloride. 240

RfD United States Environmental Protection Agency reference dose. 11

$\sum$DEHP Molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). 52, 63

SEPAGES Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé; Assessment of air pollution exposure during pregnancy and effects on health (cohort study). i–iii, v, xvi, xvii, 28, 42, 92, 93, 98, 103, 132, 133, 138, 141, 142, 160, 166–168, 171, 185, 194, 195, 204, 205, 207

SES Socioeconomic status. 191, 193

SIMEX SImulation EXtrapolation. 27, 29, 204

TDI Tolerable Daily Intake. 11, 207

Th1 T-helper 1 cell. 32, 33

Th2 T-helper 2 cell. 32–34

TNFα Tumor Necrosis Factor α. 33

VEMS Volume Expiratoire Maximum par Seconde. v, 45, 46

WHO World Health Organization. 2, 3

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List of tables [2]

2. Tables within the supplemental material of the articles are numbered separately and are not included in this list.

Chapter 1

Global introduction

> *"Many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wildlife, and humans. The consequences of such disruption can be profound because of the crucial role hormones play in controlling development."*
>
> –Meeting at the Wingspread Conference Center in Racine, Wisconsin, USA. July 1991

Millions of different man-made compounds have been produced since 1900.[202,157] Between 1940 and 1980, the production of synthetic chemical expanded 350-fold in weight, and between 1972 and 1992, it was estimated that 1000 new chemicals were newly synthesized each year, and made available in the market to be commercialized.[157]

Among these synthesized compounds, pesticides (from the English word, *pest* and the Latin word *cida*, kill) were intensively developed to improve agricultural production and to control pest organisms such as insects, rodents, fungi bacteria, snails and unwanted weeds in cities. Dichlorodiphenyl-trichloroethane (DDT) commercialized since 1939, was one of the first chemicals developed to be widely used as pesticides[1]. After the Second World War, pesticides were extensively manufactured and commercialized in industrialized countries worldwide without regulation or restriction until the 1960's for uses in crops and residential settings.[210,334] Agricultural production increased and human health benefited from this as well as from the lessening of vector-borne diseases. Paul Müller, who discovered the biocidal properties of DDT was awarded the Nobel Prize in Physiology (Medicine) in 1948.

However, meanwhile, the nontarget wildlife was also affected. The aquatic biologist Rachel Carson gathered several years of environmental research and narrated it

1. DDT was used in control of vector-borne diseases such as typhus and malaria.

for a general audience in a book, *"Silent Spring"*, published in 1962[2]. In this book, she warned the general population about the hazards of use of pesticides on wildlife and human health.[68] DDT and other organochlorine pesticides caused environmental damage through contamination of soils, water, vegetation and finally affected nontarget species (e.g., certain bird or fish species) by biomagnification and bioaccumulation in food chains. This book made the potential environmental problems of synthesized chemicals everyone's concern, triggered technical development for environmental research, and pressured politics to change management and control of pesticides in the United States of America (USA)[3] and other industrialized countries.[48] DDT and other pesticides were banned in several countries including the USA (1972) within ten years after the publication of Carson's book (Sweden in 1969 and France in 1971).

Main adverse effects reported worldwide in various wildlife species were related to the reproductive function, with for instance eggshell thinning for top predator birds, and deleterious effects on reproductive organs in fish species.[211] In 1991, several decades after *"Silent Spring"*, Theo Corborn, whose research focused on the Great Lakes of North America, made the connection between all these observed reproductive effects and a possible mode of action involving the endocrine system. With a small group of multidisciplinary scientists, she coined the term *endocrine disruptors* (EDs)[4], to point to man-made chemicals *"capable of disrupting the endocrine system of animals, including fish, wildlife, and humans"*. At the end of the twentieth century, endocrine disrupting effects were reported for chemicals such as persistent organic pollutants (POPs) including polychlorinated bisphenyls (PCBs), DDT and dioxins. Over the last decades, as the scientists and public's knowledge of EDs increased, so did the number of man-made chemicals used and released in the environment, including many potential *emergent* EDs, less persistent and less bioaccumulative than the formerly identified ones.[345,93]

The commonly accepted definition for EDs was developed in 2002 by the World Health Organization / International Program for Chemical Safety (WHO)/(IPCS), and defines:

— an ED as *"an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations"*;

2. The book title refers to the decline of bird populations and the possibly ensuing silence.
3. e.g., creation of the United States Environmental Protection Agency (EPA).
4. Meeting at the Wingspread Conference Center in Racine, Wisconsin, USA. Twenty-Five Years of Endocrine Disruption Science: remembering Theo Colborn. http://dx.doi.org/10.1289/EHP746

— a potential ED as *"an exogenous substance or mixture that possesses properties that might be expressed to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations"* (WHO/IPCS 2002).

Also, at the 2011 meeting of the Organisation for Economic Cooperation and Development (OECD), several experts proposed to add a third category to take into account substances that may satisfy only part of the definition (i.e., endocrine mechanisms or the health effects) [5]:

— a possible ED is *"a chemical that is able to alter the functioning of the endocrine system but for which information about possible adverse consequences of that alteration in an intact organism is uncertain"* (OECD 2011).

More recently the scientific committee of the European Food Safety Authority (EFSA) elaborated a definition for any substance having the ability to interfere with the endocrine system without the need of demonstrating evidence of adverse health effects:[97]

— an endocrine active substance is *"a chemical that can interact directly or indirectly with the endocrine system, and subsequently result in an effect on the endocrine system, target organs and tissues"* (EFSA 2013).

The endocrine system is a set of glands secreting hormones (see Box 1.1). It is involved in primordial body processes, from the cell differentiation at the embryonic stage to the control of organs and reproduction process in adulthood. EDs can mimic or antagonize the role of hormones; modulate their synthesis, transport and metabolism; or bind to their dedicated (cell membrane or nuclear) receptors. Also, interactions between the endocrine, nervous and immune systems exist,[96,220] blurring the boundaries between all these systems, and widening the range of possible effects of EDs. In the last decades, evidence that EDs play a role in reproductive, metabolic and immune disorders; and thyroid-related diseases (including neurodevelopmental effects), is increasing, especially from animal and experimental (cellular and molecular) studies. However, in many cases, additional literature in humans is required.[345,326]

Although the investigations regarding the first identified EDs (e.g., DDT, PCBs, tributyltin or dioxins) are still ongoing, understanding the impact of emergent EDs currently in the commerce is becoming essential, given the constant exposure of the population in thousands of synthesized chemicals.

5. Second Meeting of the Advisory Group on Endocrine Disrupter Testing and Assessment. OECD, Paris, France.

In this thesis, we focus on two families of currently commercialized man-made chemicals suspected to disrupt the endocrine system: the phenols and phthalates (detailed in the next chapters 2.2 and 4).

Many toxicological studies, as well as the unfortunate examples of diethylstilbestrol reproductive and carcinogenic consequences in prenatally-exposed daughters, methylmercury neurodevelopmental disorders in Minamata and thyroid-related disease such as cretinism have shown the importance of exposure timing, especially during the embryonic and fetal development.[178,345] We explore the impact of exposure to phenols and phthalates, during the fetal development on respiratory health. The human respiratory function is controlled by hormones for embryonic, fetal and postnatal developments (Chapters 2.4 and 4), but the effects of EDs on respiratory health are poorly studied in humans (see Vrijheid et al.[326], Chapters 2.4 and 4).

Additionally, such chemicals create new challenges for epidemiologists aiming at characterizing the impact of these substances on human health. These emergent EDs have a short biological half-life, i.e. they are nonpersistent in human organisms (see Chapter 2.2.3). This can lead to temporal variability in biomarker concentrations used for exposure assessment (detailed in Chapter 2.3.3), which is poorly characterized in the context of pregnancy. Hence, we study this within-subject variability over several time windows during pregnancy and its impact on dose-response functions (Chapters 5 and 6). While classical approaches for exposure assessment, relying for instance on biomarker concentrations in a few number of biospecimens, are efficient in most cases for persistent EDs, they are likely no longer valid for less persistent substances and innovative methods are required to study the relationships between these chemicals and health disorders in observational settings in humans. Therefore, we evaluate the efficiency of a more elaborate sampling design, the *within-subject pooling* approach, for exposure assessment to such substances (Chapter 6). Finally, thesis results are overall discussed (Chapter 7), and perspectives for future research are presented (Chapter 8).

Box 1.1 – **THE HUMAN ENDOCRINE SYSTEM, based on the State of the science of endocrine disrupting chemicals (WHO)**[345]

Basic features of the endocrine system

— The endocrine system is composed of various glands that secrete hormones into the blood system to reach distant target cells, organs or tissues. The main glands include the hypothalamus, the pituitary, thyroid parathyroid and adrenal glands, pancreas and gonads (see Fig. 1.1). Additionally, other organs and tissues (e.g., liver, heart, intestines, kidneys, adipose tissue) have also an endocrine function.[121] Several hormones such as steroids are carried into the blood via specific transport proteins.[205]

— The hormones interact with dedicated *receptors* outside (on the cell membrane, *membrane receptors*) or inside (on the nuclear membrane, *nuclear receptors*) the target cells to trigger their effects.

— Hormones act at very low concentrations, typically nano or picomolar ranges, and the dose-response functions are not always monotonic.[121,315]

— Hormones are crucial for the development stages during the embryonic and fetal life and during the whole life, as they are involved in essential body's functions.[205]

— Endocrine, nervous, and immune systems are closely intertwined.

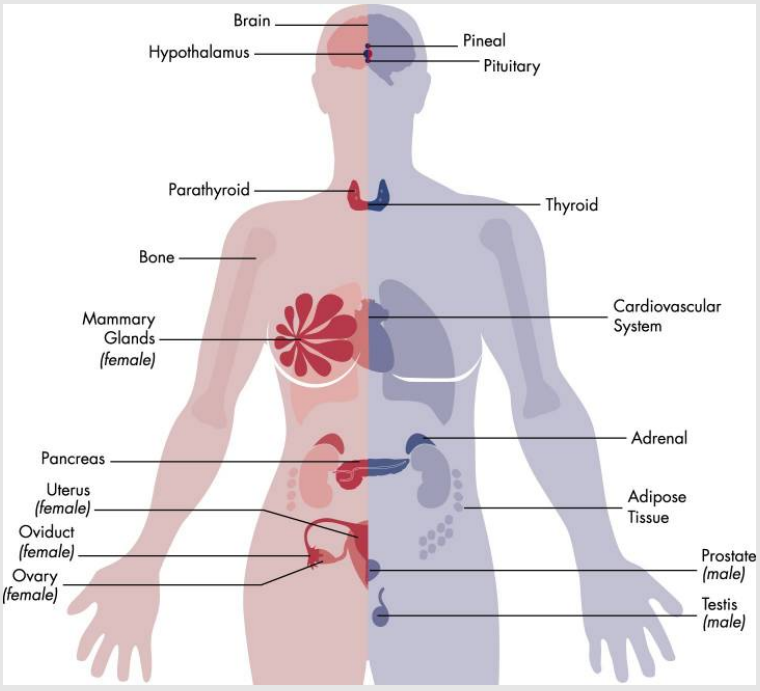


Fig 1.1 – Major human's endocrinally-sensitive organs and endocrine glands. Extracted from Gore et al.[115].

Chapter 2

Phenols, phthalates and respiratory health: background

In this chapter, we first provide an overview of the sources and of the exposure of the general population to these two families of EDs, as well as information about their metabolism in the body. In a second time, we review the implications for the assessment of exposure in epidemiological studies. Thirdly, we focus on respiratory health, lung development during embryonic and fetal life, and the possible impact of adverse exposure during this sensitive period. Finally, in the last section, we evaluate the plausibility of adverse affects of phenol and phthalate exposures on respiratory health, based on the evidence from experimental, animal and epidemiological literature.

2.1 French summary

Les phénols et phtalates sont deux familles de composés suspectés d'être des perturbateurs endocriniens avec des demi-vies courtes dans l'organisme. L'exposition de la population générale est ubiquitaire et chronique, du fait de leur utilisation dans les matériaux de construction de nos bâtiments/habitations et dans de nombreux produits de la vie quotidienne. Plusieurs de ces composés ont déjà été retrouvés dans les matrices biologiques telles que le liquide amniotique et le méconium chez l'animal et chez l'Homme, attestant d'une exposition fœtale très probable. Chez l'Homme, la phase prénatale est une période cruciale pour le bon développement de la fonction respiratoire de l'enfant et du futur adulte, mais l'impact possible d'une exposition intra-utérine aux phénols et phtalates sur la santé respiratoire durant l'enfance et à plus long terme a été très peu étudié. Les phénols et les phtalates pourraient agir sur le développement pulmonaire par interactions avec certains récepteurs (par exemple, la famille des PPARs), ou modifier la fonction respiratoire par immunomodulation ou par des mécanismes proinflammatoires.

Les études visant à identifier et quantifier les effets des phénols et des phtalates chez l'Homme reposent en majorité sur des concentrations de biomarqueurs dans un à trois échantillons d'urine pour estimer l'exposition. Cependant, les biomarqueurs urinaires des phénols et des phtalates varient probablement fortement dans le temps chez un même sujet, du fait de leur courte demi-vie et de la nature plus ou moins épisodique de l'exposition. L'étude de cette variabilité dans le contexte de la grossesse repose principalement sur deux ou trois échantillons, ce qui est probablement insuffisant. De plus, peu d'études basées sur des données réelles ont cherché à caractériser l'impact possible de cette variabilité intra-individuelle sur les relations doses-réponses pour estimer les associations entre une exposition et un effet sur la santé donnés ; ou à proposer des stratégies d'échantillonnage plus élaborées pour améliorer l'estimation de l'exposition.

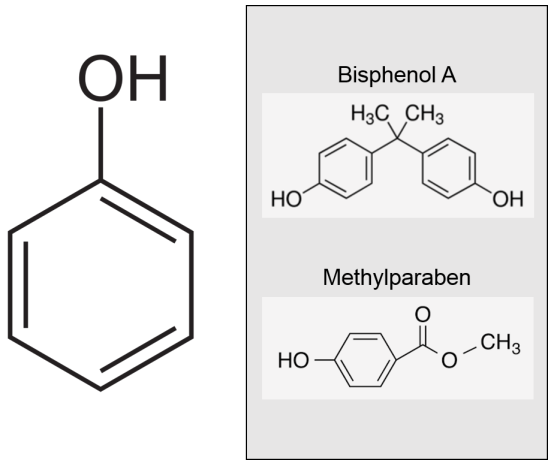
L'objectif de cette thèse est d'étudier l'exposition aux phénols et aux phtalates au cours de la grossesse et ses possibles effets sur la santé respiratoire de l'enfant. Plus précisément, ce travail vise à :

1. *étudier les associations entre l'exposition in utero aux phénols et aux phtalates, et la santé respiratoire de l'enfant au cours de ses premières années de vie ;*
2. *caractériser la variabilité temporelle intra-individuelle des phénols et des phtalates au cours de la grossesse ;*
3. *proposer une approche d'estimation de l'exposition basée sur le pooling d'échantillons intra-sujet et évaluer son efficacité en comparant avec une approche "idéale", et en quantifiant le biais et la puissance statistique dans les études épidémiologiques en fonction du nombre d'échantillons utilisés pour estimer l'exposition.*

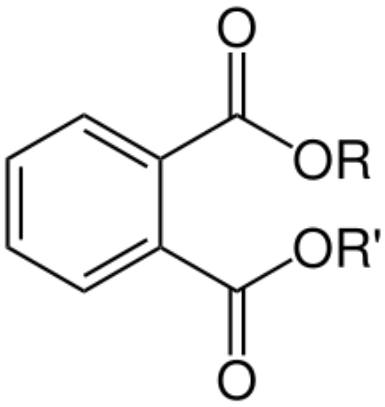
2.2 Phenols and phthalates

2.2.1 Sources

Phenolic compounds and phthalates include high volume manufactured chemicals, several of which being suspected to be EDs. Phenols are defined as chemicals with an hydroxyl group (–OH) bonded to a phenyl group (C_6H_5–, see Figure 2.1.a.). Phthalates are esters of phthalic acid (see Figure 2.1.b.), which can be classified according to their molecular weight, as *high* and *low* molecular weight phthalates (HMW and LMW phthalates, *above* and *below 250 g/mol*, respectively). The most common phenols and phthalates are listed in Box 2.1 and are studied in this thesis.



(a) Phenol and examples of phenolic compounds suspected to be EDs.



(b) The basic phthalate structure.

Figure 2.1 – Basic chemical structures of **(a)** phenols and **(b)** phthalates.

These compounds have specific properties. Some are e.g., antifungal and antimicrobial preservatives, ultraviolet-blockers, monomers adding strength and toughness in plastic polymers, plasticizers softening plastics, increasing their flexibility and durability, and solvents holding color and fixing fragrance. Consequently, compounds from the two families have a wide range of uses and can be found in many products present in our environment. Among others, such compounds are used in resins, plastic and vinyl material for consumer products (including food and cosmetic packaging), medical devices, automobile manufacturing, and building construction (bisphenols, benzophenone-3, butyl-benzyl phthalate (BBzP), di-2-ethylhexyl phthalate (DEHP), di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP), di-n-octyl phthalate (DNOP)); in personal care products (benzophenone-3, triclosan, parabens, diethyl phthalate (DEP), di-n-butyl phthalate (DnBP)); in clothing and kitchenware (triclosan, DnBP); diet (including beverages), food supplements and pharmaceuticals (parabens, benzophenone-3, DEP, DEHP, di-isobutyl phthalate (DiBP), DnBP); in paints, lacquers, rubbers,

Box 2.1 – **PHENOLS, PARENT PHTHALATES AND THEIR METABOLITES STUDIED IN THIS THESIS**

Phenols	**Abb.**
2,4-Dichlorophenol	2,4-DCP
2,5-Dichlorophenol	2,5-DCP
Bisphenol A	BPA
Bisphenol S	BPS
Benzophenone-3	BP3
Triclosan	TCS
Methylparaben	MP
Ethylparaben	EP
Propylparaben	PP
Butylparaben	BP

Phthalates	**Abb.**	**Primary metabolites**	**Abb.**	**Secondary metabolites**	**Abb.**
LMW phthalates					
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP		
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	MnBP	Mono-(3-carboxypropyl) phthalate	MCPP
Di-isobutyl phthalate	DiBP	Mono-isobutyl phthalate	MiBP		
HMW phthalates					
Di-n-octyl phthalate	DNOP			Mono-(3-carboxypropyl) phthalate	MCPP
Butyl-benzyl phthalate	BBzP	Mono-benzyl phthalate	MBzP		
Di-2-ethylhexyl phthalate	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
Di-isodecyl phthalate	DIDP			Mono-(carboxynonyl) phthalate	MCNP
Di-isononyl phthalate	DINP			Mono(carboxyoctyl) phthalate	MCOP

inks, adhesives, sealants, thermal printer paper (benzophenone-3, phthalates, bisphenols); and in insecticides, or pesticides (dichlorophenols, benzophenone-3, DNOP, DEP).[316,74,40,308,224,227,223,353,171,225] A more detailed description of phenols and phthalates sources is provided in Appendix A.

Evolution of the regulation and ongoing market changes

Exposure of the population to phenols and phthalates depends on their specific uses and on regulatory decisions. Due to growing health concerns and environmental awareness, several phenols and phthalates were banned or restricted in some products (see Table 2.1), while other ones are increasingly used, resulting in changes in exposure levels (see Figure 2.2).

Table 2.1 – Main regulations in Europe and in the USA regarding the compounds studied in this thesis.

Compound	Country	Year	Legislation	Current EFSA Tolerable daily intake (TDI) and/or EPA reference dose (RfD)
DEHP	France	2015	Ban from medical tubes in pediatrics, neonatology and maternity wards in hospitals.	TDI: 50μg/kg bw/day RfD: 20μg/kg bw/day
DEHP, BBzP, DBP	EU	2005	Ban from toys and childrencare articles (Directive 2005/84/EC)	TDI (DBP): 10μg/kg bw/day
		2007	Ban from food packaging (Directive 2007/19/EC)	RfD (DBP): 100μg/kg bw/day
		2009	Ban from cosmetics (EC Regulation 1223/2009)	
	USA	2008	Restricted to <0.1% w/w in toys and childcare articles	TDI (BBzP): 500μg/kg bw/day RfD (BBzP): 200μg/kg bw/day
DINP	EU	2005	Ban from toys that can be put in the mouth by children (Directive 2005/84/EC)	TDI: 150μg/kg bw/day RfD: 120μg/kg bw/day
	USA	2008	Interim restriction for toys that can be put in the mouth by children	
		2014	Restricted to <0.1% w/w in toys and childcare articles	
DIDP	USA	2014	Restricted to <0.1% w/w in toys and childcare articles	TDI: 150μg/kg bw/day
Bisphenol A	Canada	2009	Ban from baby bottles	TDI: 4μg/kg bw/day RfD: 50μg/kg bw/day
	France	2010	Ban from baby bottles	
	EU	2011	Ban from baby bottles (Directive 2011/8/EU and EU Regulation 321/2011)	
	USA	2012	Ban from baby bottles	
		2013	Ban from infant formula packaging	
	France	2014	Ban from material in contact with food (food packaging or kitchenware)	
Butylparaben, propylparaben	EU	2009	Restricted to <0.4% w/w for one paraben and 0.8% for a mixture of parabens in cosmetics (EC Regulation 1223/2009)	NA*
		2014	Restricted to <0.14% w/w for one paraben in cosmetics (EU regulation 1004/2014)	
Methylparaben, ethylparaben	EU	2009	Restricted to <0.4% w/w for one paraben and 0.8% for a mixture of parabens in cosmetics (EC Regulation 1223/2009)	TDI: 10μg/kg bw/day
Triclosan	EU	2009	Restricted to <0.3% w/w in cosmetics (EC Regulation 1223/2009)	TDI: NA* RfD: 300μg/kg bw/day
		2014	Restricted to <0.2% w/w in mouthwashes and 0.3% in in toothpastes, hand and body soaps/shower gels, deodorants, face powders and blemish concealers (EU Regulation 358/2014)	
	Switzerland	2015	Ban from clothing	
	USA	2015	Ban from clothing	
		2016	Ban from consumer antiseptic wash products	
	EU	2016	Ban from biocidal products for hygiene purpose (EU Commission Implementing Decision 2016/110)	
Benzophenone-3	EU	2017	Restricted to <6% w/w in cosmetic sunscreen products and <0.5% in all types of cosmetics (EU Regulation 2017/238)	NA*

EFSA: European Food Safety Authority; EPA: United States Environmental Protection Agency; EU: European Union; EC: European Commission, NA: not applicable.
* No official health-based threshold value.

2.2.2 Exposure of the general population

Exposure routes

Due to the variety of uses of phenols and phthalates, exposure of the general population occurs via multiple routes (ingestion, inhalation and dermal absorption).

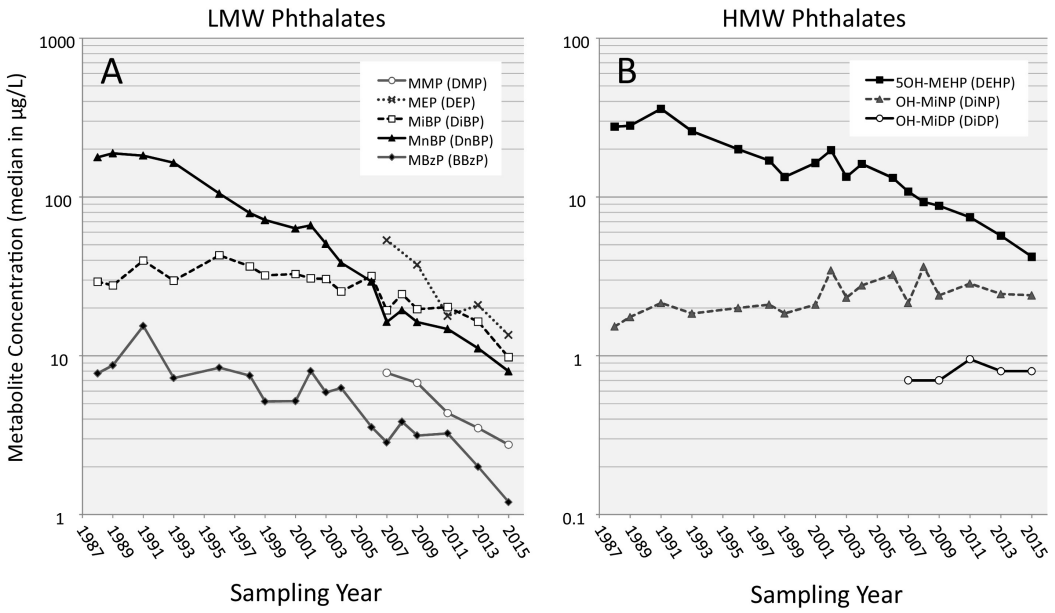


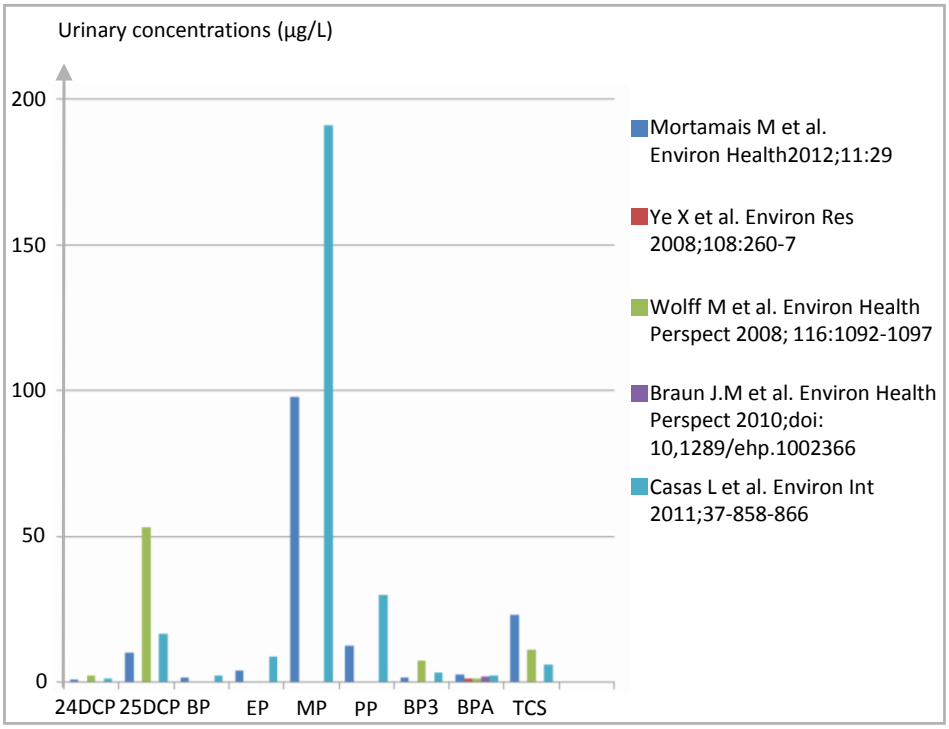
Figure 2.2 – Urinary concentrations of key LMW and HMW phthalate metabolites (median, in μg/L) in Germany over the years 1988-2015. Extracted from Koch et al.[168].

Chemicals of the two families can migrate from packaging to food and beverages and be absorbed into the body through ingestion of contaminated diet.[111,169,342] For compounds such as bisphenol A (outside France, owing to the ban of this use since 2015) and several phthalates, diet is a major exposure source.[345] Other dietary sources include drinking water for benzophenone and dichlorophenols, and beverages and food for benzophenone and parabens. Infants and children can be exposed through similar routes of exposure, and are also exposed through their hand-to-mouth activities (e.g. for phthalates and bisphenol A).[112,189] For babies and infants, ingestion of breast milk constitutes another exposure source for many phenols (e.g., bisphenol A, parabens, benzophenone-3, triclosan) and phthalates (e.g., metabolites of DEP, DBP, DEHP, BBzP).[345,342]

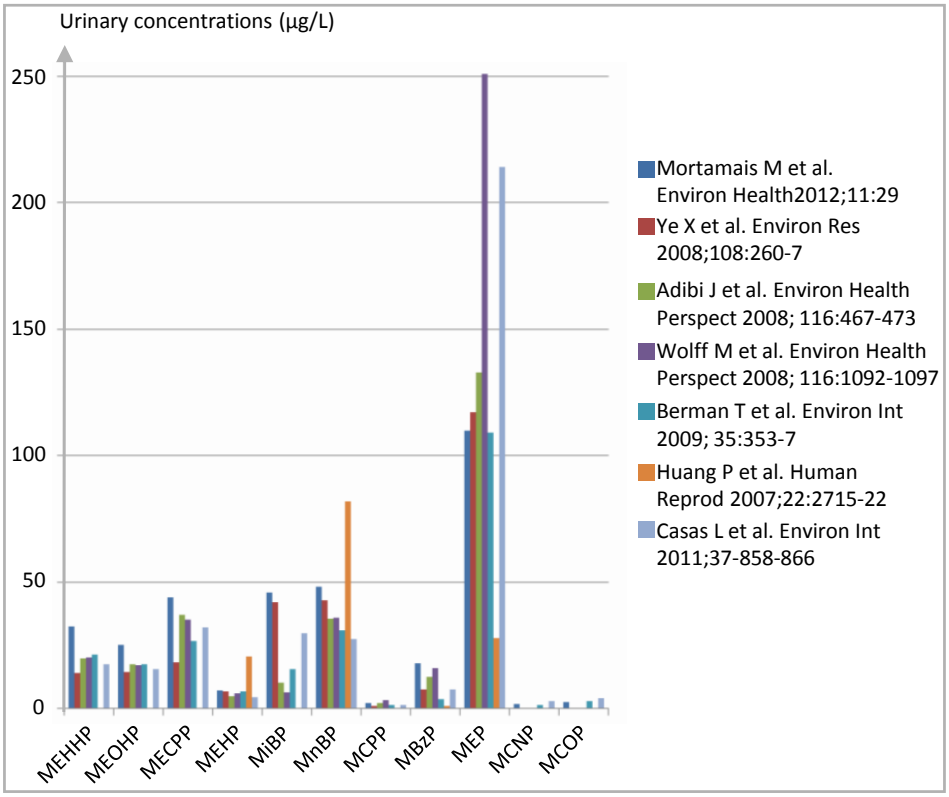
For parabens used in cosmetics and other personal care products, the exposure occurs predominantly via skin absorption.[40,20] This route of exposure is also relevant for benzophenone and phthalates that are present in some personal care products[172,171,149,147] and for bisphenols through handling of thermal printer papers.[139,95,35]

Human exposure to phthalates, benzophenone and bisphenols occurs additionally via inhalation or ingestion of contaminated dust and particles.[112,189,137,341,346,143]

Because of the ubiquity of these phenols and phthalates in the environment of Western countries, the exposure of the general population and thus of sensitive populations such as pregnant women (see Figure 2.3) and children is widespread and continuous.[71,214,88,87,69] Since pregnant women are chronically and widely exposed to these chemicals, so are fetuses.



(a) Phenols



(b) Phthalate metabolites

Figure 2.3 – Median values of urinary **(a)** phenols and **(b)** phthalate metabolites (μg/L) in selected publications among pregnant women. Adapted from Mortamais et al.[214].

2.2.3 Toxicokinetics

Exposure of the population and possible adverse health effects depends on the toxicokinetics of the compounds of interest, i.e. on the behavior of the compounds in the (human) body. Toxicokinetics includes the absorption into the body, the distribution in the systemic circulation or in specific organs, the metabolism which can transform the chemical in several metabolites and finally the excretion of compounds from the body via for example urines, feces, or the accumulation in specific tissues. Metabolism differs between phenols and phthalates and also between compounds of the same family.

Metabolism and excretion of phthalates

First, by the action of nonspecific lipases and esterases, phthalates are rapidly metabolized to hydrolytic monoester metabolites (primary metabolites). This occurs in the stomach; in the intestine after oral exposure; directly in the blood with an intravenous infusion; and during skin permeation after a percutaneous exposure.[237,164,160,303,147,148] Monoesters are rapidly absorbed from the gastrointestinal tract or the skin layers into the blood systemic circulation to reach predominantly the liver and the kidneys.[201] In a second stage (see Figure 2.4), monoesters can undergo multistep oxidation of their alkylchains which generates secondary more hydrophilic metabolites (with hydroxy-, carboxy- and oxo- groups).[167]

HMW phthalates, with the longest side-chains, undergo preferentially oxidative pathways before being excreted, while LMW phthalates are mainly excreted in their simple monoester forms.[27,282,268] Primary or secondary metabolites will preferentially be transformed in a second metabolic phase in the liver, as glucuronide and sulfate conjugates, except for DEP monoester, mostly excreted in its free form.[282,280,13,175,209] The biotransformation pathways last only several hours (less than one day) for most phthalates before the excretion of metabolites, mostly in urines. A small fraction of metabolites can also be found in bile and feces but these excretion pathways are minor in humans.[163,164,160] Bioaccumulation in the body is presumably limited, even though an accumulation in lipid reserve may exist.[90,163,148,147] Elimination half-lives are short, especially for monoesters (2-8 hours) – and therefore for LMW phthalates – and slightly longer (3-24 hours) for some secondary metabolites.[237,276,163,164,156,12,13,209,148,147] Few is known regarding metabolism and excretion of phthalates during pregnancy in humans (see below, Section 2.2.4).

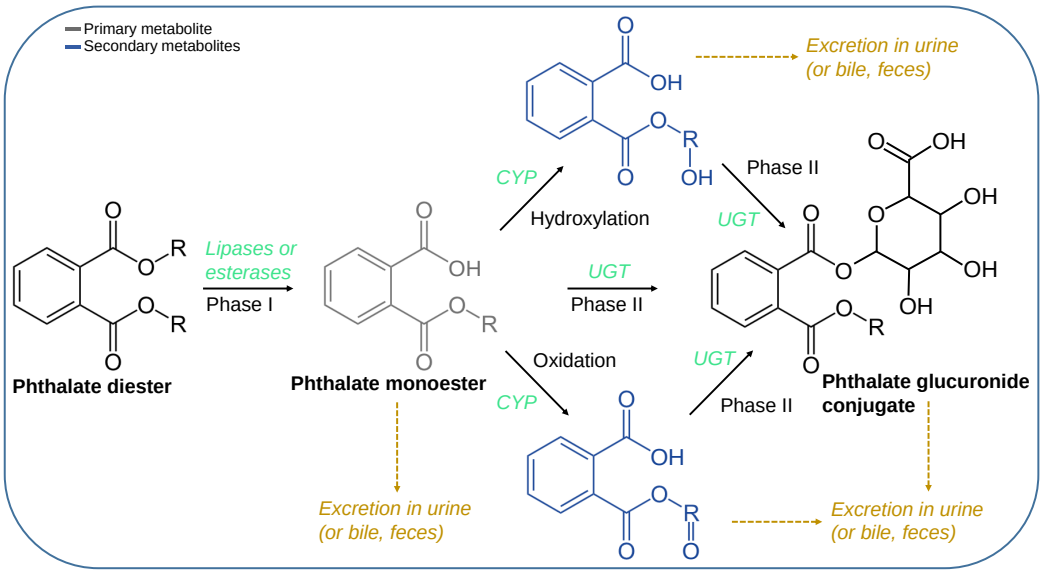


Figure 2.4 – Metabolic pathways for phthalates in humans.

Metabolism and Excretion of Phenols

Upon exposure, phenolic compounds are also readily absorbed through skin and the gastrointestinal tract. Phase I metabolism is minor for phenols, except for parabens, which are mainly transformed in a nonspecific metabolite, *p*–hydroxybenzoic acid (PHBA); and possibly benzophenone-3,[309,350,85] and a significant fraction of the parent compound is absorbed unchanged. Metabolites and parent forms are most likely conjugated to increase their solubility in water before being excreted in the urine with a short (between 1 and 20 hours) elimination half-life.[309,331,351,259,308,289,353,306,307,324] Elimination half-lives are not known in pregnant women (Section 2.2.4). As for phthalates, oxidized metabolites have also been measured in urines in non-negligible amounts for parabens with a more complex ester side chain (e.g., butylparaben), while this is not expected to happen for those with a shorter one (e.g., methylparaben).[330,213]

To summarize, based on toxicological studies, phenols and phthalates studied in this thesis are reportedly quickly absorbed (oral and dermal exposure) into the human body, and mainly excreted in urine with an elimination half-life between one and 24 hours, depending of the route of exposure, the compound, and the dose. In addition, there is no (or scant) evidence of accumulation in human tissues. Detailed information on compound-specific toxicokinetics are given in Appendix B. However, toxicokinetics data in humans is limited for dichlorophenols, benzophenone-3, some parabens, and bisphenols other than bisphenol A; and is very scarce in the context of pregnancy.

2.2.4 Toxicokinetics during pregnancy

Pregnancy induces many physiological modifications such as increased gastric pH; decrease of the intestinal motility; greater glomerular filtration rate; increase cardiac output; overexpression of some hepatic enzymes. These can alter the toxicokinetics of xenobiotics, from the absorption to the elimination of such compounds.[11,98] Besides, pregnancy-related pathologies such as hypertension, gestational diabetes, preeclampsia can occur and additionally complicate the metabolic process.

Due to increased glomerular filtration rate and renal blood flow during pregnancy, it is assumed that renal clearance is increased in pregnant women, which would lead to a shorter elimination half-life, compared to non-pregnant women.[11,2] For example, cotinine, a urinary biomarker of tobacco smoke exposure, has been found to have about twice as fast elimination half-life during pregnancy compared to postpartum.[86] However, for other substances, excretion is reportedly unaffected by pregnancy, suggesting that other mechanisms, which change during pregnancy, also contribute to renal clearance. This is for example the case of amoxicillin.[215,216] Regarding phenols and phthalates, differences in bisphenol A metabolism between pregnant and non-pregnant subjects have been reported in nonhuman primates, with a longer residence time of bisphenol A (i.e. total time spent by the compound inside the body) during pregnancy.[265]

The feto-placental unit, with its own metabolism activities, plays a critical role in the overall gestational toxicokinetics of xenobiotics since compounds are also distributed to the fetal compartment, sometimes within one hour following exposure.[265] Some phenols and phthalates are capable of crossing the placenta, and can be found in placental tissue,[318] amniotic fluid[279,246] and cord blood.[340,22,142,113] Additionally, phthalates and bisphenol A were found to have an extended elimination half-life in amniotic fluid and fetal serum,[106,317] resulting in sustained exposure of fetus.

For phenols, the free form is often considered as the bioactive one whereas the conjugated forms are assumed to be toxicologically inactive and more stable. For example, conjugated forms of bisphenol A were found to be less active than the free bisphenol A.[113] However, conjugated forms can undergo deconjugation in many tissues in the presence of non-specific β-glucuronidases and sulfatases. Among these tissues, placenta was found capable of deconjugating glucuronide bisphenol A in small amount, thus possibly increasing the fetal exposure to the biologically active bisphenol A.[232] Besides, it has been reported that metabolites can also be biologically active,[127,126,321,85] so that toxicity should probably not be predicted only on the basis of the free form concentration.

As seen above, phenols and phthalates are nonpersistent in human bodies, although data in the specific context of pregnancy are very limited. However, fetuses are exposed to phenols and phthalates (possibly continuously) that may adversely affect their pre- and postnatal health. Exposure assessment of phenols and phthalates in epidemiological studies is challenged by the nonpersistent nature of these compounds, which can creates within-subject variability in exposure levels. This aspect is developed in the next section.

2.3 Assessment of exposure to phenols and phthalates in epidemiological studies

Contrary to experimental studies, for which levels and routes of exposure (the amount of a given external chemical in the immediate body environment, and which can enter the body) are controlled by scientists, observational studies investigating the health effects of exposure to environmental toxicants in humans need to deal with multiples sources and routes of exposure, as well as mixtures of compounds, and generally without direct information on exposure. Since fetuses are protected in their mothers' womb, accurately assessing the fetal exposure during pregnancy is not straightforward.

2.3.1 Assessment in the environment

Exposure to phenols and phthalates can be assessed from environmental data, through job title, questionnaires,[145,45,176] and measurements in environmental media, such as in air or house dust.[7,8,46,170,354] However, owing to the abundance of exposure sources detailed in Sections 2.2.1, 2.2.2 and in Appendix A, exposure assessment relying on occupation title, questionnaires (also subject to memory bias), measurement in air and house dust, are probably very limited and can lead to significant exposure misclassification. None of these methods take into account all the routes of exposure nor do they provide an estimate of the amount of chemicals that actually enters the human body (dose).

2.3.2 Human biomonitoring

Human biomonitoring (formed from the contraction of "biological" and "monitoring") consists in the measurement of chemical substances, i.e. the parent compound or one of its metabolites, in the body fluids or tissues, i.e. the *biological matrix*.[229] The species measured in the biological matrix are termed *biomarkers of exposure*, as they indicate exposure to environmental toxicants.[14] Contrary to the exposure assessment methods seen above, human biomonitoring tells whether individuals are exposed to a particular environmental toxicant, and integrating all the known and unknown sources and pathways, so as to provide an estimate of the amount of the chemical that has actually entered into the body (Figure 2.5). This is why, given the multiplicity of sources and routes of exposure for phenols and phthalates, human biomonitoring is currently the preferred method to assess exposure to these chemicals.[151,165] However, this is not a direct measurement of the internal dose.

Various biological matrices can be used, including human fluids (e.g., urine, blood, saliva, breast milk, feces, seminal fluid, sweat, nails, pulmonary liquid or air) and

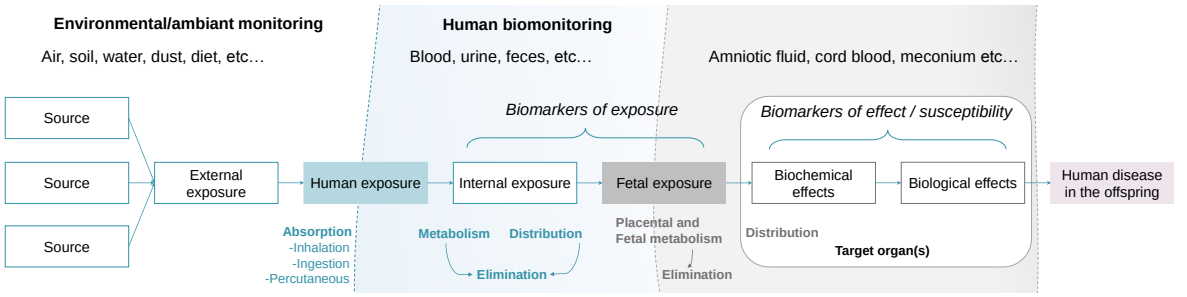


Figure 2.5 – Scheme of environmental and biological monitoring

tissues (e.g., hair, fat tissue, bone, teeth). Among these matrices, some are directly related to fetuses.

2.3.2.1 Fetus-related biological matrices

Amniotic fluid, which is mainly composed of fetal urine after the second half of pregnancy; meconium, i.e. the first stools of the newborn accumulated by the fetus from the 12th week of gestation, hair nails, and cord blood may be biologically relevant media for direct fetal exposure assessment.[312,186]

Collecting amniotic fluid requires an amniocentesis, an invasive medical procedure which can be harmful to the fetus and the mother, with a risk of miscarriage. Also, there is a continuous turn-over of the amniotic fluid, and toxicants may be eliminated via the placenta in several hours.[340] If amniotic fluid is collected at birth, there is a possibility of contamination through maternal blood, urine, feces or delivery material.[340]

While collecting meconium is non-invasive and may provide information on long and chronic exposures via its accumulation until birth,[186] meconium samples can be easily contaminated or unavailable. Indeed, the fetus can discharge meconium into amniotic fluid before birth, during the delivery or several days after birth, resulting in possible contamination of meconium samples via maternal or fetal fluids, as well as extraneous material. Analytical techniques are also less developed for meconium than for other media, such as fluids.

Newborn's hair and nails are interesting as they are easily collected and might provide information on more ancient exposures.[17,10] However, their use is limited because the toxicokinetics (bioaccumulation) of nonpersistent biomarkers in these matrices is not well documented; difficulties exist to differentiate internal and external exposure (risk of external contamination); and the amount of hair or nails requested to have a good sensitivity may be too substantial, especially for analyzing several substances simultaneously.[17,9,10,73]

Cord blood, collected at birth, may only represent a measure of the recent exposure due to the transience of metabolites in the blood, and hence may not reflect exposure

during the whole pregnancy.[151]

Therefore, relying on exposure biomarkers in amniotic fluid, meconium, cord blood, hair and nails for prenatal phenol and phthalate exposure assessment in epidemiological studies is not straightforward. Information about fetal exposure can be provided indirectly with maternal exposure. Other matrices which have been used in environmental epidemiology for phenols and phthalates exposure assessment are maternal biological matrices.

2.3.2.2 Maternal biological matrices

Maternal hair and nails might be promising matrices but suffer from the same limitations detailed for newborns. Urine and blood are the preferred media used in environmental epidemiology for many biomarkers, since they are easy to collect; not much invasive; and efficient analytical methods are already developed to quantify even trace amounts of many families of compounds.[165,281,161] Phenols and phthalates do not accumulate in the body and, with a short terminal elimination half-life predominantly through the urine (see Section 2.2.3), their presence in the blood is possibly more than in urine. Also, enzymes in blood can transform compounds such as phthalates present in the containers or the (laboratory) atmosphere into their monoesters, resulting in possible contamination of samples. On the contrary, these enzymes are not present in urine and hence, such reactions do not exist in this media. Moreover, urine collection is a less invasive procedure, and detection frequencies are usually higher in urines than in blood.[64] Thus, although the relationship between measures of exposure biomarkers in urine and fetal exposure is not defined yet – and would imply to rely on complex toxicokinetic models – urine is the preferred matrix to assess phenols and phthalates for epidemiological studies[64,165] evaluating the effects of pregnancy exposure (including pregnancy outcomes and health effects in the offspring).

2.3.2.3 Issues related to urinary biomarkers of phenols and phthalates

Urine Samples and Urine Dilution

One issue related to the use of biomarkers when measured in urine samples is that chemical concentrations are related to urine dilution. For instance, since urine volumes vary from void to void, high hydration preceding the urine collection can lead to higher urine volume and artificially lower metabolite concentrations compared to low hydration.

Creatinine or specific gravity are commonly used for adjusting urine dilution in chemical exposure assessment. Creatinine is a waste product of the body primarily excreted in the urine at a fairly constant rate by glomerular filtration, and specific

gravity is the ratio of the sample-specific density to the density of water. Urine density, and consequently specific gravity, are related to molecules present in urine, the most abundant being urea, electrolytes, creatinine, and other metabolite waste products including xenobiotic metabolites.

No consensus exists for the optimal approach to control for urine dilution as both markers have limitations. If chemicals undergo active tubular secretion, which is likely the preferred type of renal excretion for conjugated species,[42] creatinine adjustment, and hence an approach using specific gravity which is related to creatinine urinary concentration, would not correct appropriately for urine dilution.

Additionally, creatinine concentration and specific gravity can be affected by pregnancy conditions: creatinine through body mass variations and gestational (physiologic or pathologic) modifications impacting on the renal clearance; and specific gravity through possible gestational excretion of glucose or proteins in the urine.[42,28,78]

Choice of exposure biomarkers

Although the risk of measuring extraneous phthalate and phenol contamination (e.g., from the containers, reagents, equipment) is lower in urines where hydrolytic enzymes are not present,[151,161] a careful choice of the sampling material and the laboratory for assays is required. Measuring secondary oxidized metabolites for HMW phthalates is one way to limit the risk of measuring the contaminated part since they are not influenced by external contamination. However using only secondary metabolites does not take into account the small fraction of the parent compound which is excreted as its simple monoester.[27,282,161,160,165] The metabolites can be further grouped using their molar concentrations in an attempt to better reflect the overall exposure to the parent compound.[245,110]

For LMW phthalates, mainly excreted as their monoester metabolites, and for most phenols, there is no oxidative species used to limit the risk of contamination.[64] However, pharmacokinetic data (see Section 2.2.3) indicate that conjugated species represent the largest fraction of the excreted dose and are least likely to be due to external contamination.[64,160,27,282,161]

To sum up, owing to the multitude sources of exposure to phenols and phthalates and the short half-life of these compounds, measuring their metabolites in maternal urine is currently the preferred method to assess exposure in epidemiological studies investigating their impact on the offspring following pregnancy exposure. However, as for exposure assessment in the environment, relying on biomonitoring is also subject to misclassification. Biomarker quantification to estimate the individual's true exposure may include some random measurement error, with part of the error being related to the instrumental precision, and for another part to within-subject variations.[273,293]

2.3.3 Within-subject variability

2.3.3.1 Temporal variability during pregnancy

Phenols and phthalates are likely to have within-subject temporal variations over a given time window,[166,162,352,249] due to (i) their short elimination half-lives, and (ii) behavior and episodic exposures.

Studies evaluating the within-subject reproducibility of urinary concentrations of phenol and phthalate metabolites during pregnancy[65,101,314,4,37,50,51,119,153,204,246,294,103] are presented in Tables 2.2 and 2.3. The Intraclass Correlation Coefficient (ICC), which corresponds to the ratio of the between-subject variance to the total variance (see Eq 2.1) can be used to give an estimation of the within-subject variability. ICCs vary between 0 and 1, and the greater the ICC, the lower the ratio of the within-subject variance to the between-subject variance.

$$ICC = \frac{{\sigma^2}_{between-subject}}{{\sigma^2}_{between-subject} + {\sigma^2}_{within-subject}} \tag{2.1}$$

A few of these studies investigated the within-subject variability of phenols other than bisphenol A.[294,204,246,119,287,103] Variability was moderate for most phenols (ICC between 0.4 and 0.5) except bisphenol A, with higher variability (ICC below 0.3). In general, phthalates exhibited high variability, especially HMW phthalates.

2.3.3.2 Within-day and between-day variability

Most of the studies presented in Tables 2.2 and 2.3 relied on two or three spot biospecimens collected in each pregnant woman several weeks or months apart. This is not enough to conclude on the within-subject temporal variability of phenols and phthalates throughout pregnancy. Also, the heterogeneity of gestational ages at urine collection, of time of day when urine is collected, and of intervals between within-subject biospecimens may explain differences observed in their results. Additionally, the design of these studies did not allow to characterize the within-day or the within-week variability of biomarker concentrations.

Few studies, but in non-pregnant subjects, had a more comprehensive design (see Figure 2.6 for examples). Based on complete urine collections throughout four to seven consecutive days in eight non-pregnant participants, these studies described the within-subject (within-day and between-day) variability of phenols and phthalates.[162,352,21,249] The within-subject variability was high for bisphenol A and MEHHP (metabolite of DEHP) and rather limited for MEP (metabolite of DEP), some parabens, triclosan and benzophenone-3.[162,352,21,249] These studies could not describe the variability over periods longer than a week.

In the context of pregnancy, information on within-subject variability over several consecutive days is, to the best of our knowledge, limited to one study focusing on bisphenol A and phthalates concentrations from complete urine collection during one day in 66 pregnant women.[103] This study focused on within-day variability but did not investigate between-day variability, as they relied on a single day. Hence, within-subject variability of phenols and phthalates needs to be studied more deeply in pregnant women.

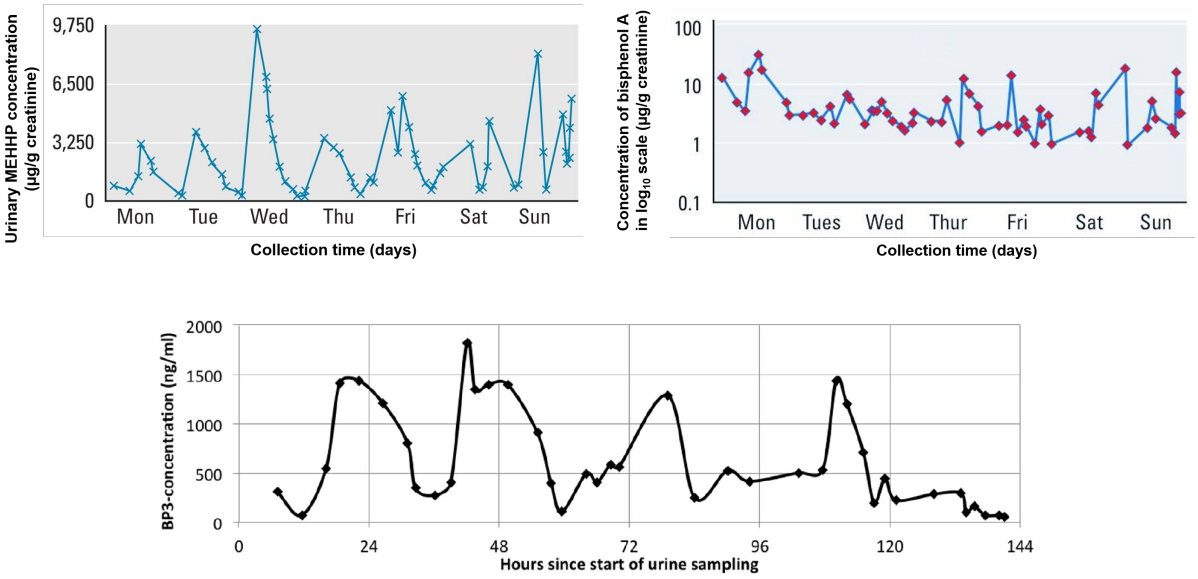


Figure 2.6 – Creatinine-corrected urinary concentrations of MEHHP (μg/g creatinine) (extracted from Preau et al.[249]); and bisphenol A in $\log_{10}$ scale (μg/g creatinine) (extracted from Ye et al.[352]); and urinary concentration of benzophenone-3 for all spot urine samples from one non-pregnant individual (extracted from Koch et al.[162]).

Table 2.2 – Publications on the variability of *phenols* levels in biospecimens collected during pregnancy.

Reference	Meeker et al.[204]	Bertelsen et al.[37] Guidry et al.[119]	Quirós-Alcalá et al.[252]	Philippat et al.[246]	Jusko et al.[153]	Braun et al.[50] Stacy et al.[294]	Braun et al.[51]	Smith et al.[287]	Fisher et al.[103]
Population	Puerto Rico PROTECT study	Norway MoBa cohort	USA Salinas Valley CHAMACOS study	USA New York SARAEH cohort	Netherlands Generation R study	USA Cincinnati HOME study	USA Massachusetts EARTH study	USA Massachusetts Fertility Center	Canada P4 study
Period	2010-2012	2007-2008	1999-2000	2005-2008	2004-2006	2003-2006	2004-2009	2005-2010	2009-2010
No of women	105	45	375	71	80	389	137	129	80
No of samples per woman	3/pregnancy	3/pregnancy	2/pregnancy	3/pregnancy	3/pregnancy	3/pregnancy	≥2 before pregnancy ≥2 during pregnancy	2-3/pregnancy	All urine voids for 1 weekday (T1) and/or 1 week-end day (T1) 1 spot (T2, T3, delivery, post-partum)
Gestational age	20 GW, 24 GW, 28 GW	17 GW, 23 GW, 29 GW	14 GW, 26 GW	18 GW, 23 GW, 33 GW	13 GW, 20 GW, 30 GW	16 GW, 26 GW, delivery	5 GW, 20 GW, 33 GW	6 GW, 21 GW, 34 GW	≤ 20 GW, 24-28 GW, 32-36 GW, delivery, 2-3 mo post-partum
Remark	Uncorrected for urine dilution	Uncorrected for urine dilution	Uncorrected for urine dilution	Uncorrected for urine dilution	Uncorrected for urine dilution	Uncorrected for urine dilution	Specific gravity correction	Specific gravity correction	Uncorrected for urine dilution
ICC (95% CI)									
2,4-DCP	0.37 (0.25, 0.50)	50% < LOD		0.47					
2,5-DCP	0.50 (0.38, 0.62)	50% < LOD		0.52					
MP	0.36 (0.24, 0.50)	0.48 (0.33, 0.61)		0.52				0.38	
EP				0.48					
PP	0.31 (0.19, 0.46)	0.55 (0.41, 0.67)		0.51				0.36	
BP	0.45 (0.33, 0.57)	0.34 (0.18, 0.49)		0.54				0.48	
TCS	0.42 (0.30, 0.55)	0.45		0.56		0.38-0.58			
BP3	0.58 (0.47, 0.68)	0.43 (0.28, 0.57)		0.57					
BPA	0.27 (0.15, 0.42)	0.24 (0.09, 0.39)	0.22	0.23	0.32 (0.18, 0.46)	0.25	0.12		0.11 (0.04, 0.26)[a] 0.31 (0.22, 0.42)[b] 0.33 (0.23, 0.44)[c]

GW, gestational weeks; mo, months; T1, T2, T3, trimesters of pregnancy; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; BPA, bisphenol A; BP3, benzophenone-3; TCS, triclosan; MP, methyl-paraben; EP, ethyl-paraben; PP, propyl-paraben; BP, butyl-paraben.

[a] ICC across all time points (5 spot samples: one in each trimester of pregnancy, one at delivery, and one in post-partum); biomarker concentrations not measured for all compounds in T2, T3, delivery and post-partum samples.

[b] Within-day (weekday) ICC.

[c] Within-day (week-end day) ICC.

Table 2.3 – Publications on the variability of *phthalate metabolites* levels in biospecimens collected during pregnancy.

Reference	Cantonwine et al.[65]	Ferguson et al.[101]	Valvi et al.[314]	Adibi et al.[4]	Braun et al.[51]	Fisher et al.[103]	
Population	Puerto Rico PROTECT study	USA Boston	Spain Sabadell INMA cohort	USA New York CCCEH study	USA Massachusetts EARTH study	Canada P4 study	
Period	2010-2012	2006-2008	2004-2006	2001-2004	2004-2009	2009-2010	
No of women	139	129	401	28	137	80	
No of samples per woman	3/pregnancy	4/pregnancy	2/pregnancy	2-4/pregnancy	≥2 before pregnancy ≥2 during pregnancy	All urine voids for 1 weekday (T1) and/or 1 week-end day (T1) 1 spot (T2, T3, delivery, post-partum)	
Gestational age	18 GW, 22 GW, 26 GW	10 GW, 18 GW, 26 GW, 35 GW	13 GW, 34 GW	33 GW, 35 GW, 37 GW, 39 GW	5 GW, 20 GW, 33 GW	≤20 GW, 24-28 GW, 32-36 GW, delivery, 2-3 mo post-partum	
Remark	Uncorrected for urine dilution	Specific-gravity correction	Creatinine correction	Uncorrected for urine dilution	Specific gravity correction	Uncorrected for urine dilution	
ICC (95% CI)							
MEP	0.43 (0.33, 0.54)	0.47 (0.42, 0.52)	0.23	0.30	0.50	0.38 (0.27, 0.51)[a]	0.66 (0.57, 0.75)[b] 0.68 (0.59, 0.76)[c]
MiBP	0.35 (0.24, 0.47)	0.52 (0.48, 0.57)	0.20	0.54	0.38	ND[a]	0.37 (0.24, 0.53)[b] 0.41 (0.27, 0.57)[c]
MnBP	0.41 (0.30, 0.59)	0.57 (0.53, 0.62)	0.19	0.62	0.45	0.30 (0.19, 0.42)[a]	0.35 (0.25, 0.46)[b] 0.38 (0.28, 0.48)[c]
MCPP	0.23 (0.13, 0.37)	0.36 (0.31, 0.41)		0.44		0.21 (0.11, 0.36)[a]	0.21 (0.14, 0.32)[b] 0.36 (0.26, 0.47)[c]
MCNP	0.09 (0.03, 0.27)						
MCOP	0.29 (0.19, 0.41)						
MBzP	0.37 (0.27, 0.49)	0.61 (0.56, 0.65)	0.24	0.66	0.25	0.24 (0.14, 0.37)[a]	0.60 (0.50, 0.69)[b] 0.60 (0.51, 0.69)[c]
MEHHP	0.25 (0.15, 0.38)	0.21 (0.17, 0.27)	0.06	0.36		0.18 (0.10, 0.32)[a]	0.34 (0.25, 0.45)[b] 0.30 (0.21, 0.41)[c]
MEOHP	0.26 (0.16, 0.39)	0.19 (0.15, 0.25)	0.07	0.34		0.22 (0.13, 0.35)[a]	0.33 (0.24, 0.44)[b] 0.29 (0.20, 0.40)[c]
MECPP	0.20 (0.11, 0.35)	0.31 (0.26, 0.36)	0.19	0.33		ND[a]	0.49 (0.34, 0.63)[b] 0.18 (0.09, 0.34)[c]
MEHP	0.35 (0.24, 0.47)	0.30 (0.25, 0.35)	0.18	0.35	0.08	0.16 (0.08, 0.29)[a]	0.28 (0.19, 0.38)[b] 0.39 (0.29, 0.50)[c]

GW, gestational weeks; mo, months; T1, T2, T3, trimesters of pregnancy; ND, no data; MEP, monoethyl phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-n-butyl phthalate; MCPP, mono (3-carboxypropyl) phthalate; MCNP, mono-(carboxynonyl) phthalate; MCOP, monocarboxy-isooctyl phthalate; MBzP, monobenzyl phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate.

[a] ICC across all time points (5 spot samples: one in each trimester of pregnancy, one at delivery, and one in post-partum); biomarker concentrations not measured for all compounds in T2, T3, delivery and post-partum samples.

[b] Within-day (weekday) ICC.

[c] Within-day (week-end day) ICC.

2.3.4 Exposure measurement error

2.3.4.1 Measurement error in exposure assessment via exposure biomarkers

When within-subject temporal variations are high (as expected for short half-lived chemicals such as phenols and phthalates and described from the few available studies), relying on biomarker concentrations measured in a few number of biospecimens per subject (e.g. one to three) is likely to imperfectly represent the true exposure over time windows longer than a few hours, such as a day, a week, or the whole pregnancy.[54] Thus, within-subject variability in biomarker concentrations is likely to be a source of measurement error in epidemiological studies examining the effects of nonpersistent compounds such as phenols and phthalates.[59,67] This issue is of importance given that most epidemiological studies investigating human health effects of phthalates or phenols exposure generally rely on only one single (occasionally three) exposure measure. Since the true exposure of the subject (e.g., global exposure over the entire pregnancy) is only estimated through surrogates of exposure, i.e. biomarker concentrations measured in a few biospecimens, the exposure measurement error is expected to be of classical type (Eq 2.2).[59,67]

Classical measurement error model, additive

$$W = X + U, \text{ with} \tag{2.2}$$

W the surrogate of exposure (e.g., one spot measure of urinary biomarkers),
X the true exposure (e.g., the pregnancy average exposure),
U the error, independent of X.

Classical-type error corresponds to a situation in which biomarker concentrations measured in spot biospecimens vary randomly around the true unmeasured exposure, so that the true exposure can be approximated by the mean of many repeated measurements over the time window of interest.[293,59,67] Such an error is expected to result in a loss of precision (and hence of statistical power), and to bias the exposure-health outcome association estimates towards the null (attenuation bias).[59,67] The attenuation in regression estimates can be related to the within-subject variability through the attenuation factor, also called reliability ratio.[59,67,269] In simple linear regression, this attenuation factor corresponds to the ICC for the classical additive error model (detailed in Appendix C). For example, in a recent simulation-based study from Perrier et al.[242], relying on only one biospecimen to assess exposure in a classical-type error setting was shown to attenuate by 80% the true effect estimate for a chemical with high within-subject variability (ICC of 0.2). The attenuation bias was lower (40%), but still

important for compounds with moderate within-subject variability (ICC of 0.6). This study is detailed in Box 2.2.

2.3.4.2 Limiting exposure measurement error in epidemiological studies

One simple approach to reduce measurement error and increase reliability of the exposure average measured with biomarkers is to increase the number of biospecimens by repeating exposure assessment for each subject in the time window of interest.[67,256] Based on Perrier et al.[242], at least 35 biospecimens for highly variable compounds (ICC of 0.2), and six samples for moderately variable compounds (ICC of 0.6) are required to limit the bias in the effect estimate to 10% or less. When biomarkers are assessed in several biospecimens per subject in at least a subgroup of subjects, statistical methods such as simulation extrapolation (SIMEX)[81,124] and regression calibration[67,123] can be used to correct for measurement error. Although repeating the collection of biospecimens is efficient to reduce measurement error, this approach increases analytical costs if biomarkers are measured in each biospecimen.

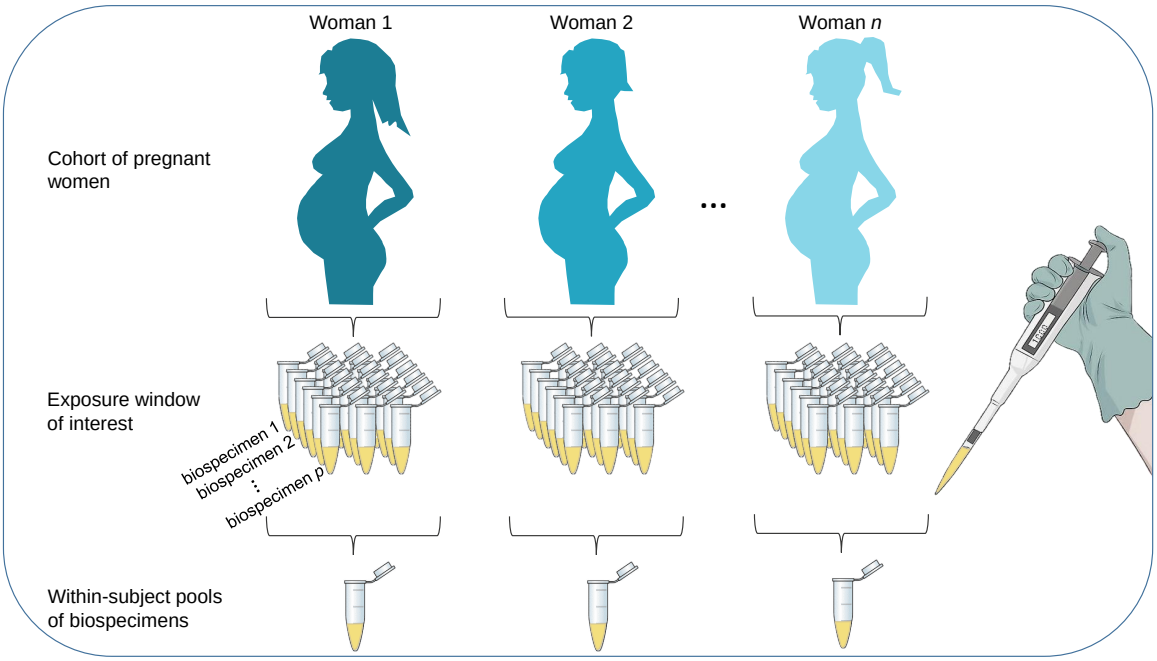


Figure 2.7 – Within-subject pooling of urine biospecimens.

Within-subject pooling of biospecimens

It is only recently that *within-subject pooling* approaches consisting in collecting several samples per subject over the time window of interest and pooling them within-subject prior to biomarker analysis (see Figure 2.7), have been proposed as cost-efficient strategies for exposure assessment.[70,129,271,242] Within-subject pooling benefits from the repeated sampling, but without increasing analytical costs since only samples pooled within-subject are assayed. The underlying hypothesis of within-subject pooling is that

the biomarker concentration measured in the pooled sample represents the average of concentrations from the spot samples over the corresponding time window (e.g., a week if all samples of a given week are pooled). This assumes, among others, that there is no chemical reaction in urine[242,271] and no influence of urine dilution (if a given volume is taken from each biospecimen). This approach appears to be theoretically efficient to decrease bias and increase statistical power.[242,336] Such an approach is used in the SEPAGES cohort, which included 484 parent-child trios recruited in Grenoble urban area at an early stage of pregnancy (before the 18th week of gestation)[1]. Although Perrier et al.[242] showed theoretically that within-subject pooling could limit misclassification bias (see Box 2.1), this approach has, to the best of our knowledge, never been applied on a large scale, and it leaves many practical questions open in terms of sampling design.

To conclude this section, phenols and phthalates are widespread in the environment, resulting in chronic and wide exposure of the general population, including pregnant women. Phenols and phthalates exposure is usually assessed through the measurements of exposure biomarkers in urines. Although these compounds are likely nonpersistent in human organisms, within-subject variability is not well characterized in pregnant women. This variability can be an important issue for epidemiological studies, and the within-subject pooling of biospecimens has been suggested to handle it, but is not tested empirically. Several phenols and phthalates can cross the placenta, which results in exposure of the offspring starting in the early stages of life. This may impact physiological functions with critical stages of development in the *in utero* period. Since the embryonic and fetal stages are of high importance for normal lung development and are controlled by the hormonal and immune systems, this thesis is focused on respiratory health as one example of physiological functions which may be adversely affected by these two families of EDs. This aspect is developed in the next section (Section 2.4).

1. http://sepages.inserm.fr/en/home/

Box 2.2 – The within-subject pooling of biospecimens, Perrier et al.[242]

The aim of this simulation study was to characterize the ability of within-subject pooling of biospecimens to reduce bias due to exposure measurement error for chemicals with high within-subject variability in biomarker concentrations

Methods

Two chemicals were considered, with distinct ICCs: 0.6 (moderate within-subject variability, chemical **A**) and 0.2 (high within-subject variability, chemical **B**). Assuming a classical additive measurement error model, they generated X the true exposure, $X \sim \mathcal{N}(0, \sigma_x^2 = 1)$.
Then, for each subject $i (i = 1, ..., n = 3,000)$, they generated 1 to 50 biospecimens, with biomarker concentrations (W_{ij}), corresponding to error-prone measures of X_i. W_{ij} were affected by the within-subject error $U_{ij} \sim \mathcal{N}(0, \sigma^2_{U_{within}})$, assumed to be due to the within-subject variability, creating random variations around the true value. The variance of the error term U_{ij} was calculated as

$$\sigma^2_{U_{within}} = \sigma_x^2\left(\frac{1}{ICC} - 1\right) \tag{2.3}$$

Then, they simulated continuous health outcomes Y_i for each subject i as

$$Y_i = \alpha + \beta_1 X_i + \epsilon, \tag{2.4}$$

with $\beta_1 = -100$g the effect of the true exposure X (biomarker urinary concentrations averaged over a given time period), i.e., the true effect; ϵ the random error $\sim \mathcal{N}(0, \sigma_\epsilon^2)$; $\alpha = 14,900$g and the standard deviation of ϵ(1,500g). $\beta_1 = 0$ was considered to characterize the impact on the risk type I error. They characterized the associations between the health outcomes and the concentration measured in the pool. This concentration was assumed to be the mean of individual samples. Binary outcomes were also simulated. Finally, statistical power and bias were estimated. Bias was estimated as

$$Bias = \frac{\beta_1 - \beta_{obs}}{\beta_1}, \tag{2.5}$$

with β_{obs} the observed effect estimate with the pooling method.

Several approaches to correct for measurement error were tested:

— *a posteriori* disattenuation. For a given number k of biospecimens in the pool:

$$\hat{\beta}_{corr} = \hat{\beta}_{obs}\left(\frac{k-1}{k} + \frac{1}{kICC}\right), \tag{2.6}$$

— Two measurement error models: Regression calibration and simulation extrapolation SIMEX.

Main results

For linear and binary outcomes, using 1 sample led to attenuation bias in the association estimate (40% for ICC=0.6; 80% for ICC=0.2), but did not increase the risk of type I error. Power was also reduced (71 and 32%, respectively for chemicals **A** and **B**). With the pooling method, increasing the number of samples in the pool increased power. Compared with pooling, correction methods did not improve power. To limit bias ≤10%, 6, 2, 2, and 1 biospecimens were required when ICC=0.6 with the pooling, SIMEX, regression calibration and pooling followed by disattenuation methods, respectively. When ICC=0.2, these values were greater: 35, 8, 2, 1 samples.

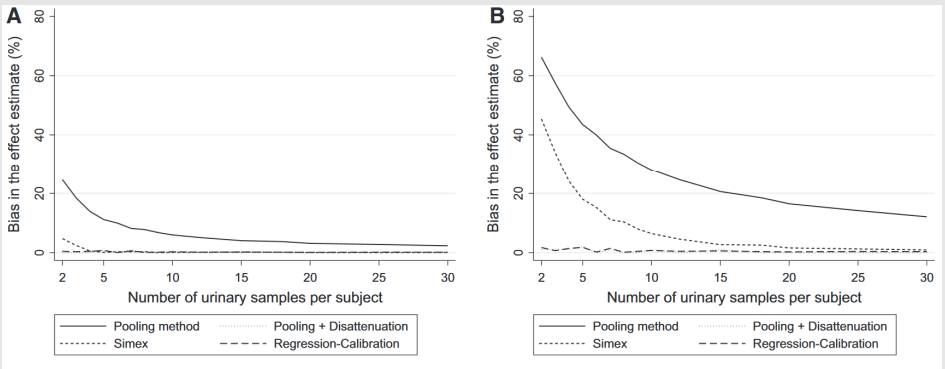


Fig 2.1 – Bias in the health effect estimate (%) according to the number of biospecimens collected per subject to assess exposure (1,000 simulations of studies with n=3,000 subjects) for Chemicals **A** (ICC=0.6) and **B** (ICC=0.2). Continuous health outcome with $\beta_1 = -100$g.

2.4 Respiratory health

Respiratory diseases are a global public health problem, currently responsible for a high social and economic burden worldwide. Asthma is a complex disease characterized by bronchial hyperresponsiveness to inhaled stimuli (irritants, aeroallergens) or exercise, and chronic airway inflammation, resulting in recurrent respiratory symptoms that vary over time, such as wheezing and coughing episodes (predominantly at night), chest tightness and breathlessness, and airflow obstruction.[222,133] Today, more than 300 million of people in the world are suffering from asthma and over recent decades, asthma has become one of the most frequent chronic disease in childhood, affecting around 10% of children in western countries.[5,133,114] Direct (e.g., hospital and physician visits, drugs) and indirect (including work disability and management of comorbidities) economic costs are considerable.[5,94,233] Pediatric morbidity and mortality associated with childhood lower tract respiratory infections (e.g., pneumonia, atypical pneumonia, bronchitis, and bronchiolitis) is worse in the lowest-income and developing countries but morbidity is still high in developed countries[159,185] and these illnesses are associated with chronic respiratory diseases such as asthma later in life.[199] There is growing evidence that chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD) have their origin in the early stages of life.[5,133,114,197,66]

2.4.1 Early-life origins of respiratory diseases

Pre- and postnatal lung development

Intra-uterine life is a critical period for development. As for many other vital functions, major developmental steps occur *in utero* for the lungs. The respiratory system starts its structural and functional development during the embryonic stage by formation of the trachea, the main bronchi and the lobes and lobules, followed by multiple fetal stages to achieve its general structure at birth with the branching of the conducting airways (pseudoglandular stage); the development of the respiratory airways with acinar structures, the production of surfactant (canalicular stage); and finally the alveolar formation and maturation (saccular-alveolar stage). Development continues through lung growth and expansion with the multiplication of alveoli (areas for gas exchanges) in early childhood, which will complete around 20 years of age (see Figure 2.8 and Stocks et al.[299] for details about the process).

This prenatal morphogenesis process is tightly regulated, particularly by the endocrine and immune systems. During specific developmental windows, precise structural and functional modifications of tissues are controlled by hormonal signaling. Thus, during these critical windows, and due to immaturity of the lungs, the immune system and other developmental events, the respiratory system is particularly

vulnerable and could be impaired *in utero* by both endogenous or exogenous factors, with possible long-term consequences in childhood, and later in adult life.[207,66,197] This is coherent with Barker's hypothesis, now known as the hypothesis of Developmental Origins of Health and Diseases (DOHaD).

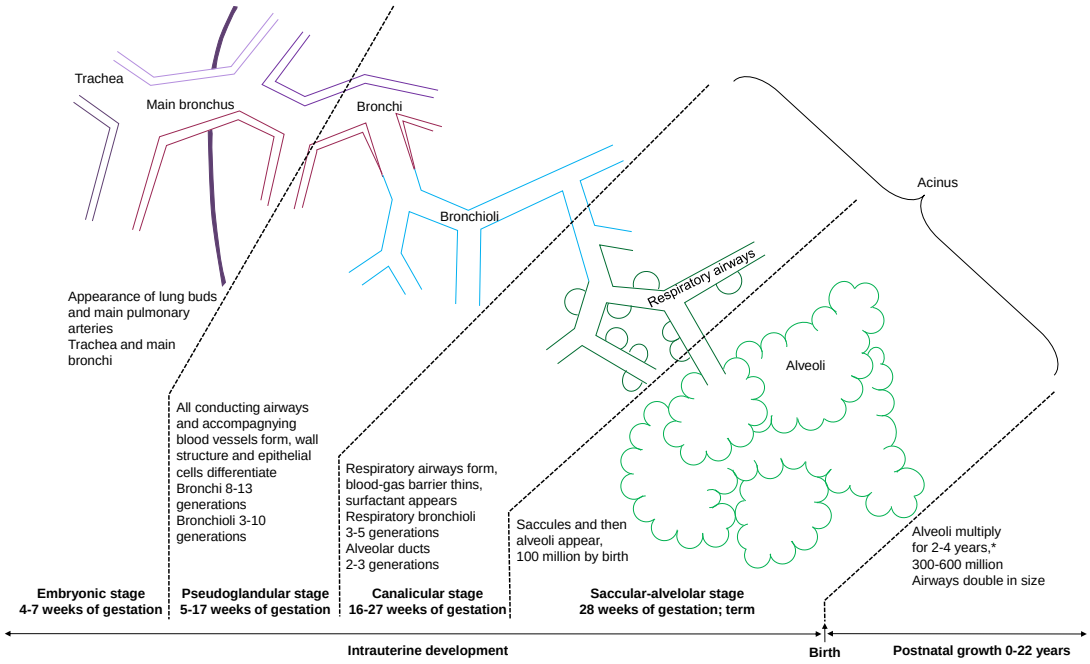


Figure 2.8 – Formation of airway and parenchymal structures during prenatal and postnatal lung development. Adapted from Stocks et al.[299].
*Although alveoli are thought to be all formed by 2-4 years of age, alveoli might have the capability to multiply beyond this age. Timings are approximate, with some overlap between the different stages.

Developmental Origins of Health and Diseases (DOHaD): implications for respiratory health

This hypothesis suggests that adverse environmental conditions occurring during early-life development and especially during fetal life, when organs and systems have most plasticity, can directly affect the susceptibility to diseases by fetal or early-life programming, i.e., by permanently changing the structure, metabolism, and physiologic functions of organs.[25,23]

Many epidemiological studies provide support to the DOHaD hypothesis in the context of respiratory health (see Martinez[197], Carraro et al.[66], Duijts et al.[92], for review). For example, pediatric respiratory illnesses, including acute lower respiratory infections and bronchopulmonary dysplasia, have been associated with a deficit in lung function, and an increased risk of chronic obstructive respiratory diseases such as asthma later in life.[24,61,197] Additionally, reduced lung function in childhood, and previous to the appearance of respiratory symptoms, has also been associated with a permanent lung function deficit in young adults and an increased risk of developing a chronic obstructive disease over the life course.[198,267,296,197,66,92]

Prenatal risk factors of childhood respiratory impairment

Endogenous factors include genetically predisposition factors inherited from parents, inducing a higher risk of allergic sensitization, lower lung function; which are known as risk factors of wheezing and asthma in later life.[356,212,134,250,288] Sex and ethnicity are other endogenous factors of susceptibility.[32]

Additionally, preterm delivery and respiratory distress syndrome at birth highly increase the risk of childhood asthma, wheezing and life-long respiratory diseases, including COPD, and a reduced lung function in adult life[290,323] (see Martinez[197], Carraro et al.[66], Duijts et al.[92] for review).

In addition to endogenous factors and birth events, *in utero* exposure to exogenous substances can also impact respiratory health in the offspring. A positive example is the administration of corticoids to the mother to reduce the risk of infant respiratory disease, which accelerates fetal production of surfactant in the lungs.[43,58] Among deleterious agents, it is now indisputable that maternal smoking during pregnancy is associated with increased risk of incident asthma and wheezing in childhood.[298,60,235,197,66,92] There is also growing evidence that air pollution in the prenatal period may play a role in the development of respiratory diseases,[181,319] but effects of prenatal and postnatal exposures to air pollution are difficult to distinguish.

Lung development being highly hormone-dependent, respiratory defects due to environmental EDs exposure, capable of crossing the human placenta (see Section 2.2) at critical developmental windows, is a relevant hypothesis, and could explain part of the asthma burden in the last decades. Among these chemicals, several phenols and phthalates are suspected.[207,45]

2.4.2 Prenatal exposure to phenols and phthalates and respiratory health

2.4.2.1 Biological plausibility

Experimental studies have suggested several biological mechanisms, by which *in utero* exposure to phenols and phthalates could adversely affect respiratory health in childhood.

Phthalates

Phthalates are suspected to modulate the immune system resulting in proallergic effects. Some phthalates such as DEHP, DnBP, DINP, may modify the balance between T-helper type 1 (Th1) and T-helper type 2 (Th2) cells, which is important in allergic

processes.[348] Disturbed Th1/Th2 balance is a major mediator of the allergic airway inflammation observed in the pathogenesis of asthma.[203] Studies conducted both *in vivo* and *in vitro* reported an adjuvant effect of these phthalates on Th2-differentiation following exposure through diet, inhalation or subcutaneous injection, and consequently on Th2-dependent production, as inflammatory cytokines and chemokines; interleukin 4 (IL4), immunoglobulin E (IgE), and G1 (IgG1).[120,77,122,266,183] It means that phthalates may act as adjuvants, inducing allergic sensitization after exposure to allergens, and might lead to allergic asthma later in life.[179,348] Some toxicological studies were reviewed in Bornehag and Nanberg[45].

Another possible mechanism involved is the modulation of inflammatory responses. DEHP, DnBP and DiBP exposures have been associated with an increase of the recruitment of eosinophils, the production of interleukin 5 (IL5) and antigen-induced degranulation in mast cells of rats. This might result in a phthalate-dependent infiltration of inflammatory cells in lung tissue, leading to the inflammation of pulmonary tissue or bronchial eosinophilic inflammation which may enhance airway hyperresponsiveness.[219,266,207,254]

Oxidative stress has been suggested as a potential biological mechanism in adverse health effects in the case of other environmental contaminants, such as air pollution.[72,332] Oxidative stress induced by phthalates has been reported in several studies, including studies among pregnant women.[355,99,102,100] Additionally, phthalate exposure might also induce oxidative stress directly in lung cells, as reported in elderly and adolescent populations.[234,105,328]

Asthma-promoting effect of some phthalates (e.g. DEHP and BBzP) may be mediated through altered DNA methylation in the offspring. In a recent study, BBzP prenatal exposure has been found to induce global DNA hypermethylation in the offspring, resulting in a reduced expression of several genes involved in the Th2 cell differentiation.[146] Also, a lower DNA methylation in the tumor necrosis factor α (TNFα) gene has been suggested by a recent study to mediate part of the association between DEHP exposure and asthma.[329]

The action of phthalates could be hormonally mediated, since estrogens or sex steroidal hormones are involved in the control of the respiratory system,[31] with therefore possible sex-specific effects. For instance, a study examining the effects of maternal exposure to DEHP in mice offspring suggested effects on allergic immune response only in male newborns.[348]

The action of phthalates may not be limited to estrogen or androgen-sensitive systems, since several phthalates may produce their effect through interaction with the peroxisome proliferator-activated receptors (PPARs) α or γ,[44,190] potentially leading to abnormal alveolar maturation and reduced surfactant production.[207] PPAR-α and

PPAR-γ are also involved in the control of the immune system by both pro- and anti-inflammatory effects.[357,358,80]

Phenols

Little is known about the possible effects of phenols on respiratory health. The plausible hypotheses mainly rely on the experimental evidence available for bisphenol A.

Experimental studies showed that prenatal exposure to bisphenol A might modulate the immune system by increasing induced Th2-polarization and increasing the IgE serum levels and the production of proallergic mediators such as the cytokine IL4 (several studies reviewed in Kwak et al.[176], Yan et al.[347], Rogers et al.[260]). In another study in mice, prenatal exposure to bisphenol A through drinking water promoted the development of postnatal allergic sensitization and allergic asthma in offspring.[218]

Moreover, prenatal exposure to bisphenol A was associated with bronchial eosinophilic inflammation and airway hyper-responsiveness in mice, suggesting a potential negative effect in lung function in humans through a proinflammatory action.[206]

As for phthalates, the immune effects might be mediated by hormonally-induced interactions between bisphenol A and estrogen and androgenic receptors or the PPARs family.[207,260] As most other phenols have also weak estrogenic or (anti)androgenic activities,[52,228,226,238,85,41,76,83,84,300,359,339,275,194,304] they might induce similar effects on respiratory health.

2.4.2.2 Plausibility from epidemiological studies

Few longitudinal studies focused on the prenatal exposure window. These studies are summarized in Table 2.4.

Phthalates

For phthalates, studies investigating the prenatal exposure to BBzP through its monoester metabolite MBzP reported deleterious effects on respiratory health, with an increased risk of current asthma or history of asthma in children between 5 and 11 years of age,[110,338] as well as an increased risk of wheezing.[110,173] Prenatal exposure to DEHP (via one of its oxidative metabolite MEHHP) was not associated with wheezing or asthma among 300 children[338] whereas the sum of several DEHP metabolites was associated with increased risk of wheeze in two studies.[110,173] Gascon et al.[110] also reported increased risks of bronchitis, asthma and a trend of higher risk of chest infections. Relying on DEHP metabolites assessed in a maternal prenatal blood sample, Smit et al.[286] did not find evidence for an association with asthma in 1,024 children aged between 5 and 9 years old, but found increased odds of current wheezing in the Ukrainian cohort (n=492). The most recent study (n=371 mother-child pairs)[146] did not observe any

Table 2.4 – Epidemiological studies on phenols and phthalates exposure during pregnancy and respiratory health in offspring (restricted to prospective cohort studies).

Reference, Location and period	Study Population (n)	Chemical(s)	Biospecimen(s)	Health outcome(s)	Model(s) Main Results (95% CI)	Main adjustment factors
Spanier et al. (2012), [285] Cincinnati OH metropolitan area, USA 2003-2006	HOME study Mother-child pairs (n=365)	BPA (Total) <LOD replaced with LOD/√2 Standardized for creatinine and ln-transformation	3 maternal spot urine samples (16, 26 GW, birth)	Wheeze: 6-, 12-, 18-, 24-, 30-, 36-months Phone and home visits, NHANES wheeze question "Has [child's name] had wheezing or whistling in his/her chest in the last 6 months?"	Adjusted OR (95% CI) from Generalized Estimating Equations (GEEs) with a logit link to account for repeated outcome measurements 16w, 26w, birth BPA concentrations and mean BPA concentration were not associated with wheeze over the 3-yr period. High BPA concentration (> median) associated with wheeze at 6 months: 2.27 (1.28, 4.06). No association with later wheeze.	Maternal allergy, mean prenatal serum cotinine concentration, survey time point (categorical), season, and intervention group
Donohue et al. (2013), [86] Whyatt et al. (2014b) South Bronx, Northern Manhattan, NY, USA 2001-2010 African American and Dominican women	CCCEH cohort Prenatal exposure: Mother-child pairs (n=375) Postnatal exposure: n=408 3yr n=401 5yr n=318 7yr	BPA (Total) Log-transformed	1 maternal spot urine sample (34 GW) 3 spot urine samples in children: 3, 5, 7 yr	IgE at 7yr Atopic if IgE≥35 UI/mL Wheeze: 5-, 6-, 7-yr from ISAAC questionnaires Current Asthma (5-12 yr): 5-, 6-, 7-, 9- and 11-yr from ISAAC questionnaires, + physician examination (includes pre/postbronchodilator spirometry) + FeNO test (7-11 yr) if no cold symptoms at the visit	Adjusted OR (95% CI) from logistic regressions (wheeze, asthma, atopy), and adjusted beta (95% CI) from linear regressions (FeNO) Prenatal BPA: Wheeze 5yr: 0.7 (0.5, 0.9) protective association No association with wheeze at 7 yr, or 9 yr and no association with asthma: 0.8 (0.5, 1.1) Response to bronchodilator: 0.6 (0.4-0.9) FeNO and atopy: no association. All postnatal BPA concentrations were associated with increased risks of wheeze and asthma and increased FeNO (7yr only). Postnatal BPA increased risk of asthma and other wheeze-related symptoms among children with higher but not lower prenatal MBzP concentrations.	Maternal asthma, environmental tobacco smoke exposure, sex, race/ethnicity, and urine specific gravity asthma analysis: additionally controlled for age at physician examination
Whyatt et al. (2014), [330] South Bronx, Northern Manhattan, NY, USA 2001-2010 African American and Dominican women	CCCEH cohort Prenatal exposure: Mother-child pairs (n=300) Postnatal exposure: n=216 3yr n=270 5yr n=154 7yr	MEHHP, MBzP, MnBP, MEP BPA < LOD replaced with LOD/2 Ln-transformed	1 maternal spot urine sample (34 GW) 3 spot urine samples in children: 3, 5, 7 yr	History of asthma-like symptoms : 5-, 6-, 7-, 9-, 11-yr from ISAAC questionnaires and Brief Respiratory Questionnaire Current Asthma (5-12 yr): 5-, 6-, 7-, 9- and 11-yr from ISAAC questionnaires, + physician examination (includes pre/postbronchodilator spirometry).	Adjusted RR (95% CI) per ln-unit increase from Poisson regression with robust standard error estimation (Generalized Estimating Equations) MBzP / MnBP History of asthma-like symptoms: 1.12 (1.01, 1.24) / 1.16 (1.02,1.33) Current asthma: 1.17 (1.01, 1.35) / 1.25 (1.04, 1.51) History of asthma-like symptoms and no current asthma: 1.33 (1.05, 1.70) / 1.17 (0.98, 1.41) No association with MEHHP or MEP.	Maternal asthma, household smoke exposure, maternal prenatal BPA, maternal prenatal demoralization, urine specific gravity, and child age at physician examination
Spanier et al. (2014), [284] Cincinnati, OH metropolitan area, USA 2003-2006	HOME study Mother-child pairs FEV_1 (n=155 4yr, n=193 5yr) Wheezing (n=360)	BPA < LOD replaced with LOD/√2 Standardized for creatinine and log_{10}-transformed	2 maternal spot urine samples (16, 26 GW) Children: 1 urine sample each year	FEV_1 (percent predicted) (spirometry) Wheeze: every 6 months until 5 yr from NHANES questionnaire	Adjusted beta (95% CI) from linear regression Mean maternal BPA concentration FEV_1% 4yr: -14.2 (-24.5, -3.9) FEV_1% 5yr: 0.04 (-9.04, 9.12) Adjusted OR (95% CI) from Generalized Estimating Equations (GEEs) with a logit link Wheezing: 1.55 (0.91, 2.63) Postnatal BPA: no association with FEV1% or wheeze.	Prenatal tobacco exposure, season, breastfeeding history, family history of asthma, family history of allergy, child eczema, child allergy, birth weight, maternal parity, pet ownership, and cockroach exposure
Smit et al. (2015), [279] Greenland, Ukraine, Poland 2002-2004	INUENDO cohort Mother-child pairs (n=1024) n= 492 Ukrainian n=532 Greenlandic mother-child pairs	MEHHP, MEOHP, MECPP, MHiNP, MOiNP, MCiOP < LOD: single imputation from a log-normal probability distribution	1 maternal blood (serum) sample	Ever/current wheeze (5-9 yr) Ever asthma (5-9 yr) Ever/current eczema (5-9 yr) from ISAAC questionnaires	Adjusted OR (95% CI) from multiple logistic regression models in each population and standard fixed-effects meta-analysis for the overall effect. DEHP metabolites associated with increased odds of current wheeze in Ukrainian children: 1.56 (1.03, 2.37) Meta-analyses: DiNP metabolites associated with lower odds of current eczema. No association with the other phthalates.	Maternal allergy, maternal education, maternal smoking, maternal age, child sex, child age at follow-up, gestational age at blood sampling, breastfeeding, birthweight

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Table 2.4 – Continued

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Reference, Location and period	Study Population (n)	Chemical(s)	Biospecimen(s)	Health outcome(s)	Model(s) Main Results (95% CI)	Main adjustment factors
Gascon et al. (2015), [105] Sabadell, Spain 2004-2008	INMA cohort Mother-child pairs BPA: n= 462 (n=175 with IgE measurement) Phthalates: n=391 (n=176 with IgE measurement)	BPA (Total) MEHHP, MEHP, MEOHP, MECPP, MBzP, MEP, MiBP, MnBP Sum of DEHP metabolites Sum of LMW phthalates Sum of HMW phthalates < LOD: multiple imputation Log2 transformation	2 maternal spot urine samples (12, 32 GW)	Wheeze: 6-, 14-months and 4- 7-yr Chest infections, bronchitis and eczema: 6-, 14-months and 4- 7-yr Asthma: 7-yr Interviewer-led questionnaires IgE levels at 4-yr	Adjusted RR (95% CI) from Generalized Estimating Equations (GEEs) (wheeze, chest infections, bronchitis) and logistic regressions (asthma) BPA: No association with eczema or atopy. Wheeze 1.20 (1.03, 1.40) Chest infections 1.15 (1.00, 1.32) Bronchitis 1.18 (1.01, 1.37) Asthma 1.21 (0.94, 1.57) Higher RR with 2nd sample sumDEHP: No association with eczema or atopy. Wheeze 1.25 (1.04, 1.50) Chest infections 1.14 (0.97, 1.35) Bronchitis 1.20 (1.01, 1.43) Asthma 1.38 (1.05, 1.82) MBzP: No association with respiratory infections, eczema or atopy. Wheeze 1.15 (1.00, 1.33) Asthma 1.26 (1.01, 1.82) Higher RR with 1st sample (DEHP, MBzP) LMW phthalates not associated with any outcomes.	Maternal education, number of siblings, and maternal smoking during pregnancy, and phthalate models were additionally adjusted for maternal history of asthma/allergy and maternal body mass index.
Ku et al. (2015), [166] Taiwan 2000-2001	TMICS cohort Mother-child pairs (n=136)	MEHP, MBzP, MBP, MEP, Sum of DEHP metabolites Standardization for creatinine and log_{10}-transformation	1 maternal spot urine sample (3rd trimester) 3 urine samples in children: 2, 5, 8 yr.	Wheezing history 8 yr Doctor-diagnosed Asthma 8 yr from ISAAC questionnaires IgE levels 8 yr	Adjusted OR (95% CI) (wheeze, asthma) or adjusted beta (95% CI) (IgE) for the upper quintile compared to the reference group from logistic and linear regression models Prenatal concentrations: sumDEHP associated with wheezing: 3.12 (0.98, 9.98), higher odds in boys. No association with asthma MEHP associated with increased IgE in allergic children MBzP associated with wheezing in boys only: 4.95 (1.08, 22.63) and with increased IgE in both sexes Other phthalates not associated with wheeze, asthma nor IgE. Postnatal concentrations: MEHP associated with increased risk of asthma in boys only: 6.14 (1.17, 32.13) sumDEHP and MEP at 5 yr associated with increased risk of asthma, greater risk in boys compared with girls.	Symptoms analysis: parental allergies and family members' smoking status. IgE analysis: sex and parental allergies.
Jahreis et al. (2017), [139] Leipzig, Germany, 2006-2008	LINA cohort Mother-child pairs (n=371)	MEP, MiBP, MnBP, MCPrP, MBzP, MEHP, MEHHP, MEOHP, MECPP, BPA Standardization for creatinine	1 maternal spot urine sample (34 GW)	Doctor-diagnosed Asthma 6 yr from questionnaires IgE in children	Adjusted OR (95% CI) from logistic regression models OR (95% CI) for 100 ng/mg increase: Asthma IgE MnBP 1.24 (1.02, 1.50) 1.21 (1.04, 1.41) MEP 1.27 (0.98, 1.64) 1.14 (1.02, 1.26) No association for the other phthalates and BPA.	Gender, siblings, smoking during pregnancy, environmental tobacco smoke exposure after birth, cat keeping, parental history of atopy, parental educational level

association with doctor-diagnosed asthma in the first six years of life [2]. DnBP prenatal exposure assessed through MnBP measurement in one maternal spot urine sample increased significantly the odds of asthma in two mother-child cohorts (follow-up until 11 years of age, n=300 mother-child pairs;[338] and 420 mother-child pairs followed up until age 6 years,[146]) whereas DnBP prenatal exposure assessed through the mean MnBP concentration of two urine samples was not associated with respiratory symptoms in the first seven years of life.[110] DEP prenatal exposure assessed via measurement of MEP in spot urine sample(s) was not associated with respiratory symptoms and atopy in three studies at ages seven, eleven and eight, respectively,[110,338,173] but Jahreis et al.[146] observed increased asthma odds at 6 years of age and higher IgE levels.

No prospective study has investigated the impact of prenatal exposure to phthalates on lung function. Whyatt et al.[338] used pre/postbronchodilator spirometry but only as a criteria for diagnosis of current asthma. Two cross-sectional studies have investigated associations of several phthalates and pulmonary function. Cakmak et al.[63] reported deleterious associations of spirometric measurements (forced Expiratory Volume in 1 second (FEV_1), forced vital capacity (FVC), and the FEV_1 / FVC ratio) with DnBP, BBzP, and DEHP metabolites among 3,071 individuals, but only DnBP metabolites were associated with reduced lung function parameters in children of 6-16 years old (n=1,642). The associations were stronger in males. Hoppin et al.[138] observed reduced FEV_1 and FVC for increases in MBP but did not report an association with MEHP (DEHP metabolite) or MBzP (BBzP metabolite).

Phenols

The epidemiological literature studying the associations between prenatal exposure to phenols and respiratory outcomes is very scarce and is only focused on bisphenol A (studies summarized in Table 2.4). Regarding associations between prenatal bisphenol A exposure and questionnaire-based respiratory outcomes, Donohue et al.[91] reported a protective association of prenatal bisphenol A with wheezing between 5 and 7 years of age (n=375 children), whereas two other studies with bisphenol A assessed from two maternal urine samples during pregnancy reported trends of increased risk of wheezing until 5 (n=360 mother-child pairs[292]) and 7 years of age (n=462 mother-child pairs[110]). Additionally, Gascon et al.[110] observed deleterious associations with asthma at age 7 and respiratory infections but two other studies did not report association with asthma during the 6-year follow-up (n=420 mother-child pairs[146]), and between 5 and 11 years of age (n=375 mother-child pairs[91]).

Only one study in 208 children aimed at studying the effects of prenatal exposure to bisphenol A on lung function.[292] An increase in prenatal bisphenol A concentration

2. This article was published after the acceptation of the article in this thesis and was therefore not discussed in Chapter 4

was associated with a decrease in FEV_1 in percent predicted (FEV_1%) at 4 years of age (beta, -14%; 95% confidence interval, CI: -25, -4 for an increase by one $\log_{10}$ unit), which totally disappeared at 5 years of age, with a beta of 0.04% (95% CI, -9, 9).

To our knowledge, no prospective study investigated the effects of prenatal exposure to other phenols on respiratory health. A cross-sectional study in 837 children aged 6-18 years investigated the effects of triclosan and parabens on allergen sensitization, wheeze in the past 12 months, and doctor-diagnosed asthma.[291] Higher triclosan concentrations were associated with increased odds of asthma and wheeze, but only in atopic children, and methylparaben decreased the odds of nonatopic wheeze. In 623 10-year-old Norwegian children, increases in triclosan led to increased odds of allergic sensitization, current rhinitis but no association was observed with current asthma.[36] Regarding dichlorophenols, in a NHANES-based cross-sectional study of children aged 6 years or more (n=2,211 children), no association was reported in non-atopic wheezers, while higher 2,5-dichlorophenol concentrations were significantly associated with doctor-diagnosed asthma, and higher 2,4-dichlorophenol levels were associated with more wheezing morbidity in atopic wheezers.[150] Both chemicals were associated with increased levels of at least one allergen-specific IgE.

Overall, only few prospective studies aimed at investigating the effects on respiratory health of fetal exposure to phenols and phthalates. Comparability of results is limited by discrepancies in the postnatal follow-up (e.g., years of follow-up, criteria for asthma diagnosis), and the metabolites assessed for phthalate exposures. Additionally, the limited number of samples used for the exposure assessment may lead to strong exposure misclassification (see Section 2.3.4). Except for bisphenol A, no study sought to evaluate the impact on the lung function despite landmark articles, which demonstrated early decrements in lung function parameters before any respiratory symptoms.[198,267,296]

To summarize this chapter, we have presented phenols and phthalates, two families of nonpersistent EDs; the assessment of such chemicals in epidemiological studies with its main issues; and the possible impact of prenatal exposure to these environmental agents on respiratory health. This raises several research questions:

— What is the impact of *in utero* exposure to phenols and phthalates on childhood respiratory health? (Compounds with the strongest *a priori* hypotheses of an adverse effect on respiratory health are bisphenol A, DEHP, and, to a lesser extent, DnBP, BBzP and DINP).

— Can we better characterize the within-subject variability of these compounds in pregnant women?

— How does this variability impact dose-response functions in epidemiological studies?

— Is the within-subject pooling approach efficient in practice?

The aims of the thesis, developed to address these specific questions, are presented in the next chapter.

Chapter 3

Objectives of the thesis

3.1 Thesis subject

The detailed aims of this thesis (see Figure 3.1) are:

1. To investigate the potential effects of in utero exposure to phenols and phthalates on respiratory health in childhood (**Aim 1**, Chapter 4):
   (a) On the onset of wheezing, asthma, and bronchiolitis or bronchitis episodes until age 5 years;
   (b) On the pulmonary function at 5 years of age.
2. To characterize the within-subject temporal variability of phenol biomarkers in urine of pregnant women for different time periods (**Aim 2**, Chapter 5):
   (a) within a day;
   (b) between days within a week;
   (c) over the whole pregnancy (between specific weeks).
3. To empirically validate the within-subject biospecimens pooling approach (**Aim 3**, Chapter 6):
   (a) By evaluating the efficiency of a *degraded* within-subject biospecimens pooling design relying on the collection of three repeated daily spot urine samples. This degraded design was compared to the *ideal* approach consisting in collecting all the urine biospecimens to assess the average exposure over specific time periods of pregnancy;
   (b) By characterizing the bias and statistical power in dose-response functions due to the empirically observed specific measurement error structure of select urine phenol biomarkers of exposure.

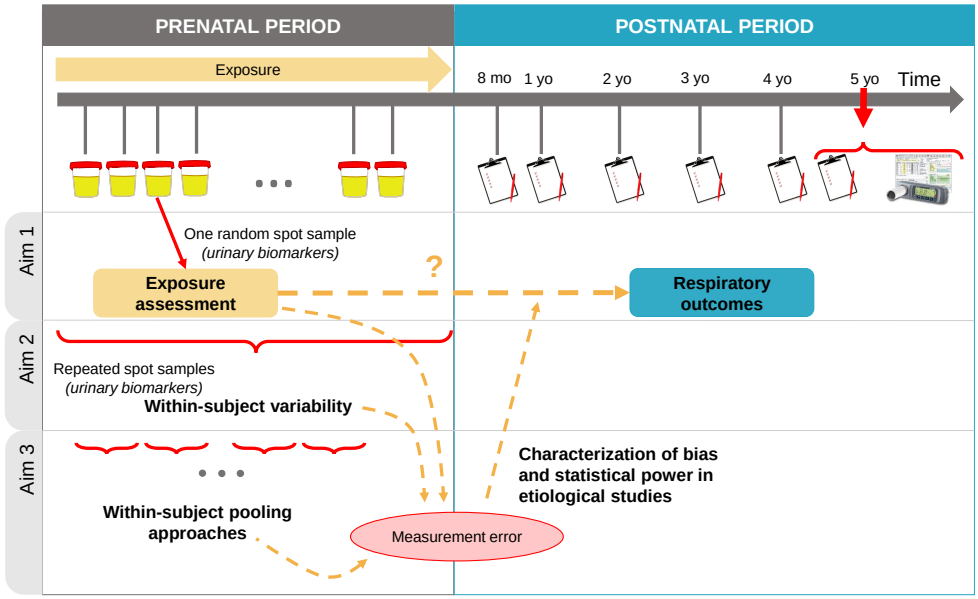

Figure 3.1 – Overview of the aims of the thesis

3.2 Contributions

Celine Vernet wrote the drafts of the articles (Chapters 4, 5 and 6), conducted all the statistical analyses (Chapters 4, 5 and 6), drew the draft of the protocols for studies relying on the SEPAGES-feasibility cohort (Chapters 5 and 6), prepared the pooled samples (Chapters 5 and 6), and took part in the data management of the phenols dataset for the SEPAGES-feasibility study (Chapters 5 and 6). Publications and communications are listed in Appendix D.

Chapter 4

In utero exposure to select phenols and phthalates and respiratory health in five-year-old boys: a prospective study

Céline Vernet, [1] Isabelle Pin, [1,2] Lise Giorgis-Allemand, [1] Claire Philippat, [1] Meriem Benmerad, [1] Joane Quentin, [1,2] Antonia M. Calafat, [3] Xiaoyun Ye, [3] Isabella Annesi-Maesano, [4] Valérie Siroux, [1] * Rémy Slama, [1] * and the EDEN mother-child cohort study group.

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Short running title:
In utero EDs exposure and respiratory health

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The EDEN Mother-Child Cohort Study Group includes: I. Annesi-Maesano, J. Botton, M.-A. Charles, P. Dargent-Molina, B. de Lauzon-Guillain, P. Ducimetière, M. de Agostini, B. Foliguet, A. Forhan, X. Fritel, A. Germa, V. Goua, R. Hankard, B. Heude, M. Kaminski, B. Larroque, N. Lelong, J. Lepeule, G. Magnin, L. Marchand, C. Nabet, R. Slama, M.J. Saurel-Cubizolles, M. Schweitzer and O. Thiebaugeorge.

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Conflicts of interest: none

4.1 French summary

Introduction

Certains phénols et phtalates sont suspectés d'être des perturbateurs endocriniens chez l'animal et chez l'Homme. Des études in vitro et in vivo chez différentes espèces de rongeurs ont suggéré des effets pro-inflammatoires, des effets sur le système immunitaire par la modulation de la production de cytokines. Ces modifications pourraient affecter le système respiratoire notamment après une exposition périnatale, i.e. pendant la gestation ou pendant la période néonatale. Chez l'Homme, très peu d'études se sont intéressées à l'impact d'une exposition in utero à ces substances sur la santé respiratoire, ce qui ne permet pas de conclure.

Objectif

Notre but est de caractériser les effets de l'exposition aux phénols et aux phtalates au cours de la grossesse sur la santé respiratoire des enfants mâles.

Méthodes

Notre étude est basée sur les 587 femmes enceintes de la cohorte mères-enfants EDEN qui ont eu un dosage de 9 phénols et 11 phtalates dans un échantillon d'urines recueilli au cours de la grossesse, et qui ont rempli au moins un questionnaire dédié à la santé respiratoire de leur enfant après sa naissance. Les dosages ayant été réalisés uniquement chez les mères de garçons inclus dans le cadre d'études précédentes, notre étude est centrée sur les enfants de sexe masculin. Les informations sur les symptômes respiratoires étaient recueillies par questionnaires remplis par les parents pendant les 5 premières années de vie. Au cours de la cinquième année de vie, les enfants ont réalisé un test de la fonction pulmonaire par spirométrie nous permettant d'analyser le Volume Expiratoire Maximum par Seconde (VEMS) chez 228 garçons. Les associations entre l'exposition prénatale aux composés des deux familles et l'apparition de symptômes respiratoires d'après les questionnaires sont étudiées par des modèles de survie avec censure par intervalles. L'association avec le VEMS en pourcents prédits (VEMS%) est étudiée par régression linéaire. Les modèles sont ajustés sur des facteurs de confusion potentiels sélectionnés d'après la littérature. Les valeurs manquantes des covariables sont imputées 100 fois par une méthode d'imputation multiple par équations chaînées (MICE).

Résultats

Aucun des phénols ou phtalates étudiés n'est associé statistiquement avec plusieurs

paramètres respiratoires. L'augmentation d'une unité de la concentration log-transformée d'éthylparabène est associée à une augmentation du ratio du taux d'incidence de l'asthme diagnostiqué par un médecin (rapport des risques instantanés ou Hazard Rate [HR] : 1.10 ; avec un intervalle de confiance [IC], à 95% : 1.00, 1.21) et a tendance à être associé avec une diminution du VEMS% (beta, -0.59 ; IC à 95% : -1.24, 0.05). Le bisphénol A avait tendance à augmenter le risque d'asthme (HR : 1.23 ; IC à 95% : 0.97, 1.55) et de bronchiolite ou bronchite (HR : 1.13 ; IC à 95% : 0.99, 1.30). Des tendances d'associations délétères avec un unique paramètre respiratoire sont aussi observées entre le 2,5-dichlorophénol et le risque de sifflements dans la poitrine, et entre le mono-(carboxynonyl) phthalate (MCNP), un métabolite du di-isodécyl phtalate (DIDP) et le risque de sifflements.

Conclusion

L'exposition à l'éthylparabène, le bisphénol A, le 2,5-dichlorophénol, et au DIDP a tendance à être associée à des effets délétères sur la santé respiratoire des garçons au cours des cinq premières années de vie. Les tendances d'associations sont plutôt cohérentes entre les paramètres de santé respiratoire pour l'éthylparabène et le bisphénol A. Ces tendances d'associations délétères du bisphénol A avec les risques d'asthme et de bronchiolite ou bronchite sont cohérentes avec ceux d'une précédente étude de cohorte européenne chez des garçons et des filles.[9]

4.2 Abstract

Background

Phenols and phthalates may have immunomodulatory and pro-inflammatory effects and thereby adversely affect respiratory health.

Objective

We estimated the associations between gestational exposure to select phthalates and phenols and respiratory health in boys.

Methods

Among 587 pregnant women from the EDEN (Etude des Déterminants pré et post natals du développement et de la santé de l'Enfant) cohort who delivered a boy, 9 phenols and 11 phthalates metabolites were quantified in spot pregnancy urine samples. Respiratory outcomes were followed-up by questionnaires until age 5, when Forced Expiratory Volume in 1 second (FEV_1) was measured by spirometry. Adjusted associations of urinary metabolites log-transformed concentrations with respiratory outcomes and FEV_1 in percent predicted (FEV_1%) were estimated by survival and linear regression models, respectively.

Results

No phenol or phthalate metabolite exhibited clear deleterious associations simultaneously with several respiratory outcomes. Ethyl-paraben was associated with increased asthma rate (Hazard Rate, HR: 1.10; 95% Confidence Interval, CI: 1.00, 1.21) and tended to be negatively associated with FEV_1% (beta: -0.59; 95% CI: -1.24, 0.05); bisphenol A tended to be associated with increased rates of asthma diagnosis (HR: 1.23; 95% CI: 0.97, 1.55) and bronchiolitis/bronchitis (HR: 1.13; 95% CI: 0.99, 1.30). Isolated trends for deleterious associations were also observed between 2,5-dichlorophenol and wheezing and between mono-(carboxynonyl) phthalate (MCNP), a metabolite of di-isodecyl phthalate (DIDP) and wheezing.

Conclusion

Ethyl-paraben, bisphenol A, 2,5-dichlorophenol, and DIDP tented to be associated with altered respiratory health, with ethyl-paraben and bisphenol A exhibiting some consistency across respiratory outcomes. The trends between bisphenol A pregnancy level and increased asthma and bronchiolitis/bronchitis rates in childhood were coherent with a previous cohort study.

4.3 Introduction

Asthma is now the most frequent chronic childhood disease, affecting around 10% of children in Western countries.[1] Changes in the prevalence of exposure to environmental factors in the 20th Century, including synthetic chemicals, have been suggested to contribute to the increased asthma prevalence.[3] Concern exists specifically regarding phenols and phthalates, two families of suspected endocrine disruptors.

Phenols and phthalates are produced in large volumes. Bisphenol A is found in food packaging or epoxy resins (such uses were banned in France in 2015). Other phenols, such as parabens, benzophenone-3, and triclosan are found in cosmetics, sunscreens, and antibacterial soaps, while some dichlorophenols are intermediates in the production of herbicides and room deodorizers. Phthalates are mainly used as plasticizers and are present in many plastic products, such as polyvinyl chloride floor covering, toys, and food packaging. Some phthalates are components of solvents and personal care products (e.g., soap, nail polish, lotion, fragrances), and are used as excipients in pharmaceuticals.[15]

Due to immaturity of the lungs, and of the immune system and due to the physiology of development, early-life exposures may have long-term adverse effect on respiratory health.[20] Experimental evidence suggests that bisphenol A and phthalates such as di(2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DINP) and butylbenzyl phthalate (BBzP) or their monoester metabolites can cross the placenta and may have proallergic properties.[3,17] In mice, prenatal exposure to bisphenol A has been associated with increased allergic sensitization and bronchial inflammation.[22] In humans, few longitudinal studies focused on the prenatal exposure window and have reported increased rates of asthma, wheeze and respiratory tract infections with bisphenol A, BBzP and DEHP metabolites.[8,9,16,35,38,37,40,41] No prospective study evaluated the impact of phenols other than bisphenol A on respiratory health; regarding phthalates, DINP and di-isodecyl phthalate (DIDP) which are increasingly used as DEHP substitutes, have not been studied. Only one study examined the association between pulmonary function in childhood and prenatal exposure to phenols or phthalates; the study related bisphenol A to spirometric tests; this study suggested an adverse association between bisphenol A level and spirometric tests at 4 years but not at 5 years of age.[38] To our knowledge, no study investigated effects of prenatal exposure to other phenols or phthalates on pulmonary function measurements.

Our aim was to characterize associations between prenatal exposure to select phenols and phthalates and the development of respiratory pathologies in the first five

years of life and pulmonary function in male offspring aged 5 years. Compounds with the highest a priori likelihood in favour of an effect were, on the basis of animal studies DEHP, bisphenol A, and, to a lesser extent, DINP.

4.4 Methods

4.4.1 Data source

This study relied on a subgroup of the EDEN (Etude des Déterminants pré et post natals du développement et de la santé de l'Enfant) mother-child cohort. Briefly, 2,002 pregnant women were recruited before 24 gestational weeks in two Nancy and Poitiers (France) University hospitals between 2003 and 2006. Exclusion criteria included pre-pregnancy diabetes, multiple gestation, inability to read or speak French, and moving outside the region planned within the next three years. The detailed study protocol has been described previously.[25]

The present study included all male offspring with metabolites of pregnancy phenols and phthalates measured in a maternal spot urine sample and for whom at least one completed respiratory questionnaire or an acceptable pulmonary function test (spirometry) was available. Phenols and phthalates biomarkers were originally quantified in the urines of male offspring's mothers only, as part of previous studies investigating the impact of maternal exposure to endocrine disruptors on male genital anomalies and further on male fetal and postnatal growth.[7,25] All participants provided written informed consent for themselves and their offspring for biological measurements and data collection. The EDEN cohort was approved by the following ethics committees: Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale (CCP-PRB) of Kremlin Bicêtre on 12 December 2002 and from the Commission Nationale Informatique et Liberté (CNIL), which is the French data privacy institution.[12] The involvement of the Centers for Disease Control and Prevention (CDC) did not constitute engagement in human subject research.

4.4.2 Exposure assessment

Between 23 and 29 gestational weeks, pregnant women were asked to come for a clinical examination with a sample of their first morning void. If forgotten, the urine sample was collected during the study visit. Polypropylene containers were used to avoid any contamination and urines were stored at -80°C before shipments for analyses to the CDC laboratory in Atlanta (Georgia, USA) at two distinct periods.

Urinary concentrations of creatinine, nine phenols and 11 phthalates metabolites (listed in Tables 4.1 and 4.5) were measured. Molar concentrations were summed for four parabens ($\sum parabens$), 2,4 and 2,5-dichlorophenols ($\sum dichlorophenols$) and four DEHP metabolites ($\sum DEHP$), total low molecular weight (< 250 g/mol) phthalates

($\sum LMW$) and high molecular weight (> 250 g/mol) phthalates ($\sum HMW$). Concentrations under the limit of detection were replaced by instrumental reading values, or by the compound-specific lowest instrumental reading value divided by square root of two when the instrumental reading value was missing. Biomarkers and creatinine concentrations were quantified following identical analytical methodology in 2008 (n = 191) and in 2011 (n = 413).[25] A two-step standardization approach was applied to reduce the undesirable variability in biomarker urinary concentrations owed to sampling conditions. First, linear regressions were conducted to estimate the effects of sampling conditions (day and hour of sampling, gestational age at urine collection, storage duration at room temperature before freezing, and year of biomarker analysis) and level of creatinine on each ln-transformed biomarker concentration. Second, these regression estimates were used to predict standardized concentrations that would have been observed if all samples had been collected under identical conditions.[21]

4.4.3 Respiratory health

The French-enriched version of the International Study of Allergy and Asthma in Children (ISAAC) self-report questionnaires was used to assess doctor-diagnosed asthma, wheezing at age 8 months and at each year until age 5 years and bronchiolitis/bronchitis episode until age 3 years in offspring. Doctor-diagnosed asthma and wheezing were defined from the question "Did your child ever have a medical diagnosis of asthma?" and "In the last 12 months (or since birth for the first questionnaire), has your child had wheezing in the chest?" Bronchiolitis/bronchitis was defined from the question: "In the last 12 months (or since birth for the first questionnaire), has your child had a bronchiolitis or bronchitis?"

Spirometry was performed using SpiroBank® G Spirometer by MIR (Rome, Italy) at about 5 years of age by trained personnel, following the American Thoracic Society / European Respiratory Society (ATS/ERS) guidelines. Boys were seated, wearing nose clips. Between three and eight forced expiratory manoeuvres were performed. Results were classified by a paediatric pulmonologist as acceptable or unacceptable in accordance with ATS/ERS recommendations for preschool children.[2] Criteria for an acceptable manoeuvre were: a rapid rise and a smooth or convex descending limb in the flow-volume curve, without artefact (glottic closure, cough, leaks), with forced expiration times larger than 1 second. We considered the Forced Expiratory Volume in 1 second (FEV_1), a standardized and reproducible test.[23] The highest FEV_1 from any of the satisfactory manoeuvres was expressed as a percentage of the age-, height-, sex- and ethnic-specific predicted value ($FEV_1\%$) calculated with the Global Lung Initiative (GLI) equations.[27]

Table 4.1 – Raw and standardized urinary concentrations of phthalate and phenol biomarkers among pregnant women from included population (n = 587, EDEN cohort).

Analyte	LOD (μg/L)	>LOD (%)	Raw concentrations (μg/L)[a]			Standardized concentrations (μg/L)[b]			Spearman correlation[c]
			5th	50th	95th	5th	50th	95th	
2,4-Dichlorophenol	0.2	97	0.3	1.0	9.9	0.3	1.0	9.0	0.95
2,5-Dichlorophenol	0.2	100	1.6	9.8	278.0	1.8	9.4	279.4	0.97
$\sum$Dichlorophenols (μmol/L)			0.0	0.1	1.8	0.0	0.1	1.8	0.96
Bisphenol A	0.4	99	0.6	2.6	10.7	0.8	2.4	8.9	0.86
Benzophenone-3	0.4	91	0.2	2.1	81.2	0.3	2.3	75.4	0.97
Triclosan	2.3	81	0.1	29.3	744.0	0.2	27.6	697.9	>0.99
Methyl-paraben	1.0	100	7.8	118.0	1730.0	7.9	111.4	1152.2	0.96
Ethyl-paraben	1.0	72	0.1	4.5	74.4	0.1	3.4	68.6	0.94
Propyl-paraben	0.2	98	0.5	16.1	289.0	0.5	14.3	258.3	0.97
Butyl-paraben	0.2	82	0.1	1.9	59.7	0.1	1.9	57.6	0.96
$\sum$Parabens (μmol/L)			0.1	1.0	13.8	0.1	0.9	9.9	0.96
$\sum$LMW (μmol/L)			0.3	1.3	8.4	0.4	1.1	6.2	0.85
MEP	0.8	100	20.6	113.0	1050.0	22.0	99.0	703.2	0.90
MnBP	0.6	100	8.1	52.4	515.0	12.5	44.4	444.8	0.89
MiBP	0.3	100	8.9	45.1	218.0	11.8	40.2	167.6	0.84
$\sum$HMW (μmol/L)			0.1	0.5	2.2	0.2	0.5	1.7	0.84
MCPP	0.2	99	0.5	2.3	11.2	0.7	1.9	9.3	0.87
MBzP	0.3	100	3.0	20.0	135.0	4.6	18.2	105.5	0.85
MCNP	0.6	97	0.5	1.5	12.9	0.5	1.3	10.2	0.90
MCOP	0.7	98	0.9	3.7	18.4	1.2	4.0	19.6	0.90
MEHHP	0.7	100	5.4	30.4	124.0	6.8	26.7	99.2	0.87
MEOHP	0.7	100	4.2	24.2	105.0	5.4	22.3	84.2	0.87
MECPP	0.6	100	9.9	43.0	183.0	12.3	38.2	156.9	0.88
MEHP	1.2	96	0.9	8.3	40.7	1.3	7.4	34.4	0.90
$\sum$DEHP (μmol/L)			0.1	0.4	1.5	0.1	0.3	1.2	0.87

LOD = Limit of Detection; 5th 25th 50th 75th 95th = percentiles; $\sum$Dichlorophenols = molar sum of Dichlorophenols (2,4-Dichlorophenol, 2,5-Dichlorophenol); $\sum$Parabens = molar sum of parabens (Methyl-, Ethyl-, Propyl-, Butyl-parabens); $\sum$LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); MEP = Monoethyl phthalate; MnBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; $\sum$HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); MCPP = Mono (3-carboxypropyl) phthalate; MBzP = Monobenzyl phthalate; MCNP = Mono-(carboxynonyl) phthalate, MCOP = Monocarboxyoctyl phthalate; MEHHP = Mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = Mono(2-ethyl-5-oxohexyl) phthalate; MECPP = Mono(2-ethyl-5-carboxypentyl) phthalate; MEHP = Mono(2-ethylhexyl) phthalate; $\sum$DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). Parent phthalates detailed in Table 4.5.

[a] Biomarker concentrations <LOD were replaced by instrumental reading values. Machine values equal to 0 were replaced by the lowest machine value divided by square root of 2.
[b] Standardized for urine sampling conditions (creatinine level, day and hour of sampling, gestational age, storage duration at room temperature and year of analysis), as detailed in Mortamais et al.[21].
[c] Spearman correlation between measured and standardized biomarkers concentrations

4.4.4 Statistical analysis

Associations between each biomarker standardized concentration and the first occurrence of respiratory outcomes were investigated by distinct discrete-time survival models with a complementary log-log link function.[14] $FEV_1\%$ followed approximately a normal distribution and was analysed by distinct linear regression models in which biomarker concentrations were considered separately as ln-transformed continuous concentrations. To describe the dose-response relationship, exposure was additionally categorized into tertiles of biomarker concentrations. We calculated *p*-values for trends using a variable with three categories whose values corresponded to the median value in each tertile, coded in models as a continuous variable.[32]

Adjustment variables were identified *a priori* from a review of the literature. Variables were retained in the model if they were associated with the outcome ($p \leq 0.20$) and/or if their removal or addition changed the regression coefficients of the associations between phenols or phthalates biomarkers and the outcome by >10%. Selected variables included: centre of recruitment (Nancy / Poitiers); residence area (city-centre / urban area / rural area); maternal country of birth (mainland France / others); parental history of asthma, rhinitis, eczema or food allergies; the highest parental education level ($\leq$ high school+1 year / high school+2 years / $\geq$ high-school+3 years); passive or active maternal smoking during pregnancy (yes / no); presence of older siblings; child-care attendance before 1 year; and postnatal passive smoking (yes / no, time-varying covariate in survival models). $FEV_1\%$ models were additionally adjusted for offspring's age (continuous), and height (restricted cubic splines coding) at pulmonary function assessment. Missing data in covariates (between 0.17 and 24.7%) were imputed with multiple imputation methods.[39]

We did not formally test statistical significance nor correct for multiple comparison, but in interpreting results, we looked for consistency of deleterious associations across several respiratory phenotypes.

We conducted further sensitivity analyses to address the robustness of the results a) to the standardization of the concentrations by repeating analyses with non-standardized concentrations, and b) after excluding offspring with major risk factors of respiratory symptoms: preterm birth, smoking mother, or parental history of asthma.

Analyses were conducted using STATA 12.1 (Stata Corp, College Station, Texas).

4.5 Results

4.5.1 Population

The EDEN cohort included 995 live-born male offspring. From previous studies that investigated the effects of maternal exposure to endocrine disruptors on male genital anomalies and male foetal and postnatal growth, 604 boys had phenol and phthalate biomarkers measured in maternal urines. At least one respiratory questionnaire that had been completed by a parent was available for 587 boys who were included in the analyses of respiratory outcomes. The numbers of boys with complete follow-up data were 428 (73%) for bronchiolitis/bronchitis (until 3 years of age), 350 (60%) and 447 (76%) for wheezing and asthma diagnosis, respectively (follow-up until 5 years of age). One value for at least one covariate was missing for 277 boys. A spirometric test has been performed in 397 (68%) out of the 587 boys. For 95% of these boys, spirometry occurred between 5.4 and 6.0 years of age; 39% out of 397 boys were excluded due to insufficient forced expiratory times and 4% did not meet the criteria considered acceptable for at least one of their spirometric tests, so that 228 boys (57%) were included in the present study for $FEV_1\%$ analysis. Participants included in the analyses were comparable to the excluded EDEN male offspring with regard to most characteristics, but were more likely to have highly educated parents, to be born from French metropolitan mothers and to live in a non-smoking environment (Table 4.2).

Of the compounds measured, those with the highest (raw or standardized) urinary concentrations were for phenols, triclosan and methyl-paraben and, for phthalates, monoethyl phthalate (MEP) and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) (Table 4.1). Within compounds, crude and standardized concentrations were highly correlated ($r \geq 83\%$). Standardized concentrations of dichlorophenols, parabens and DEHP metabolites were highly correlated within each family of compounds ($r \geq 0.80$). Strong correlations ($r \geq 0.83$) existed also between molar sums and associated compounds. The other correlation coefficients between standardized concentrations were below 0.66 (Tables 4.6-4.7).

Asthma was diagnosed in 112 boys by age 5 years (cumulative incidence rate (CIR) at 5 years, 20.4%; 95% Confidence Interval (CI): 17.2, 24.0%) and parents reported wheezing in 254 boys (CIR = 45.0%; 95% CI: 41.0, 49.3%). Bronchiolitis/bronchitis cumulative incidence was 70.4% at 3 years (95% CI: 66.6, 74.2%, Figure 4.3). Average $FEV_1\%$ was 91.0% (5^{th}-95^{th} percentiles: 72.7-107.5%) and tended to be lower in children with doctor-diagnosed asthma, history of wheezing or bronchiolitis/bronchitis (not detailed).

Table 4.2 – Characteristics of included and excluded boys in the two analyses from the EDEN cohort [N (%) or mean±SD].

Characteristic	Spirometry analysis			Repiratory outcomes analysis		
	Included (n=228)	Excluded (n=767)	P value	Included (n=587)	Excluded (n=408)	P value
Centre of recruitment						
Nancy	93 (41)	371 (48)	0.04	247 (42)	217 (53)	0.001
Poitiers	135 (59)	396 (52)		340 (58)	191 (47)	
Living area						
Rural area	85 (37)	238 (31)	0.11	200 (34)	123 (30)	0.41
Urban area	100 (44)	346 (45)		258 (44)	188 (46)	
City-center	42 (18)	183 (24)		128 (22)	97 (24)	
Missing	1 (<1)	ND		1 (<1)	ND	
Maternal country of birth						
Mainland France	221 (97)	717 (93)	0.11	563 (96)	375 (92)	0.02
All others	6 (3)	39 (5)		19 (3)	26 (6)	
Missing	1 (<1)	11 (1)		5 (<1)	7 (2)	
Parental higher education level						
≤ High school + 1y	71 (31)	274 (36)	0.25	193 (33)	152 (37)	0.04
High school + 2y	56 (25)	161 (21)		128 (22)	89 (22)	
≥ High school + 3y	93 (41)	278 (36)		241 (41)	130 (32)	
Missing	8 (4)	54 (7)		8 (4)	37 (9)	
Pregnancy maternal active smoking (cig/day)						
0	173 (76)	561 (73)	0.06	449 (76)	285 (70)	<0.001
1-5	38 (17)	106 (14)		89 (15)	55 (13)	
≥6	17 (7)	100 (13)		49 (8)	68 (17)	
Passive smoking during pregnancy						
Yes	143 (63)	437 (57)	0.13	372 (63)	199 (49)	<0.001
No	85 (37)	329 (43)		215 (37)	208 (51)	
Missing	ND	1 (<1)		ND	1 (<1)	
Parental history of asthma/allergies						
Yes	98 (43)	339 (44)	0.68	253 (43)	184 (45)	0.44
No	130 (57)	422 (55)		333 (57)	219 (54)	
Missing	ND	6 (<1)		1 (<1)	5 (1)	
Gestational duration (weeks)	39.7±1.5	39.6±1.9	0.41	39.8±1.5	39.4±2.6	0.002
Presence of older siblings						
Yes	104 (46)	325 (42)	0.39	324 (55)	242 (59)	0.20
No	124 (54)	442 (58)		263 (45)	166 (41)	
Day-care attendance before 1 year						
Yes	38 (17)	124 (16)	0.39	104 (18)	58 (14)	0.39
No	179 (78)	490 (64)		453 (77)	216 (53)	
Missing	11 (5)	153 (20)		30 (5)	134 (33)	
Postnatal passive smoking						
Yes	112 (49)	364 (47)	0.005	295 (50)	181 (44)	<0.001
No	109 (48)	228 (30)		262 (45)	75 (18)	
Missing	7 (3)	175 (23)		30 (5)	152 (37)	
Age at spirometry (years)	5.7±0.1	5.6±0.1	0.05			
Height at spirometry (cm)	115.7±4.9	115.1±4.6	0.13			

SD = standard deviation, ND = no data.

4.5.2 Phenols and respiratory health

Ln-transformed ethyl-paraben standardized concentration was associated with increased rate of doctor-diagnosed asthma (Hazard Rate, HR for one-unit increase in ln-transformed concentration, 1.10; 95% CI: 1.00, 1.21, p = 0.04) and reduced mean FEV_1% (beta for one-unit increase in ln-transformed concentration, -0.59%; 95% CI: -1.24, 0.05; p = 0.07). Bisphenol A tended to be associated with increased rates of asthma (HR, 1.23; 95% CI: 0.97, 1.55; p = 0.09) and of bronchiolitis/bronchitis (HR, 1.13; 95% CI: 0.99, 1.30; p = 0.08). Ln-transformed 2,5-dichlorophenol concentration was associated with an increased incidence of wheeze (HR, 1.08; 95% CI: 1.00, 1.17; p = 0.04). Methyl-paraben was associated with reduced rates of bronchiolitis/bronchitis (HR, 0.94; 95% CI: 0.88, 1.00; p = 0.05) and of wheezing (HR, 0.92; 95% CI: 0.85, 1.00; p = 0.05). Similar trends were found for propyl-paraben (Table 4.3, Figure 4.1A). Benzophenone-3 tended to be associated with reduced rate of wheezing (HR, 0.93; 95% CI: 0.86, 1.01; p = 0.08). Models with concentrations coded in tertiles showed coherent results (Table 4.3). No clear association with any respiratory outcome was observed with the other phenols.

Table 4.3 – Adjusted associations between pregnancy phenols standardized concentrations and respiratory outcomes (n = 587) and $FEV_1\%$ (n = 228) in boys.

	Wheezing [a] (until age 5y) N = 587			Asthma diagnosis [a] (until age 5y) N = 587			Bronchiolitis/Bronchitis [a] (until age 3y) N = 587			$FEV_1\%$ [b] N = 228		
Phenol [c]	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	beta (95% CI)	p_{het} [e]	*p*-value
2,4-Dichlorophenol												
Continuous[d]	1.06 (0.95, 1.19)		0.27	1.02 (0.87, 1.20)		0.79	0.98 (0.89, 1.08)		0.68	0.29 (-0.94, 1.54)		0.64
T1	1.00	0.64	0.74[f]	1.00	0.49	0.96[f]	1.00	0.50	0.40[f]	0.00	0.50	0.93[f]
T2	1.16 (0.85, 1.58)			1.30 (0.83, 2.05)			1.08 (0.84, 1.38)			2.01 (-1.57, 5.60)		
T3	1.09 (0.81, 1.49)			1.07 (0.66, 1.72)			0.93 (0.73, 1.19)			0.45 (-3.04, 3.94)		
2,5-Dichlorophenol												
Continuous[d]	1.08 (1.00, 1.17)		0.04	1.04 (0.93, 1.16)		0.52	1.00 (0.94, 1.07)		0.97	0.14 (-0.71, 1.00)		0.74
T1	1.00	0.01	0.13[f]	1.00	0.24	0.35[f]	1.00	0.82	0.54[f]	0.00	0.93	0.71[f]
T2	1.66 (1.20, 2.28)			1.46 (0.90, 2.34)			1.03 (0.81, 1.33)			0.03 (-3.58, 3.64)		
T3	1.51 (1.10, 2.08)			1.41 (0.88, 2.25)			1.08 (0.85, 1.38)			0.59 (-2.94, 4.11)		
$\sum$Dichlorophenols												
Continuous[d]	1.08 (1.00, 1.17)		0.048	1.04 (0.92, 1.17)		0.55	1.00 (0.93, 1.07)		0.99	0.17 (-0.72, 1.07)		0.70
T1	1.00	0.03	0.08[f]	1.00	0.24	0.24[f]	1.00	0.76	0.47[f]	0.00	0.95	0.78[f]
T2	1.45 (1.05, 1.99)			1.41 (0.87, 2.27)			1.00 (0.78, 1.29)			-0.16 (-3.81, 3.48)		
T3	1.47 (1.07, 2.01)			1.45 (0.91, 2.32)			1.08 (0.85, 1.39)			0.38 (-3.14, 3.91)		
Bisphenol A												
Continuous[d]	0.97 (0.82, 1.15)		0.75	1.23 (0.97, 1.55)		0.09	1.13 (0.99, 1.30)		0.08	-0.51 (-2.33, 1.32)		0.58
T1	1.00	0.99	0.90[f]	1.00	0.28	0.12[f]	1.00	0.52	0.26[f]	0.00	0.46	0.62[f]
T2	0.98 (0.72, 1.33)			1.04 (0.63, 1.71)			1.06 (0.83, 1.36)			-2.20 (-5.69, 1.27)		
T3	0.98 (0.72, 1.34)			1.39 (0.87, 2.22)			1.15 (0.90, 1.48)			-1.38 (-4.99, 2.23)		
Benzophenone-3												
Continuous[d]	0.93 (0.86, 1.01)		0.08	0.97 (0.87, 1.09)		0.60	1.00 (0.94, 1.06)		0.91	-0.42 (-1.31, 0.47)		0.36
T1	1.00	0.03	0.01[f]	1.00	0.26	0.16[f]	1.00	0.65	0.85[f]	0.00	0.83	0.56[f]
T2	0.87 (0.65, 1.17)			0.81 (0.52, 1.26)			0.89 (0.70, 1.14)			-0.60 (-4.10, 2.90)		
T3	0.66 (0.48, 0.91)			0.68 (0.42, 1.09)			0.93 (0.73, 1.19)			-1.08 (-4.55, 2.40)		
Triclosan												
Continuous[d]	0.98 (0.93, 1.03)		0.46	0.99 (0.92, 1.07)		0.86	1.00 (0.96, 1.04)		0.83	0.17 (-0.40, 0.73)		0.56
T1	1.00	0.79	0.57[f]	1.00	0.91	0.75[f]	1.00	0.80	0.62[f]	0.00	0.96	0.91[f]
T2	0.94 (0.69, 1.27)			1.06 (0.67, 1.66)			1.05 (0.82, 1.34)			0.48 (-2.97, 3.94)		
T3	0.90 (0.67, 1.22)			0.96 (0.60, 1.52)			0.97 (0.76, 1.23)			0.69 (-3.13, 3.84)		
Methyl-paraben												
Continuous[d]	0.92 (0.85, 1.00)		0.051	1.00 (0.89, 1.13)		0.99	0.94 (0.88, 1.00)		0.046	-0.46 (-1.30, 0.37)		0.28

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Table 4.3 – Continued

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Phenol [c]	Wheezing [a] (until age 5y) N = 587			Asthma diagnosis [a] (until age 5y) N = 587			Bronchiolitis/Bronchitis [a] (until age 3y) N = 587			FEV_1% [b] N = 228		
	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	beta (95% CI)	p_{het} [e]	*p*-value
T1	1.00	0.03	0.17[f]	1.00	0.90	0.94[f]	1.00	0.02	0.01[f]	0.00	0.19	0.18[f]
T2	0.68 (0.50, 0.92)			0.90 (0.57, 1.43)			0.80 (0.64, 1.03)			1.66 (-1.89, 5.21)		
T3	0.74 (0.55, 1.00)			0.99 (0.62, 1.58)			0.70 (0.54, 0.89)			-1.63 (-5.06, 1.78)		
Ethyl-paraben												
Continuous[d]	1.01 (0.95, 1.07)		0.72	1.10 (1.00, 1.21)		0.04	0.99 (0.94, 1.03)		0.53	-0.59 (-1.24, 0.05)		0.07
T1	1.00	0.63	0.65[f]	1.00	0.047	0.08[f]	1.00	0.004	0.15[f]	0.00	0.52	0.31[f]
T2	1.15 (0.85, 1.57)			1.66 (1.02, 2.71)			1.40 (1.10, 1.79)			-1.13 (-4.61, 2.36)		
T3	1.13 (0.83, 1.55)			1.81 (1.10, 2.98)			0.97 (0.76, 1.26)			-2.02 (-5.51, 1.47)		
Propyl-paraben												
Continuous[d]	0.95 (0.89, 1.02)		0.14	0.99 (0.90, 1.09)		0.85	0.95 (0.91, 1.00)		0.08	-0.22 (-0.92, 0.49)		0.55
T1	1.00	0.58	0.44[f]	1.00	0.91	0.89[f]	1.00	0.13	0.12[f]	0.00	0.63	0.38[f]
T2	0.88 (0.66, 1.19)			0.91 (0.58, 1.45)			0.83 (0.65, 1.06)			-0.43 (-3.06, 3.91)		
T3	0.86 (0.63, 1.17)			1.00 (0.63, 1.59)			0.79 (0.61, 1.00)			-1.23 (-4.71, 2.25)		
Butyl-paraben												
Continuous[d]	0.98 (0.92, 1.04)		0.49	1.01 (0.92, 1.11)		0.89	0.98 (0.93, 1.03)		0.36	-0.41 (-1.09, 0.27)		0.23
T1	1.00	0.24	0.15[f]	1.00	0.59	0.68[f]	1.00	0.02	0.01[f]	0.00	0.30	0.15[f]
T2	1.13 (0.84, 1.53)			1.26 (0.79, 2.01)			1.13 (0.89, 1.44)			-1.07 (-4.50, 2.36)		
T3	0.86 (0.62, 1.20)			1.22 (0.75, 2.01)			0.79 (0.61, 1.03)			-2.75 (-6.26, 0.76)		
$\sum$Parabens												
Continuous[d]	0.92 (0.85, 1.00)		0.05	1.00 (0.89, 1.13)		0.98	0.94 (0.88, 1.00)		0.054	-0.46 (-1.30, 0.38)		0.29
T1	1.00	0.06	0.11[f]	1.00	0.94	0.75[f]	1.00	0.05	0.10[f]	0.00	0.06	0.13[f]
T2	0.73 (0.54, 0.99)			0.95 (0.61, 1.50)			0.77 (0.61, 0.98)			2.58 (-0.92, 6.09)		
T3	0.73 (0.54, 0.99)			0.92 (0.57, 1.48)			0.77 (0.60, 0.98)			-1.61 (-5.04, 1.82)		

HR = hazard rate; FEV_1% = forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; $\sum$Dichlorophenols = molar sum of Dichlorophenols (2,4-, 2,5-dichlorophenols); $\sum$Parabens = molar sum of parabens (Methyl-, Ethyl-, Propyl-, Butyl-parabens).

[a] Models adjusted for centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings and child-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the multiple imputation by chained equations (MICE) method (100 imputations were performed). [b] additionally adjusted for child's height and age.

[c] Standardized for urine sampling conditions (creatinine level, day and hour of sampling, gestational age, storage duration at room temperature and year of analysis), as detailed in Mortamais et al.[21].

[d] Estimates for 1 unit increase in ln-transformed standardized concentration.

[e] p-values of heterogeneity test.

[f] p-values of monotonic trend test.

In sensitivity analyses, very small variations in regression estimates were observed when standardized biomarkers concentrations were replaced by raw concentrations. Analyses restricted to full-term boys (n = 562 and 217 for survival analyses and FEV_1% analyses, respectively), to boys from non-smoking mothers (n = 447 and 171), or non-asthmatic parents (n = 470 and 185), led to similar trends of deleterious associations between ethyl-paraben and both asthma diagnosis and FEV_1%, between bisphenol A and asthma as well as bronchiolitis/bronchitis and between 2,5-dichlorophenol and wheezing. Similarly, trends for protective associations between methyl-paraben and wheezing or bronchiolitis/bronchitis remained (Tables 4.8-4.14).

4.5.3 Phthalates and respiratory health

No phthalate metabolite was clearly associated with several respiratory outcomes, but monocarboxynonyl phthalate (MCNP) tended to be associated with increased rate of wheezing (HR for one-unit increase in ln-transformed concentration, 1.11; 95% CI: 0.98, 1.24; p = 0.09) and asthma (HR, 1.13; 95% CI: 0.95, 1.35; p = 0.16) and monocarboxyoctyl (MCOP) tended to be associated with increased bronchiolitis/bronchitis rate (HR, 1.09; 95% CI: 0.97, 1.22; p = 0.17) and decreased FEV_1% (beta, -1.25; 95% CI: -2.86, 0.35; p = 0.13). Mono-isobutyl phthalate (MiBP) and DEHP metabolites tended to be associated with reduced FEV_1% (p between 0.12 and 0.26, Table 4.4, Figure 4.1B). Mono(3-carboxypropyl) phthalate (MCPP) tended to be associated with a reduced rate of bronchiolitis/bronchitis (HR, 0.89; 95% CI: 0.79, 1.02). When biomarker concentrations were categorized into tertiles, we observed the same trends of associations (Table 4.4).

Sensitivity analyses led to similar results for the phthalates metabolites, with consistent hazard rates and beta coefficients (Tables 4.9-4.15).

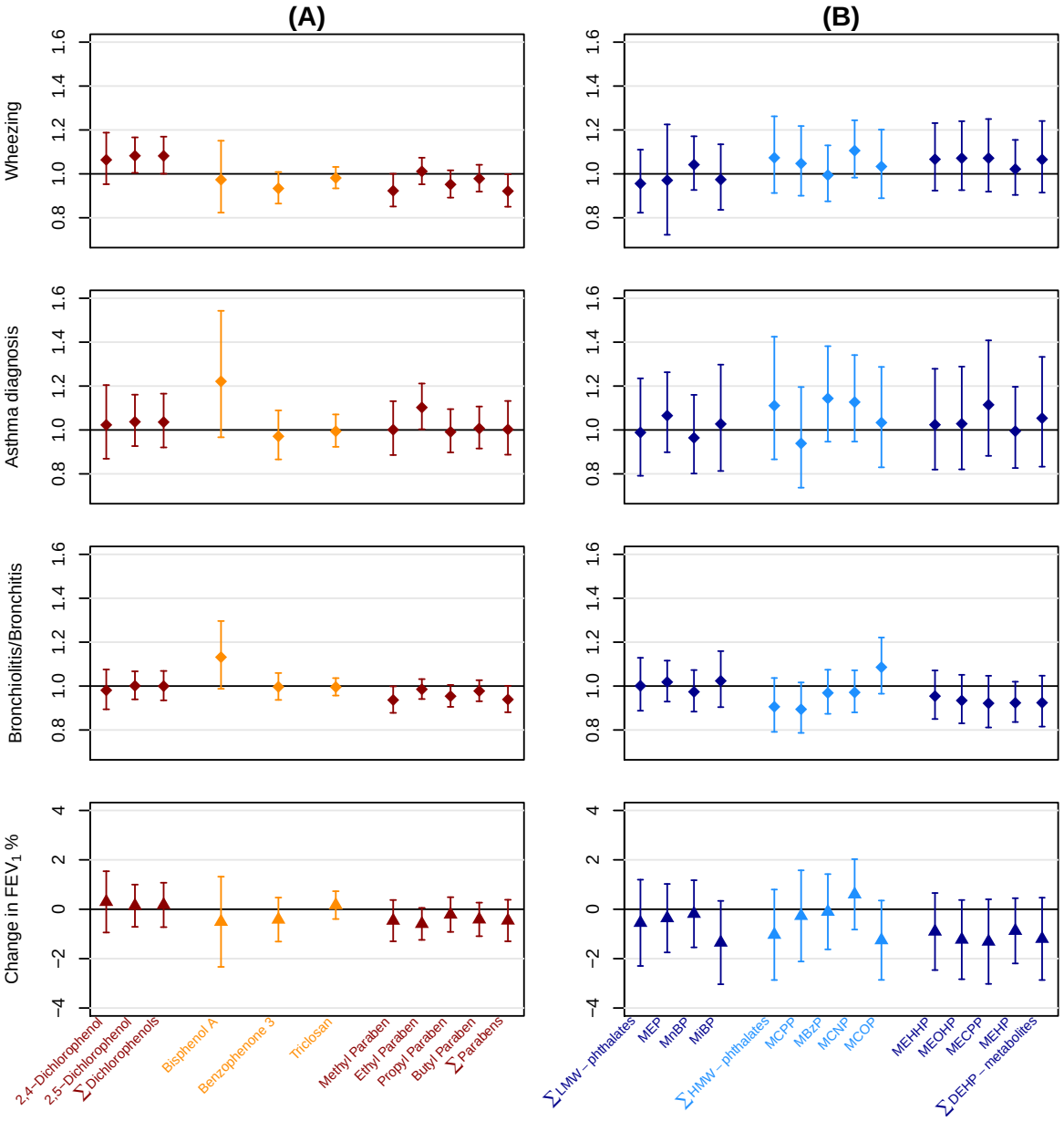

Figure 4.1 – Adjusted associations of phenols (A) and phthalates metabolites (B) ln-transformed standardized concentrations with respiratory outcomes (HR, n = 587) and FEV_1% in boys (beta, n = 228, EDEN cohort).

Effect estimates for 1 unit increase in ln-transformed standardized concentrations. Adjusted for centre, residence area, parental history of asthma/allergies, maternal ethnicity, maximal parental education level, passive or active smoking during pregnancy, postnatal passive smoking, older siblings, child-care (and additionally adjusted for boy's height and age in spirometry analysis). Multiple imputation was used to handle missing values in covariates (100 imputations were performed). Phenols and phthalates metabolites concentrations were standardized for urine sampling conditions (see methods section). Diamond and triangle markers represent HR and beta values, respectively; with error bars for 95% CI.

Table 4.4 – Adjusted associations between pregnancy phthalate metabolites standardized concentrations and respiratory outcomes (n = 587) and $FEV_1\%$ (n = 228) in boys.

	Wheezing [a] (until age 5y) N = 587			Asthma diagnosis [a] (until age 5y) N = 587			Bronchiolitis/Bronchitis [a] (until age 3y) N = 587			$FEV_1\%$[b] N = 228		
Phthalate [c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
∑LMW												
Continuous[d]	0.96 (0.82, 1.11)		0.55	0.99 (0.80, 1.24)		0.96	1.00 (0.89, 1.13)		0.99	-0.55 (-2.30, 1.19)		0.54
T1	1.00	0.92	0.77[f]	1.00	0.53	0.91[f]	1.00	0.86	0.75[f]	0.00	0.17	0.06[f]
T2	1.03 (0.76, 1.40)			1.29 (0.82, 2.02)			1.07 (0.84, 1.37)			-0.37 (-3.83, 3.09)		
T3	0.97 (0.71, 1.33)			1.09 (0.68, 1.76)			1.05 (0.82, 1.35)			-3.02 (-6.46, 0.42)		
MEP												
Continuous[d]	0.97 (0.86, 1.09)		0.62	1.07 (0.90, 1.27)		0.44	1.02 (0.93, 1.12)		0.70	-0.36 (-1.75 1.02)		0.61
T1	1.00	0.87	0.97[f]	1.00	0.56	0.38[f]	1.00	0.84	0.63[f]	0.00	0.74	0.44[f]
T2	0.92 (0.68, 1.25)			0.90 (0.56, 1.46)			1.06 (0.83, 1.35)			-0.35 (-3.79, 3.09)		
T3	0.97 (0.72, 1.32)			1.16 (0.74, 1.83)			1.07 (0.84, 1.37)			-1.34 (-4.86, 2.19)		
MnBP												
Continuous[d]	1.04 (0.93, 1.17)		0.49	0.97 (0.80, 1.16)		0.72	0.97 (0.88, 1.07)		0.59	-0.18 (-1.54, 1.17)		0.79
T1	1.00	0.53	0.33[f]	1.00	0.75	0.57[f]	1.00	0.52	0.51[f]	0.00	0.92	0.83[f]
T2	0.95 (0.69, 1.29)			0.87 (0.55, 1.36)			0.88 (0.69, 1.12)			0.43 (-3.06, 3.92)		
T3	1.12 (0.83, 1.52)			0.85 (0.54, 1.35)			0.90 (0.70, 1.14)			-0.27 (-3.84, 3.29)		
MiBP												
Continuous[d]	0.97 (0.84, 1.13)		0.74	1.03 (0.82, 1.30)		0.79	1.02 (0.90, 1.16)		0.71	-1.35 (-3.04, 0.34)		0.12
T1	1.00	0.76	0.94[f]	1.00	0.83	0.74[f]	1.00	0.99	0.99[f]	0.00	0.14	0.05[f]
T2	0.89 (0.65, 1.22)			1.09 (0.70, 1.71)			1.02 (0.79, 1.30)			-0.40 (-3.91, 3.10)		
T3	0.96 (0.72, 1.31)			0.95 (0.59, 1.52)			1.00 (0.78, 1.28)			-3.26 (-6.73, 0.21)		
∑HMW												
Continuous[d]	1.07 (0.91, 1.26)		0.39	1.11 (0.87, 1.43)		0.40	0.91 (0.79, 1.04)		0.15	-1.03 (-2.87, 0.80)		0.27
T1	1.00	0.25	0.89[f]	1.00	0.97	0.98[f]	1.00	0.50	0.29[f]	0.00	0.26	0.47[f]
T2	1.29 (0.95, 1.75)			1.06 (0.67, 1.69)			0.91 (0.71, 1.16)			-2.80 (-6.22, 0.61)		
T3	1.09 (0.79, 1.49)			1.02 (0.64, 1.62)			0.87 (0.68, 1.11)			-1.85 (-5.32, 1.62)		
MCPP												
Continuous[d]	1.05 (0.90, 1.22)		0.55	0.94 (0.74, 1.20)		0.62	0.89 (0.79, 1.02)		0.09	-0.27 (-2.12, 1.58)		0.77

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Table 4.4 – Continued

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Phthalate [c]	Wheezing [a] (until age 5y) N = 587			Asthma diagnosis [a] (until age 5y) N = 587			Bronchiolitis/Bronchitis [a] (until age 3y) N = 587			FEV_1% [b] N = 228		
	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	beta (95% CI)	p_{het} [e]	*p*-value
T1	1.00	0.66	0.37[f]	1.00	0.78	0.58[f]	1.00	0.37	0.23[f]	0.00	0.31	0.73[f]
T2	1.06 (0.77, 1.44)			0.88 (0.56, 1.38)			1.05 (0.83, 1.33)			-2.22 (-5.64, 1.21)		
T3	1.15 (0.85, 1.57)			0.86 (0.54, 1.37)			0.88 (0.69, 1.13)			0.18 (-3.29, 3.64)		
MBzP												
Continuous[d]	1.00 (0.87, 1.13)		0.93	1.15 (0.95, 1.39)		0.15	0.97 (0.87, 1.07)		0.55	-0.10 (-1.63, 1.42)		0.90
T1	1.00	0.32	0.45[f]	1.00	0.39	0.97[f]	1.00	0.46	0.21[f]	0.00	0.20	0.25[f]
T2	1.18 (0.87, 1.60)			1.37 (0.86, 2.19)			0.99 (0.77, 1.26)			-2.85 (-6.35, 0.66)		
T3	0.94 (0.69, 1.29)			1.11 (0.69, 1.79)			0.86 (0.68, 1.11)			-2.63 (-6.11, 0.84)		
MCNP												
Continuous[d]	1.11 (0.98, 1.24)		0.09	1.13 (0.95, 1.35)		0.16	0.97 (0.88, 1.07)		0.56	0.60 (-0.82, 2.03)		0.41
T1	1.00	0.22	0.18[f]	1.00	0.19	0.26[f]	1.00	0.69	0.88[f]	0.00	0.72	0.84[f]
T2	1.24 (0.91, 1.70)			1.51 (0.93, 2.46)			1.10 (0.87, 1.41)			-1.30 (-4.75, 2.15)		
T3	1.29 (0.95, 1.76)			1.46 (0.91, 2.35)			1.01 (0.79, 1.30)			-0.15 (-3.63, 3.32)		
MCOP												
Continuous[d]	1.03 (0.89, 1.20)		0.67	1.03 (0.83, 1.29)		0.76	1.09 (0.97, 1.22)		0.17	-1.25 (-2.86, 0.35)		0.13
T1	1.00	0.81	0.52[f]	1.00	0.52	0.77[f]	1.00	0.90	0.92[f]	0.00	0.42	0.22[f]
T2	0.96 (0.71, 1.31)			0.80 (0.50, 1.27)			1.05 (0.82, 1.34)			0.02 (-3.55, 3.59)		
T3	0.90 (0.67, 1.23)			1.01 (0.65, 1.58)			1.00 (0.78, 1.28)			-1.99 (-5.42, 1.43)		
MEHHP												
Continuous[d]	1.07 (0.92, 1.23)		0.38	1.02 (0.82, 1.28)		0.83	0.95 (0.85, 1.07)		0.43	-0.90 (-2.46, 0.66)		0.26
T1	1.00	0.70	0.71[f]	1.00	0.41	0.62[f]	1.00	0.71	0.54[f]	0.00	0.33	0.35[f]
T2	1.14 (0.84, 1.56)			1.26 (0.80, 2.00)			1.05 (0.82, 1.33)			-2.54 (-5.95, 0.87)		
T3	1.09 (0.80, 1.48)			0.95 (0.59, 1.53)			0.94 (0.73, 1.20)			-1.72 (-5.27, 1.83)		
MEOHP												
Continuous[d]	1.07 (0.93, 1.24)		0.36	1.03 (0.82, 1.29)		0.80	0.93 (0.83, 1.05)		0.26	-1.23 (-2.83, 0.37)		0.13
T1	1.00	0.39	0.70[f]	1.00	0.89	0.68[f]	1.00	0.38	0.45[f]	0.00	0.10	0.10[f]
T2	1.24 (0.91, 1.69)			1.02 (0.65, 1.62)			1.11 (0.87, 1.41)			-3.35 (-6.79, 0.09)		
T3	1.11 (0.81, 1.51)			0.92 (0.58, 1.46)			0.94 (0.73, 1.20)			-3.10 (-6.56, 0.35)		

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Table 4.4 – Continued

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Phthalate [c]	Wheezing [a] (until age 5y) N = 587			Asthma diagnosis [a] (until age 5y) N = 587			Bronchiolitis/Bronchitis [a] (until age 3y) N = 587			FEV_1% [b] N = 228		
	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	HR (95% CI)	p_{het} [e]	*p*-value	beta (95% CI)	p_{het} [e]	*p*-value
MECPP												
Continuous[d]	1.07 (0.92, 1.25)		0.38	1.11 (0.88, 1.41)		0.36	0.92 (0.81, 1.05)		0.21	-1.31 (-3.02, 0.40)		0.13
T1	1.00	0.18	0.52[f]	1.00	0.42	0.81[f]	1.00	0.88	0.62[f]	0.00	0.12	0.15[f]
T2	1.34 (0.98, 1.82)			1.36 (0.85, 2.15)			0.98 (0.77, 1.24)			-3.33 (-6.80, 0.14)		
T3	1.17 (0.85, 1.61)			1.13 (0.70, 1.83)			0.94 (0.74, 1.20)			-2.89 (-6.36, 0.57)		
MEHP												
Continuous[d]	1.02 (0.90, 1.15)		0.73	0.99 (0.83, 1.20)		0.95	0.92 (0.84, 1.02)		0.12	-0.87 (-2.19, 0.45)		0.19
T1	1.00	0.59	0.36[f]	1.00	0.69	0.44[f]	1.00	0.27	0.48[f]	0.00	0.35	0.15[f]
T2	0.97 (0.71, 1.33)			0.97 (0.61, 1.55)			0.82 (0.64, 1.04)			-0.72 (-4.14, 2.70)		
T3	1.13 (0.83, 1.53)			1.17 (0.74, 1.83)			0.89 (0.69, 1.13)			-2.50 (-5.97, 0.97)		
$\sum$DEHP												
Continuous[d]	1.06 (0.91, 1.24)		0.42	1.05(0.83, 1.33)		0.66	0.92 (0.82, 1.05)		0.22	-1.20 (-2.87, 0.47)		0.16
T1	1.00	0.19	0.86[f]	1.00	0.61	0.77[f]	1.00	0.70	0.44[f]	0.00	0.27	0.49[f]
T2	1.32 (0.97, 1.79)			1.20 (0.76, 1.90)			1.01 (0.79, 1.28)			-2.84 (-6.28, 0.61)		
T3	1.09 (0.80, 1.50)			0.98 (0.61, 1.57)			0.92 (0.72, 1.17)			-1.50 (-4.96, 1.97)		

HR = hazard rate; FEV_1% = forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; $\sum$LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); $\sum$HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); $\sum$DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). Parent compounds and associated metabolites are detailed in Table 4.5 (Supplemental material).

[a] Models adjusted for centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the multiple imputation by chained equations (MICE) method (100 imputations were performed). [b] additionally adjusted for child's height and age.

[c] Standardized for urine sampling conditions (creatinine level, day and hour of sampling, gestational age, storage duration at room temperature and year of analysis), as detailed in Mortamais et al.[21].

[d] Estimates for 1 unit increase in ln-transformed standardized concentration.

[e] p-values of heterogeneity test.

[f] p-values of monotonic trend test.

4.6 Discussion

This study evaluated possible deleterious effects of prenatal exposure to phthalates and phenols on respiratory health in childhood. In our male population, associations were not in the same direction across chemicals. First, increased levels of ethyl-paraben, bisphenol A, 2,5-dichlorophenol, and DIDP tented to be associated with altered respiratory health, with ethyl-paraben and bisphenol A exhibiting some consistency across respiratory outcomes. Inversely, and contrary to our a priori hypothesis, we observed reduced rates of bronchiolitis/bronchitis and wheezing with increased exposure to methyl-, propyl-parabens and benzophenone-3. MCPP, a metabolite of di-*n*-octylphthalate (DNOP), di-*n*-butyl phthalate (DnBP) and several HMW phthalates, tended to reduce the rate of bronchiolitis/bronchitis.

Regarding bisphenol A, a study of 208 children reported a strong inverse association between prenatal bisphenol A concentration and $FEV_1\%$ at age 4 years (beta, -14%; 95% CI: -25, -4 for an increase by one $\log_{10}$ unit) which totally disappeared at age 5 years (beta, 0.04; 95% CI: -9, 9).[38] The latter wide CI is consistent with our results at age 5 years estimated for a $\log_{10}$ unit (beta, -1, 95% CI: -5, 3). Regarding associations between prenatal bisphenol A exposure and questionnaire-based respiratory outcomes, Donohue et al.[8] reported no association with asthma status evaluated once between ages 5 and 12 years, whereas Gascon et al.[9] reported trends of deleterious association with asthma, bronchitis and chest infections until age 7 years (point estimates for relative risks for one-unit increase in $\log_2$-transformed concentration varied from 1.15 to 1.21 across phenotypes). In a larger population with a similar bisphenol A concentration range, our results also suggested elevated rates of doctor-diagnosed asthma and bronchiolitis/bronchitis of similar effect sizes to those in Gascon et al.[9]. Regarding wheezing, our study did not evidence any association and results from the previous studies are inconsistent, with studies reported either "protective" association[8] or trend for deleterious association.[9,37,38] Spanier et al.[37] showed that the association was stronger considering concentrations from urine samples collected at 16 gestational weeks compared to 26 gestational weeks, suggesting that the exposure window may play a role in the association with wheezing. In mice, prenatal exposure to bisphenol A through drinking water promoted the development of allergic asthma in offspring.[22] Bisphenol A may affect the immune functions and increase IgE serum levels or the production of proallergic mediators such as cytokine IL4.[17,30] Such immune effects might be mediated by interactions with oestrogen receptors or the family of peroxisome proliferator-activated receptors (PPARs).[30]

The literature regarding other phenols is limited to cross-sectional studies. In

our study, 2,5-dichlorophenol tended to be associated with wheezing rate, an association never considered, to our knowledge, in a prospective setting. The protective associations we observed between methyl-paraben or propyl-paraben and wheezing or bronchiolitis/bronchitis are in line with those previously reported with non-atopic wheezing.[34,36] One proposed hypothesis might be an effect through their antimicrobial properties.[34] To our knowledge, no previous experimental or human study considered association between benzophenone-3 and risk of wheezing.

Only one cross-sectional study has investigated associations of several phthalates and pulmonary function, reporting deleterious associations of spirometric measurements with MCPP, mono-*n*-butyl phthalate (MnBP) and DEHP metabolites.[4] In a population somewhat older than ours (boys were 6-16 year-old), FEV_1% decreased with urinary concentrations of DEHP metabolites, consistently with the trend observed in our study; associations were stronger with the forced vital capacity (FVC) and the ratio FEV_1/FVC, outcomes that we could not consider. We also observed suggestive associations between MCOP and MiBP concentrations and decreased FEV_1% (p, 0.12 and p, 0.13, respectively, for the log-transformed concentrations), associations which have, to our knowledge, never been considered so far.

Combining two cohorts, Smit et al.[35] did not observe any association between serum oxidative metabolites of DEHP, DINP and wheeze or asthma in children evaluated at one-time point between 5 and 9 years of age. Whyatt et al.[40], reported elevated risks of asthma or asthma-like symptoms between 5 and 11 years associated with urinary metabolites of BBzP and DnBP (represented by MBzP and MnBP respectively), which were not observed in the boys from our study. Gascon et al.[9] showed deleterious associations of BBzP and DEHP urinary metabolites with asthma, wheeze, chest infections and bronchitis until age 7 years, which we did not confirm in our male population followed until 5 years. Ku et al.[16] reported deleterious associations between urinary metabolites of BBzP or DEHP and wheeze at 8 years in boys, which, again, we could not confirm until age 5. The strongest deleterious association of phthalates metabolites with respiratory outcomes was observed for MCNP (DIDP metabolite), not investigated in the previous longitudinal studies, and for which our study provides only limited evidence in favour of associations with wheezing (p, 0.09 for log-transformed coding) and asthma diagnosis (p, 0.16). Currently, DIDP and DINP are increasingly used as substitutes to DEHP and are the most commonly used plasticizers in Western Europe.[13]

Experimental studies suggested that phthalates as DINP and DEHP could release proinflammatory mediators in lung cells and have an adjuvant effect on immune response in mice or rats, following dietary exposure,[6,10,33] inhalation[11] or subcutaneous

injection.[18] These mechanisms may enhance airway hyperresponsiveness by the infiltration of inflammatory cells in lung tissue.[20,28] DEHP may also interact with the PPARs nuclear receptors superfamily and lead to abnormal alveolar maturation and reduced surfactant production.[20]

We relied on discrete time survival modelling to assess associations with respiratory diseases. This approach was justified by the prospective nature of our study and has the advantage of allowing for efficiently taking into account subjects lost to follow-up and timing of disease occurrence as well as incorporating time-varying adjustment factors such as postnatal passive smoking. Few previous studies on this topic (e.g. Gascon et al.[9]) had relied on survival modelling, to our knowledge.

The present study considered an objective measure of the pulmonary function at an early age. FEV_1 is the most widely used lung function measurement in epidemiology, with strong reproducibility.[23] However, obtaining satisfactory forced expiratory manoeuvres is difficult in children under 6 years of age, as very young children are not always able to produce prolonged expirations.[2] We therefore did not analyse the FVC and the ratio FEV_1/FVC and 39% of the eligible children were not considered in the analysis of FEV_1 because of exhalation times shorter than one second. Still, like in a previous study in a similar age range population,[38] mean FEV_1% (90.0%) was lower than expected. In young children, $FEV_{0.5}$ or $FEV_{0.75}$ could be of interest but were not available in our study. From the questionnaires, we were not able to differentiate bronchiolitis and bronchitis occurrences since only one question was asked for these diseases. Additionally, our study did not take into account the well-known wheezing phenotypic heterogeneity relying on age at onset and symptoms persistency[19] as it would require a larger sample size to ensure a satisfactory statistical power.

The initial purpose of phenols and phthalates assays in the EDEN cohort was to investigate the impact of maternal exposure to ubiquitous endocrine disruptors on male genital organogenesis.[7] This was followed by studies on foetal and postnatal growth in male offspring.[26,25] Hence, data on prenatal exposures were available in boys only. Thus, our study was unable to address the existence of sex-specific effects suggested by previous studies.[4,9,16] However, focusing on one sex does not bias results, which only apply to boys and should not be generalized to girls. From a statistical point of view, focusing on a single sex is a way to optimize the study accuracy (limiting variance) in a context of limited total sample size (defined by our budget for chemicals assays), and possible sex-specific effects suggested by previous studies. From a public health point of view, identifying effects in a single sex, or in a specific sensitive subgroup, should be enough to support risk management decisions. Our analyses were conducted in a

relatively well-educated population with a majority of non-smoking mothers during pregnancy, but representativeness is not a condition of validity in etiological studies such as ours,[31] and focusing on rather homogeneous populations might actually limit bias due to unmeasured confounders. However, we cannot rule out the possibility of selection bias.

Reliance on a single maternal urine sample generally leads to exposure misclassification in the case of chemicals with high intra-individual temporal variability.[24] If we assume that error is of classical type, then the expected impact corresponds to attenuation bias, its amplitude being highest for bisphenol A and DEHP metabolites, the compounds with the largest within-subject variability.[24,29] For this reason, the lack of significant association with most exposure biomarkers, in particular bisphenol A and DEHP metabolites (those with the lowest intra-class correlations and hence the largest attenuation bias), should not be seen as strong evidence of a lack of effect of these compounds. We had no information on postnatal exposures which might be correlated to maternal pregnancy levels, so that in theory, any of the associations reported here could be due to postnatal (and not specifically prenatal) exposures. However, it has been shown that phenols and phthalates biomarker concentrations measured postnatally in children were poorly to moderately correlated with those from their mother during pregnancy.[5,40]

In conclusion, our prospective study relying on respiratory outcomes and pulmonary function tests showed possible adverse associations between prenatal urinary concentrations of 2,5-dichlorophenol, ethyl-paraben, bisphenol A, and DIDP biomarkers and respiratory health in boys until age 5 years, with ethyl-paraben and bisphenol A exhibiting some consistency across respiratory outcomes. The associations of bisphenol A pregnancy level with asthma diagnosis and bronchiolitis/bronchitis have been previously reported in a cohort study among boys and girls.[9] Our results add to an emerging literature on respiratory health impacts of early exposure to several phenols and phthalates.

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4.8 Supplemental material

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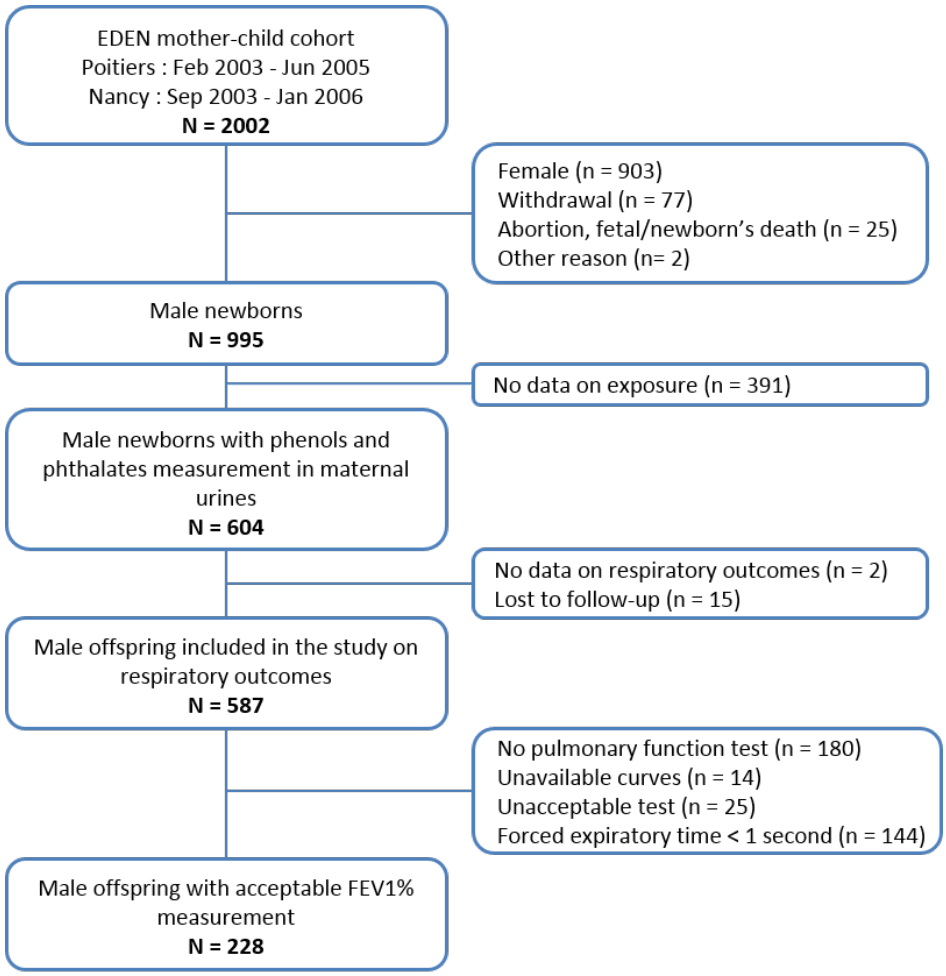

Figure 4.2 – Flow chart of the study in the EDEN mother-child cohort (not included in the online supplemental material).

Table 4.5 – Parent phthalates and associated urinary metabolites in the EDEN cohort.

Parent compounds	**Abbreviation**	**Urinary metabolites**	**Abbreviation**
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	MnBP
Di-isobutyl phthalate	DiBP	Mono-isobutyl phthalate	MiBP
Di-n-butyl phthalate	DnBP	Mono-(3-carboxypropyl) phthalate	MCPP
Di-*n*-octylphthalate	DNOP		
Other high molecular weight phthalates	/		
Butylbenzyl phthalate	BBzP	Mono-benzyl phthalate	MBzP
Di-isodecyl phthalate	DIDP	Mono-(carboxynonyl) phthalate	MCNP
Di-isononyl phthalate	DINP	Mono(carboxyoctyl) phthalate	MCOP
Di-2-ethylhexyl phthalate	DEHP	Mono-(2-ethyl-5-hydroxyhexyl phthalate	MEHHP
		Mono-(2-ethyl-5-oxohexyl) phthalate	MEOHP
		Mono-(2-ethyl-5-carboxypentyl) phthalate	MECPP
		Mono-2-ethylhexyl phthalate	MEHP

Table 4.6 – Spearman correlation between ln-transformed standardized [a] phenol concentrations.

	2,4-DCP	2,5-DCP	∑-DCP	BPA	BP3	TCS	MP	EP	PP	BP	∑PB
2,4-DCP	1.00										
2,5-DCP	0.82	1.00									
∑2-DCP	0.86	1.00	1.00								
BPA	0.01	0.05	0.05	1.00							
BP3	0.06	0.04	0.04	0.03	1.00						
TCS	0.13	-0.11	-0.08	-0.05	0.01	1.00					
MP	-0.02	-0.09	-0.08	0.07	0.20	0.15	1.00				
EP	0.08	-0.04	-0.02	-0.03	0.15	0.16	0.55	1.00			
PP	0.00	-0.07	-0.06	0.00	0.12	0.22	0.81	0.49	1.00		
BP	0.03	-0.11	-0.09	0.00	0.16	0.23	0.55	0.68	0.53	1.00	
∑PB	-0.01	-0.09	-0.08	0.05	0.19	0.17	0.99	0.59	0.84	0.58	1.00

2,4-DCP = 2,4-Dichlorophenol; 2,5-DCP = 2,5-Dichlorophenol; ∑-DCP = molar sum of Dichlorophenols (2,4-DCP, 2,5-DCP); BPA = Bisphenol A; BP3 = Benzophenone-3; TCS = Triclosan; MP = Methyl-paraben; EP = Ethyl-paraben; PP = Propyl-paraben; BP = Butyl-paraben; ∑PB = molar sum of parabens (MP, EP, PP, BP).

[a] Standardized for urine sampling conditions (creatinine level, day and hour of sampling, gestational age, storage duration at room temperature and year of analysis), as detailed in Mortamais et al. (2012).

Table 4.7 – Spearman correlation between ln-transformed standardized [a] phthalate metabolites concentrations.

	∑LMW	MEP	MnBP	MiBP	∑HMW	MCPP	MBzP	MCNP	MCOP	MEHHP	MEOHP	MECPP	MEHP	∑DEHP
∑LMW	1.00													
MEP	0.78	1.00												
MnBP	0.59	0.08	1.00											
MiBP	0.33	0.03	0.39	1.00										
∑HMW	0.16	0.00	0.33	0.37	1.00									
MCPP	0.41	0.04	0.64	0.24	0.31	1.00								
MBzP	0.27	0.12	0.37	0.43	0.57	0.23	1.00							
MCNP	0.05	0.03	0.06	0.10	0.28	0.29	0.13	1.00						
MCOP	0.06	0.02	0.06	0.12	0.44	0.23	0.25	0.44	1.00					
MEHHP	0.11	0.01	0.25	0.31	0.93	0.21	0.38	0.21	0.35	1.00				
MEOHP	0.11	0.00	0.26	0.35	0.95	0.23	0.41	0.22	0.37	0.98	1.00			
MECPP	0.03	-0.04	0.18	0.26	0.93	0.18	0.32	0.23	0.37	0.94	0.95	1.00		
MEHP	0.10	-0.01	0.22	0.36	0.83	0.19	0.38	0.16	0.33	0.82	0.84	0.80	1.00	
∑DEHP	0.08	-0.02	0.23	0.31	0.95	0.20	0.37	0.22	0.36	0.98	0.99	0.98	0.86	1.00

∑LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); MEP = Monoethyl phthalate; MnBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; ∑HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); MCPP = Mono (3-carboxypropyl) phthalate; MBzP = Monobenzyl phthalate; MCNP = Mono-(carboxynonyl) phthalate, MCOP = Monocarboxyoctyl phthalate; MEHHP = Mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = Mono(2-ethyl-5-oxohexyl) phthalate; MECPP = Mono(2-ethyl-5-carboxypentyl) phthalate; MEHP = Mono(2-ethylhexyl) phthalate; ∑DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP).

[a] Standardized for urine sampling conditions (creatinine level, day and hour of sampling, gestational age, storage duration at room temperature and year of analysis), as detailed in Mortamais et al. (2012).

Table 4.8 – Adjusted associations between pregnancy phenols raw (non-standardized) concentrations and respiratory outcomes (n=587) and $FEV_1\%$ (n=228) in boys. Models additionally adjusted for creatinine.

	Wheezing (until age 5y)[a]			Asthma diagnosis (until age 5y)[a]			Bronchiolitis/Bronchitis (until age 3y)[a]			$FEV_1\%$[b]		
	N = 587			N = 587			N = 587			N = 228		
Phenol[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
2,4-Dichlorophenol												
Continuous[d]	1.06 (0.95, 1.18)		0.27	1.02 (0.87, 1.20)		0.79	0.98 (0.89, 1.08)		0.69	0.31 (-0.93, 1.55)		0.62
T1	1.00	0.31	*0.23*	1.00	0.85	*0.64*	1.00	0.93	*0.71*	0.00	0.91	*0.85*
T2	0.89 (0.64, 1.22)			0.94 (0.58, 1.52)			1.00 (0.78, 1.29)			0.54 (-3.11, 4.18)		
T3	1.13 (0.83, 1.55)			1.08 (0.67, 1.74)			0.96 (0.74, 1.24)			-0.20 (-3.93, 3.54)		
2,5-Dichlorophenol												
Continuous[d]	1.08 (1.00, 1.16)		0.04	1.04 (0.93, 1.16)		0.53	1.00 (0.94, 1.07)		0.97	0.18 (-0.67, 1.03)		0.68
T1	1.00	0.02	*0.39*	1.00	0.22	*0.50*	1.00	0.84	*0.67*	0.00	0.82	*0.85*
T2	1.57 (1.14, 2.16)			1.51 (0.94, 2.42)			1.06 (0.82, 1.37)			1.15 (-2.50, 4.80)		
T3	1.38 (1.00, 1.91)			1.37 (0.85, 2.23)			1.07 (0.84, 1.38)			0.87 (-2.82, 4.56)		
∑Dichlorophenols												
Continuous[d]	1.08 (1.00, 1.17)		0.05	1.04 (0.92, 1.17)		0.56	1.00 (0.94, 1.07)		1.00	0.21 (-0.69, 1.11)		0.65
T1	1.00	0.02	*0.41*	1.00	0.17	*0.53*	1.00	0.67	*0.51*	0.00	0.76	*0.85*
T2	1.59 (1.15, 2.19)			1.57 (0.97, 2.53)			1.09 (0.85, 1.41)			1.36 (-2.35, 5.06)		
T3	1.39 (1.00, 1.93)			1.39 (0.85, 2.27)			1.12 (0.87, 1.44)			0.99 (-2.72, 4.70)		
Bisphenol A												
Continuous[d]	0.98 (0.83, 1.15)		0.77	1.21 (0.96, 1.53)		0.11	1.13 (0.99, 1.30)		0.07	-0.55 (-2.34, 1.23)		0.54
T1	1.00	0.14	*0.71*	1.00	0.51	*0.43*	1.00	0.11	*0.13*	0.00	0.61	*0.46*
T2	1.33 (0.97, 1.82)			1.30 (0.79, 2.14)			1.27 (0.98, 1.63)			-1.52 (-5.13, 2.09)		
T3	1.05 (0.74, 1.49)			1.32 (0.79, 2.19)			1.29 (0.99, 1.70)			-1.87 (-5.84, 2.10)		
Benzophenone-3												
Continuous[d]	0.94 (0.87, 1.01)		0.10	0.96 (0.85, 1.07)		0.46	0.99 (0.93, 1.05)		0.82	-0.32 (-1.19, 0.54)		0.46
T1	1.00	0.14	*0.05*	1.00	0.15	*0.27*	1.00	0.73	*0.43*	0.00	0.80	*0.50*
T2	0.96 (0.71, 1.30)			0.67 (0.43, 1.07)			0.98 (0.77, 1.25)			-0.09 (-3.61, 3.42)		
T3	0.74 (0.54, 1.02)			0.68 (0.42, 1.09)			0.91 (0.70, 1.17)			-1.09 (-4.68, 2.50)		
Triclosan												
Continuous[d]	0.98 (0.94, 1.03)		0.50	0.99 (0.92, 1.07)		0.86	0.99 (0.96, 1.03)		0.79	0.18 (-0.38, 0.75)		0.52
T1	1.00	0.74	*0.85*	1.00	0.83	*0.81*	1.00	0.95	*0.82*	0.00	0.97	*0.86*
T2	0.89 (0.65, 1.21)			1.13 (0.72, 1.77)			0.97 (0.76, 1.24)			-0.25 (-3.72, 3.22)		
T3	0.93 (0.69, 1.25)			1.00 (0.63, 1.60)			0.96 (0.76, 1.23)			0.19 (-3.31, 3.69)		
Methyl-paraben												
Continuous[d]	0.93 (0.85, 1.00)		0.06	0.99 (0.88, 1.12)		0.91	0.94 (0.88, 1.00)		0.05	-0.38 (-1.22, 0.46)		0.37
T1	1.00	0.11	*0.21*	1.00	0.56	*0.70*	1.00	0.02	*0.01*	0.00	0.65	*0.44*
T2	0.75 (0.55, 1.02)			1.23 (0.78, 1.95)			0.82 (0.65, 1.05)			0.65 (-2.85, 4.14)		
T3	0.76 (0.56, 1.04)			0.99 (0.60, 1.62)			0.68 (0.53, 0.89)			-1.03 (-4.70, 2.63)		
Ethyl-paraben												
Continuous[d]	1.01 (0.96, 1.08)		0.63	1.09 (1.00, 1.20)		0.06	0.99 (0.94, 1.03)		0.56	-0.55 (-1.19, 0.10)		0.10
T1	1.00	0.82	*0.55*	1.00	0.18	*0.31*	1.00	0.02	*0.05*	0.00	0.20	*0.08*
T2	0.98 (0.71, 1.34)			1.51 (0.93, 2.44)			1.22 (0.96, 1.56)			0.19 (-3.41, 3.80)		
T3	1.07 (0.78, 1.48)			1.51 (0.91, 2.51)			0.87 (0.67, 1.13)			-2.65 (-6.25, 0.96)		
Propyl-paraben												
Continuous[d]	0.96 (0.90, 1.02)		0.18	0.98 (0.89, 1.08)		0.71	0.96 (0.91, 1.01)		0.09	-0.12 (-0.82, 0.59)		0.74
T1	1.00	0.53	*0.31*	1.00	0.43	*0.37*	1.00	0.03	*0.05*	0.00	0.68	*0.38*
T2	0.91 (0.67, 1.23)			1.19 (0.75, 1.86)			0.77 (0.61, 0.99)			-0.37 (-3.94, 3.20)		
T3	0.84 (0.61, 1.14)			0.87 (0.53, 1.42)			0.73 (0.57, 0.94)			-1.56 (-5.18, 2.07)		
Butyl-paraben												
Continuous[d]	0.98 (0.92, 1.04)		0.54	1.00 (0.91, 1.10)		0.98	0.97 (0.93, 1.02)		0.29	-0.37 (-1.05, 0.30)		0.28
T1	1.00	0.43	*0.20*	1.00	1.00	*0.96*	1.00	0.003	*0.002*	0.00	0.67	*0.58*
T2	1.01 (0.75, 1.37)			1.02 (0.64, 1.61)			1.15 (0.90, 1.46)			-1.30 (-4.79, 2.19)		
T3	0.84 (0.61, 1.16)			1.00 (0.61, 1.63)			0.75 (0.58, 0.97)			-1.48 (-5.01, 2.04)		
∑Parabens												
Continuous[d]	0.93 (0.86, 1.00)		0.06	0.99 (0.88, 1.12)		0.93	0.94 (0.88, 1.00)		0.06	-0.37 (-1.21, 0.47)		0.39
T1	1.00	0.13	*0.07*	1.00	0.34	*0.46*	1.00	0.02	*0.004*	0.00	0.81	*0.56*
T2	0.83 (0.62, 1.13)			1.27 (0.81, 2.00)			0.90 (0.71, 1.14)			0.28 (-3.19, 3.74)		
T3	0.72 (0.52, 0.99)			0.92 (0.55, 1.52)			0.69 (0.53, 0.89)			-0.88 (-4.57, 2.80)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑Dichlorophenols = molar sum of Dichlorophenols (2,4-, 2,5-dichlorophenols); ∑Parabens = molar sum of parabens (Methyl-, Ethyl-, Propyl-, Butyl-parabens).

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.9 – Adjusted associations between pregnancy phthalate metabolites raw (non-standardized) concentrations and respiratory outcomes (n=587) and $FEV_1\%$ (n=228) in boys. Models additionally adjusted for creatinine.

	Wheezing (until age 5y)[a]			**Asthma diagnosis (until age 5y)[a]**			**Bronchiolitis/Bronchitis (until age 3y)[a]**			**$FEV_1\%$[b]**		
	N = 587			N = 587			N = 587			N = 228		
Phthalate[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
∑LMW												
Continuous[d]	0.97 (0.84, 1.12)		0.69	0.97 (0.78, 1.21)		0.80	1.00 (0.89, 1.12)		1.00	-0.43 (-2.14, 1.27)		0.62
T1	1.00	0.80	*0.86*	1.00	0.94	*0.75*	1.00	0.71	*0.72*	0.00	0.16	*0.06*
T2	1.10 (0.79, 1.52)			0.94 (0.58, 1.52)			0.92 (0.71, 1.19)			0.35 (-3.23, 3.92)		
T3	1.01 (0.71, 1.43)			0.91 (0.54, 1.53)			1.01 (0.77, 1.34)			-3.00 (-6.96, 0.96)		
MEP												
Continuous[d]	0.99 (0.88, 1.11)		0.86	1.03 (0.87, 1.22)		0.72	1.02 (0.94, 1.12)		0.63	-0.10 (-1.45, 1.24)		0.88
T1	1.00	0.87	*0.80*	1.00	0.99	*0.90*	1.00	0.75	*0.99*	0.00	0.67	*0.51*
T2	1.07 (0.77, 1.47)			0.99 (0.61, 1.60)			1.10 (0.85, 1.42)			0.87 (-2.65, 4.39)		
T3	0.99 (0.71, 1.38)			1.02 (0.62, 1.69)			1.04 (0.80, 1.36)			-0.77 (-4.72, 3.19)		
MnBP												
Continuous[d]	1.03 (0.92, 1.16)		0.62	1.00 (0.83, 1.19)		0.96	0.98 (0.89, 1.07)		0.61	-0.35 (-1.69, 0.99)		0.61
T1	1.00	0.64	*0.36*	1.00	0.64	*0.89*	1.00	0.69	*0.98*	0.00	0.41	*0.34*
T2	1.01 (0.73, 1.40)			1.23 (0.76, 1.96)			0.90 (0.69, 1.16)			1.47 (-2.13, 5.09)		
T3	1.15 (0.81, 1.64)			1.04 (0.61, 1.79)			0.96 (0.73, 1.28)			-0.89 (-4.89, 3.11)		
MiBP												
Continuous[d]	0.98 (0.84, 1.14)		0.76	1.03 (0.81, 1.30)		0.82	1.02 (0.90, 1.16)		0.72	-1.34 (-3.02, 0.34)		0.12
T1	1.00	0.28	*0.22*	1.00	0.36	*0.47*	1.00	0.92	*0.96*	0.00	0.14	*0.06*
T2	0.90 (0.65, 1.25)			0.78 (0.48, 1.27)			0.95 (0.74, 1.22)			0.53 (-3.07, 4.14)		
T3	1.17 (0.83, 1.66)			1.11 (0.65, 1.87)			0.99 (0.75, 1.31)			-3.01 (-7.05, 1.03)		
∑HMW												
Continuous[d]	1.05 (0.90, 1.23)		0.54	1.14 (0.90, 1.45)		0.28	0.90 (0.79, 1.03)		0.13	-1.26 (-3.06, 0.55)		0.17
T1	1.00	0.07	*0.21*	1.00	0.13	*0.20*	1.00	0.12	*0.09*	0.00	0.09	*0.03*
T2	1.46 (1.05, 2.03)			1.64 (1.00, 2.69)			1.05 (0.82, 1.36)			-0.36 (-3.97, 3.24)		
T3	1.36 (0.95, 1.93)			1.57 (0.91, 2.68)			0.81 (0.61, 1.08)			-4.03 (-8.08, -0.02)		
MCPP												
Continuous[d]	1.03 (0.89, 1.20)		0.68	0.98 (0.77, 1.24)		0.85	0.90 (0.80, 1.02)		0.11	-0.49 (-2.32, 1.35)		0.60
T1	1.00	0.55	*0.28*	1.00	0.20	*0.94*	1.00	0.77	*0.92*	0.00	0.15	*0.87*
T2	1.08 (0.78, 1.49)			1.50 (0.93, 2.41)			0.91 (0.70, 1.18)			-3.59 (-7.19, 0.01)		
T3	1.21 (0.86, 1.69)			1.13 (0.65, 1.94)			0.96 (0.73, 1.26)			-2.00 (-5.89, 1.90)		
MBzP												
Continuous[d]	0.98 (0.86, 1.11)		0.75	1.17 (0.97, 1.41)		0.10	0.97 (0.87, 1.07)		0.51	-0.23 (-1.73, 1.25)		0.75
T1	1.00	0.87	*0.63*	1.00	0.14	*0.12*	1.00	0.28	*0.84*	0.00	0.82	*0.86*
T2	0.96 (0.71, 1.37)			1.51 (0.91, 2.49)			1.22 (0.94, 1.58)			-1.20 (-4.94, 2.54)		
T3	1.07 (0.76, 1.51)			1.68 (0.98, 2.86)			1.06 (0.79, 1.40)			-0.79 (-4.79, 3.22)		
MCNP												
Continuous[d]	1.10 (0.98, 1.24)		0.10	1.15 (0.97, 1.36)		0.12	0.98 (0.89, 1.08)		0.64	0.51 (-0.90, 1.92)		0.48
T1	1.00	0.05	*0.02*	1.00	0.20	*0.07*	1.00	0.62	*0.56*	0.00	0.12	*0.79*
T2	1.05 (0.76, 1.46)			1.14 (0.68, 1.89)			1.12 (0.87, 1.45)			-3.50 (-7.17, 0.17)		
T3	1.44 (1.03, 2.01)			1.54 (0.93, 2.55)			1.12 (0.86, 1.47)			-0.72 (-4.58, 3.15)		
MCOP												
Continuous[d]	1.03 (0.89, 1.20)		0.67	1.04 (0.84, 1.29)		0.72	1.08 (0.96, 1.22)		0.19	-1.17 (-2.77, 0.42)		0.15
T1	1.00	0.84	*0.78*	1.00	0.75	*0.71*	1.00	0.86	*0.87*	0.00	0.64	*0.37*
T2	1.10 (0.80, 1.51)			1.20 (0.74, 1.93)			1.07 (0.83, 1.38)			-0.36 (-3.92, 3.20)		
T3	1.07 (0.77, 1.50)			1.14 (0.69, 1.90)			1.04 (0.79, 1.36)			-1.70 (-5.51, 2.10)		
MEHHP												
Continuous[d]	1.05 (0.91, 1.20)		0.54	1.07 (0.86, 1.32)		0.54	0.95 (0.85, 1.07)		0.41	-1.12 (-2.64, 0.40)		0.15
T1	1.00	0.21	*0.37*	1.00	0.32	*0.66*	1.00	0.68	*0.87*	0.00	0.06	*0.03*
T2	1.34 (0.97, 1.85)			1.43 (0.89, 2.30)			1.10 (0.86, 1.42)			-3.14 (-6.61, 0.32)		
T3	1.25 (0.89, 1.75)			1.21 (0.72, 2.04)			1.01 (0.77, 1.32)			-4.49 (-8.30, -0.68)		
MEOHP												
Continuous[d]	1.05 (0.91, 1.21)		0.51	1.07 (0.86, 1.33)		0.54	0.93 (0.83, 1.04)		0.21	-1.42 (-2.98, 0.14)		0.08
T1	1.00	0.27	*0.24*	1.00	0.55	*0.72*	1.00	0.77	*0.48*	0.00	0.25	*0.09*
T2	1.28 (0.92, 1.77)			1.30 (0.81, 2.10)			0.98 (0.76, 1.27)			-1.03 (-4.64, 2.57)		
T3	1.28 (0.91, 1.80)			1.17 (0.69, 1.98)			0.91 (0.70, 1.20)			-3.30 (-7.29, 0.69)		
MECPP												
Continuous[d]	1.07 (0.92, 1.25)		0.38	1.11 (0.88, 1.40)		0.37	0.92 (0.81, 1.05)		0.20	-1.38 (-3.08, 0.32)		0.11
T1	1.00	0.30	*0.57*	1.00	0.41	*0.62*	1.00	0.84	*0.55*	0.00	0.32	*0.15*
T2	1.30 (0.93, 1.80)			1.40 (0.86, 2.27)			0.98 (0.76, 1.27)			-1.93 (-5.67, 1.81)		
T3	1.19 (0.84, 1.70)			1.25 (0.74, 2.14)			0.92 (0.70, 1.22)			-3.10 (-7.15, 0.96)		
MEHP												
Continuous[d]	1.01 (0.89, 1.14)	0.43	0.90	1.03 (0.85, 1.23)		0.79	0.92 (0.83, 1.01)		0.09	-1.02 (-2.33, 0.28)		0.12
T1	1.00		*0.30*	1.00	0.62	*0.45*	1.00	0.52	*0.64*	0.00	0.27	*0.17*
T2	0.93 (0.67, 1.29)			0.92 (0.57, 1.49)			0.86 (0.67, 1.11)			0.23 (-3.33, 3.80)		
T3	1.14 (0.82, 1.58)			1.16 (0.72, 1.88)			0.91 (0.70, 1.19)			-3.10 (-6.25, 1.22)		
∑DEHP												
Continuous[d]	1.05 (0.90, 1.22)		0.52	1.08 (0.86, 1.36)		0.50	0.92 (0.82, 1.04)		0.20	-1.38 (-3.02, 0.26)		0.10
T1	1.00	0.09	*0.33*	1.00	0.23	*0.60*	1.00	0.94	*0.78*	0.00	0.24	*0.19*
T2	1.45 (1.04, 2.01)			1.52 (0.94, 2.47)			0.96 (0.74, 1.24)			-2.71 (-6.28, 0.87)		
T3	1.30 (0.92, 1.84)			1.29 (0.75, 2.20)			0.96 (0.73, 1.26)			-3.05 (-7.02, 0.91)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); ∑HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); ∑DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). Parent compounds and associated metabolites are detailed in Table S1 (Supplemental material)

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.10 – Adjusted associations between pregnancy phenols standardized concentrations and respiratory outcomes (n=447) and $FEV_1\%$ (n=171) in boys from non-smoking mothers.

	Wheezing (until age 5y)[a]			Asthma diagnosis (until age 5y)[a]			Bronchiolitis/Bronchitis (until age 3y)[a]			$FEV_1\%$[b]		
	N = 447			N = 447			N = 447			N = 171		
Phenol[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
2,4-Dichlorophenol												
Continuous[d]	1.04 (0.92, 1.18)		0.53	1.04 (0.86, 1.25)		0.72	0.95 (0.85, 1.06)		0.36	0.76 (-0.76, 2.28)		0.32
T1	1.00	0.46	*0.90*	1.00	0.80	*0.99*	1.00	0.58	*0.30*	0.00	0.58	*0.97*
T2	1.25 (0.88, 1.80)			1.19 (0.70, 2.02)			0.96 (0.72, 1.27)			2.23 (-2.14, 6.61)		
T3	1.11 (0.77, 1.59)			1.06 (0.62, 1.81)			0.86 (0.65, 1.14)			0.70 (-3.57, 4.97)		
2,5-Dichlorophenol												
Continuous[d]	1.06 (0.98, 1.16)		0.16	1.05 (0.92, 1.20)		0.46	0.98 (0.91, 1.05)		0.52	0.46 (-0.58, 1.51)		0.38
T1	1.00	0.02	*0.29*	1.00	0.13	*0.43*	1.00	0.96	*0.86*	0.00	0.97	*0.90*
T2	1.70 (1.17, 2.48)			1.76 (1.01, 3.05)			1.03 (0.77, 1.38)			0.54 (-3.79, 4.87)		
T3	1.46 (1.01, 2.12)			1.50 (0.86, 2.62)			0.99 (0.74, 1.32)			0.43 (-3.84, 4.70)		
∑Dichlorophenols												
Continuous[d]	1.06 (0.97, 1.16)		0.19	1.05 (0.91, 1.20)		0.52	0.97 (0.90, 1.05)		0.48	0.51 (-0.58, 1.60)		0.36
T1	1.00	0.11	*0.43*	1.00	0.11	*0.54*	1.00	0.92	*1.00*	0.00	0.97	*0.99*
T2	1.48 (1.02, 2.15)			1.80 (1.04, 3.12)			1.06 (0.79, 1.42)			0.52 (-3.77, 4.80)		
T3	1.32 (0.92, 1.91)			1.45 (0.83, 2.54)			1.02 (0.77, 1.36)			0.16 (-4.10, 4.42)		
Bisphenol A												
Continuous[d]	1.02 (0.83, 1.24)		0.87	1.23 (0.93, 1.62)		0.15	1.16 (0.98, 1.37)		0.08	-0.25 (-2.43, 1.93)		0.82
T1	1.00	0.70	*0.43*	1.00	0.26	*0.10*	1.00	0.38	*0.20*	0.00	0.60	*0.35*
T2	1.11 (0.77, 1.59)			1.14 (0.63, 2.05)			1.00 (0.75, 1.33)			-1.92 (-6.12, 2.28)		
T3	1.17 (0.81, 1.69)			1.54 (0.89, 2.66)			1.19 (0.89, 1.58)			-1.86 (-6.18, 2.46)		
Benzophenone-3												
Continuous[d]	0.95 (0.87, 1.04)		0.29	1.00 (0.88, 1.14)		0.99	0.99 (0.93, 1.07)		0.88	-0.58 (-1.60, 0.44)		0.26
T1	1.00	0.11	*0.15*	1.00	0.14	*0.52*	1.00	0.94	*0.75*	0.00	0.36	*0.40*
T2	0.75 (0.53, 1.07)			0.59 (0.35, 1.02)			0.98 (0.74, 1.31)			-2.59 (-6.81, 1.62)		
T3	0.69 (0.48, 1.00)			0.69 (0.40, 1.16)			0.95 (0.71, 1.27)			-2.73 (-6.97, 1.51)		
Triclosan												
Continuous[d]	0.99 (0.94, 1.05)		0.78	0.99 (0.91, 1.08)		0.79	1.01 (0.96, 1.05)		0.77	0.08 (-0.61, 0.76)		0.83
T1	1.00	0.77	*0.48*	1.00	0.71	*0.44*	1.00	0.53	*0.71*	0.00	0.92	*0.69*
T2	0.96 (0.68, 1.37)			1.06 (0.64, 1.75)			1.16 (0.87, 1.53)			-0.03 (-4.18, 4.12)		
T3	0.88 (0.62, 1.25)			0.84 (0.49, 1.46)			1.01 (0.76, 1.34)			0.71 (-3.50, 4.99)		
Methyl-paraben												
Continuous[d]	0.91 (0.83, 1.00)		0.05	0.98 (0.85, 1.12)		0.72	0.96 (0.89, 1.03)		0.24	-0.48 (-1.45, 0.49)		0.33
T1	1.00	0.09	*0.24*	1.00	0.92	*0.93*	1.00	0.12	*0.09*	0.00	0.24	*0.18*
T2	0.70 (0.49, 0.99)			1.12 (0.66, 1.89)			0.81 (0.61, 1.07)			1.62 (-2.59, 5.82)		
T3	0.75 (0.53, 1.06)			1.05 (0.61, 1.82)			0.75 (0.56, 1.00)			-1.98 (-6.02, 2.07)		
Ethyl-paraben												
Continuous[d]	0.99 (0.93, 1.07)		0.88	1.10 (0.99, 1.23)		0.08	1.00 (0.96, 1.06)		0.98	-0.57 (-1.32, 0.19)		0.14
T1	1.00	0.82	*0.53*	1.00	0.08	*0.05*	1.00	0.03	*0.38*	0.00	0.55	*0.28*
T2	1.04 (0.72, 1.49)			1.47 (0.83, 2.60)			1.41 (1.06, 1.87)			-0.35 (-4.56, 3.87)		
T3	1.12 (0.78, 1.62)			1.91 (1.08, 3.37)			1.03 (0.76, 1.39)			-2.11 (-6.26, 2.04)		
Propyl-paraben												
Continuous[d]	0.97 (0.90, 1.04)		0.40	1.01 (0.90, 1.14)		0.83	0.96 (0.91, 1.03)		0.25	-0.30 (-1.11, 0.52)		0.47
T1	1.00	0.71	*0.57*	1.00	0.75	*0.97*	1.00	0.34	*0.17*	0.00	0.91	*0.86*
T2	1.09 (0.77, 1.55)			1.22 (0.72, 2.07)			0.91 (0.69, 1.20)			-0.91 (-5.03, 3.20)		
T3	0.94 (0.66, 1.35)			1.08 (0.63, 1.86)			0.81 (0.61, 1.07)			-0.57 (-4.71, 3.56)		
Butyl-paraben												
Continuous[d]	0.97 (0.90, 1.05)		0.45	1.00 (0.89, 1.11)		0.93	0.98 (0.93, 1.04)		0.56	-0.43 (-1.23, 0.37)		0.29
T1	1.00	0.34	*0.35*	1.00	0.67	*0.85*	1.00	0.08	*0.08*	0.00	0.47	*0.34*
T2	1.21 (0.85, 1.73)			1.28 (0.75, 2.19)			1.20 (0.90, 1.60)			-1.72 (-5.85, 2.41)		
T3	0.94 (0.64, 1.38)			1.19 (0.67, 2.12)			0.87 (0.64, 1.17)			-2.56 (-6.74, 1.62)		
∑Parabens												
Continuous[d]	0.91 (0.83, 1.00)		0.04	0.98 (0.85, 1.13)		0.79	0.96 (0.89, 1.03)		0.26	-0.45 (-1.43, 0.52)		0.36
T1	1.00	0.15	*0.16*	1.00	0.82	*0.67*	1.00	0.18	*0.27*	0.00	0.52	*0.31*
T2	0.75 (0.53, 1.07)			1.10 (0.66, 1.83)			0.79 (0.60, 1.05)			0.73 (-3.48, 4.95)		
T3	0.73 (0.51, 1.05)			0.92 (0.53, 1.60)			0.81 (0.61, 1.07)			-1.63 (-5.70, 2.44)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑Dichlorophenols = molar sum of Dichlorophenols (2,4-, 2,5-dichlorophenols); ∑Parabens = molar sum of parabens (Methyl-, Ethyl-, Propyl-, Butyl-parabens).

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.11 – Adjusted associations between pregnancy phthalate metabolites standardized concentrations and respiratory outcomes (n=447) and $FEV_1\%$ (n=171) in boys from non-smoking mothers.

	Wheezing (until age 5y)[a]			Asthma diagnosis (until age 5y)[a]			Bronchiolitis/Bronchitis (until age 3y)[a]			$FEV_1\%$[b]		
	N = 447			N = 447			N = 447			N = 171		
Phthalate[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
∑LMW												
Continuous[d]	0.93 (0.78, 1.11)		0.42	1.01 (0.78, 1.32)		0.93	1.05 (0.91, 1.21)		0.49	-0.13 (-2.36, 2.10)		0.91
T1	1.00	0.74	*0.89*	1.00	0.05	*0.90*	1.00	0.64	*0.36*	0.00	0.64	*0.35*
T2	1.15 (0.81, 1.64)			1.84 (1.09, 3.10)			1.07 (0.80, 1.43)			-0.56 (-4.77, 3.65)		
T3	1.06 (0.74, 1.52)			1.18 (0.66, 2.12)			1.15 (0.86, 1.52)			-1.93 (-6.02, 2.17)		
MEP												
Continuous[d]	0.97 (0.84, 1.11)		0.62	1.10 (0.90, 1.34)		0.36	1.03 (0.92, 1.15)		0.60	-0.37 (-2.11, 1.37)		0.67
T1	1.00	0.97	*0.87*	1.00	0.55	*0.35*	1.00	0.92	*0.68*	0.00	0.77	*0.72*
T2	0.98 (0.69, 1.39)			1.25 (0.72, 2.15)			1.01 (0.76, 1.35)			0.81 (-3.26, 4.50)		
T3	1.02 (0.71, 1.46)			1.34 (0.78, 2.32)			1.06 (0.80, 1.41)			-0.69 (-4.91, 3.53)		
MnBP												
Continuous[d]	1.04 (0.91, 1.19)		0.52	1.01 (0.83, 1.24)		0.91	1.03 (0.92, 1.15)		0.65	0.22 (-1.36, 1.79)		0.79
T1	1.00	0.73	*0.47*	1.00	0.71	*0.91*	1.00	0.93	*0.84*	0.00	0.93	*0.75*
T2	0.97 (0.67, 1.39)			1.23 (0.73, 2.08)			0.95 (0.72, 1.27)			0.09 (-4.14, 4.31)		
T3	1.11 (0.78, 1.58)			1.04 (0.60, 1.81)			0.96 (0.72, 1.27)			-0.64 (-4.88, 3.60)		
MiBP												
Continuous[d]	0.95 (0.79, 1.14)		0.58	1.01 (0.77, 1.33)		0.92	0.98 (0.84, 1.14)		0.80	-0.51 (-2.62, 1.60)		0.63
T1	1.00	0.83	*0.94*	1.00	0.64	*0.71*	1.00	1.00	*0.95*	0.00	0.46	*0.23*
T2	0.89 (0.62, 1.29)			1.21 (0.71, 2.06)			1.00 (0.75, 1.34)			-0.11 (-4.34, 4.11)		
T3	0.96 (0.67, 1.37)			0.95 (0.55, 1.64)			1.01 (0.75, 1.35)			-2.28 (-6.36, 1.80)		
∑HMW												
Continuous[d]	1.08 (0.90, 1.29)		0.44	1.12 (0.84, 1.48)		0.45	0.92 (0.78, 1.07)		0.27	-0.79 (-2.99, 1.41)		0.48
T1	1.00	0.28	*0.64*	1.00	0.64	*0.45*	1.00	0.85	*0.68*	0.00	0.65	*0.61*
T2	1.34 (0.93, 1.92)			0.91 (0.53, 1.58)			1.04 (0.78, 1.37)			-1.96 (-6.19, 2.27)		
T3	1.16 (0.80, 1.68)			1.17 (0.69, 1.97)			0.95 (0.71, 1.27)			-1.23 (-5.39, 2.93)		
MCPP												
Continuous[d]	0.98 (0.82, 1.17)		0.85	0.94 (0.72, 1.23)		0.65	0.92 (0.79, 1.06)		0.25	0.08 (-2.08, 2.25)		0.94
T1	1.00	0.99	*0.90*	1.00	0.87	*0.62*	1.00	0.18	*0.14*	0.00	0.75	*0.83*
T2	0.99 (0.69, 1.40)			0.93 (0.55, 1.58)			1.11 (0.84, 1.46)			-0.95 (-5.14, 3.23)		
T3	0.98 (0.68, 1.40)			0.87 (0.50, 1.49)			0.85 (0.63, 1.14)			0.28 (-3.87, 4.83)		
MBzP												
Continuous[d]	1.01 (0.87, 1.17)		0.90	1.22 (0.99, 1.51)		0.06	0.96 (0.85, 1.08)		0.46	0.20 (-1.62, 2.01)		0.83
T1	1.00	0.80	*1.00*	1.00	0.28	*0.38*	1.00	0.67	*0.39*	0.00	0.10	*0.31*
T2	1.13 (0.78, 1.62)			1.56 (0.88, 2.77)			1.00 (0.75, 1.33)			-4.34 (-8.55, -0.13)		
T3	1.03 (0.72, 1.49)			1.42 (0.82, 2.47)			0.89 (0.67, 1.19)			-3.39 (-7.52, 0.73)		
MCNP												
Continuous[d]	1.04 (0.90, 1.19)		0.60	0.99 (0.80, 1.22)		0.91	0.93 (0.82, 1.04)		0.19	1.36 (-0.28, 3.01)		0.10
T1	1.00	0.70	*0.40*	1.00	0.24	*0.41*	1.00	0.95	*0.88*	0.00	0.09	*0.19*
T2	1.02 (0.71, 1.46)			1.60 (0.92, 2.80)			0.95 (0.72, 1.27)			-2.71 (6.83, 1.41)		
T3	1.15 (0.81, 1.64)			1.43 (0.82, 2.49)			0.97 (0.73, 1.28)			1.97 (-2.21, 6.14)		
MCOP												
Continuous[d]	0.97 (0.82, 1.16)		0.77	0.95 (0.73, 1.22)		0.67	1.06 (0.93, 1.22)		0.37	-1.29 (-3.10, 0.53)		0.16
T1	1.00	0.50	*0.31*	1.00	0.62	*0.82*	1.00	0.33	*0.82*	0.00	0.46	*0.37*
T2	0.86 (0.60, 1.22)			0.76 (0.44, 1.31)			1.21 (0.91, 1.61)			-2.54 (-6.75, 1.68)		
T3	0.81 (0.57, 1.16)			0.89 (0.53, 1.50)			1.01 (0.75, 1.36)			-1.89 (-6.03, 2.25)		
MEHHP												
Continuous[d]	1.09 (0.92, 1.28)		0.32	1.04 (0.80, 1.34)		0.78	0.97 (0.85, 1.11)		0.64	-0.67 (-2.57, 1.23)		0.49
T1	1.00	0.37	*0.21*	1.00	0.93	*0.85*	1.00	0.89	*0.94*	0.00	0.63	*0.63*
T2	1.22 (0.84, 1.75)			1.07 (0.63, 1.83)			1.06 (0.80, 1.41)			-1.98 (-6.04, 2.08)		
T3	1.29 (0.90, 1.85)			0.97 (0.56, 1.67)			1.00 (0.75, 1.34)			-1.14 (-5.37, 3.10)		
MEOHP												
Continuous[d]	1.08 (0.92, 1.28)		0.34	1.05 (0.81, 1.36)		0.70	0.95 (0.83, 1.09)		0.49	-1.06 (-3.01, 0.89)		0.29
T1	1.00	0.48	*0.31*	1.00	0.69	*0.85*	1.00	0.95	*0.92*	0.00	0.43	*0.21*
T2	1.20 (0.83, 1.72)			0.81 (0.47, 1.40)			1.05 (0.79, 1.39)			-2.04 (-6.17, 2.10)		
T3	1.24 (0.86, 1.78)			1.00 (0.59, 1.68)			1.02 (0.77, 1.36)			-2.63 (-6.87, 1.60)		
MECPP												
Continuous[d]	1.05 (0.88, 1.25)		0.58	1.07 (0.82, 1.41)		0.60	0.95 (0.82, 1.10)		0.48	-1.15 (-3.17, 0.86)		0.26
T1	1.00	0.40	*0.36*	1.00	0.97	*0.80*	1.00	0.96	*0.79*	0.00	0.12	*0.04*
T2	1.26 (0.88, 1.81)			1.04 (0.60, 1.79)			1.00 (0.75, 1.33)			-2.35 (-6.48, 1.78)		
T3	1.23 (0.85, 1.77)			1.07 (0.63, 1.83)			1.04 (0.78, 1.38)			-4.24 (-8.30, -0.18)		
MEHP												
Continuous[d]	1.05 (0.91, 1.22)		0.50	1.00 (0.81, 1.25)		0.97	0.95 (0.84, 1.06)		0.35	-1.08 (-2.70, 0.53)		0.19
T1	1.00	0.68	*0.40*	1.00	0.69	*0.71*	1.00	0.70	*0.54*	0.00	0.49	*0.29*
T2	1.09 (0.76, 1.57)			0.84 (0.49, 1.44)			0.90 (0.68, 1.20)			0.25 (-3.94, 4.43)		
T3	1.17 (0.82, 1.67)			1.05 (0.63, 1.76)			0.90 (0.68, 1.20)			-2.05 (-6.20, 2.11)		
∑DEHP												
Continuous[d]	1.07 (0.90, 1.28)		0.42	1.05 (0.80, 1.37)		0.72	0.95 (0.82, 1.09)		0.45	-1.04 (-3.03, 0.96)		0.30
T1	1.00	0.30	*0.40*	1.00	0.97	*0.93*	1.00	0.89	*0.90*	0.00	0.44	*0.34*
T2	1.32 (0.92, 1.90)			0.95 (0.56, 1.63)			1.06 (0.80, 1.41)			-2.53 (-6.61, 1.54)		
T3	1.23 (0.85, 1.77)			1.01 (0.60, 1.71)			0.99 (0.75, 1.33)			-1.98 (-6.15, 2.19)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); ∑HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); ∑DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). Parent compounds and associated metabolites are detailed in Table S1 (Supplemental material)

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.12 – Adjusted associations between pregnancy phenols standardized concentrations and respiratory outcomes (n=470) and $FEV_1\%$ (n=185) in boys from non-asthmatic parents.

	Wheezing (until age 5y)[a] N = 470			Asthma diagnosis (until age 5y)[a] N = 470			Bronchiolitis/Bronchitis (until age 3y)[a] N = 470			$FEV_1\%$[b] N = 185		
Phenol[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
2,4-Dichlorophenol												
Continuous[d]	1.06 (0.94, 1.20)		0.36	0.95 (0.78, 1.16)		0.62	0.95 (0.86, 1.06)		0.38	-0.30 (-1.77, 1.17)		0.69
T1	1.00	0.73	*0.91*	1.00	0.34	*0.38*	1.00	0.52	*0.34*	0.00	0.91	*0.76*
T2	1.15 (0.81, 1.63)			1.28 (0.76, 2.14)			1.06 (0.80, 1.39)			-0.73 (-4.73, 3.28)		
T3	1.06 (0.75, 1.51)			0.86 (0.49, 1.49)			0.90 (0.69, 1.18)			-0.84 (-4.81, 3.14)		
2,5-Dichlorophenol												
Continuous[d]	1.08 (0.99, 1.18)		0.08	1.01 (0.88, 1.16)		0.86	0.99 (0.92, 1.07)		0.86	-0.02 (-1.00, 1.03)		0.97
T1	1.00	0.11	*0.22*	1.00	0.23	*0.43*	1.00	0.76	*0.58*	0.00	0.96	*0.78*
T2	1.42 (0.99, 2.03)			1.59 (0.91, 2.76)			1.08 (0.82, 1.44)			0.06 (-3.91, 4.03)		
T3	1.40 (0.98, 2.00)			1.46 (0.84, 2.54)			1.11 (0.84, 1.46)			0.50 (-3.42, 4.42)		
∑Dichlorophenols												
Continuous[d]	1.08 (0.99, 1.18)		0.10	1.00 (0.87, 1.16)		0.95	0.99 (0.92, 1.07)		0.80	-0.02 (-1.09, 1.04)		0.97
T1	1.00	0.11	*0.16*	1.00	0.20	*0.28*	1.00	0.79	*0.49*	0.00	0.99	*0.91*
T2	1.39 (0.97, 1.99)			1.57 (0.90, 2.75)			1.02 (0.77, 1.35)			0.21 (-3.77, 4.19)		
T3	1.42 (1.00, 2.02)			1.54 (0.89, 2.68)			1.10 (0.83, 1.45)			0.27 (-3.67, 4.21)		
Bisphenol A												
Continuous[d]	0.94 (0.77, 1.15)		0.56	1.25 (0.93, 1.66)		0.14	1.11 (0.95, 1.29)		0.18	-1.40 (-3.47, 0.67)		0.18
T1	1.00	0.86	*0.84*	1.00	0.47	*0.22*	1.00	0.50	*0.26*	0.00	0.45	*0.25*
T2	0.91 (0.64, 1.28)			1.12 (0.63, 1.98)			1.10 (0.83, 1.45)			-1.79 (-5.69, 2.10)		
T3	0.95 (0.66, 1.34)			1.38 (0.80, 2.39)			1.18 (0.89, 1.57)			-2.49 (-6.47, 1.49)		
Benzophenone-3												
Continuous[d]	0.95 (0.87, 1.03)		0.21	0.96 (0.84, 1.10)		0.57	1.00 (0.93, 1.07)		0.97	-0.80 (-1.82, 0.22)		0.12
T1	1.00	0.13	*0.05*	1.00	0.36	*0.17*	1.00	0.78	*0.83*	0.00	0.36	*0.16*
T2	0.89 (0.63, 1.24)			0.86 (0.52, 1.43)			0.91 (0.69, 1.19)			-0.75 (-4.65, 3.14)		
T3	0.69 (0.48, 0.99)			0.67 (0.38, 1.16)			0.93 (0.71, 1.24)			-2.73 (-6.61, 1.15)		
Triclosan												
Continuous[d]	0.99 (0.93, 1.04)		0.62	0.99 (0.91, 1.08)		0.89	0.99 (0.95, 1.04)		0.70	0.07 (-0.57, 0.71)		0.83
T1	1.00	0.97	*0.80*	1.00	1.00	*0.93*	1.00	0.94	*0.73*	0.00	0.99	*0.94*
T2	0.98 (0.70, 1.39)			1.00 (0.59, 1.70)			0.99 (0.75, 1.30)			-0.16 (-4.11, 3.79)		
T3	0.96 (0.68, 1.35)			0.98 (0.58, 1.67)			0.95 (0.73, 1.25)			0.18 (-4.07, 3.70)		
Methyl-paraben												
Continuous[d]	0.93 (0.85, 1.02)		0.11	1.02 (0.89, 1.17)		0.75	0.94 (0.87, 1.01)		0.10	-0.46 (-1.40, 0.49)		0.34
T1	1.00	0.09	*0.16*	1.00	0.87	*0.94*	1.00	0.03	*0.01*	0.00	0.18	*0.31*
T2	0.71 (0.50, 1.00)			1.14 (0.68, 1.91)			0.81 (0.62, 1.06)			2.44 (-1.47, 6.35)		
T3	0.73 (0.52, 1.03)			1.01 (0.58, 1.75)			0.68 (0.52, 0.90)			-1.21 (-5.02, 2.59)		
Ethyl-paraben												
Continuous[d]	1.02 (0.95, 1.09)		0.58	1.11 (0.99, 1.23)		0.08	0.99 (0.94, 1.04)		0.69	-0.85 (-1.57, -0.13)		0.02
T1	1.00	0.49	*0.82*	1.00	0.08	*0.17*	1.00	0.001	*0.08*	0.00	0.10	*0.04*
T2	1.23 (0.87, 1.75)			1.80 (1.03, 3.16)			1.51 (1.15, 1.98)			-1.98 (-5.79, 1.83)		
T3	1.13 (0.79, 1.61)			1.82 (1.02, 3.25)			0.95 (0.71, 1.26)			-4.28 (-8.15, -0.41)		
Propyl-paraben												
Continuous[d]	0.94 (0.88, 1.02)		0.12	1.00 (0.90, 1.12)		0.95	0.96 (0.91, 1.02)		0.19	-0.19 (-0.98, 0.60)		0.63
T1	1.00	0.62	*0.45*	1.00	0.91	*0.75*	1.00	0.27	*0.22*	0.00	0.46	*0.46*
T2	0.88 (0.63, 1.24)			0.92 (0.55, 1.61)			0.84 (0.64, 1.11)			1.80 (-2.06, 5.66)		
T3	0.85 (0.60, 1.20)			1.06 (0.62, 1.81)			0.81 (0.61, 1.06)			-0.62 (-4.52, 3.28)		
Butyl-paraben												
Continuous[d]	0.99 (0.92, 1.06)		0.77	0.99 (0.88, 1.11)		0.87	0.98 (0.93, 1.03)		0.45	-0.91 (-1.68, -0.14)		0.02
T1	1.00	0.46	*0.31*	1.00	0.98	*0.84*	1.00	0.004	0.01	0.00	0.15	*0.06*
T2	1.11 (0.79, 1.57)			1.00 (0.59, 1.70)			1.26 (0.96, 1.66)			-1.30 (-5.12, 2.52)		
T3	0.89 (0.62, 1.29)			1.05 (0.60, 1.85)			0.79 (0.59, 1.06)			-3.78 (-7.63, 0.07)		
∑Parabens												
Continuous[d]	0.93 (0.85, 1.01)		0.09	1.02 (0.89, 1.17)		0.79	0.95 (0.88, 1.02)		0.13	-0.48 (-1.43, 0.47)		0.32
T1	1.00	0.13	*0.14*	1.00	0.89	*0.73*	1.00	0.07	*0.06*	0.00	0.43	*0.67*
T2	0.75 (0.54, 1.06)			1.06 (0.64, 1.78)			0.78 (0.60, 1.02)			2.07 (-1.88, 6.01)		
T3	0.73 (0.52, 1.04)			0.93 (0.54, 1.61)			0.74 (0.56, 0.98)			-0.38 (-4.19, 3.44)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑Dichlorophenols = molar sum of Dichlorophenols (2,4-, 2,5-dichlorophenols); ∑Parabens = molar sum of parabens (Methyl-, Ethyl-, Propyl-, Butyl-parabens).

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.13 – Adjusted associations between pregnancy phthalate metabolites standardized concentrations and respiratory outcomes (n=447) and $FEV_1\%$ (n=171) in boys from non-smoking mothers.

	Wheezing (until age 5y)[a]			Asthma diagnosis (until age 5y)[a]			Bronchiolitis/Bronchitis (until age 3y)[a]			$FEV_1\%$[b]		
	N = 470			N = 470			N = 470			N = 185		
Phthalate[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
∑LMW												
Continuous[d]	0.90 (0.76, 1.07)		0.25	0.92 (0.71, 1.20)		0.55	0.97 (0.85, 1.11)		0.71	-1.29 (-3.26, 0.68)		0.20
T1	1.00	0.42	*0.26*	1.00	0.88	*0.67*	1.00	0.84	*0.61*	0.00	0.10	*0.03*
T2	0.85 (0.60, 1.19)			1.04 (0.62, 1.74)			0.94 (0.72, 1.24)			-0.32 (-4.18, 3.54)		
T3	0.80 (0.56, 1.13)			0.90 (0.52, 1.56)			0.92 (0.70, 1.22)			-3.71 (-7.50, -0.07)		
MEP												
Continuous[d]	0.95 (0.83, 1.08)		0.43	1.01 (0.82, 1.24)		0.94	0.99 (0.90, 1.10)		0.91	-1.19 (-2.75, 0.36)		0.13
T1	1.00	0.65	*0.35*	1.00	0.80	*0.51*	1.00	0.69	*0.66*	0.00	0.43	*0.26*
T2	0.95 (0.67, 1.34)			0.92 (0.54, 1.57)			1.09 (0.83, 1.43)			-2.01 (-5.87, 1.86)		
T3	0.85 (0.60, 1.21)			0.83 (0.48, 1.43)			0.97 (0.73, 1.28)			-2.36 (-6.24, 1.51)		
MnBP												
Continuous[d]	1.00 (0.88, 1.14)		0.97	0.97 (0.78, 1.19)		0.74	0.95 (0.85, 1.06)		0.31	-0.18 (-1.65, 1.30)		0.81
T1	1.00	0.97	*0.85*	1.00	0.74	*0.56*	1.00	0.10	*0.34*	0.00	0.42	*0.51*
T2	1.04 (0.74, 1.47)			0.85 (0.50, 1.42)			0.75 (0.57, 0.98)			1.76 (-2.20, 5.72)		
T3	1.04 (0.73, 1.49)			0.83 (0.48, 1.42)			0.80 (0.61, 1.06)			-0.72 (-4.75, 3.32)		
MiBP												
Continuous[d]	1.01 (0.85, 1.20)		0.88	1.15 (0.87, 1.51)		0.33	1.08 (0.94, 1.24)		0.29	-0.77 (-2.68, 1.14)		0.43
T1	1.00	0.81	*0.83*	1.00	0.98	*0.97*	1.00	0.86	*0.59*	0.00	0.38	*0.23*
T2	0.91 (0.63, 1.30)			1.06 (0.62, 1.80)			1.03 (0.78, 1.37)			0.80 (-3.11, 4.71)		
T3	1.01 (0.71, 1.43)			1.02 (0.60, 1.75)			1.08 (0.82, 1.43)			-1.88 (-5.76, 2.01)		
∑HMW												
Continuous[d]	1.12 (0.93, 1.34)		0.23	1.15 (0.86, 1.53)		0.35	0.95 (0.82, 1.10)		0.49	-1.31 (-3.47, 0.84)		0.23
T1	1.00	0.39	*0.89*	1.00	0.93	*0.81*	1.00	0.55	*0.37*	0.00	0.23	*0.76*
T2	1.24 (0.88, 1.76)			1.06 (0.62, 1.80)			0.88 (0.67, 1.16)			-3.33 (-7.18, 0.52)		
T3	1.03 (0.72, 1.47)			0.95 (0.56, 1.64)			0.87 (0.66, 1.14)			-1.46 (-5.36, 2.44)		
MCPP												
Continuous[d]	0.99 (0.84, 1.17)		0.90	0.97 (0.75, 1.27)		0.85	0.87 (0.76, 1.00)		0.04	-0.28 (-2.28, 1.72)		0.78
T1	1.00	0.56	*0.80*	1.00	0.98	*0.86*	1.00	0.15	*0.08*	0.00	0.23	*0.50*
T2	1.18 (0.83, 1.65)			0.98 (0.57, 1.66)			1.05 (0.81, 1.38)			-2.66 (-6.47, 1.15)		
T3	1.00 (0.70, 1.43)			0.95 (0.56, 1.63)			0.81 (0.61, 1.07)			0.38 (-3.51, 4.27)		
MBzP												
Continuous[d]	1.01 (0.87, 1.16)		0.95	1.16 (0.92, 1.45)		0.20	1.00 (0.89, 1.12)		0.98	-0.82 (-2.60, 0.95)		0.36
T1	1.00	0.75	*0.53*	1.00	0.62	*0.95*	1.00	0.97	*0.96*	0.00	0.13	*0.05*
T2	1.04 (0.74, 1.48)			1.29 (0.75, 2.23)			0.97 (0.74, 1.28)			-2.00 (-5.91, 1.92)		
T3	0.92 (0.64, 1.31)			1.08 (0.61, 1.89)			1.00 (0.76, 1.31)			-3.92 (-7.77, -0.08)		
MCNP												
Continuous[d]	1.12 (0.98, 1.28)		0.09	1.12 (0.92, 1.36)		0.27	0.98 (0.88, 1.09)		0.69	-0.06 (-1.74, 1.62)		0.94
T1	1.00	0.31	*0.15*	1.00	0.60	*0.46*	1.00	0.96	*0.98*	0.00	0.51	*0.61*
T2	1.16 (0.81, 1.65)			1.27 (0.73, 2.20)			1.04 (0.79, 1.37)			-2.14 (-6.02, 1.73)		
T3	1.31 (0.93, 1.85)			1.29 (0.75, 2.21)			1.02 (0.77, 1.33)			-1.82 (-5.79, 2.15)		
MCOP												
Continuous[d]	1.07 (0.91, 1.27)		0.40	1.09 (0.85, 1.38)		0.50	1.15 (1.02, 1.31)		0.03	-1.78 (-3.50, -0.06)		0.04
T1	1.00	0.68	*0.69*	1.00	0.81	*0.66*	1.00	0.63	*0.66*	0.00	0.11	*0.04*
T2	1.12 (0.79, 1.58)			0.91 (0.53, 1.57)			1.14 (0.87, 1.50)			-0.87 (-4.74, 3.00)		
T3	0.96 (0.68, 1.37)			1.09 (0.65, 1.85)			1.09 (0.83, 1.44)			-3.91 (-7.77, -0.05)		
MEHHP												
Continuous[d]	1.14 (0.96, 1.36)		0.13	1.10 (0.85, 1.42)		0.48	1.00 (0.88, 1.14)		0.98	-0.86 (-2.70, 0.97)		0.35
T1	1.00	0.21	*0.58*	1.00	0.37	*0.76*	1.00	0.62	*0.82*	0.00	0.22	*0.75*
T2	1.37 (0.97, 1.95)			1.38 (0.81, 2.35)			1.12 (0.85, 1.47)			-3.20 (-6.98, 0.58)		
T3	1.18 (0.82, 1.69)			1.00 (0.57, 1.74)			0.99 (0.75, 1.31)			-0.79 (-4.73, 3.15)		
MEOHP												
Continuous[d]	1.13 (0.95, 1.33)		0.16	1.08 (0.83, 1.41)		0.56	0.97 (0.85, 1.11)		0.68	-1.25 (-3.13, 0.63)		0.19
T1	1.00	0.09	*0.66*	1.00	0.72	*0.54*	1.00	0.25	*0.62*	0.00	0.10	*0.51*
T2	1.46 (1.03, 2.07)			1.07 (0.64, 1.81)			1.19 (0.91, 1.57)			-4.19 (-8.02, -0.35)		
T3	1.15 (0.80, 1.66)			0.86 (0.50, 1.49)			0.96 (0.73, 1.28)			-1.74 (-5.62, 2.13)		
MECPP												
Continuous[d]	1.13 (0.95, 1.34)		0.17	1.13 (0.87, 1.49)		0.36	0.95 (0.82, 1.10)		0.48	-1.21 (-3.22, 0.80)		0.24
T1	1.00	0.11	*0.47*	1.00	0.78	*0.92*	1.00	0.91	*0.66*	0.00	0.28	*0.52*
T2	1.45 (1.02, 2.06)			1.21 (0.70, 2.07)			0.98 (0.75, 1.29)			-3.18 (-7.10, 0.74)		
T3	1.23 (0.86, 1.76)			1.07 (0.62, 1.85)			0.94 (0.72, 1.24)			-1.91 (-5.81, 2.00)		
MEHP												
Continuous[d]	1.04 (0.91, 1.20)		0.55	1.06 (0.86, 1.32)		0.57	0.93 (0.83, 1.04)		0.21	-0.81 (-2.32, 0.70)		0.29
T1	1.00	0.39	*0.19*	1.00	0.31	*0.17*	1.00	0.25	*0.75*	0.00	0.69	*0.39*
T2	1.00 (0.70, 1.43)			0.93 (0.53, 1.64)			0.81 (0.62, 1.07)			-0.34 (-4.21, 3.53)		
T3	1.23 (0.87, 1.73)			1.36 (0.81, 2.81)			0.99 (0.76, 1.30)			-1.61 (-5.51, 2.29)		
∑DEHP												
Continuous[d]	1.13 (0.95, 1.34)		0.18	1.10 (0.84, 1.45)		0.48	0.96 (0.83, 1.11)		0.57	-1.14 (-3.10, 0.82)		0.25
T1	1.00	0.10	*0.78*	1.00	0.66	*0.62*	1.00	0.69	*0.80*	0.00	0.16	*0.70*
T2	1.45 (1.02, 2.07)			1.16 (0.68, 1.98)			1.10 (0.84, 1.45)			-3.37 (-7.23, 0.48)		
T3	1.14 (0.79, 1.63)			0.91 (0.53, 1.57)			0.99 (0.75, 1.30)			-0.30 (-4.21, 3.60)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); ∑HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); ∑DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). Parent compounds and associated metabolites are detailed in Table S1 (Supplemental material)

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.14 – Adjusted associations between pregnancy phenols standardized concentrations and respiratory outcomes (n=562) and $FEV_1\%$ (n=217) in full-term boys.

	Wheezing (until age 5y)[a] N = 562			Asthma diagnosis (until age 5y)[a] N = 562			Bronchiolitis/Bronchitis (until age 3y)[a] N = 562			$FEV_1\%$[b] N = 217		
Phenol[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
2,4-Dichlorophenol												
Continuous[d]	1.04 (0.93, 1.17)		0.45	1.07 (0.90, 1.27)		0.45	0.98 (0.89, 1.08)		0.63	0.62 (-0.69, 1.93)		0.35
T1	1.00	0.38	*0.97*	1.00	0.28	*0.91*	1.00	0.39	*0.42*	0.00	0.62	*0.95*
T2	1.24 (0.90, 1.70)			1.45 (0.91, 2.31)			1.12 (0.87, 1.44)			1.83 (-1.91, 5.57)		
T3	1.06 (0.77, 1.46)			1.14 (0.70, 1.86)			0.94 (0.73, 1.21)			0.67 (-2.93, 4.26)		
2,5-Dichlorophenol												
Continuous[d]	1.07 (0.99, 1.16)		0.08	1.07 (0.95, 1.20)		0.25	1.00 (0.94, 1.07)		1.00	0.27 (-0.62, 1.16)		0.55
T1	1.00	0.01	*0.19*	1.00	0.20	*0.20*	1.00	0.65	*0.57*	0.00	0.93	*0.80*
T2	1.68 (1.21, 2.33)			1.45 (0.89, 2.35)			0.92 (0.71, 1.19)			-0.44 (-4.13, 3.24)		
T3	1.49 (1.07, 2.07)			1.50 (0.93, 2.43)			1.03 (0.80, 1.33)			0.25 (-3.36, 3.86)		
∑Dichlorophenols												
Continuous[d]	1.07 (0.99, 1.16)		0.10	1.07 (0.95, 1.21)		0.27	1.00 (0.93, 1.07)		0.97	0.32 (-0.62, 1.26)		0.50
T1	1.00	0.06	*0.18*	1.00	0.21	*0.19*	1.00	0.71	*0.42*	0.00	0.87	*0.89*
T2	1.44 (1.04, 1.99)			1.42 (0.87, 2.30)			1.00 (0.77, 1.29)			-0.89 (-4.61, 2.82)		
T3	1.40 (1.01, 1.93)			1.51 (0.93, 2.44)			1.10 (0.85, 1.41)			-0.08 (-3.68, 3.53)		
Bisphenol A												
Continuous[d]	0.98 (0.83, 1.16)		0.83	1.19 (0.94, 1.51)		0.15	1.14 (0.99, 1.31)		0.06	-0.66 (-2.53, 1.20)		0.48
T1	1.00	1.00	*0.98*	1.00	0.45	*0.21*	1.00	0.39	*0.18*	0.00	0.59	*0.63*
T2	1.01 (0.74, 1.38)			1.07 (0.65, 1.76)			1.10 (0.86, 1.42)			-1.85 (-5.45, 1.76)		
T3	1.01 (0.73, 1.39)			1.32 (0.83, 2.12)			1.20 (0.93, 1.54)			-1.28 (-4.98, 2.42)		
Benzophenone-3												
Continuous[d]	0.92 (0.85, 1.00)		0.06	0.94 (0.83, 1.06)		0.30	1.00 (0.93, 1.06)		0.90	-0.32 (-1.29, 0.65)		0.69
T1	1.00	0.03	*0.01*	1.00	0.11	*0.07*	1.00	0.72	*0.79*	0.00	0.89	*0.75*
T2	0.88 (0.65, 1.20)			0.76 (0.49, 1.20)			0.90 (0.70, 1.16)			-0.71 (-4.33, 2.91)		
T3	0.65 (0.47, 0.90)			0.59 (0.36, 0.97)			0.93 (0.72, 1.20)			-0.78 (-4.34, 2.78)		
Triclosan												
Continuous[d]	0.98 (0.94, 1.04)		0.62	0.98 (0.91, 1.06)		0.63	1.00 (0.96, 1.04)		0.94	0.10 (-0.48, 0.69)		0.73
T1	1.00	0.88	*0.63*	1.00	0.88	*0.75*	1.00	0.98	*0.95*	0.00	0.91	*0.82*
T2	0.97 (0.71, 1.32)			1.09 (0.69, 1.72)			1.02 (0.80, 1.31)			0.52 (-3.04, 4.08)		
T3	0.92 (0.68, 1.26)			0.97 (0.60, 1.55)			1.00 (0.78, 1.29)			-0.25 (-3.88, 3.38)		
Methyl-paraben												
Continuous[d]	0.93 (0.85, 1.01)		0.07	1.01 (0.89, 1.14)		0.93	0.95 (0.89, 1.02)		0.14	-0.42 (-1.29, 0.44)		0.33
T1	1.00	0.08	*0.14*	1.00	0.96	*0.99*	1.00	0.06	*0.03*	0.00	0.26	*0.22*
T2	0.74 (0.54, 1.00)			0.94 (0.59, 1.49)			0.85 (0.66, 1.08)			1.57 (-2.08, 5.22)		
T3	0.74 (0.54, 1.01)			0.99 (0.61, 1.59)			0.73 (0.57, 0.95)			-1.51 (-5.03, 2.01)		
Ethyl-paraben												
Continuous[d]	1.01 (0.95, 1.07)		0.86	1.10 (1.00, 1.22)		0.04	0.99 (0.95, 1.04)		0.80	-0.52 (-1.19, 0.15)		0.13
T1	1.00	0.65	*0.83*	1.00	0.05	*0.10*	1.00	0.01	*0.29*	0.00	0.53	*0.31*
T2	1.16 (0.85, 1.59)			1.67 (1.02, 2.74)			1.38 (1.07, 1.78)			-1.05 (-4.65, 2.55)		
T3	1.10 (0.79, 1.51)			1.79 (1.08, 2.98)			1.01 (0.78, 1.31)			-2.05 (-5.64, 1.54)		
Propyl-paraben												
Continuous[d]	0.95 (0.89, 1.02)		0.18	0.99 (0.89, 1.09)		0.79	0.97 (0.92, 1.03)		0.29	-0.18 (-0.91, 0.56)		0.63
T1	1.00	0.24	*0.31*	1.00	0.72	*0.72*	1.00	0.37	*0.22*	0.00	0.83	*0.55*
T2	0.79 (0.58, 1.08)			0.83 (0.52, 1.32)			0.90 (0.70, 1.15)			0.05 (-3.56, 3.66)		
T3	0.80 (0.58, 1.09)			0.87 (0.54, 1.40)			0.84 (0.65, 1.08)			-0.95 (-4.53, 2.63)		
Butyl-paraben												
Continuous[d]	0.98 (0.92, 1.05)		0.60	1.00 (0.90, 1.10)		0.95	0.99 (0.94, 1.04)		0.73	-0.36 (-1.07, 0.35)		0.32
T1	1.00	0.12	*0.13*	1.00	0.58	*0.78*	1.00	0.02	0.03	0.00	0.47	*0.53*
T2	1.23 (0.90, 1.67)			1.28 (0.80, 2.06)			1.22 (0.95, 1.57)			-1.94 (-5.50, 1.62)		
T3	0.88 (0.63, 1.23)			1.20 (0.72, 1.99)			0.85 (0.65, 1.10)			-1.97 (-5.60, 1.66)		
∑Parabens												
Continuous[d]	0.92 (0.85, 1.00)		0.06	1.01 (0.89, 1.14)		0.93	0.95 (0.89, 1.02)		0.16	-0.42 (-1.28, 0.45)		0.35
T1	1.00	0.07	*0.08*	1.00	0.90	*0.73*	1.00	0.11	*0.19*	0.00	0.19	*0.26*
T2	0.76 (0.56, 1.03)			1.05 (0.66, 1.65)			0.79 (0.62, 1.02)			2.15 (-1.47, 5.78)		
T3	0.72 (0.52, 0.98)			0.94 (0.58, 1.52)			0.80 (0.62, 1.03)			-1.20 (-4.73, 2.32)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑Dichlorophenols = molar sum of Dichlorophenols (2,4-, 2,5-dichlorophenols); ∑Parabens = molar sum of parabens (Methyl-, Ethyl-, Propyl-, Butyl-parabens).

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

Table 4.15 – Adjusted associations between pregnancy phthalate metabolites standardized concentrations and respiratory outcomes (n=562) and $FEV_1\%$ (n=217) in full-term boys.

	Wheezing (until age 5y)[a]			Asthma diagnosis (until age 5y)[a]			Bronchiolitis/Bronchitis (until age 3y)[a]			$FEV_1\%$[b]		
	N = 562			N = 562			N = 562			N = 217		
Phthalate[c]	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	HR (95% CI)	p_{het}[e]	*p*-value	beta (95% CI)	p_{het}[e]	*p*-value
∑LMW												
Continuous[d]	0.94 (0.81, 1.10)		0.46	0.99 (0.79, 1.24)		0.93	1.00 (0.88, 1.13)		0.96	-0.18 (-1.98, 1.63)		0.85
T1	1.00	0.75	*0.58*	1.00	0.67	*0.92*	1.00	0.84	*0.72*	0.00	0.26	*0.10*
T2	1.06 (0.77, 1.44)			1.21 (0.77, 1.90)			1.07 (0.83, 1.38)			-0.65 (-4.22, 2.93)		
T3	0.93 (0.68, 1.29)			1.02 (0.63, 1.66)			1.06 (0.82, 1.37)			-2.82 (-6.35, 0.71)		
MEP												
Continuous[d]	0.97 (0.86, 1.10)		0.64	1.10 (0.92, 1.31)		0.29	1.02 (0.93, 1.12)		0.67	-0.01 (-1.46, 1.44)		0.98
T1	1.00	1.00	*0.94*	1.00	0.51	*0.33*	1.00	0.60	*0.50*	0.00	0.99	*0.95*
T2	1.01 (0.74, 1.38)			0.91 (0.56, 1.48)			1.12 (0.87, 1.44)			0.16 (-3.40, 3.71)		
T3	1.01 (0.74, 1.39)			1.19 (0.75, 1.89)			1.12 (0.86, 1.44)			-0.08 (-3.69, 3.54)		
MnBP												
Continuous[d]	1.03 (0.91, 1.16)		0.62	0.93 (0.77, 1.12)		0.43	0.97 (0.88, 1.07)		0.54	-0.27 (-1.66, 1.12)		0.70
T1	1.00	0.61	*0.42*	1.00	0.56	*0.31*	1.00	0.51	*0.51*	0.00	0.95	*0.82*
T2	0.95 (0.69, 1.30)			0.87 (0.55, 1.37)			0.87 (0.68, 1.12)			0.27 (-3.33, 3.87)		
T3	1.10 (0.81, 1.51)			0.77 (0.48, 1.24)			0.89 (0.69, 1.15)			-0.33 (-4.00, 3.34)		
MiBP												
Continuous[d]	0.97 (0.83, 1.13)		0.67	1.00 (0.79, 1.27)		0.99	1.01 (0.89, 1.15)		0.85	-1.30 (-3.02, 0.41)		0.14
T1	1.00	0.75	*0.96*	1.00	0.69	*0.53*	1.00	0.93	*0.85*	0.00	0.09	*0.03*
T2	0.88 (0.64, 1.22)			1.09 (0.69, 1.73)			1.03 (0.80, 1.33)			-0.81 (-4.42, 2.81)		
T3	0.96 (0.70, 1.32)			0.88 (0.54, 1.44)			0.99 (0.76, 1.27)			-3.77 (-7.32, -0.23)		
∑HMW												
Continuous[d]	1.07 (0.91, 1.26)		0.43	1.09 (0.84, 1.40)		0.53	0.92 (0.80, 1.05)		0.21	-1.03 (-2.90, 0.84)		0.28
T1	1.00	0.30	*0.99*	1.00	0.91	*0.90*	1.00	0.60	*0.38*	0.00	0.24	*0.43*
T2	1.27 (0.92, 1.73)			1.11 (0.69, 1.78)			0.91 (0.71, 1.17)			-2.99 (-6.51, 0.54)		
T3	1.06 (0.77, 1.46)			1.05 (0.66, 1.69)			0.88 (0.68, 1.14)			-2.04 (-5.62, 1.54)		
MCPP												
Continuous[d]	1.04 (0.89, 1.21)		0.63	0.88 (0.69, 1.13)		0.32	0.90 (0.79, 1.03)		0.12	-0.50 (-2.40, 1.40)		0.60
T1	1.00	0.72	*0.46*	1.00	0.58	*0.30*	1.00	0.46	*0.30*	0.00	0.44	*0.89*
T2	1.09 (0.79, 1.49)			0.97 (0.61, 1.53)			1.05 (0.82, 1.34)			-2.21 (-5.76, 1.34)		
T3	1.14 (0.83, 1.57)			0.79 (0.48, 1.28)			0.90 (0.69, 1.16)			-0.57 (-4.14, 2.99)		
MBzP												
Continuous[d]	0.99 (0.87, 1.13)		0.88	1.13 (0.94, 1.38)		0.20	0.96 (0.87, 1.07)		0.51	-0.11 (-1.68, 1.45)		0.89
T1	1.00	0.43	*0.43*	1.00	0.78	*0.92*	1.00	0.34	*0.14*	0.00	0.13	*0.30*
T2	1.13 (0.83, 1.54)			1.19 (0.74, 1.92)			0.96 (0.74, 1.23)			-3.58 (-7.19, 0.03)		
T3	0.92 (0.67, 1.27)			1.07 (0.67, 1.73)			0.83 (0.65, 1.07)			-2.70 (-6.29, 0.89)		
MCNP												
Continuous[d]	1.10 (0.98, 1.25)		0.11	1.13 (0.95, 1.34)		0.18	1.00 (0.90, 1.10)		0.99	0.49 (-0.98, 1.95)		0.51
T1	1.00	0.22	*0.17*	1.00	0.29	*0.24*	1.00	0.55	*0.66*	0.00	0.94	*0.89*
T2	1.25 (0.91, 1.73)			1.39 (0.85, 2.29)			1.15 (0.89, 1.48)			-0.60 (-4.15, 2.96)		
T3	1.30 (0.95, 1.78)			1.43 (0.88, 2.30)			1.09 (0.85, 1.41)			-0.43 (-3.99, 3.13)		
MCOP												
Continuous[d]	1.04 (0.89, 1.21)		0.63	1.03 (0.82, 1.28)		0.83	1.11 (0.99, 1.25)		0.08	-1.31 (-2.95, 0.34)		0.12
T1	1.00	0.79	*0.57*	1.00	0.45	*0.90*	1.00	0.89	*0.95*	0.00	0.45	*0.21*
T2	0.92 (0.67, 1.26)			0.75 (0.46, 1.21)			1.06 (0.83, 1.37)			-0.59 (-4.28, 3.09)		
T3	0.90 (0.66, 1.23)			0.97 (0.61, 1.52)			1.02 (0.79, 1.32)			-2.21 (-5.75, 1.34)		
MEHHP												
Continuous[d]	1.06 (0.92, 1.23)		0.40	1.01 (0.80, 1.26)		0.96	0.96 (0.86, 1.08)		0.54	-0.86 (-2.45, 0.73)		0.29
T1	1.00	0.56	*0.60*	1.00	0.32	*0.70*	1.00	0.81	*0.67*	0.00	0.26	*0.33*
T2	1.19 (0.86, 1.64)			1.34 (0.84, 2.15)			1.04 (0.81, 1.34)			-2.88 (-6.39, 0.64)		
T3	1.12 (0.82, 1.54)			0.98 (0.60, 1.60)			0.96 (0.74, 1.23)			-1.93 (-5.57, 1.72)		
MEOHP												
Continuous[d]	1.07 (0.92, 1.24)		0.37	1.01 (0.80, 1.27)		0.95	0.94 (0.84, 1.06)		0.34	-1.26 (-2.89, 0.37)		0.13
T1	1.00	0.36	*0.67*	1.00	0.88	*0.68*	1.00	0.56	*0.53*	0.00	0.08	*0.08*
T2	1.26 (0.92, 1.73)			1.03 (0.65, 1.64)			1.08 (0.84, 1.39)			-3.58 (-7.13, -0.04)		
T3	1.11 (0.81, 1.54)			0.92 (0.57, 1.47)			0.94 (0.73, 1.21)			-3.35 (-6.89, 0.20)		
MECPP												
Continuous[d]	1.06 (0.91, 1.24)		0.44	1.08 (0.86, 1.38)		0.50	0.93 (0.82, 1.06)		0.28	-1.31 (-3.06, 0.44)		0.14
T1	1.00	0.14	*0.85*	1.00	0.44	*0.94*	1.00	0.92	*0.73*	0.00	0.12	*0.10*
T2	1.36 (0.99, 1.85)			1.32 (0.83, 2.11)			1.01 (0.79, 1.30)			-3.12 (-6.67, 0.45)		
T3	1.11 (0.80, 1.53)			1.05 (0.65, 1.72)			0.96 (0.75, 1.24)			-3.35 (-6.91, 0.21)		
MEHP												
Continuous[d]	1.02 (0.90, 1.15)		0.76	0.98 (0.81, 1.18)		0.84	0.93 (0.84, 1.03)		0.16	-0.83 (-2.18, 0.52)		0.23
T1	1.00	0.71	*0.41*	1.00	0.77	*0.48*	1.00	0.24	*0.65*	0.00	0.31	*0.16*
T2	1.02 (0.74, 1.40)			1.01 (0.63, 1.63)			0.81 (0.63, 1.04)			0.04 (-3.51, 3.58)		
T3	1.13 (0.83, 1.54)			1.17 (0.73, 1.85)			0.91 (0.71, 1.16)			-2.36 (-5.92, 1.20)		
∑DEHP												
Continuous[d]	1.06 (0.91, 1.24)		0.46	1.03 (0.81, 1.31)		0.81	0.94 (0.82, 1.06)		0.30	-1.18 (-2.88, 0.52)		0.17
T1	1.00	0.23	*0.98*	1.00	0.60	*0.70*	1.00	0.84	*0.59*	0.00	0.16	*0.32*
T2	1.30 (0.95, 1.78)			1.19 (0.75, 1.91)			1.01 (0.79, 1.30)			-3.41 (-6.94, 0.12)		
T3	1.06 (0.77, 1.47)			0.96 (0.59, 1.55)			0.94 (0.73, 1.21)			-1.99 (-5.56, 1.58)		

HR = hazard ratio; $FEV_1\%$= forced expiratory volume in 1 second expressed in percent predicted; CI = confidence interval; ∑LMW = molar sum of Low Molecular Weight phthalates (MEP, MnBP, MiBP); ∑HMW = molar sum of High Molecular Weight phthalates (MCPP, MBzP, MCNP, MCOP, MEHHP, MEOHP, MECPP, MEHP); ∑DEHP = molar sum of di(2-ethylhexyl) phthalate metabolites (MEHHP, MEOHP, MECPP, MEHP). Parent compounds and associated metabolites are detailed in Table S1 (Supplemental material)

[a] Models adjusted for creatinine, centre, residence area, parental history of asthma or allergies, maternal ethnicity, maximal parental education level, maternal or passive smoking during pregnancy, postnatal passive smoking, older siblings, day-care. Missing values in covariates were imputed for at least one covariate in 277 boys, using the MICE multiple imputation method (100 imputations were performed) [b] additionally adjusted for child's height and age.
[c] Crude concentrations.
[d] Estimates for 1 unit increase in ln-transformed standardized concentration.
[e] p-values of heterogeneity test.
Italicized p-values are p-values of monotonic trend test.

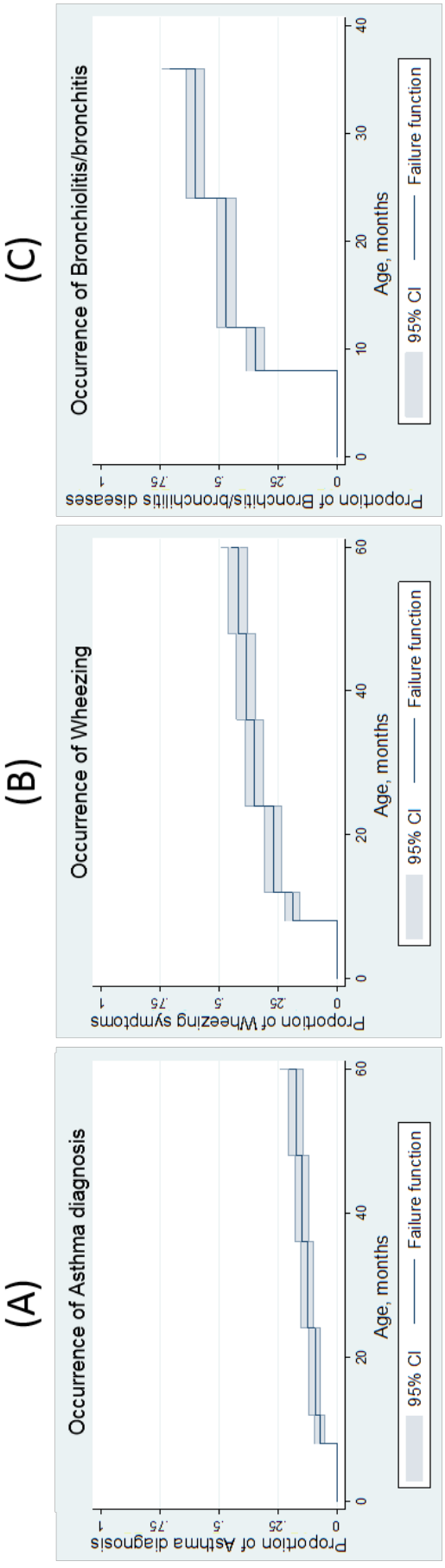

Figure 4.3 – Occurrence of doctor-diagnosed asthma (A), wheezing (B) and bronchiolitis/bronchitis (C), Kaplan-Meier estimates.

4.9 Additional results and discussion to Chapter 4

This section presents additional results of the article presented in Chapter 4, non-included in the submitted version to *Environmental Health Perspectives*. As suggested in the Discussion section (Section 4.6) of the article, measurement error may introduce attenuation bias in the regression estimates. In the following, we propose to use the method described in the article from Perrier et al.[3], to correct the results for measurement error. This *a posteriori* disattenuation takes into account the within-subject variance as part of the measurement error.

4.9.1 Methods

Corrected effect estimates are obtained by dividing the estimated regression coefficients (beta) by the compound-specific intraclass correlation coefficient (ICC). For HRs, linear (beta) regression coefficients are divided by the ICC before exponentiation to result in corrected HRs (see Eq 4.1).

$$\beta_{corr} = \frac{\beta}{ICC} \quad \text{and} \quad HR_{corr} = \exp\left(\frac{\beta}{ICC}\right) = \exp\left(\beta_{corr}\right) \tag{4.1}$$

Confidence intervals (CI) are corrected by using the standard error of the linear regression estimate divided by the compound-specific ICC before calculating the upper and lower bounds (Eq 4.2 and 4.3).

$$CI_{(\beta)_{corr}} = \beta_{corr} \pm t_{(df,\alpha/2)} * \frac{se}{ICC} \tag{4.2}$$

$$CI_{(HR)_{corr}} = \exp\left(\beta_{corr} \pm t_{(df,\alpha/2)} * \frac{se}{ICC}\right), \text{ with} \tag{4.3}$$

$t_{(df,\alpha/2)}$ the Student's t-distribution critical value given a significance level $\alpha/2$ ($\alpha = 0.05$);
df the degrees of freedom;
se the standard error of the regression estimate.

Without repeated assays, we were not able to estimate ICCs internal to the EDEN population. We used ICCs from two studies, in which several urine samples were collected during pregnancy.[4,1] ICCs were 0.1 for MCNP, 0.2 for bisphenol A, MCPP and DEHP metabolites; 0.3 for MCOP, 0.4 for MEP, MnBP, MiBP and MBzP, and 0.6 for dichlorophenols, triclosan, benzophenone-3 and parabens.

Additionally, in order to take into account the uncertainty of ICC estimates, we also corrected regression estimates and confidence intervals by using the lower and upper confidence limits for ICC values instead of the ICC (95% confidence interval). AS an example, results are shown for the associations between DEHP metabolites and

$FEV_1\%$, given the ICCs values and their confidence intervals previously reported for phthalates.[1]

4.9.2 Corrected results and discussion

As expected, HRs and betas corrected for exposure measurement error using the disattenuation were greater in absolute value than the uncorrected ones (Figures 4.4 and 4.5). Bisphenol A, MCNP, MCOP, MCPP, and DEHP metabolites exhibited the greatest corrected estimates in absolute value, as well as the widest confidence intervals, even after taking into account of the incertainty in of ICC estimates. This was partly expected from the low values of the ICCs for these compounds. We observed that in some cases, the corrected effect estimates were very big in absolute value. Some care is needed when using ICCs from external studies, as we show in Chapter 6 that relying on ICCs external to the study may result is potentially untrustworthy estimates. We assumed the transportability of ICCs values but differences in the period and time at urine collection between the present and the two external studies[4,1] may greatly limit the appropriateness of using external data. Inaccuracy in ICCs might partly explain the somewhat extreme corrected results for some compounds. Additionally, this approach was shown to work well for simple regression models (no adjustment factors)[3] but is too simplifying for adjusted models, when a more complex attenuation factor should be used.[2] This may explain the extreme corrected estimates and should lead to a cautious interpretation of the results.

Correction of the effect estimates might be relevant in the context of pooled analyses or meta-analyses to combine studies.

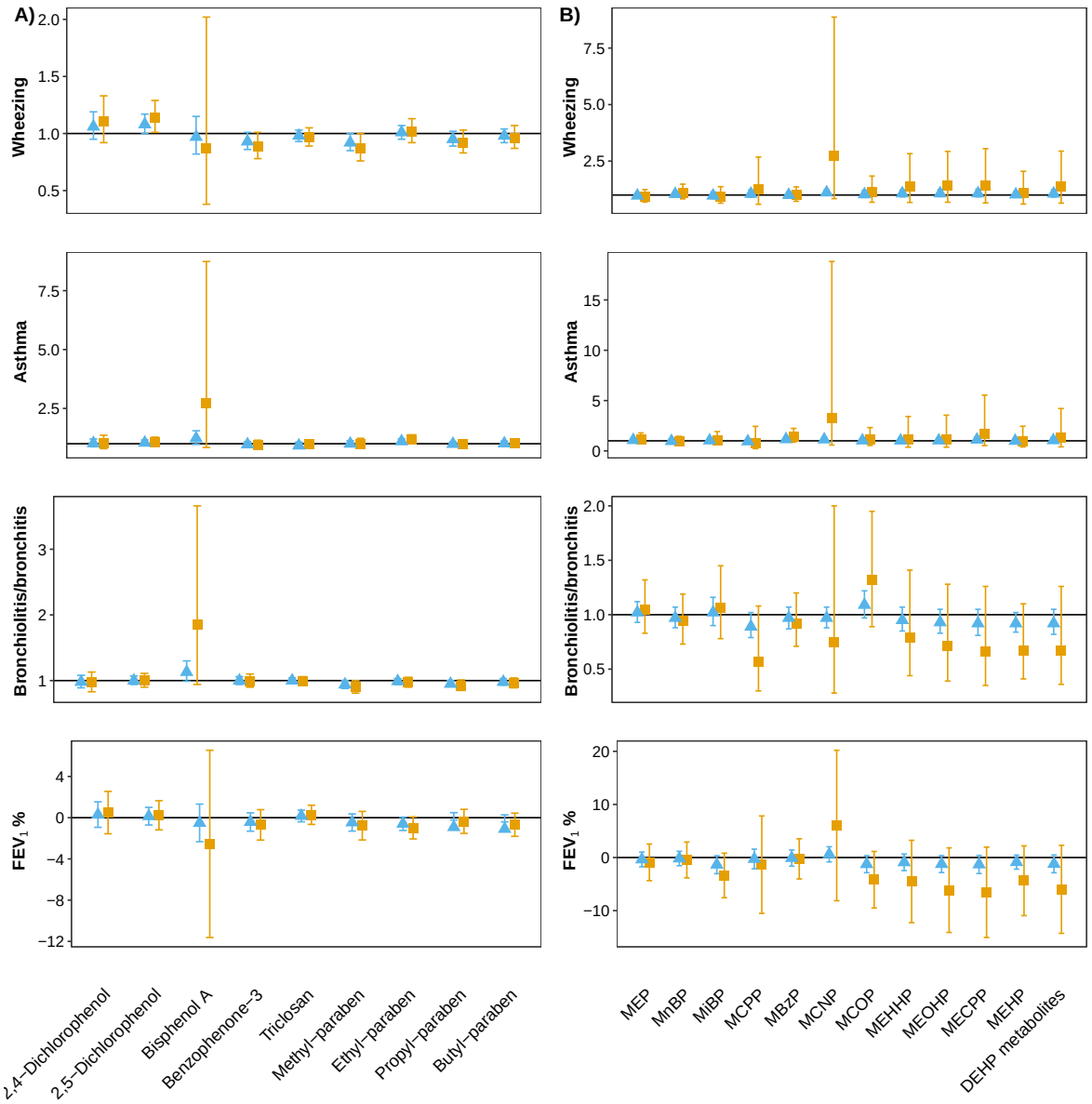

Figure 4.4 – Adjusted associations of phenols A) and phthalates metabolites B) ln-transformed standardized concentrations with respiratory outcomes (HR, n = 587) and FEV_1% in boys (beta, n = 228, EDEN cohort), before (blue triangles) and after (orange squares) correction for exposure measurement error using the *a posteriori* disattenuation method.

Effect estimates for 1 unit increase in ln-transformed standardized concentrations. Phenols and phthalates metabolites concentrations were standardized for urine sampling conditions (see methods section). Blue triangle and orange square markers represent, respectively uncorrected and corrected HR (or beta for FEV_1%) values; with error bars for 95% CI.

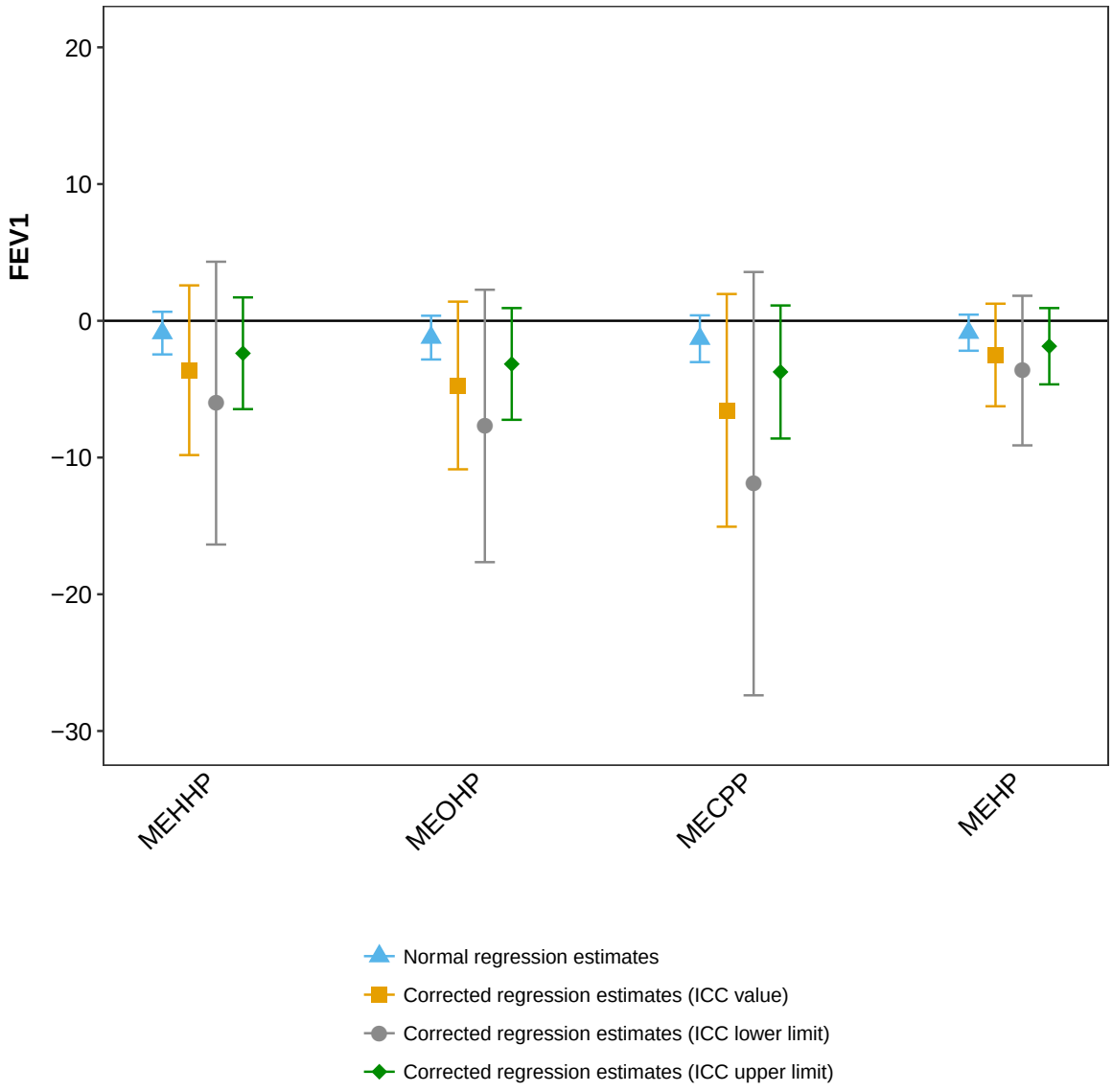

Figure 4.5 – Adjusted associations of DEHP metabolites concentrations with FEV_1% in boys (beta, n = 228, EDEN cohort), before (orange squares) and after correction for exposure measurement error using the *a posteriori* disattenuation method with ICC estimates (blue triangles) or lower (grey circles) and upper (green diamonds) limits of ICC 95% confidence intervals.

Effect estimates for 1 unit increase in ln-transformed concentrations. DEHP metabolites concentrations were standardized for urine sampling conditions (see methods section). Blue triangle, orange square, grey circle and green diamond markers represent beta value, uncorrected, corrected using ICC estimate, corrected using lower and upper confidence limits, respectively; with error bars for 95% CI.

4.9.3 References of this section

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Chapter 5

Within-day, between-day and between-week variability of urinary concentrations of phenol biomarkers in pregnant women

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Short running title:
Temporal variability of phenols during pregnancy

keywords:
2,4-dichlorophenol; 2,5-dichlorophenol; butylparaben; ethylparaben; methylparaben; propylparaben; bisphenol A; bisphenol S; benzophenone-3; triclosan; phenols; urine; biomarkers; human; variability; pregnancy; endocrine disruptors; exposure; epidemiology.

Published in Environmental Health Perspectives [1]

1. https://ehp.niehs.nih.gov/ehp1994/

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The findings expressed in this article are the opinions of the authors and do not necessarily reflect the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

Conflicts of interest: none

5.1 French summary

Introduction

Les phénols et les phtalates sont des composés très largement présents dans notre environnement. De nombreuses études chez l'animal, notamment chez le rongeur, rapportent des effets délétères de l'exposition à certains phénols et phtalates sur la santé, particulièrement lorsque l'exposition a lieu au cours du développement périnatal. Cependant, comme le montre la première étude de cette thèse, la confirmation de ces effets chez l'Homme est limitée, ce qui pourrait être dû à l'estimation de l'exposition. Les phénols et les phtalates sont des composés non-persistants, i.e., rapidement métabolisés par l'organisme et éliminés principalement dans les urines après quelques heures. De ce fait, l'estimation de l'exposition au cours de la grossesse via la mesure des concentrations des biomarqueurs dans un échantillon unique d'urine est probablement limitée. La variabilité de ces composés chez la femme enceinte est principalement caractérisée par des études reposant sur des échantillons uniques collectés à deux ou trois moments de la grossesse, ce qui ne permet pas d'explorer la variabilité intra-individuelle intra-jour ou inter-jour. Avant cette thèse, une seule étude s'est intéressée à la variabilité intra-jour pendant la grossesse. Reposant sur toutes les urines d'un jour, cette étude a exploré une quinzaine de phtalates et le bisphénol A, mais, à notre connaissance les autres phénols n'ont jamais été étudiés dans le contexte de la grossesse.

Objectif

L'objectif de cette étude est de caractériser la variabilité intra-individuelle intra-jour, inter-jour et inter-semaine de plusieurs phénols au cours de la grossesse.

Méthodes

Trente femmes enceintes ont recueilli toutes leurs urines pendant une semaine à trois occasions au cours de la grossesse (moyenne±écart-type, 15±2, 24±2, and 32±1 semaines de grossesse), dans le cadre de l'étude de faisabilité de SEPAGES. Nous avons sélectionné les huit femmes enceintes avec le moins d'échantillons manquants (60 échantillons collectés en moyenne par femme et par semaine). Parmi les huit femmes enceintes, deux ont fait un recueil exhaustif de leurs urines. Pour chaque participante, les aliquotes sont poolés par jour, et les pools "jour" par semaine. Les concentrations des biomarqueurs urinaires de 10 phénols sont mesurées dans ces pools. Par ailleurs, pour les deux femmes ayant collecté toutes leurs urines, les concentrations sont aussi mesurées dans tous les échantillons individuels de la première semaine de collection. Des coefficients de corrélation intra-classe (CCI) sont calculés pour caractériser la variabilité intra-jour avec les échantillons individuels, la variabilité inter-jour avec les

pools "jour", et la variabilité inter-semaine avec les pools "semaine".

Résultats

Pour la plupart des phénols, la variabilité intra-jour est forte, avec des CCI entre 0.03 et 0.5. La variabilité entre les jours d'une même semaine est plus faible, avec des CCI supérieurs à 0.6 sauf pour le bisphénol S (CCI, 0.14, intervalle de confiance à 95% 0.00-0.39). La variabilité entre les semaines de collection varie selon les phénols étudiés. La variabilité est la plus faible pour le 2,5-dichlorophénol (CCI supérieur à 0.9) ; et la plus forte pour le triclosan et le bisphénol S (CCI inférieur à 0.3).

Conclusion

Pendant la grossesse, les phénols ont une variabilité intra-individuelle très marquée au cours de la journée, alors que la variabilité des moyennes journalières entre les différents jours d'une même semaine est beaucoup plus faible. Mesurer la concentration des biomarqueurs urinaires des phénols dans un seul échantillon est insuffisant pour estimer correctement l'exposition. Collecter plusieurs échantillons d'une même semaine pourrait être une approche suffisante pour représenter correctement l'exposition sur toute la grossesse pour certains phénols, par exemple, le 2,5-dichlorophénol, mais pas pour tous.

5.2 Abstract

Background

Toxicology studies have shown adverse effects of developmental exposure to industrial phenols. Evaluation in humans is challenged by potentially marked within-subject variability of phenol biomarkers in pregnant women, which is poorly characterized.

Objectives

To characterize within-day, between-day and between-week variability of phenol urinary biomarker concentrations during pregnancy.

Methods

In eight French pregnant women, we collected all urine voids over one week (average, 60 samples per week per woman) at three occasions (mean±standard deviation, 15±2, 24±2, and 32±1 gestational weeks) in 2012-2013. Aliquots of each day and of the whole week were pooled within-subject. We assayed concentrations of ten phenols in these pools, and, for two women, in all spot (unpooled) samples collected during one week. We characterized variability using intraclass correlation coefficients (ICCs) with spot samples (within-day variability), daily pools (between-day variability) and weekly pools (between-week variability).

Results

For most biomarkers, the within-day variability was high (ICCs between 0.03 and 0.50). The between-day variability, based on samples pooled within each day, was much lower, with ICCs above 0.60 except for bisphenol S (ICC, 0.14, 95% confidence interval, 0.00-0.39). The between-week variability differed between compounds, triclosan and bisphenol S having the lowest ICCs (below 0.3) and 2,5-dichlorophenol the highest (ICC above 0.9).

Conclusion

During pregnancy, phenol biomarkers showed a strong within-day variability, while the variability between days of a given week was more limited. One biospecimen is not enough to efficiently characterize exposure; collecting biospecimens during a single week may be enough to represent well the whole pregnancy exposure for some but not all phenols.

5.3 Introduction

Phenols include high-production-volume chemicals with widespread uses in daily life products. For example, bisphenols are employed in the manufacture of epoxy resins and certain polymer plastics used in food and beverage containers, and in other consumer products.[9,15,24] Parabens are used as preservatives in cosmetics, food, beverages and pharmaceuticals; benzophenone-3, an ultraviolet-filter, is used in plastics and cosmetics; triclosan is used for its antibacterial properties in personal care products, clothing or kitchenware;[11,19,23] 2,4-dichlorophenol is used in the production of certain pesticides and 2,5-dichlorophenol is a major metabolite of 1,4-dichlorobenzene, which is used in moth balls and room deodorizers.[8,22]

Concern exists regarding the health effects of phenols, which are potential endocrine disruptors, particularly following exposure during foetal life.[6] In terms of study design, most biomarker-based studies in humans relied on biomarker concentrations assessed in very few (one to three) spot biospecimens per pregnant woman. For chemicals with strong within-subject temporal variations, relying on a small number of biospecimens is expected to imperfectly characterize the average exposure (e.g. over a day, a week or more), to lead to exposure misclassification, and consequently bias dose-response functions.[26,7] The biological half-life of phenols in pregnant women is not known, and could strongly differ from that of non-pregnant women, as is the case for e.g. urinary biomarkers of tobacco smoke exposure such as cotinine, which has been found to have about twice as fast elimination half-life during pregnancy compared to postpartum.[12] Studies based on non-pregnant adults reported a short (less than 12 hours) half-life for some phenols.[16,29,32] Consequently, the relevance of relying on one spot biospecimen to provide a proxy of exposure for time windows of one day or longer is probably limited. This issue is of importance given the expected impact of exposure misclassification on bias in dose-response functions relating biomarker levels to health parameters.[26]

Several studies evaluated the reproducibility of urinary phenol concentrations during pregnancy.[2,4,5,14,17,21,28,31] These studies relied on generally two or three spot biospecimens collected from each pregnant woman several weeks or months apart. Such a design did not allow characterizing the within-day or the within-week variability in biomarkers concentrations. Based on complete urine collections throughout several days in eight non-pregnant participants, two studies reported high within-subject and between-day variability for bisphenol A,[18,34] while this variability was relatively small for some parabens, triclosan and benzophenone-3.[18] High within-day variability of bisphenol A concentrations was also reported in 66 pregnant women with complete

urine collection during one or two days.[13] In the context of pregnancy, estimations of the within-subject variability of phenols other than bisphenol A are lacking.

Accurate description of the variability of phenol urinary concentrations during pregnancy is crucial for adopting biospecimens sampling strategies that limits exposure misclassification in etiological studies. Our aim was consequently to characterize the within-day, between-day (within a week) and between-week variability of urinary concentrations of ten phenols in pregnant women.

5.4 Methods

5.4.1 Study participants

This study relied on a subgroup of the feasibility study conducted between July 2012 and July 2013 in the planning of the SEPAGES cohort (Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé; Assessment of air pollution exposure during pregnancy and effects on health). In this feasibility study, 40 women with a singleton pregnancy and living in the Grenoble urban area (France) were recruited from private obstetrical practices, before 17 gestational weeks (calculated from the date of the last menstrual period). The exclusion criteria included inability to write or speak French, being under 18 years of age, planning to give birth outside of one of the four maternity hospitals of the Grenoble urban area, and not being enrolled in the French social security system. All participating women and their partners provided written informed consent for themselves and their offspring for biological measurements and data collection.[25] SEPAGES-feasibility cohort was approved by the appropriate ethical committees (CPP, Comité de Protection des Personnes Sud-Est; CNIL, Commission Nationale de l'Informatique et des Libertés; CCTIRS, Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé; ANSM, Agence Nationale de Sécurité du Médicament et des produits de santé). The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subject research.

5.4.2 Study design and urine collection

The urine collection protocol is described in Figure 5.1. Urine collection took place during seven consecutive days at three periods of pregnancy (1st collection week, median: 13 gestational weeks, min–max: 10-18 gestational weeks; 2nd collection week, median: 23, min–max: 21-26; and 3rd collection week, median: 32, min–max: 29-33, Table 5.1). Thirty out of the 40 women participating in SEPAGES-feasibility study were asked to collect about 60 mL of each urine void, and to report in daily diaries micturition time for collected and missing voids. The remaining 10 participating women were asked to collect about 60 mL of only three urine voids per day, and were therefore not considered in the present study. Women collected urine in polypropylene containers and stored it in a refrigerator (4°C) in their home. When they were not at home, collected urine was stored in a cooler with ice packs. Specimens were retrieved two or three times a week by the study staff and brought in coolers to Inserm research centre (Institute for Advanced Biosciences, Grenoble, France). Each sample was aliquoted

into 2 mL polypropylene cryovials (up to five vials per sample) and frozen at -80°C until pooling procedure or shipping for analysis. Because of costs constraints, we only quantified phenol biomarkers in the subgroup of eight women with the smallest rate of missed voids. Among these women, two had managed to collect a sample of each of their urine voids (no missing void, subgroup 1), while the other six women collected more than 95% of their weekly urine voids (subgroup 2).

5.4.3 Pooling procedure

We thawed at 4°C and vortexed aliquots in polypropylene containers and pooled them according to the protocol detailed in Figure 5.1. For each woman and each study day, we took equal volumes of urine from all samples of the day and combined them within woman, leading to seven within-subject daily pools for a 1-week period (days 1 to 7). For each subject, we then prepared three weekly pools by combining an equal volume of the seven daily pools from each collection week (weeks 1, 2 and 3).

Immediately after preparation, pooled samples were placed in 2-mL polypropylene cryovials and frozen at -80°C. The pools and all aliquots from spot samples to be analysed were kept frozen until shipment on dry ice to the CDC laboratory in Atlanta (Georgia, USA). At the CDC laboratory, all urine samples were stored at or below -70°C until analysis.

5.4.4 Phenols, creatinine and specific gravity measurements

The total urinary concentrations of 2,4- and 2,5-dichlorophenols, benzophenone-3, bisphenol A, bisphenol S, triclosan, and butyl, methyl, ethyl, and propylparabens were quantified at the CDC using a modification of an online solid-phase extraction high-performance liquid chromatography-isotope dilution-tandem mass spectrometry method.[35] Limits of detection (LODs) are listed in Table 5.2. The coefficients of variation of quality control measurements ranged, according to compounds and concentration ranges, between 3.4 and 14.7%. It was higher for methylparaben, bisphenol A, benzophenone-3 and triclosan, for which the range was 5.8-14.7% and lower for the other compounds (3.4 to 6.4%). Moreover, the team in charge of urine collection added eight replicates to the samples assayed for phenols, in a way that was blinded to the lab. Correlation coefficients between biomarker concentrations in samples and their replicates ranged from 0.95 (bisphenols A and S) to 1.00 (2,4-dichlorophenol, ethyl, propyl and butylparabens and triclosan). Two urine dilution markers were also quantified in the same samples: urinary creatinine measured at the CDC using a Roche/Hitachi MODULAR ANALYTICS Urine Work Area (SWA) P (photometric analysis) module

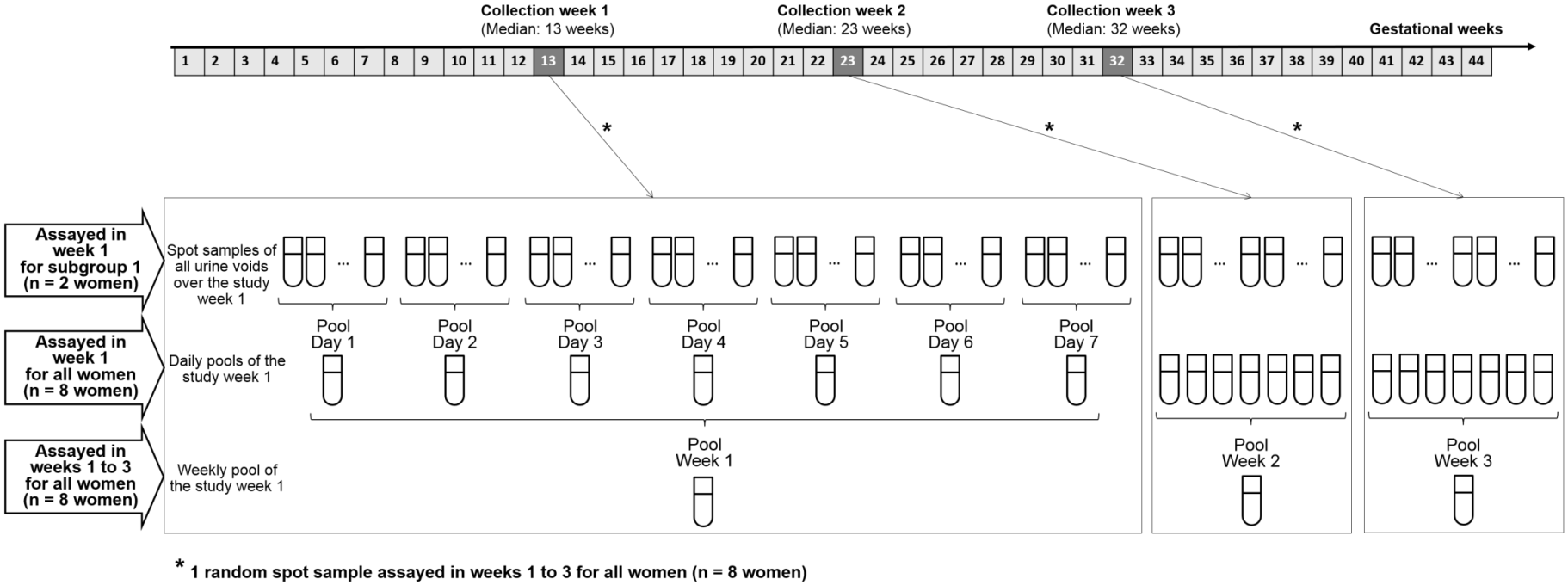

Figure 5.1 – Urine collection, pooling procedure and biomarker assays in all the study population (n = 8 women) and in the nested subgroup 1 (n = 2 women).

(Roche Diagnostics, Indianapolis, IN, USA); and urinary specific gravity, measured at room temperature using a handheld Atago PAL 10-S refractometer (Atago, Bellevue, WA, USA) at Inserm Grenoble laboratory.

We analysed a total of 216 samples (136 spot samples, 8×7=56 daily pools, 8×3=24 weekly pools, see Figure 5.1) for phenol biomarkers, creatinine and specific gravity. For all women, we analysed the seven daily pools of the first study week (56 daily pools) and the three weekly pools (24 weekly pools). Additionally, for the 2 women in subgroup 1, we also analysed all spot samples of week 1 (total, 114 samples) and two spot samples randomly selected among those from the two other collection weeks (one random spot sample in each collection week). Finally for each of the six participants in subgroup 2, we analysed three spot samples randomly selected among all the samples from the three collection weeks (one in each week of collection, Figure 5.1), so that, for all 8 women, we could assay one random spot sample for each of the three collection weeks.

5.4.5 Statistical analyses

Concentrations below the LOD were replaced by instrumental readings, or by the compound-specific lowest non-zero instrumental reading divided by the square root of two when the instrumental reading was zero. We $\log_{10}$-transformed the urinary concentrations of phenol biomarkers to achieve approximate normality in the distributions. Correlations of biomarker concentrations between types of sample (unpooled samples, daily and weekly pools) for a given biomarker and between biomarker concentrations for a given type of sample were calculated using Spearman correlation coefficients.

Our assessment of variability relied on intraclass correlation coefficients (ICCs) estimated using one-way random-effect ANOVA models. The approach was identical for all ICCs estimations. ICCs close to zero indicate poor reproducibility of a concentration within the considered period while values close to one indicate high reproducibility. In the case of negative ICC estimate (which can happen with ANOVA models),[33] we considered the ICC not to be computable and only reported the 95% Confidence Interval (CI), truncating its lower bound to zero.

To characterize the within-day variability, we defined ICC as the ratio of the between-day variance to the total variance (sum of within- and between-day variances). We relied on women of subgroup 1 (a total of 114 samples, collected during the first collection week for the two women). The woman- and compound-specific weekly mean was subtracted from the spot concentrations to correct for the between-subject variability before estimating ICCs representing the within-day variability.

We assessed the within-week (between-day) and between-week variability with ICCs calculated as the ratio of the between-subject variance to the total variance (sum of within- and between-subject variance). For the between-day variability, we ran models based on all 56 daily pools of the study week 1 (eight women, each with seven daily pools). For the between-week variability, we used all 24 weekly pools (eight women, each with three weekly pools).

To allow comparisons with previous studies that relied on two to three spot samples collected during pregnancy, we additionally estimated ICCs based on the three random spot samples collected during pregnancy (each sample being randomly selected in each collection week for the eight women).

To assess the robustness of the findings to the statistical methods, we also computed ICCs using random intercept linear mixed models (maximum likelihood estimates) instead of ANOVA models. To assess the potential impact of urinary dilution on variance estimates for phenol biomarkers, the ANOVA analyses were repeated using phenol biomarker concentrations corrected for creatinine (ratio of the phenol biomarker concentrations to the creatinine concentration in the same sample) and for specific gravity using a formula previously described,[28] or by including creatinine concentration or specific gravity as a covariate in the random intercept linear mixed models.

Data were analyzed using STATA 12.1 (Stata Corp, College Station, Texas).

5.5 Results

5.5.1 Study population and samples

Table 5.1 – Characteristics of the population (40 pregnant women from SEPAGES feasibility study, out of which 8 participated in the current study).

Characteristic	Included (n = 8) N (%) or *mean ± SD*	Excluded (n = 32) N (%) or *mean ± SD*	P-value*
Maternal age at enrolment (years)	*29.6 ± 3.8*	*30.7 ± 3.6*	0.34
Civil status			
Married	5 (63)	12 (37)	0.25
Cohabitating	3 (37)	20 (63)	
Maternal education			
High school or less	0	2 (6)	1.00
Up to 3 years of college	4 (50)	14 (44)	
> 3 years of college	4 (50)	16 (50)	
Smoking history during pregnancy			
Yes	1 (12)	6 (19)	1.00
No	7 (88)	21 (66)	
Missing	0	5 (15)	
Parity			
Primiparous	5 (63)	19 (59)	1.00
Multiparous	3 (37)	13 (41)	
Gestational age (weeks)			
Week 1 of urine collection	*15.0 ± 1.9*	*12.9 ± 1.5*	0.01
Week 2 of urine collection	*24.0 ± 1.6*	*23.3 ± 1.4*	0.21
Week 3 of urine collection	*32.3 ± 0.7*	*31.8 ± 0.9*	0.16
Time between two weeks of urine collection (weeks)			
Week 1 – Week 2	*9.0 ± 1.9*	*10.5 ± 1.8*	0.06
Week 2 – Week 3	*8.3 ± 1.2*	*8.5 ± 1.7*	0.88

SD: standard deviation
* p-values of chi-square or Mann-Whitney U-tests comparing the characteristics of included and non-included women.

At enrolment, women were 29.6 years old on average (standard deviation, SD: 3.8); most of them were primiparous (63%), did not smoke during pregnancy (88%) and all of them had college education (Table 5.1). Women collected from three to 15 urine samples per day (total from 132 to 240 samples per woman). Detection frequencies were generally between 79% (triclosan) and 100% (methyl and propylparabens), except for benzophenone-3, which was only detected in 35% of the samples. The highest (between-compound) coefficients of correlation were observed between structurally similar compounds (e.g., between the two dichlorophenols and between the four parabens), and between creatinine and specific gravity (see Table 5.6), regardless of the type of sample (unpooled, daily or weekly pools). Correlation between creatinine and

urinary concentrations of phenol biomarkers ranged from -0.06 (with propylparaben) to 0.83 (2,4-dichlorophenol) in spot samples of subgroup 1, from -0.28 (triclosan) to 0.61 (bisphenol A) in daily pools of all studied women, and from -0.15 (triclosan) to 0.56 (2,4-dichlorophenol) in weekly pools.

5.5.2 Within-day variability

Table 5.2 – Within-day variability - Descriptive statistics of the non-transformed biomarker concentrations (μg/L) for the unpooled spot samples from subgroup 1 (2 women, n=114 spot samples collected over the first week of collection) and ICCs based on $\log_{10}$-transformed phenol biomarker concentrations, creatinine concentration and specific gravity. Values were not standardized for creatinine or specific gravity.

Biomarker	LOD (μg/L)	Results above the LOD, N (%)	Percentiles					Within-day ICC (95% CI)[b]
			5th	25th	50th	75th	95th	
Phenols (μg/L)[a]								
2,4-dichlorophenol	0.1	113 (99)	0.1	0.2	0.3	0.3	0.5	0.12 (0.00, 0.28)
2,5-dichlorophenol	0.1	111 (97)	0.1	0.2	0.4	0.7	1.2	0.11 (0.00, 0.27)
Butyl paraben	0.1	113 (99)	0.1	1.1	4.2	21.7	92.7	0.10 (0.00, 0.25)
Ethyl paraben	1.0	105 (92)	<LOD	2.4	10.7	43.1	126.2	0.03 (0.00, 0.15)
Methyl paraben	1.0	114 (100)	37	90.4	217.3	1329.4	5000.0	0.27 (0.05, 0.49)
Propyl paraben	0.1	114 (100)	0.8	4.8	36.7	139.0	895.1	0.28 (0.05, 0.50)
Benzophenone-3	0.2	31 (27)	<LOD	<LOD	<LOD	1.4	10.7	0.26 (0.04, 0.48)
Bisphenol A	0.1	113 (99)	0.3	0.9	1.6	2.4	7.5	0.21 (0.01, 0.41)
Bisphenol S	0.1	112 (98)	0.1	0.2	0.3	0.5	1.3	0.50 (0.26, 0.73)
Triclosan	1.0	90 (79)	<LOD	1.0	2.1	3.3	4.8	0.30 (0.08, 0.53)
Urine dilution markers								
Creatinine (mg/dL)	NA	114 (100)	37.2	72.5	102.5	139.2	223.1	0.10 (0.00, 0.26)
Specific gravity	NA	114 (100)	1.009	1.014	1.018	1.021	1.027	0.03 (0.00, 0.15)

ICC: intraclass correlation coefficient; LOD: limit of detection; NA: not applicable.
[a] Concentrations below the LOD were replaced by instrumental reading values. For each phenol biomarker, instrumental reading values equal to 0 were replaced by the lowest non-zero instrumental reading value divided by the square root of 2.
[b] ICCs were estimated from ANOVA with a random effect on day (14 days) and within-woman mean-centering of the log-transformed phenols concentrations.

For the spot samples, urinary concentrations of most phenols and of creatinine varied within woman by several orders of magnitude throughout the first collection week and within a day (Figure 5.2). For all biomarkers including creatinine and specific gravity, ICCs were low to moderate (Table 5.2 and Figure 5.5), with the highest ICC observed for bisphenol S (0.50; 95% CI: 0.26, 0.73), and the lowest for ethylparaben (0.03; 95% CI: 0.00, 0.15) and specific gravity (0.03; 95% CI: 0.00, 0.15). Creatinine or specific gravity standardisation, respectively, slightly increased (by 0.02 to 0.07) ICCs for five and nine compounds out of 10 (Table 5.7). Using linear mixed models instead

of ANOVA methods to estimate ICCs (Tables 5.8 and 5.9) led to similar results.

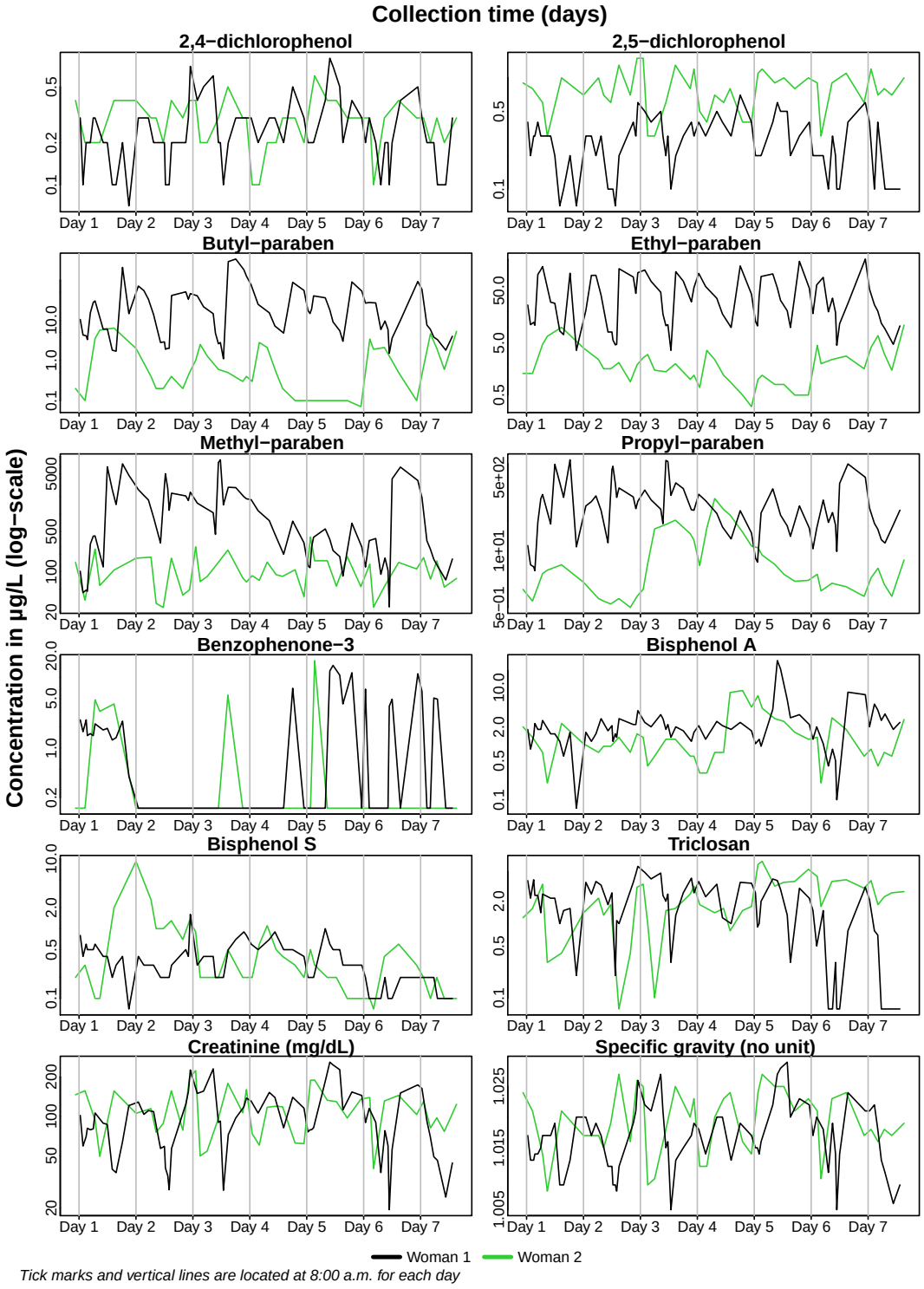

Figure 5.2 – Within-day and within-week variability - Urinary concentrations of 10 phenols (μg/L), creatinine concentration (mg/dL) and specific gravity in $\log_{10}$-scale in the unpooled spot samples from subgroup 1 (2 women, n=114 spot samples collected over the first week of collection). Note that to facilitate visualisation, each biomarker is displayed on a specific scale.

5.5.3 Between-day (within a week) variability

Table 5.3 – Between-day variability - Descriptive statistics of the non-transformed biomarker concentrations (μg/L) for the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection) and ICCs based on $\log_{10}$-transformed phenol biomarker concentrations, creatinine concentration and specific gravity. Values were not standardized for creatinine or specific gravity.

Biomarker	LOD (μg/L)	Results above the LOD, N (%)	Percentiles					Between-day ICC (95% CI)[b]
			5th	25th	50th	75th	95th	
Phenols (μg/L)[a]								
2,4-dichlorophenol	0.1	56 (100)	0.1	0.2	0.3	0.4	6.0	0.91 (0.82, 1.00)
2,5-dichlorophenol	0.1	56 (100)	0.2	0.3	0.8	3.6	269.6	0.98 (0.95, 1.00)
Butyl paraben	0.1	45 (80)	0.1	0.1	0.2	1.1	35.2	0.80 (0.61, 0.99)
Ethyl paraben	1.0	39 (70)	<LOD	<LOD	2.8	12.9	45.5	0.85 (0.70, 1.00)
Methyl paraben	1.0	56 (100)	2.4	9.2	33.0	89.7	1350.8	0.84 (0.69, 1.00)
Propyl paraben	0.1	56 (100)	0.1	0.4	2.2	39.9	161.8	0.90 (0.80, 1.00)
Benzophenone-3	0.2	28 (50)	<LOD	<LOD	0.5	3.1	33.8	0.73 (0.50, 0.96)
Bisphenol A	0.1	56 (100)	0.4	1.0	1.9	3.5	8.2	0.60 (0.30, 0.89)
Bisphenol S	0.1	56 (100)	0.1	0.2	0.2	0.4	2.8	0.14 (0.00, 0.39)
Triclosan	1.0	45 (80)	<LOD	1.1	2.1	3.0	63.7	0.89 (0.78, 1.00)
Urine dilution markers								
Creatinine (mg/dL)	NA	56 (100)	30.6	60.4	77.9	102.2	146.8	0.60 (0.30, 0.89)
Specific gravity	NA	56 (100)	1.009	1.013	1.016	1.02	1.029	0.61 (0.32, 0.90)

ICC: intraclass correlation coefficient; LOD: limit of detection; NA: not applicable.
[a] Concentrations below the LOD were replaced by instrumental reading values. For each phenol biomarker, instrumental reading values equal to 0 were replaced by the lowest non-zero instrumental reading value divided by the square root of 2.
[b] ICCs were estimated from ANOVA with a random effect on woman.

The remaining analyses are based on the whole group of 8 women. Detection frequencies were quite similar in daily pools (median over all compounds, 100%) compared to unpooled spot samples (median, 99%) for most of the compounds, except for butylparaben (80% detection in daily pools, compared to 99% in spot samples), ethylparaben (70% versus 92%) and benzophenone-3 (50% versus 27%, Table 5.3). ICCs based on daily pools were high for most compounds (above 0.80), except benzophenone-3 (ICC, 0.73; 95% CI, 0.50 to 0.96), bisphenol A (0.60; 95% CI: 0.30, 0.89) and bisphenol S (0.14; 95% CI: 0.00, 0.39; Table 5.3 and Figures 5.3 and 5.5). The highest ICCs were observed for 2,4-dichlorophenol (ICC: 0.91; 95% CI: 0.82, 1.00) and 2,5-dichlorophenol (0.98; 95% CI: 0.95, 1.00). ICCs for creatinine and specific gravity were greater in daily pools (0.60 and 0.61, respectively) compared to spot samples (0.10 and 0.03, respectively). For five compounds, correction for creatinine slightly increased (by 0.01

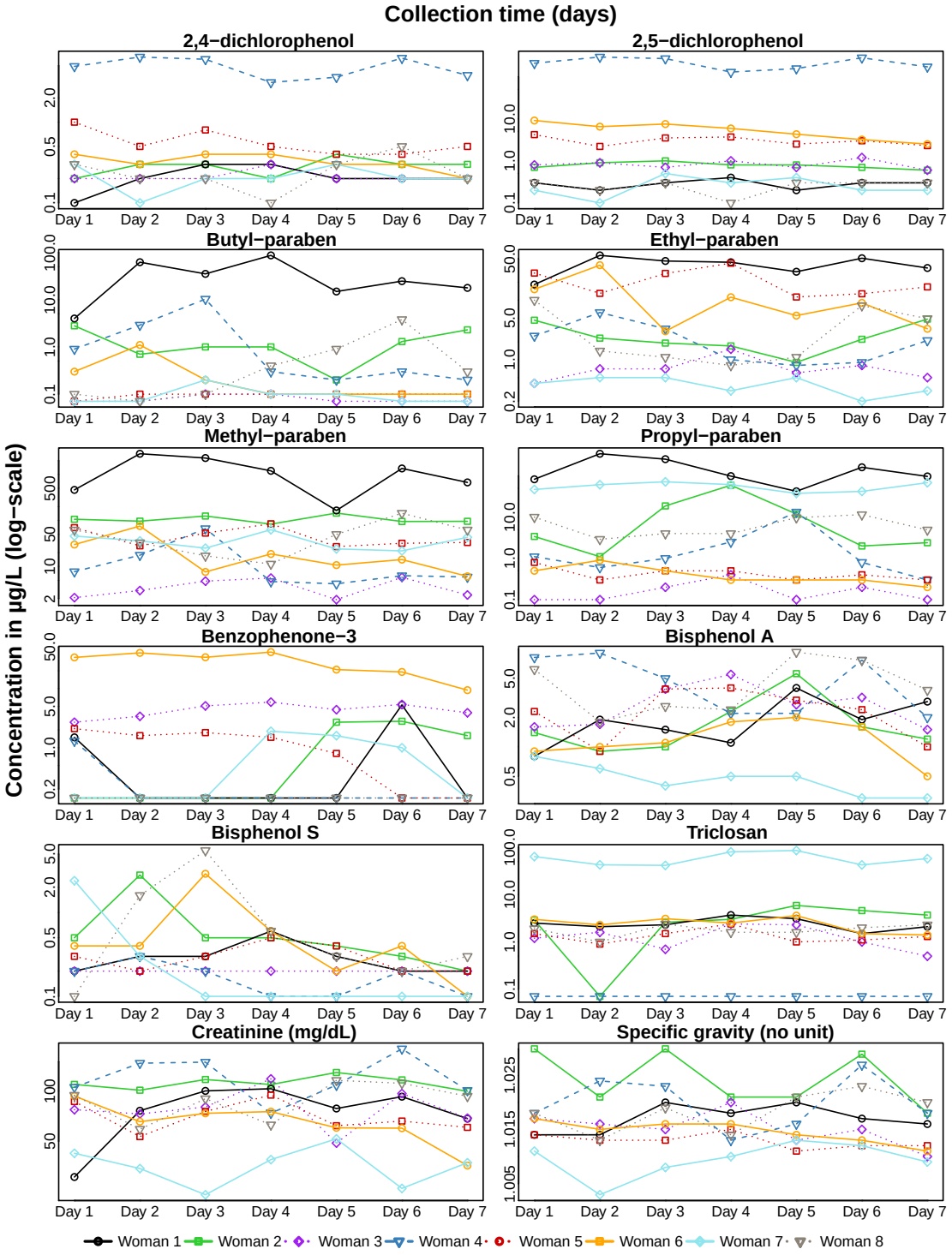

Figure 5.3 – Between-day (within a week) variability of pooled daily samples - Urinary concentrations of 10 phenols (μg/L), creatinine concentration (mg/dL) and specific gravity in $\log_{10}$-scale in the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection). Note that to facilitate visualisation, each biomarker is displayed on a specific scale.

to 0.05) ICCs, which stayed within ranges of the uncorrected confidence intervals (Table 5.10). We observed similar results using linear mixed model instead of ANOVA methods to estimate ICCs (Tables 5.8 and 5.11).

5.5.4 Between-week variability based on weekly pools

Table 5.4 – Between-week variability - Descriptive statistics of the non-transformed biomarker concentrations (μg/L) for the within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection) and ICCs based on $\log_{10}$-transformed phenol biomarker concentrations, creatinine concentration and specific gravity. Values were not standardized for creatinine or specific gravity.

Biomarker	LOD (μg/L)	Results above the LOD, N (%)	Percentiles					Between-week ICC (95% CI)[b]
			5th	25th	50th	75th	95th	
Phenols (μg/L)[a]								
2,4-dichlorophenol	0.1	24 (100)	0.1	0.2	0.3	0.6	2.2	0.65 (0.32, 0.99)
2,5-dichlorophenol	0.1	24 (100)	0.3	0.4	0.6	2.2	73.0	0.93 (0.86, 1.00)
Butyl paraben	0.1	21 (88)	0.1	0.1	0.3	0.7	24.0	0.84 (0.67, 1.00)
Ethyl paraben	1.0	19 (79)	<LOD	1.1	11.4	19.5	56.5	0.33 (0.00, 0.79)
Methyl paraben	1.0	24 (100)	3.9	23.7	44.4	99.5	1673.7	0.81 (0.60, 1.00)
Propyl paraben	0.1	24 (100)	0.2	0.4	4.8	44.4	173.6	0.86 (0.70, 1.00)
Benzophenone-3	0.2	9 (38)	<LOD	<LOD	<LOD	4.3	28.5	0.60 (0.23, 0.97)
Bisphenol A	0.1	24 (100)	0.5	1.3	1.9	3.3	5.7	0.59 (0.22, 0.97)
Bisphenol S	0.1	24 (100)	0.1	0.2	0.3	0.7	14.4	0.26 (0.00, 0.73)
Triclosan	1.0	19 (79)	<LOD	1.3	2.5	7.1	83.7	(0.00, 0.44)
Urine dilution markers								
Creatinine (mg/dL)	NA	24 (100)	49.6	66.0	84.1	111.8	142.3	0.83 (0.64, 1.00)
Specific gravity	NA	24 (100)	1.009	1.012	1.016	1.021	1.023	0.49 (0.07, 0.91)

ICC: intraclass correlation coefficient; LOD: limit of detection; NA: not applicable.
[a] Concentrations below the LOD were replaced by instrumental reading values. For each phenol biomarker, instrumental reading values equal to 0 were replaced by the lowest non-zero instrumental reading value divided by the square root of 2.
[b] ICCs were estimated from ANOVA with a random effect on woman. For triclosan, we give only the confidence interval truncated to zero due to negative estimate of ICC.

On average 9.0 weeks (SD: 1.9) elapsed between collection weeks 1 and 2, and 8.3 weeks (SD: 1.2) between collection weeks 2 and 3 (range, 5.6 to 12.0 weeks). For seven of the 10 phenol biomarkers, detection frequencies in weekly pools were similar to those in daily pools (above 79%, Table 5.4), while they were somewhat higher in weekly pools for butyl and ethylparabens (88 and 79%, compared to 80% and 70%, respectively, in daily pools), and lower for benzophenone-3 (38% versus 50% in daily pools). Between two study weeks, concentrations of almost all phenols varied by several orders of magnitude for some women (Figure 5.4). ICCs for 2,5-dichlorophenol, butyl,

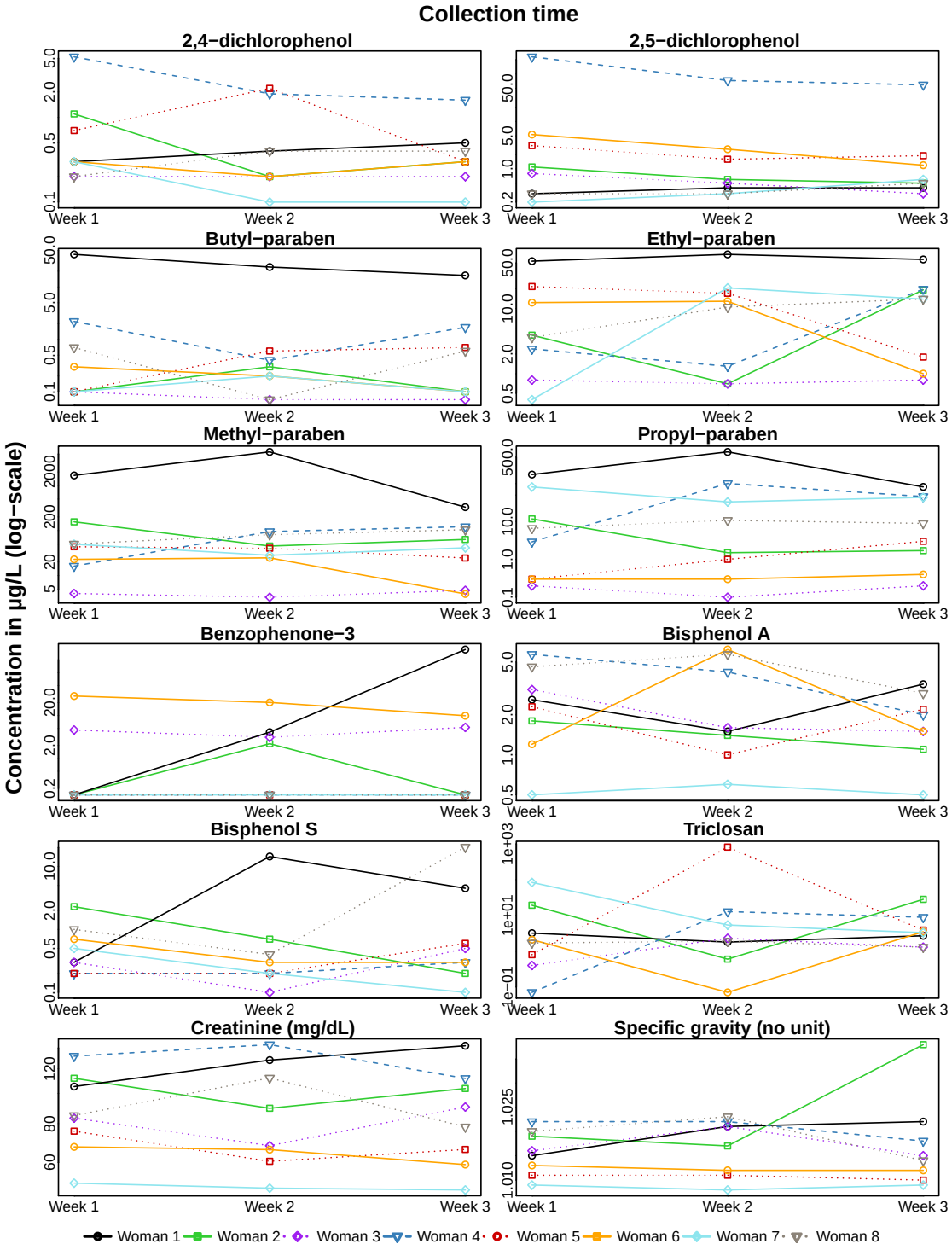

Figure 5.4 – Between-week variability of weekly samples - Urinary concentrations of 10 phenols (μg/L), creatinine concentration (mg/dL) and specific gravity in $\log_{10}$-scale in the within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection). Note that to facilitate visualisation, each biomarker is displayed on a specific scale.

methyl, propylparabens and creatinine were above 0.8, while they were below 0.6 for the other biomarkers (Table 5.4, Figure 5.5), and lowest for bisphenol S (0.26; 95% CI: 0.00, 0.73). ICC for triclosan could not be computed, but was probably in the low range (95% CI, 0.00 to 0.44). ICCs slightly decreased (by 0.01 to 0.2) for most compounds when using creatinine or specific gravity corrected concentrations, but stayed within ranges of the uncorrected confidence intervals (Table 5.10). Adjusting for creatinine or specific gravity did not change the results (Tables 5.8 and 5.11).

5.5.5 Between-week variability based on three random spot samples

Table 5.5 – Alternative estimate of between-week variability based on 3 random spot samples - Descriptive statistics of the non-transformed biomarker concentrations (μg/L) for the random spot samples from subgroup 1 and subgroup 2 (8 women, n=24 random spot samples, one sample in each of the 3 weeks of collection) and ICCs based on $\log_{10}$-transformed phenol biomarker concentrations, creatinine concentration and specific gravity. Values were not standardized for creatinine or specific gravity.

Biomarker	LOD (μg/L)	Results above the LOD, N (%)	Percentiles					Between-week ICC (95% CI)[b]
			5th	25th	50th	75th	95th	
Phenols ($\mu g/L$)[a]								
2,4-dichlorophenol	0.1	24 (100)	0.1	0.2	0.4	0.5	2.2	0.50 (0.08, 0.92)
2,5-dichlorophenol	0.1	23 (96)	0.1	0.3	0.8	1.4	70.3	0.85 (0.69, 1.00)
Butyl paraben	0.1	19 (79)	0.1	0.1	0.1	3.8	8.0	0.42 (0.00, 0.87)
Ethyl paraben	1.0	16 (67)	<LOD	<LOD	2.4	10.5	21.1	0.40 (0.00, 0.85)
Methyl paraben	1.0	24 (100)	2.3	5.0	15.4	106.3	276.7	0.85 (0.68, 1.00)
Propyl paraben	0.1	24 (100)	0.1	0.5	4.2	34.5	99.3	0.70 (0.40, 1.00)
Benzophenone-3	0.2	13 (54)	<LOD	<LOD	0.8	4.5	47.4	0.28 (0.00, 0.75)
Bisphenol A	0.1	23 (96)	0.1	0.4	1.1	2.6	8.0	0.38 (0.00, 0.83)
Bisphenol S	0.1	22 (92)	0.1	0.1	0.2	0.4	12.3	0.33 (0.00, 0.80)
Triclosan	1.0	13 (54)	<LOD	<LOD	1.1	2.4	29.9	0.11 (0.00, 0.58)
Urine dilution markers								
Creatinine (mg/dL)	NA	24 (100)	13.90	28.65	67.43	125.94	156.25	0.54 (0.14, 0.94)
Specific gravity	NA	24 (100)	1.005	1.007	1.016	1.021	1.029	0.69 (0.39, 1.00)

ICC: intraclass correlation coefficient; LOD: limit of detection; NA: not applicable.
[a] Concentrations below the LOD were replaced by instrumental reading values. For each phenol biomarker, instrumental reading values equal to 0 were replaced by the lowest non-zero instrumental reading value divided by the square root of 2.
[b] ICCs were estimated from AOVA with a random effect on woman. For triclosan analysis, one sample with extreme value excluded (n=23 samples).

Detection frequencies of biomarker concentrations in the three random spot samples collected in each woman were above 79%, except for ethylparaben (70%) and triclosan (54%). ICCs computed using these random spots samples were lower than 0.6, except for 2,5-dichlorophenol (ICC, 0.85; 95% CI, 0.69 to 1.00), methylparaben (0.85; 95% CI: 0.68, 1.00) and propylparaben (0.70; 0.40, 1.00, Table 5.5). As for weekly pools, ICCs slightly decreased (by 0.01 to 0.19) when using creatinine- or specific gravity-corrected concentrations (Table 5.10). Adjustment for creatinine or specific gravity did not change the results (Tables 5.8 and 5.11).

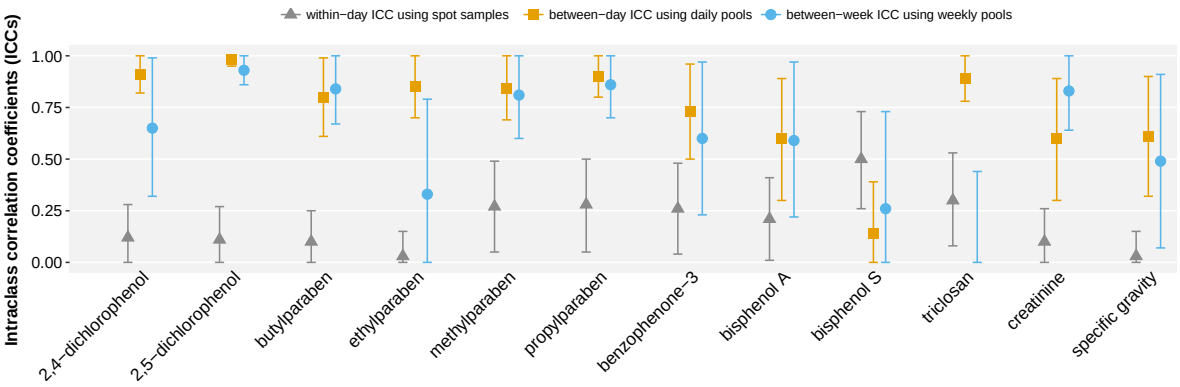

Figure 5.5 – Intraclass correlation coefficients (error bars for 95% confidence intervals) for the within-day variability using the unpooled spot samples from subgroup 1 (2 women, n=114 spot samples collected over the first week of collection, triangle markers), the between-day variability using the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection, square markers) and the between-week variability in the within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection, circle markers). For triclosan, we give only the confidence interval truncated to zero due to negative estimate of between-week ICC.

5.6 Discussion

To our knowledge, this study is the first to evaluate the within-day, between-day and between-week variability of ten phenol biomarkers as well as of creatinine and specific gravity in pregnant women. Most compounds showed very high variability over the course of a day (ICCs generally below 0.3), while the between-day variability of the daily averages over the course of a week was much lower. This pattern was opposite for bisphenol S, which had a stronger between-day than within-day variability. The variability of the weekly averages considered several weeks apart exhibited more contrasted patterns across compounds, with low between-week variability for some compounds (2,5-dichlorophenol, butylparaben, propylparaben, methylparaben) and a high variability for others (ethylparaben, bisphenol S, triclosan). Urinary dilution or creatinine levels did not explain much of the observed within-subject variability in phenol biomarkers.

5.6.1 Strengths and limitations

A key strength of our study is the reliance on pregnant women who agreed to collect samples from each micturition over three weeks. This study considered a large number of phenols, including bisphenol S and others for which the literature is rather sparse. Also, contrary to previous studies, our design allowed characterization of the temporal variability of phenol biomarkers over several time-windows during pregnancy, and in particular within the day and between the days of a week. From eight blinded samples analysed in duplicate, we observed very high (above 0.95) correlation between two analyses of the same urine sample for all biomarkers, making it very unlikely that the reported ICCs were strongly influenced by analytical error. A limitation relates to the fact that our estimate of the within-day variability relied on samples collected by only two women, contrary to the estimates of the between-day and between-week variability, which relied on 8 women. Volumes of urine voids were not collected, preventing us to calculate excretion rate. Caution is required in interpreting estimates for benzophenone-3, which was the compound with the lowest detection frequency in all samples (27 to 54%), and in interpreting the results related to the analyses of the weekly pools, given the large confidence intervals. Even though we restricted our study population to women with only a few missed voids (below 5%), pools were created using all available urine samples and these missing voids may be a source of error. Given that the present study was restricted to a specific population and specific chemicals, generalization of our results to other populations or other compounds should be considered with great caution.

5.6.2 Study population

Our study relied on a small population of women who agreed to collect repeated urine samples for several weeks. It was not meant to be representative of the general population or of all pregnant women from France or even the Grenoble area. Among those approached to participate, women with a high education level or interest in environmental or health issues were more likely to participate. Consequently, the behaviours (use of personal care products, diet, etc.) of our population are unlikely to represent those of all pregnant women. One might anticipate that this possibly led to an underestimation of the between-woman variability in urinary concentrations for some of the considered compounds, although over-representing women using few personal care products (as may be the case for some highly educated women aware of the health concerns regarding the use of healthcare products during pregnancy) or with a diet low in industrial phenols may also have led to underestimating the within-subject variability. Other factors such as physical activity, which might influence the toxicokinetics of xenobiotics,[27] may also have differed in our population. Most phenol urinary concentrations were lower than those reported in previous cohorts of pregnant women[14,21,28,30] and in 1,230 US non-pregnant women in 2011-2012.[8] In addition to differences in behaviours, composition of consumers' products, regulation of chemicals in each country, and analytical methods across laboratories performing the assays may also have differed across studies.

5.6.3 Variability over the course of pregnancy

Our analyses based on three random spot samples were meant to describe the ability of a simple sampling approach to capture the whole pregnancy exposure, and to allow comparison with previous studies, which relied on up to three spot samples per participant to assess biomarker concentration variability during pregnancy.[4,14,17,21,28,30,31,5,2] None of these studies investigated bisphenol S. The moderate ICCs (between 0.4 and 0.5) observed for 2,4-dichlorophenol, butyl and ethylparaben urinary concentrations were consistent with previous reports,[14,28,21,30] while we observed greater ICCs for 2,5-dichlorophenol and methylparaben (above 0.8 compared to 0.4-0.5 in these previous studies). Also, considering their rather large confidence intervals, ICCs for bisphenol A (0.4; 95% CI: 0.0, 0.8) and propylparaben (0.7; 95% CI: 0.4, 1.0) were within the range of previous studies (around 0.3 for bisphenol A and from 0.3-0.6 for propylparaben), although at the upper end of the range. In contrast, ICCs for benzophenone-3 (ICC, 0.3; 95% CI, 0.0 to 0.8) and triclosan (ICC, 0.1, 95% CI, 0.0 to 0.6) were at the lower end of the range of previously reported results, which were between 0.3 and 0.6.[2,21,28,31] Detection rates in our population were low (below 55%) for these two compounds, that

might have decreased ICCs compared to studies with higher detection rates, due to more homogeneity between women, and hence, a proportionally larger within-subject variability.

Our study is, to the best of our knowledge, the first to rely on within-subject weekly pools instead of random spot samples to describe the variability of select phenols during pregnancy. Compared to the results based on random spot samples from our study, ICCs based on weekly pools tended to be higher but the overall conclusion was similar to that with the three-sample approach: variability was contrasted between compounds; it was low for seven of the 10 phenols (ICCs above 0.59), and high (ICCs below 0.4), for the other compounds (ethylparaben, bisphenol S and triclosan).

5.6.4 Between-day (within-week) variability

For most biomarkers, the between-day variability of the urinary concentrations over a week was low (ICCs above 0.7 for eight of the 10 phenols, and ICC of 0.6 for bisphenol A). For bisphenol S, between-day variability was high (ICC, 0.14; 95% CI, 0.0 to 0.39). To the best of our knowledge, no study had relied on within-subject daily pools to investigate the variability of phenol urinary concentrations over several consecutive days. Two previous studies had relied on 24-hour simulated urine concentrations (volume-weighted averages of all daily urine voids) in a non-pregnant population of eight males and females (who collected all their complete urine voids and recorded urine volumes) to characterize the between-day variability of bisphenol A over a week[34] and of several phenols over four consecutive days.[18] We observed a somewhat lower variability of bisphenol A urinary concentration in daily pools (ICC, 0.6; 95% CI, 0.3 to 0.89) compared to these previous studies in non-pregnant subjects (ICCs between 0.12 and 0.28), while for ethyl, methyl and propylparabens, triclosan and benzophenone-3, ICCs (0.73 – 0.98) were in close agreement with those reported by Koch et al.[18] (between 0.71 and 0.99). These findings suggest a good reproducibility of daily averages of urinary concentrations over a week for most target phenols but the bisphenols.

5.6.5 Within-day variability

Within-day variability was high for all phenol biomarkers (ICCs $\leq$ 0.50), showing that a random spot sample collected within a day does not accurately represent the daily average. For bisphenol A (ICC: 0.21, 95% CI: 0.01, 0.41), this result is in line with findings from Ye et al.[34] who measured urinary bisphenol A concentration in all spot urine samples collected in one week from eight non-pregnant participants (ICCs, 0.12 – 0.21 for the within-day variability). This is also in agreement with a reported low reproducibility of bisphenol A concentrations in urine samples from one day (ICC,

0.31 – 0.33) in a study in pregnant women who provided all their urine voids during one or two days (66 women) as well as spot samples at different time points during and after pregnancy.[13] The high within-day variations in phenol urinary concentrations are probably related to the (expectedly) very low half-life of phenols in pregnant women, as can be deduced from studies in human adults,[16,29,32] and to exposure being episodic, with the main suspected exposure sources being diet (for bisphenols in food containers, for parabens used as preservatives in some industrial food preparation) and personal care products (for parabens, triclosan, benzophenone-3). The fact that the within-day variability was higher than the between-day variability for most compounds could be due to the behaviours driving exposure tending to be similar from one day to another.

5.6.6 Urine dilution and within-subject pooling

Creatinine and specific gravity are commonly used to adjust for urine dilution in studies relying on urinary biomarkers.[1,3] The two markers were strongly correlated (coefficient of correlation, 0.86 - 0.92) in spot samples, daily and weekly pools. As previously reported for creatinine, creatinine concentration and specific gravity had high within-subject variations throughout one week.[3] In our study, correcting phenol concentrations by either creatinine or specific gravity did not greatly improve ICCs, suggesting that these parameters do not explain much of the biomarkers' variability. Because the total urine volumes were unknown, we created within-subject daily pools using an equal volume of each spot sample collected each day and results might have differed if pooling volumes had been based on specific gravity or creatinine concentration. Creatinine is a body waste product primarily excreted by glomerular filtration.[1,3,10] Excretion profiles of phenols are not well characterized in humans and specifically in pregnant women. However, the low correlation between creatinine or specific gravity and most of our exposure biomarkers may reflect that urinary excretion processes for creatinine and these phenols might differ, and that the dilution of urine samples may not affect substantially biomarker concentrations. Also, in some areas of research, as a replacement for creatinine standardization, correction for urine dilution relies on parameters other than creatinine or specific gravity. For example, the Integral Quotient Normalization approach in metabolomics relies on adjustment by the median value of all biomarkers.[20]

5.6.7 Implications for sampling strategies in etiological studies

We confirmed that studies aiming at characterizing the health effects of pregnancy exposure to compounds with a high within-subject variability such as most of those

considered here should generally collect several biospecimens per subject to reduce exposure misclassification.[26] More importantly, we report for the first time period-specific ICCs for select phenols, which can be used to refine the urine sampling scheme in epidemiologic studies aiming at characterizing the health effects of such exposures. As shown in Table 5.4, for some compounds (e.g. 2,5-dichlorophenol, several parabens), if a good estimate of the exposure averaged over a specific pregnancy week is available (e.g., through collection of a sample of all urine voids over this week), then this can conveniently be used as an estimate of the average exposure over all pregnancy weeks. For triclosan and bisphenol S, for which the between-week ICCs are below 0.3, assessing exposure during a small number of weeks is unlikely to provide a reasonable estimate of the whole pregnancy exposure average. For these compounds, focusing on a few specific weeks of pregnancy may be inefficient, and one may rather consider collecting random samples during pregnancy to estimate the pregnancy average. Relying on the simulation by Perrier et al.[26] and on the ICCs estimated in the current study, for compounds such as triclosan and bisphenol S, two to three dozen urine samples would be required, while for compounds such as 2,4-dichlorophenol, with an ICC close to 0.6 for the pregnancy window, pooling approximately five urine samples would allow to strongly limit bias in the dose-response function. Relying on a spot urine sample, is, under the assumption of classical-type error, likely to induce an attenuation bias by 30% (for propylparaben) to 50% or more in the dose-response function if a single spot sample is used in etiological studies.[26] It is only for 2,5-dichlorophenol and methylparaben, for which ICCs based on three random spot samples during pregnancy (Table 5.5) were equal to 0.85, that using one or two spot urine samples collected randomly during pregnancy may provide a reasonable estimate of the whole pregnancy exposure average. If one is interested in an exposure window of a length of a week (for example the week when a specific foetal organ starts developing or at the end of which some biological parameter is assessed in the mother), then for dichlorophenols, triclosan and parabens, assessing exposure during a single day of the week should do the job, while for benzophenone-3, bisphenols A and S, it is safer to assess exposure during several days of the week. If one is now interested in assessing exposures over a specific day, collecting one spot sample is likely not enough for all of the studied phenols, since the within-day ICCs were all below 0.5.

For biomonitoring (and not etiological studies), there is no issue related to bias in dose-response functions, and collecting a spot biospecimen might be a good option if the population is large enough; collecting a random sample rather than the first morning void is likely to provide a much better estimate of the population average.

5.7 Conclusion

Biospecimens sampling strategy for accurate exposure assessment is a key issue in epidemiological studies based on short half-lived chemicals such as phenols. Our findings confirm that exposure misclassification may be high when collecting a small number of random spot samples. Future etiological studies should adopt a carefully-thought design for the biospecimen sampling instead of using the default option of a single biospecimen per subject. Our results suggest that collecting more than one biospecimen per day for preferably several days during pregnancy is likely to allow reduction in exposure misclassification.

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5.9 Supplemental material

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Table 5.6 – Spearman correlation matrix between urinary biomarkers. Each cell outside the diagonal contains the correlations in spot samples (subgroup 1, 2 women, n=114 samples), daily pools (8women, n=56 samples) and weekly pools (8 women, n=24 samples), reported in this order.

Note: The diagonal (highlighted cells and bold font) shows the spearman correlation coefficients for a given biomarker between (*) spot samples and associated daily pools (subgroup 1, 2 women, 114 samples), (§) spot samples and the associated weekly pool (subgroup 1, 2 women, 114 samples) and between (†) daily pools and the associated weekly pool (8 women, 56 samples).

Biomarker	2,4-DCP	2,5-DCP	BP	EP	MP	PP	BP3	BPA	BPS	TCS	Creatinine	Specific gravity
2,4-dichlorophenol	**0.36 ***											
	0.20 §											
	0.63 †											
2,5-dichlorophenol	0.67	**0.68 ***										
	0.76	**0.74 §**										
	0.47	**0.94 †**										
Butyl paraben	0.08	-0.38	**0.71 ***									
	0.13	0.01	**0.74 §**									
	0.39	0.18	**0.63 †**									
Ethyl paraben	0.11	-0.4	0.94	**0.69 ***								
	0.29	0.19	0.55	**0.8 §**								
	0.28	-0.02	0.5	**0.91 †**								
Methyl paraben	0.1	-0.36	0.58	0.62	**0.70 ***							
	-0.08	-0.39	0.62	0.57	**0.62 §**							
	0.42	-0.25	0.48	0.58	**0.87 †**							
Propyl paraben	-0.08	-0.51	0.57	0.64	0.8	**0.75 ***						
	-0.27	-0.6	0.5	0.08	0.69	**0.68 §**						
	0.3	-0.26	0.54	0.44	0.81	**0.94 †**						
Benzophenone-3	0.04	-0.12	0.19	0.24	0.03	0.09	**0.11 ***					
	-0.03	0.32	-0.31	0.02	-0.33	-0.54	**0 §**					
	-0.29	0.03	-0.09	-0.13	-0.34	-0.4	**0.79 †**					
Bisphenol A	0.5	0.1	0.24	0.33	0.28	0.29	0.15	**0.32 ***				
	0.36	0.24	0.19	0.2	-0.07	-0.19	-0.13	**0.29 §**				
	0.29	0.22	0.26	0.03	0.14	0	0.05	**0.72 †**				
Bisphenol S	0.42	0.36	0.16	0.19	0.09	0.04	0.04	0.23	**0.46 ***			
	-0.01	-0.05	0.18	0.29	0.32	0.04	-0.03	0.08	**0.01 §**			
	0.09	-0.28	0.33	0.05	0.43	0.24	0.28	0.17	**0.38 †**			
Triclosan	0.53	0.49	0.14	0.15	0.13	-0.02	-0.06	0.26	0.31	**0.43 ***		
	-0.4	-0.5	-0.01	-0.14	0.37	0.49	0.1	-0.46	0.15	**0.09 §**		
	0.23	-0.08	-0.02	0.1	0.27	0.42	-0.41	-0.53	-0.18	**0.82 †**		
Creatinine	0.83	0.67	0.14	0.12	0.15	-0.06	0.05	0.44	0.43	0.59	**0.32 ***	
	0.46	0.34	0.51	0.19	0.2	0.01	-0.19	0.61	0.22	-0.28	**0.15 §**	
	0.56	0.07	0.35	0.18	0.55	0.36	-0.01	0.55	0.28	-0.15	**0.74 †**	
Specific gravity	0.79	0.67	-0.01	-0.02	0.03	-0.12	0.01	0.44	0.32	0.48	0.92	**0.37 ***
	0.27	0.18	0.62	0.23	0.31	0.1	-0.19	0.49	0.3	-0.11	0.9	**0.23 §**
	0.36	0	0.11	0.02	0.34	0.13	0.07	0.48	0.13	-0.14	0.86	**0.70 †**

2,4-DCP = 2,4-dichlorophenol; 2,5-DCP = 2,5-dichlorophenol; BP = butylparaben; EP = ethylparaben; MP = methylparaben; PP = propylparaben; BP3 = benzophenone-3; BPA = bisphenol A; BPS = bisphenol S; TCS = triclosan.

Table 5.7 – Creatinine and specific gravity corrected ICCs – Within-day variability (ICC_1).

Note that ICC_1 is based on log_{10}-transformed creatinine-corrected (ICC_{creat}) and specific gravity-corrected (ICC_{SG}) phenol biomarker concentrations in the unpooled spot samples from subgroup 1 (2 women, n=114 spot samples collected over the first week of collection). Uncorrected ICCs were also reported in bold font to allow for comparison.

		Within-day variability, ICC_1 (based on unpooled spot samples)	
Biomarker	**$ICC_{uncorrected}$**	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]
Phenols[a]			
2,4-dichlorophenol	**0.12 (0.00, 0.28)**	0.05 (0.00, 0.18)	0.12 (0.00, 0.28)
2,5-dichlorophenol	**0.11 (0.00, 0.27)**	0.04 (0.00, 0.16)	0.15 (0.00, 0.33)
Butyl paraben	**0.10 (0.00, 0.25)**	0.08 (0.00, 0.23)	0.11 (0.00, 0.27)
Ethyl paraben	**0.03 (0.00, 0.15)**	0.05 (0.00, 0.18)	0.06 (0.00, 0.20)
Methyl paraben	**0.27 (0.05, 0.49)**	0.31 (0.08, 0.53)	0.31 (0.08, 0.54)
Propyl paraben	**0.28 (0.05, 0.50)**	0.26 (0.04, 0.48)	0.27 (0.05, 0.49)
Benzophenone-3	**0.26 (0.04, 0.48)**	0.31 (0.08, 0.54)	0.27 (0.05, 0.49)
Bisphenol A	**0.21 (0.01, 0.41)**	0.26 (0.04, 0.47)	0.25 (0.03, 0.46)
Bisphenol S	**0.50 (0.26, 0.73)**	0.49 (0.26, 0.73)	0.55 (0.32, 0.78)
Triclosan	**0.30 (0.08, 0.53)**	0.37 (0.14, 0.61)	0.36 (0.12, 0.59)

ICC: intraclass correlation coefficient

[a] Concentrations <LOD were replaced by instrumental reading values. Instrumental reading values equal to 0 replaced by the non-zero lowest machine value divided by square root of 2.

[b] ICCs were estimated from ANOVA model with a random effect on day (14 days), adjusted for creatinine and within-woman mean-centering of the data.

[c] ICCs were estimated from ANOVA model with a random effect on day (14 days), adjusted for specific gravity and within-woman mean-centering of the data.

Table 5.8 – Random intercept linear mixed models analyses. Within-day (ICC_1), between-day (ICC_2), between-week variability (ICC_3) and alternative estimate of between-week variability based on 3 random spot samples (ICC_4).

Note that ICC_1, ICC_2, ICC_3 and ICC_4 are respectively based on log_{10}-transformed phenol biomarker concentrations, creatinine concentration and specific gravity in the unpooled spot samples from subgroup 1 (2 women, n=114 spot samples collected over the first week of collection, ICC_1), in the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection, ICC_2), the within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection, ICC_3), the random spot samples from subgroup 1 and subgroup 2 (8 women, n=24 random spot sample, one sample in each of the 3 weeks of collection, ICC_4). Random intercept linear mixed models (maximum likelihood estimates).

	Within-day variability (based on unpooled spot samples)	Between-day variability (based on daily pools)	Between-week variability (based on weekly pools)	Alternative between-week variability (based on three random spot samples)
Biomarker	ICC_1 (95% CI)[b]	ICC_2 (95% CI)[c]	ICC_3 (95% CI)[c]	ICC_4 (95% CI)[c]
Phenols[a]				
2,4-dichlorophenol	0.11 (0.03, 0.35)	0.90 (0.75, 0.96)	0.61 (0.27, 0.87)	0.45 (0.13, 0.82)
2,5-dichlorophenol	0.10 (0.02, 0.33)	0.97 (0.93, 0.99)	0.93 (0.78, 0.98)	0.84 (0.59, 0.95)
Butyl paraben	0.08 (0.01, 0.42)	0.78 (0.53, 0.91)	0.82 (0.57, 0.94)	0.38 (0.09, 0.80)
Ethyl paraben	NA[d]	0.83 (0.62, 0.94)	0.28 (0.04, 0.78)	0.35 (0.07, 0.79)
Methyl paraben	0.23 (0.09, 0.45)	0.83 (0.61, 0.93)	0.78 (0.50, 0.93)	0.83 (0.57, 0.95)
Propyl paraben	0.27 (0.11, 0.52)	0.89 (0.73, 0.96)	0.84 (0.60, 0.95)	0.67 (0.33, 0.89)
Benzophenone-3	0.23 (0.09, 0.47)	0.70 (0.43, 0.88)	0.56 (0.22, 0.85)	0.23 (0.02, 0.79)
Bisphenol A	0.20 (0.07, 0.45)	0.56 (0.29, 0.81)	0.55 (0.21, 0.85)	0.33 (0.06, 0.79)
Bisphenol S	0.52 (0.30, 0.73)	0.11 (0.01, 0.55)	0.21 (0.02, 0.80)	0.28 (0.04, 0.78)
Triclosan	0.28 (0.12, 0.52)	0.88 (0.71, 0.95)	NA[d]	0.05 (0.00, 1.00)[e]
Urine dilution markers				
Creatinine	0.10 (0.02, 0.32)	0.58 (0.30, 0.82)	0.83 (0.58, 0.95)	0.52 (0.18, 0.84)
Specific gravity	0.03 (0.00, 0.40)	0.57 (0.29, 0.81)	0.45 (0.13, 0.82)	0.66 (0.32, 0.89)

ICC: intraclass correlation coefficient, NA: not applicable.
[a] Concentrations <LOD were replaced by instrumental reading values. Instrumental reading values equal to 0 replaced by the lowest non-zero machine value divided by square root of 2.
[b] ICCs were estimated from random intercept linear mixed model with a random effect on day (14 days) and within-woman mean-centering of the data.
[c] ICCs were estimated from random intercept linear mixed model with a random effect on woman.
[d] No estimate and confidence interval given by the model due to estimates equal to zero.
[e] One sample with extreme value excluded (n=23 samples) for the analysis of triclosan.

Table 5.9 – Random intercept linear mixed models analyses adjusted for creatinine (ICC_{creat}) or specific gravity (ICC_{SG}) – Within-day variability (ICC_1).

Note that ICC_1 is based on log_{10}-transformed phenol biomarker concentrations in the unpooled spot samples from subgroup 1 (2 women, n=114 spot samples collected over the first week of collection). Models adjusted for creatinine (ICC_{creat}) or specific gravity (ICC_{SG}, maximum likelihood estimates).

	Within-day variability, ICC_1 (based on unpooled spot samples)	
Biomarker	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]
Phenols[a]		
2,4-dichlorophenol	0.03 (0.00, 0.81)	0.16 (0.05, 0.42)
2,5-dichlorophenol	0.11 (0.03, 0.37)	0.20 (0.07, 0.45)
Butyl paraben	0.06 (0.00, 0.50)	0.08 (0.01, 0.43)
Ethyl paraben	NA[d]	0.03 (0.00, 0.89)
Methyl paraben	0.24 (0.10, 0.47)	0.25 (0.11, 0.48)
Propyl paraben	0.27 (0.11, 0.52)	0.26 (0.11, 0.52)
Benzophenone-3	0.28 (0.12, 0.52)	0.25 (0.10, 0.49)
Bisphenol A	0.24 (0.09, 0.50)	0.23 (0.09, 0.49)
Bisphenol S	0.59 (0.38, 0.78)	0.59 (0.37, 0.78)
Triclosan	0.34 (0.16, 0.58)	0.34 (0.16, 0.57)

ICC: intraclass correlation coefficient

[a] Concentrations <LOD were replaced by instrumental reading values. Instrumental reading values equal to 0 replaced by the lowest non-zero machine value divided by square root of 2.

[b] ICCs were estimated from random intercept linear mixed model with a random effect on day (14 days), adjusted for creatinine and within-woman mean-centering of the data.

[c] ICCs were estimated from random intercept linear mixed model with a random effect on day (14 days), adjusted for specific gravity and within-woman mean-centering of the data.

[d] No estimate and confidence interval given by the model due to estimates equal to zero.

Table 5.10 – Creatinine and specific gravity corrected ICCs – Between-day (ICC_2), between-week variability (ICC_3) and alternative estimate of between-week variability based on 3 random spot samples (ICC_4).

Note that ICC_2, ICC_3 and ICC_4 are respectively based on log_{10}-transformed creatinine-corrected (ICC_{creat}) and specific gravity-corrected (ICC_{SG}) phenol biomarker concentrations in the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection, ICC_2), the within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection, ICC_3), the random spot samples from subgroup 1 and subgroup 2 (8 women, n=24 random spot samples, one sample in each of the 3 weeks of collection, ICC_4). Models with concentrations corrected for creatinine (ICCcreat) or specific gravity (ICC_{SG}). Uncorrected ICCs were also reported in bold font to allow for comparison..

	Between-day variability, ICC_2 (based on daily pools)			Between-week variability, ICC_3 (based on weekly pools)			Alternative between-week variability, ICC_4 (based on three random spot samples)		
Biomarker	**$ICC_{uncorrected}$**	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]	**$ICC_{uncorrected}$**	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]	**$ICC_{uncorrected}$**	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]
Phenols[a]									
2,4-dichlorophenol	**0.91 (0.82, 1.00)**	0.94 (0.87, 1.00)	0.94 (0.87, 1.00)	**0.65 (0.32, 0.99)**	0.55 (0.16, 0.95)	0.58 (0.21, 0.96)	**0.50 (0.08, 0.92)**	0.44 (0.00, 0.88)	0.52 (0.11, 0.93)
2,5-dichlorophenol	**0.98 (0.95, 1.00)**	0.98 (0.95, 1.00)	0.98 (0.97, 1.00)	**0.93 (0.86, 1.00)**	0.93 (0.85, 1.00)	0.93 (0.83, 1.00)	**0.85 (0.69, 1.00)**	0.83 (0.63, 1.00)	0.89 (0.76, 1.00)
Butyl paraben	**0.80 (0.61, 0.99)**	0.80 (0.62, 0.99)	0.79 (0.59, 0.98)	**0.84 (0.67, 1.00)**	0.77 (0.53, 1.00)	0.79 (0.56, 1.00)	**0.42 (0.00, 0.87)**	0.33 (0.00, 0.80)	0.29 (0.00, 0.76)
Ethyl paraben	**0.85 (0.70, 1.00)**	0.87 (0.73, 1.00)	0.84 (0.69, 1.00)	**0.33 (0.00, 0.79)**	0.30 (0.00, 0.76)	0.37 (0.00, 0.83)	**0.40 (0.00, 0.85)**	0.40 (0.00, 0.85)	0.34 (0.00, 0.80)
Methyl paraben	**0.84 (0.69, 1.00)**	0.89 (0.78, 1.00)	0.85 (0.70, 1.00)	**0.81 (0.60, 1.00)**	0.77 (0.53, 1.00)	0.77 (0.52, 1.00)	**0.85 (0.68, 1.00)**	0.78 (0.55, 1.00)	0.78 (0.54, 1.00)
Propyl paraben	**0.90 (0.80, 1.00)**	0.91 (0.82, 1.00)	0.90 (0.79, 1.00)	**0.86 (0.70, 1.00)**	0.85 (0.68, 1.00)	0.84 (0.67, 1.00)	**0.70 (0.40, 1.00)**	0.60 (0.24, 0.97)	0.63 (0.28, 0.98)
Benzophenone-3	**0.73 (0.50, 0.96)**	0.75 (0.53, 0.97)	0.75 (0.53, 0.97)	**0.60 (0.23, 0.97)**	0.62 (0.26, 0.98)	0.60 (0.23, 0.97)	**0.28 (0.00, 0.75)**	0.38 (0.00, 0.84)	0.31 (0.00, 0.78)
Bisphenol A	**0.60 (0.30, 0.89)**	0.45 (0.12, 0.78)	0.47 (0.15, 0.79)	**0.59 (0.22, 0.97)**	0.46 (0.03, 0.90)	0.36 (0.00, 0.82)	**0.38 (0.00, 0.83)**	(0.00, 0.42) [d]	(0.00, 0.42) [d]
Bisphenol S	**0.14 (0.00, 0.39)**	0.20 (0.00, 0.48)	0.09 (0.00, 0.31)	**0.26 (0.00, 0.73)**	0.17 (0.00, 0.64)	0.12 (0.00, 0.57)	**0.33 (0.00, 0.80)**	0.14 (0.00, 0.60)	0.20 (0.00, 0.68)
Triclosan	**0.89 (0.78, 1.00)**	0.92 (0.84, 1.00)	0.92 (0.83, 1.00)	**(0.00, 0.44)** [d]	0.01 (0.00, 0.43)	0.05 (0.00, 0.48)	**0.11 (0.00, 0.58)**	0.10 (0.00, 0.55)	0.03 (0.00, 0.46)

ICC: intraclass correlation coefficient.
[a] Concentrations <LOD were replaced by instrumental reading values. Instrumental reading values equal to 0 replaced by the lowest non-zero machine value divided by square root of 2.
[b] ICCs were estimated from ANOVA model with a random effect on woman and adjusted for creatinine concentration.
[c] ICCs were estimated from ANOVA model with a random effect on woman and adjusted for specific gravity.
[d] We give only the confidence interval truncated to zero due to negative estimate of ICC.

Table 5.11 – Random intercept linear mixed models analyses adjusted for creatinine (ICC_{creat}) or specific gravity (ICC_{SG}) – Between-day (ICC_2), between-week variability (ICC_3) and alternative estimate of between-week variability based on 3 random spot samples (ICC_4).

Note that ICC_2, ICC_3 and ICC_4 are respectively based on log_{10}-transformed phenol biomarker concentrations in the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection, ICC_2), the within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection, ICC_3), the random spot samples from subgroup 1 and subgroup 2 (8 women, n=24 random spot sample, one sample in each of the 3 weeks of collection, ICC_4). Models adjusted for creatinine (ICC_{creat}) or specific gravity (ICC_{SG}, maximum likelihood estimates).

	Between-day variability, ICC_2 (based on daily pools)		Between-week variability, ICC_3 (based on weekly pools)		Alternative between-week variability, ICC_4 (based on three random spot samples)	
Biomarker	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]	ICC_{creat} (95% CI)[b]	ICC_{SG} (95% CI)[c]
Phenols[a]						
2,4-dichlorophenol	0.93 (0.81, 0.97)	0.92 (0.81, 0.97)	0.47 (0.15, 0.83)	0.59 (0.24, 0.87)	0.47 (0.15, 0.82)	0.48 (0.15, 0.83)
2,5-dichlorophenol	0.98 (0.94, 0.99)	0.98 (0.95, 0.99)	0.92 (0.77, 0.98)	0.93 (0.79, 0.98)	0.83 (0.58, 0.95)	0.90 (0.72, 0.97)
Butyl paraben	0.78 (0.54, 0.92)	0.76 (0.52, 0.91)	0.94 (0.80, 0.99)	0.86 (0.63, 0.96)	0.34 (0.06, 0.79)	0.25 (0.02, 0.83)
Ethyl paraben	0.85 (0.66, 0.94)	0.84 (0.64, 0.94)	0.23 (0.02, 0.79)	0.31 (0.05, 0.79)	0.39 (0.09, 0.80)	0.29 (0.04, 0.79)
Methyl paraben	0.88 (0.72, 0.96)	0.84 (0.63, 0.94)	0.71 (0.35, 0.92)	0.79 (0.50, 0.94)	0.82 (0.54, 0.94)	0.77 (0.43, 0.93)
Propyl paraben	0.90 (0.75, 0.96)	0.89 (0.73, 0.96)	0.82 (0.54, 0.94)	0.85 (0.62, 0.95)	0.67 (0.32, 0.89)	0.63 (0.27, 0.89)
Benzophenone-3	0.71 (0.43, 0.88)	0.70 (0.43, 0.88)	0.62 (0.26, 0.89)	0.56 (0.22, 0.85)	0.30 (0.04, 0.81)	0.23 (0.02, 0.79)
Bisphenol A	0.44 (0.18, 0.73)	0.50 (0.23, 0.77)	0.45 (0.13, 0.81)	0.50 (0.15, 0.85)	NA[d]	NA[d]
Bisphenol S	0.11 (0.01, 0.55)	0.07 (0.00, 0.62)	0.12 (0.00, 0.89)	0.25 (0.02, 0.87)	0.12 (0.00, 0.92)	0.21 (0.02, 0.80)
Triclosan	0.90 (0.74, 0.96)	0.90 (0.75, 0.96)	NA[d]	NA[d]	0.23 (0.02, 0.85)[e]	0.07 (0.00, 0.99)[e]

ICC: intraclass correlation coefficient, NA: not applicable.

[a] Concentrations <LOD were replaced by instrumental reading values. Instrumental reading values equal to 0 replaced by the lowest non-zero machine value divided by square root of 2.

[a] ICCs were estimated from random intercept linear mixed model with a random effect on woman and adjusted for creatinine concentration.

[b] ICCs were estimated from random intercept linear mixed model with a random effect on woman and adjusted for specific gravity.

[d] No estimate and confidence interval given by the model due to estimates equal to zero.

[e] One sample with extreme value excluded (n=23 samples) for the analysis of triclosan.

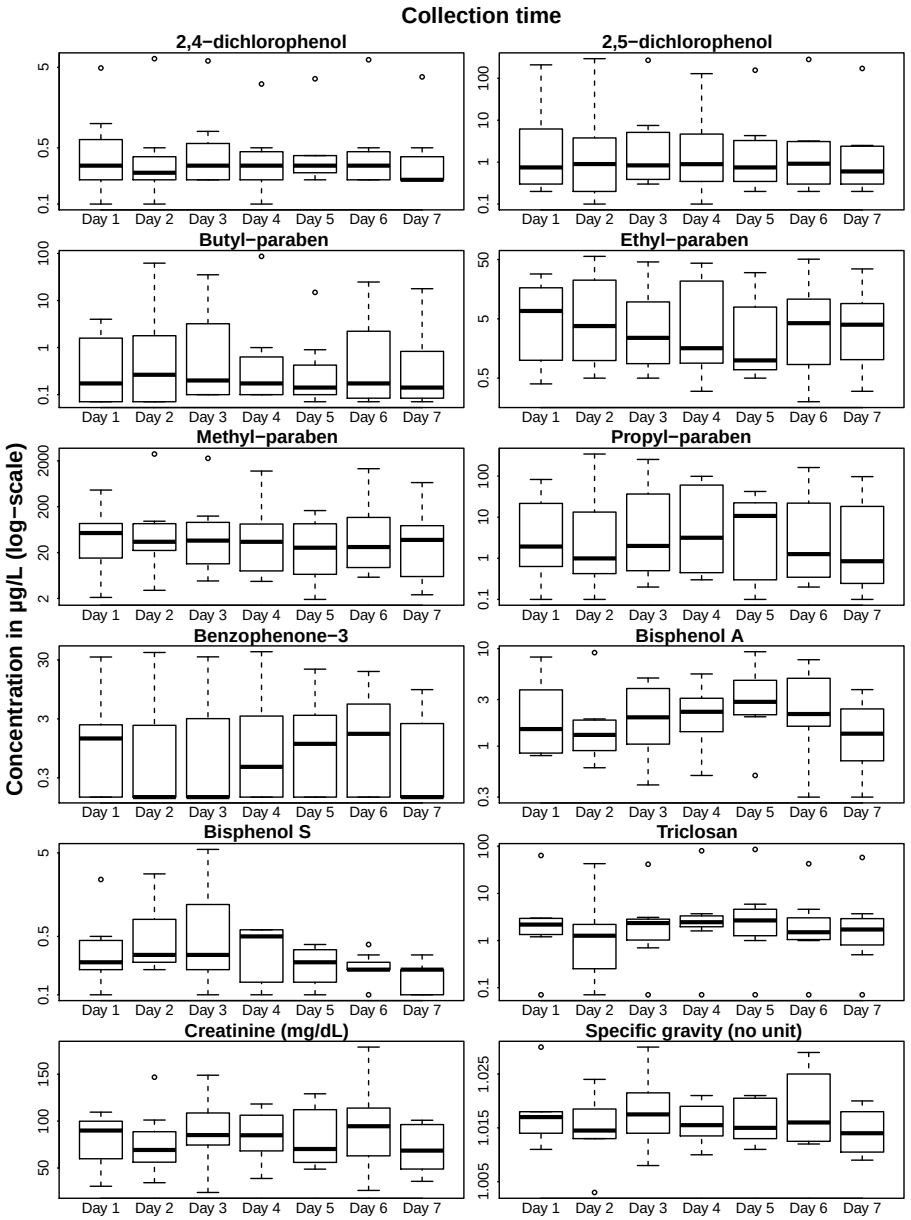

Figure 5.6 – Box plots of the distribution of urinary concentrations of 10 phenols (μg/L, $\log_{10}$-scale), creatinine concentration (mg/dL) and specific gravity in the within woman daily-pooled samples from subgroup 1 and subgroup 2 (8 women, n=56 daily pools, one daily pool for each day of the first week of collection). Boxes extend from the 25^{th} to the 75^{th} percentile, horizontal bars represent the median, whiskers extend 1.5 times the length of the interquartile range (IQR) above and below the 75^{th} and 25^{th} percentiles, respectively, and outliers are represented as points.

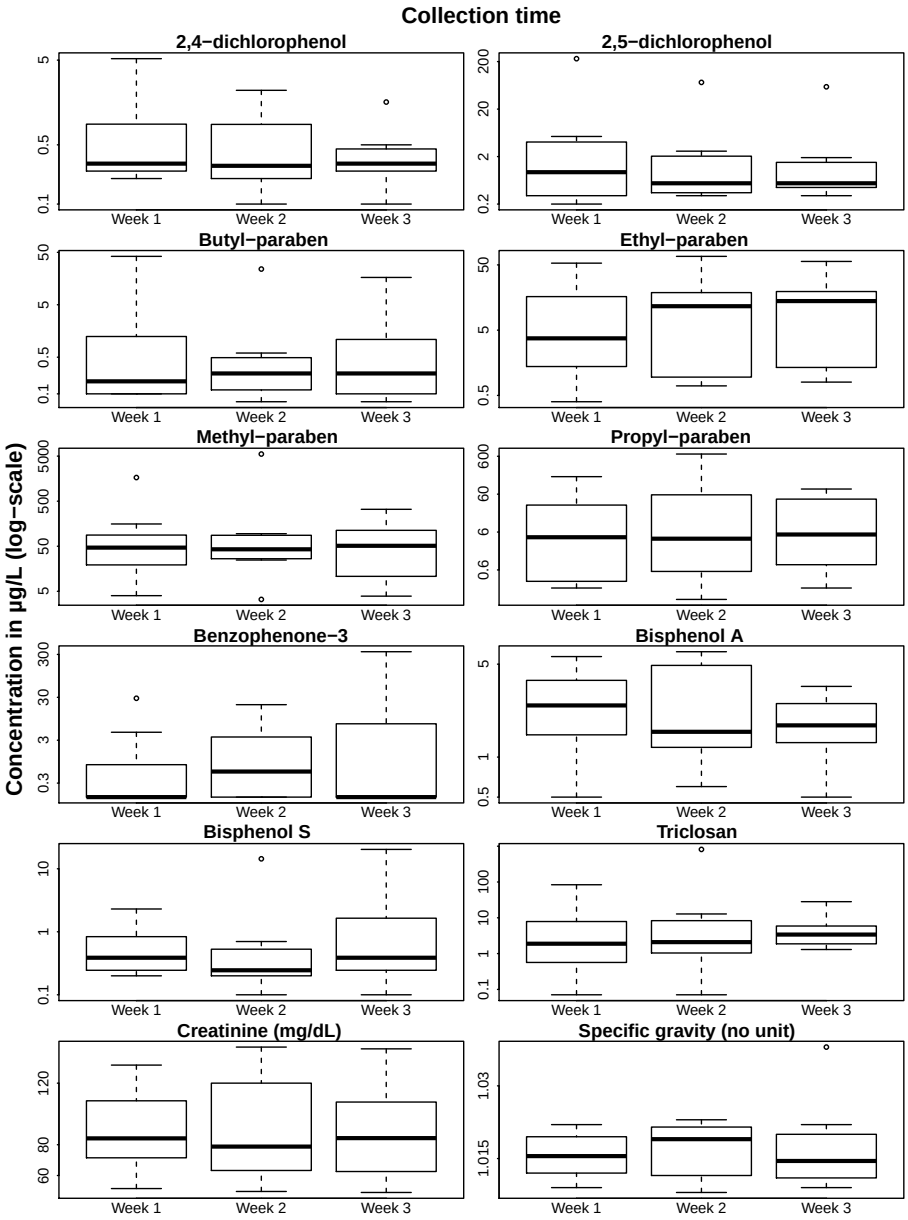

Figure 5.7 – Box plots of the distribution of urinary concentrations of 10 phenols (μg/L, $\log_{10}$-scale), creatinine concentration (mg/dL) and specific gravity in within woman weekly-pooled samples from subgroup 1 and subgroup 2 (8 women, n=24 weekly pools, one weekly pool for each of the 3 weeks of collection). Boxes extend from the 25^{th} to the 75^{th} percentile, horizontal bars represent the median, whiskers extend 1.5 times the length of the interquartile range (IQR) above and below the 75^{th} and 25^{th} percentiles, respectively, and outliers are represented as points.

Chapter 6

An empirical validation of the biospecimens within-subject pooling approach

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2,4-dichlorophenol; 2,5-dichlorophenol; butylparaben; ethylparaben; methylparaben; propylparaben; bisphenol A; bisphenol S; benzophenone-3; triclosan; phenols; urine; biomarkers; human; within-subject pooling; pregnancy; endocrine disruptors; exposure; epidemiology.

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The findings expressed in this article are the opinions of the authors and do not necessarily reflect the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

Conflicts of interest: none

6.1 French summary

Contexte

Dans l'étude précédente, nous avons observé une forte variabilité journalière des phénols au cours de la grossesse ce qui limite l'efficacité d'une mesure des concentrations des biomarqueurs dans un échantillon unique pour estimer l'exposition. Par ailleurs, un travail de Perrier et al.[26] démontre théoriquement que le pooling intra-sujet de biospécimens peut réduire le biais dans les études explorant les relations doses-réponses, lorsque l'erreur de mesure est de type classique. L'erreur de type classique correspond à une situation où la concentration du biomarqueur dans un échantillon varie aléatoirement autour de l'exposition réelle, non mesurée. Ainsi, cette exposition réelle peut être approximée par la moyenne des concentrations mesurées dans de nombreux échantillons. La validité d'une telle approche n'a jamais été validée empiriquement, et le recueil journalier de nombreux biospécimens est très fastidieux pour les participants d'une étude de cohorte.

Objectif

L'objectif principal de cette étude est de valider empiriquement l'intérêt du pooling de biospécimens intra-sujet, 1) en proposant d'évaluer l'efficacité d'une méthode de pooling intra-sujet allégée (uniquement 3 biospécimens par jour) par rapport à l'approche "idéale", qui consiste à prendre tous les biospécimens pour estimer l'exposition moyenne d'un jour, d'une semaine ou de la grossesse; et 2) en estimant le biais retrouvé dans les estimations des relations doses-réponses, lorsque l'exposition est estimée en recueillant un ou plusieurs biospécimens.

Méthodes

Notre étude se base sur les échantillons d'urine de 16 femmes enceintes de l'étude de faisabilité de la cohorte SEPAGES, qui ont recueilli toutes leurs urines pendant plusieurs semaines. Dans cette étude, nous comparons les concentrations de biomarqueurs urinaires de 10 phénols mesurées dans les pools "jour", "semaine", et "grossesse", réalisés en mélangeant, chez chaque participante, soit 1) tous les échantillons d'urine de la journée, soit 2) seulement 3 échantillons.

Nous avons ensuite réalisé une étude de simulation basée sur des données réelles d'exposition chez les mêmes femmes. Cette simulation repose sur les données d'exposition de deux phénols : le méthylparabène avec une variabilité intra-individuelle plutôt faible, et le bisphénol A, avec une variabilité intra-individuelle forte. Le biais dans les relations doses-réponses est estimé pour une mesure d'exposition qui repose sur la concentration du biomarqueur mesurée dans un et jusqu'à 20 biospécimens.

Résultats

Les estimations d'exposition moyenne de la journée, de la semaine et de la grossesse, obtenues par la méthode pooling intra-sujet allégée ou la méthode de pooling intra-sujet idéale sont très corrélées (coefficient de corrélation de Pearson supérieurs à 0.8), excepté pour l'estimation des moyennes d'exposition journalières de benzophénone-3 et triclosan.

L'utilisation d'un biospécimen unique pour estimer l'exposition au méthylparabène entraine un biais d'atténuation de 30% dans les estimations des relations doses-réponses. Ce biais est encore plus important pour le bisphénol A (68%). L'utilisation d'au moins quatre et 18 biospécimens, respectivement pour le méthylparabène et le bisphénol A, est nécessaire pour passer ce biais sous le seuil de 10%.

Conclusion

En prenant l'exemple des phénols, nous observons que, pour des composés non-persistants, le recueil et le pooling de seulement 3 échantillons par jour chez chaque participant est efficace pour estimer l'exposition au cours de fenêtres de temps allant de la journée à la grossesse. Le recueil de plusieurs dizaines de biospécimens est parfois nécessaire pour limiter de manière suffisante le biais d'atténuation pour les composés les plus variables (par exemple le bisphénol A), ce qui démontre un peu plus l'utilité d'une telle approche.

6.2 Abstract

Background

Within-subject biospecimens pooling was shown to be theoretically efficient in reducing bias in dose-response functions in presence of classical-type error. Its validity was never tested empirically, and collecting all daily urine samples is cumbersome. We evaluated the validity of a within-subject pooling approach relying on the collection of a small number of voids every day.

Methods

In 16 pregnant women who collected their urines over several weeks, we compared biomarker concentrations of 10 phenols in daily, weekly and pregnancy within-subject pools obtained using either three or every daily urine samples. Then, a simulation study using real data from the same women allowed us to estimate the bias in dose-response functions when relying on one to 20 urine samples per subject to assess exposures to methylparaben (moderate within-subject variability) and bisphenol A (high variability).

Results

Regarding the estimation of daily, weekly and pregnancy exposures, correlations between pools of all or only three urine voids were above 0.8, except for benzophenone-3 and triclosan (daily time-window). Using one biospecimen resulted in an attenuation bias in the dose-response functions of 30% (methylparaben) and 68% (bisphenol A); four and 18 samples, respectively, were required to reduce bias under a 10% threshold.

Conclusion

For short half-lived compounds, collecting and pooling three instead of all daily voids allows efficient estimation of exposures over time windows of a week or more. Collecting a few dozen urine samples allows to strongly limit attenuation bias for highly temporally variable chemicals such as bisphenol A. This provides further (empirical) validation of the within-subject pooling approach.

6.3 Introduction

Phenols (phenolic chemicals) are used in consumer products, such as epoxy resins and polymer plastics for food and beverage containers (bisphenols); cosmetics and personal care products (triclosan and parabens); ultraviolet filters (benzophenone-3); pesticides (2,4-dichlorophenol) and room deodorizers (2,5-dichlorophenol).[14,12,11,22,16,19,23,24] This leads to widespread exposure in the general population of industrialized countries. Toxicological studies suggest harmful effects of compounds from this family on a variety of pathways and biological or clinical endpoints.[6,37] Investigating the impact of these compounds on human health requires an accurate estimation of exposure over the relevant time-window.[8,28] For chemicals with a high (within-subject) temporal variability, in the case of classical-type error, relying on few biospecimens leads to attenuation bias in the dose-response relationships.[28,7] Classical type measurement error corresponds to a situation in which spot biomarker concentrations vary around the unmeasured true value, which can be approximated by the mean of many measurements repeated throughout the time window of interest for an individual.[10] Within the additive classical-type error framework, simulation studies described a relationship between the variability of a given chemical (characterized through its intraclass coefficient of correlation, ICC) and the bias in the dose-response functions.[26,28,1] However, the measurement error structure may in practice not correspond to additive classical-type error, so that it is important to confirm the magnitude of the bias induced by actually observed within-subject variation structures in biomarker levels, in particular in sensitive populations such as pregnant women.

While increasing the size of the study population is not expected to cure such bias, improving the exposure assessment by increasing the number of biospecimens collected in each subject decreases the bias in the dose-response function.[26,28] One can assay biomarkers levels in each biospecimen and use measurement error models to efficiently limit bias,[13,33,10] but this approach increases analytical costs. An alternative consists in pooling biospecimens within subject before assaying chemicals. This approach benefits from the information contained in each subject, but without increasing analytical costs since only samples pooled within-subject are assayed. This so-called *within-subject biospecimens pooling* approach has been validated theoretically,[26] but an empirical validation is lacking.

The within-subject biospecimens pooling approach also raises practical issues. Indeed, such a sampling frame is inconvenient for large-scale epidemiological studies, in particular for long exposure windows such as the entire pregnancy. In designing future epidemiological studies, it would be valuable to test if less cumbersome approaches for participants and the survey team could be efficient in estimating exposures.

In this study, our aim was two-fold:

1. to compare a sampling design relying on collection of three urine samples per day with a richer but more cumbersome design consisting in collecting all urine voids in their efficiency to approximate the true exposure to short half-lived chemicals (here, phenols) over daily, weekly and whole pregnancy exposure windows;
2. to empirically investigate the effect of within-subject temporal variability in phenols biomarkers levels observed in actual populations on bias in dose-response functions.

6.4 Methods

6.4.1 Overview

Both aims relied on a population of pregnant women, recruited as part of SEPAGES (*Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé*; Assessment of air pollution exposure during pregnancy and effects on health) cohort feasibility study,[25] in which all urine samples have been collected for three weeks during the pregnancy. Phenols levels were assayed in these urine samples with or without pooling (Figure 6.1). From this unique dataset of phenols assays, urine samples have been pooled in different ways (Figure 6.1), which allowed us to assess the correlation between two daily, weekly and pregnancy exposure estimates: the first relying on pools made up from all daily urine samples, and the second on degraded pools made up from fewer samples collected for each subject (**aim 1**). From the same exposure data, we generated a fictitious study, paralleling a previous simulation study in which exposure levels were not based on real data.[26] We assumed phenols impacted a health outcome, and characterized the impact of relying on an increasing number of urine samples to assess exposure (**aim 2**).

6.4.2 Study population

This study relied on urinary biospecimens assayed for phenol biomarkers in the feasibility study of the SEPAGES cohort conducted between July 2012 and July 2013.[35] The cohort was approved by the appropriate ethical committees (CPP, Comité de Protection des Personnes Sud-Est; CNIL, Commission Nationale de l'Informatique et des Libertés; CCTIRS, Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé; ANSM, Agence Nationale de sécurité du Médicament et des produits de santé). All participants provided written informed consent for biological measurements and data collection.

6.4.3 Urine collection

The urine collection protocol has previously been detailed.[35] During three non-consecutive weeks in pregnancy (in median at 13, 23, and 32 gestational weeks), 30 women living in the Grenoble urban area (France) collected a spot sample of each urine void in polypropylene containers. Samples were kept in the participants' refrigerators before study staff retrieval (every two days). Each sample was aliquoted into polypropylene cryovials and frozen at -80°C at Inserm research center (Institute for Advanced Biosciences, Grenoble, France). Women were asked to record any missed

void on a paper questionnaire they carried with them (at all times). Phenol biomarkers were quantified in a subgroup of 16 women with the smallest missed voids rate. Among these women, two had collected a sample of each of their urine voids (no missing void, *group A1*), six collected more than 95% of their voids (*group A2*) and another eight between 80 and 95% of their voids (*group B*).

6.4.4 Phenol biomarkers

The total (free plus conjugated forms) urinary concentrations of 2,4-dichlorophenol, 2,5-dichlorophenol, benzophenone-3, bisphenol A, bisphenol S, triclosan, butylparaben, methylparaben, ethylparaben, and propylparaben were quantified at the CDC using a modified online solid-phase extraction high-performance liquid chromatography-isotope dilution-tandem mass spectrometry method.[38] Limits of detection (LODs) are listed in Table 6.1.

6.4.5 Aim 1: Assessing the efficiency of a degraded within-subject pooling protocol

We compared the efficiency (in terms of exposure assessment) of an approach using three daily urine samples, a *degraded pooling* approach, with that of an *ideal pooling* approach using all the daily samples to provide an estimate of exposure to ten phenols over windows of days, weeks and the whole pregnancy. We additionally compared exposure estimates based on one to eight random spot samples (other *degraded* approaches), randomly selected from all the available spot samples collected during the three measurement weeks for each woman, to that of the ideal approach. This section relied on eight to 16 pregnant women, depending on the considered exposure window.

6.4.5.1 Urine pools

We pooled individual samples to average concentrations over specific time windows, as detailed in Figure 6.1. From equal volume urine samples from each subject, we prepared (i) within-subject daily pools (seven days per subject and per week), obtained from all urine voids of a given day (there were on average eight voids per day); (ii) within-subject weekly pools, obtained by pooling all daily pools of a given week (three weeks per subject); and (iii) within-subject pregnancy pools, obtained by pooling all weekly pools. The ideal approach corresponded to *Protocol 1*.

In the degraded pooling approach, *Protocol 2*, the daily pools were prepared using three samples from each subject, instead of all daily individual samples. The weekly and pregnancy pools were prepared from these simpler daily pools. The three samples

were obtained by randomly selecting one sample in the morning (after midnight–1159 hours), one in the afternoon (1200–1800 hours) and one in the evening (1801–2359 hours).

Pools and spot samples were kept frozen at -80°C in 2-mL polypropylene cryovials until shipment on dry ice to the CDC laboratory in Atlanta (Georgia, USA), where all biospecimens were stored at or below -70°C until analysis.

6.4.5.2 Statistical analysis

All biomarker concentrations were ln-transformed. Concentrations were replaced by instrumental readings when below LODs, and, when the instrumental reading equaled zero, by the compound-specific non-null lowest instrumental reading divided by $\sqrt{2}$.

Three additional degraded approaches were compared to Protocol 1:

— *Protocol 3* relied on one random spot sample;

— *Protocol 4* averaged biomarker concentrations over three random spot samples;

— *Protocol 5* averaged biomarker concentrations over eight random spot samples.

These protocols were meant to provide an estimate of the pregnancy exposure average, while Protocols 1 and 2 provided estimates of concentrations during daily, weekly and pregnancy time window.

Phenols were not assayed in all samples due to cost constraints (see Figure 6.1 for a summary of samples assayed for phenols). Comparison of Protocols 1 and 2, relied on:

— all daily pools of the first collection week in the two women in group A1, and one random daily pool from the first collection week in the six women of group A2 (n = 20 daily pools);

— all weekly pools for the eight women in groups A1 and A2 (n = 24 weekly pools);

— the pregnancy pools for the 16 women (groups A1, A2 and B, n = 16 pregnancy pools).

The pregnancy average biomarker concentrations from Protocols 3 to 5 were additionally compared to that of Protocol 1 for each of the eight women in groups A1 and A2.

For each daily, weekly and whole pregnancy time window, we compared averages of phenol concentrations from Protocol 1 and the others, using Pearson (r) and Spearman (ρ) correlation coefficients, paired t-tests, and we assessed the concordance between exposure estimates categorized in tertiles through Cohen's Kappa coefficients (K). Scatter plots and Bland-Altman plots[2,3] were used for visual comparison.

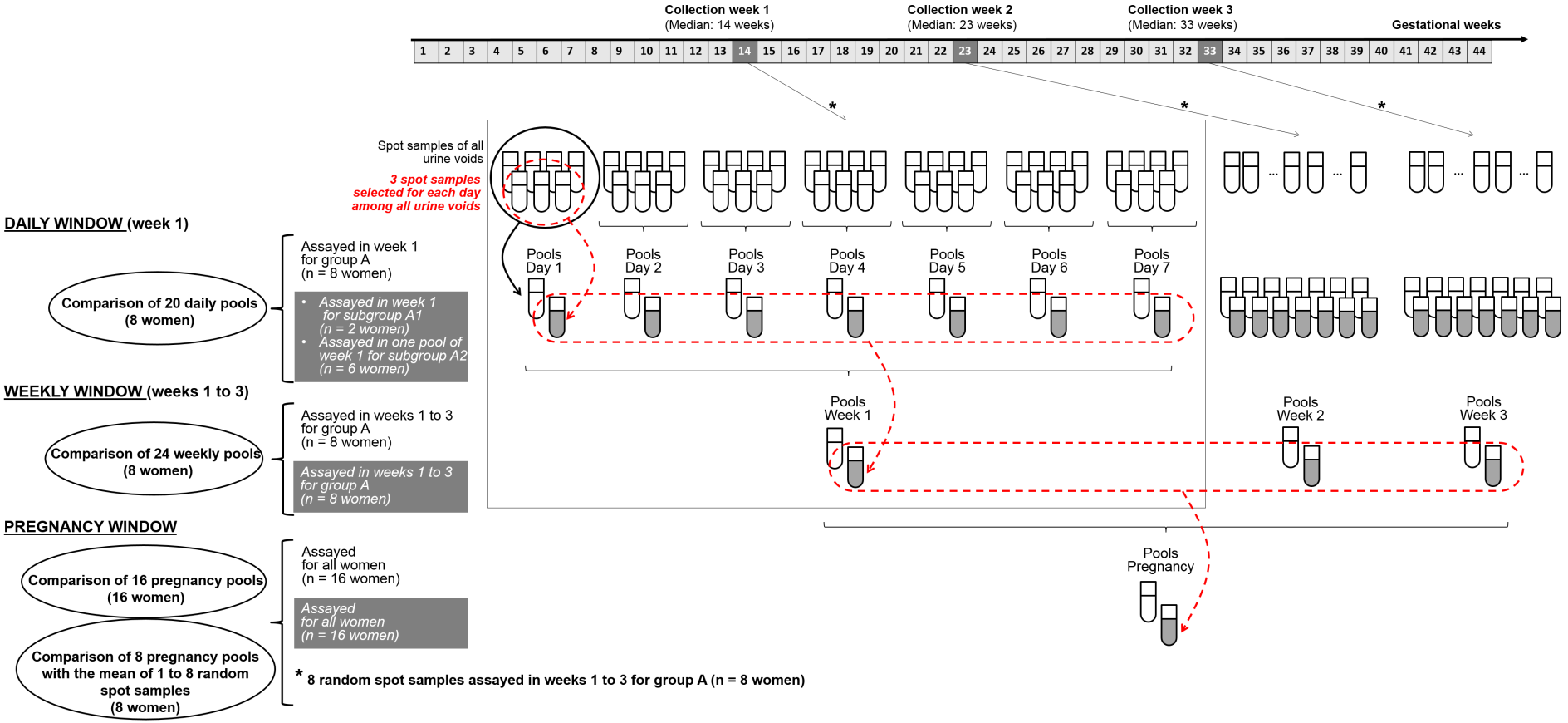

Figure 6.1 – Study design, urine collection protocol and pooling procedure in the three study groups (n = 16 pregnant women from SEPAGES cohort feasibility study).

6.4.6 Aim 2: Impact of the within-subject biomarker variability on dose-response functions

We relied on phenol urinary concentrations assayed in eight random spot samples collected throughout pregnancy (randomly selected from individual biospecimens of the three measurement weeks) in eight women of the SEPAGES-feasibility study (n = 64 samples).

A bootstrap approach was used to generate populations of 3,000 subjects with, for exposure assessment, one to 20 biospecimens each. We quantified bias and statistical power of epidemiological studies aiming at relating exposure to two error-prone phenol biomarkers to a continuous health outcome (child weight at age 3 years). We chose two phenols, methylparaben and bisphenol A, because of their contrasted ICCs; methylparaben having a rather low, and bisphenol A a high within-subject variability in the studied population of eight women (pregnancy-specific ICC was 0.85 for methylparaben and 0.38 for bisphenol A).[35] Exposure was assumed to be assessed from biomarker concentration in one random spot sample or within-subject pools of an increasing number of biospecimens, as in the theoretical study from Perrier et al.[26]. Methods were adapted from this previous simulation study and are detailed in the Appendix 1 of the Supplemental material (Section 6.9).

Bias was estimated as the difference in percent between the mean effect estimate (β) over 1,000 studies for the surrogates of exposure and the true effect (β_{true}) divided by true effect. Negative values of bias correspond to a situation where β is lower in absolute value than the true effect β_{true} (attenuation), and positive values to a situation where β is greater in absolute value than the true effect. Statistical power was calculated as the fraction of the 1,000 studies with a p-value for the association below 0.05.

We additionally reported *a posteriori* disattenuated effect estimates.[26,28] These estimates were obtained by dividing the estimated regression coefficients by the compound-specific ICC. We used two possible values of the pregnancy-specific ICC: ICC_1, corresponding to the value estimated in our study population of eight women[35] and ICC_2, corresponding to the average from previously published studies in pregnant women (ICC_2 was 0.45 and 0.20 for methylparaben and bisphenol A, respectively).[4,5,18,21,27,34] ICC_1 was assumed to correspond to the ideal value, but we also tested ICC_2 since without repeated assays, one cannot estimate ICCs specific to the study population.

Data were analyzed using STATA 12.1 (Stata Corp, College Station, Texas).

6.5 Results

6.5.1 Population

Women collected between three and 15 urine specimens per day (median, 7, 25th-75th centiles, 6-10), resulting in a total of 111 to 240 (median, 160, 25th-75th centiles, 136-188) samples per woman over the three collection weeks. Women from group B (with the highest rate of missed voids) tended to collect fewer samples per day than women from groups A1 and A2. The median interval between successive collection weeks was 8.9 weeks (25th-75th centiles, 8.1-10.2). Additional characteristics of the 16 pregnant women are presented in Table 6.6.

6.5.2 Assessing the efficiency of degraded within-subject sampling protocols

Daily exposure window

Daily pools (Protocol 1) were based on 8 urine samples per woman on average. Detection frequencies were above 75% for the daily pools for all phenols except benzophenone-3, for which detection frequency was 45%. With only three urine voids per day (Protocol 2) detection rates were fairly similar, except for triclosan (50%) and, to a lesser extent, benzophenone-3 (30%, Table 6.1). Distributions in biomarker concentrations were coherent between daily pools from Protocols 1 and 2 for almost all the compounds, except for triclosan (mean±SD ln-transformed concentration, 0.70±1.48 and -0.51±1.98 in Protocols 1 and 2 daily pools, respectively, $p<0.001$) and bisphenol S (-1.11±0.69 and -0.90±0.75 in Protocols 1 and 2 daily pools, respectively, $p<0.001$). Pearson correlations between the ln-transformed biomarker concentrations from Protocols 1 and 2 were above 0.80 ($p<0.001$), except for benzophenone-3 (r=0.57) and triclosan (r=0.68), the highest being observed for the four parabens ($r\geq0.96$, Table 6.1 and Figure 6.2). The Bland-Altman plots suggested a rather good agreement between the two protocols, although it was less obvious for triclosan, for which there was a tendency to underestimate the daily averages for the Protocol 2 daily pools compared to Protocol 1 daily pools (Figure 6.6).

Weekly exposure window

For the weekly exposure window, detection frequencies in weekly pools tended to be similar to those observed with the daily pools, except for butylparaben (for which detection rate was lower in the weekly pools, Table 6.2). Weekly averages using pools of three samples per day (Protocol 2) were coherent with those based on all urine voids (Protocol 1), except for 2,5-dichlorophenol ($p=0.04$), propylparaben ($p=0.01$) and bisphenol

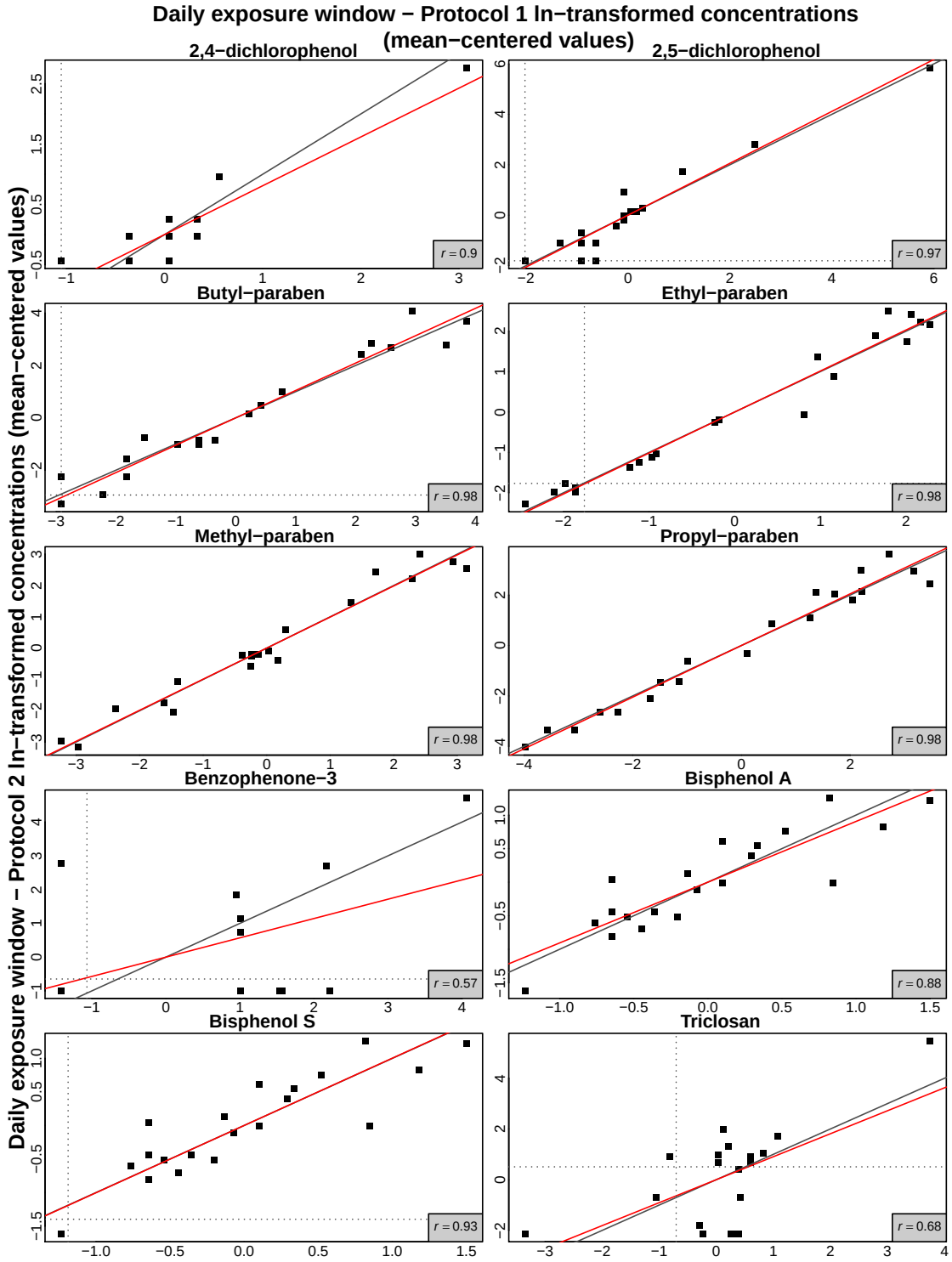

Figure 6.2 – Daily exposure window – Scatter plots of exposure estimates from Protocol 2 (equal volumes of three urines voids were within-subject pooled) against those from Protocol 1 (equal volumes of all urine voids were within-subject pooled) (mean-centered ln-transformed biomarker concentrations, n = 8 women, N = 20 samples). The filled red line represents the regression line and the filled black line the identity line. Horizontal and vertical dotted lines indicate the compound-specific limit of detection.

Table 6.1 – Daily exposure window – Descriptive statistics of the biomarker concentrations for the 20 daily pools and agreement between estimates from Protocols 1 (pooling of all urine samples/day) and 2 (pooling of 3 urine samples/day). Biomarker concentrations were ln-transformed.

		Daily pools (Protocol 1)[a] (n=8 women, n=20 samples)				Daily pools (Protocol 2)[b] (n=8 women, n=20 samples)				Agreement between Protocols 1 and 2			
			Percentiles (μg/L)				Percentiles (μg/L)			Pearson	Spearman	Kappa	
Phenolic compound	LOD (μg/L)	% > LOD	5th	50th	95th	% > LOD	5th	50th	95th	(r)	(ρ)	(K)	p-value[c]
2,4-dichlorophenol	0.1	90	<LOD	0.30	3.35	100	0.20	0.25	2.85	0.90	0.61	0.22	0.37
2,5-dichlorophenol	0.1	95	0.15	0.65	145.55	85	<LOD	0.45	110.00	0.97	0.90	0.54	0.06
Butylparaben	0.1	85	<LOD	1.15	74.45	85	<LOD	0.85	94.50	0.98	0.97	0.77	0.76
Ethylparaben	1.0	75	<LOD	4.70	53.55	80	<LOD	4.65	68.40	0.98	0.97	1.00	0.86
Methylparaben	1.0	100	5.55	101.35	2547.85	100	5.45	94.25	2067.90	0.98	0.96	0.85	0.61
Propylparaben	0.1	100	0.25	15.30	299.25	100	0.30	17.60	321.70	0.98	0.98	0.85	0.58
Benzophenone-3	0.2	45	<LOD	<LOD	19.45	30	<LOD	<LOD	24.45	0.57	0.44	0.26	0.25
Bisphenol A	0.1	100	0.65	1.55	6.65	100	0.65	2.00	6.95	0.88	0.84	0.62	0.06
Bisphenol S	0.1	95	0.15	0.30	1.70	95	0.15	0.40	2.05	0.93	0.88	0.55	<0.001
Triclosan	1.0	80	<LOD	2.55	45.80	50	<LOD	1.05	73.45	0.68	0.48	0.10	<0.001

LOD, limit of detection.
r indicates Pearson correlation coefficient, ρ indicates Spearman correlation coefficient, K indicates Kappa coefficient (based on biomarker concentration categorized into tertiles).
[a] All individual urine specimens of a day were within-subject pooled in equal volumes for daily pools.
[b] 3 individual urine specimens of a day were within-subject pooled in equal volumes for daily pools.
[c] p-value of Student's t-test comparing biomarker ln-transformed concentrations from Protocol 1 and Protocol 2 daily pools.

A (p=0.02). Ln-transformed weekly biomarker concentrations were highly correlated between Protocols 1 and 2 (all r coefficients above 0.8, p<0.001), the lowest being observed for benzophenone-3 (r=0.81) and the highest ($r \geq 0.98$) for three parabens and 2,5-dichlorophenol (Table 6.2 and Figure 6.3). Bland-Altman plots (Figure 6.7) showed a good agreement between the two protocols but a slightly tendency for Protocol 2 to overestimate weekly propylparaben averages.

Pregnancy exposure

For pregnancy pools, detection frequencies were very similar between Protocols 1 (median, 97%, 25^{th}-75^{th} centiles, 83-100) and 2 (median, 97%, 25^{th}-75^{th} centiles, 90-100). They were lower in general for Protocol 3, which relied on one random spot sample for pregnancy exposure assessment, (median, 69%, 25^{th}-75^{th} centiles, 50-88), except for benzophenone-3, for which detection rate was 63% with Protocol 3 compared to 31% in Protocols 1 and 2 (Table 6.3). For all compounds, pregnancy averages (n = 16 women) were in close agreement between Protocols 1 and 2 ($r \geq 0.86$, p<0.001), while Protocols 3 to 5 differed from Protocol 1 for most compounds (comparison based on eight women). The larger the number of spot samples used to assess pregnancy exposure, the higher the correlation with Protocol 1 pregnancy averages (r=−0.67 to 0.74, r=0.60 to 0.92 and r=0.68 to 0.98, respectively for Protocols 3, 4, and 5, see Table 6.3 and Figure 6.4). The scatter plots and Bland-Altman plots mainly suggested underestimation of pregnancy exposure when using one or few random spot samples (Protocols 3-5) to estimate mean pregnancy exposure, compared to Protocol 1. The agreement was good between the two pooling approaches (see Figures 6.8-6.9).

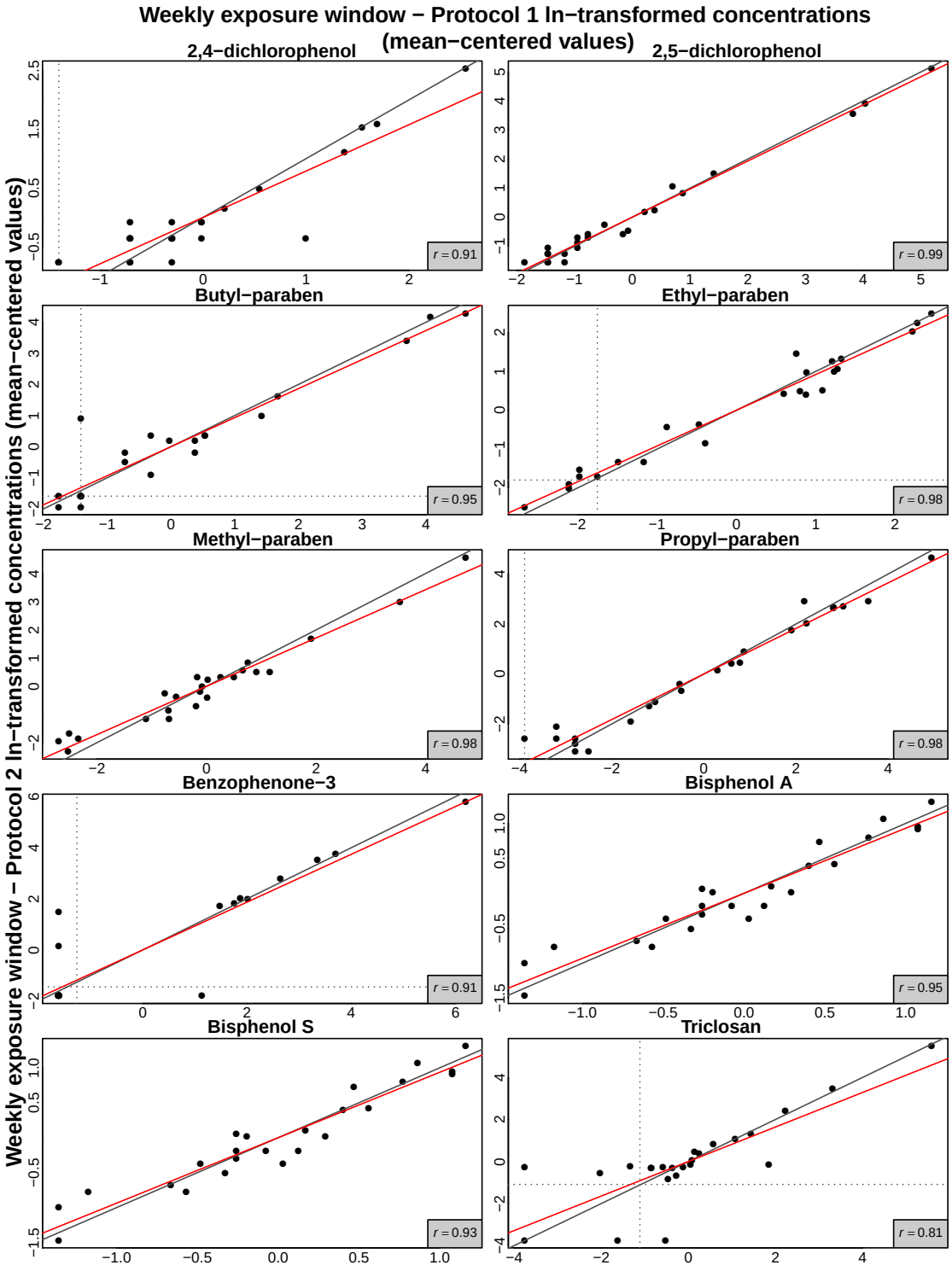

Figure 6.3 – Weekly exposure window – Scatter plots of exposure estimates from Protocol 2 (equal volumes of three urine voids were within-subject pooled for daily pools) against those from Protocol 1 (equal volumes of all urine voids were within-subject pooled) (mean-centered ln-transformed biomarker concentrations, n = 8 women, N = 24 samples). The filled red line represents the regression line and the filled black line the identity line. Horizontal and vertical dotted lines indicate the compound-specific limit of detection.

Table 6.2 – Weekly exposure window – Descriptive statistics of the biomarker concentrations for the 24 weekly pools and agreement between estimates from Protocols 1 (pooling of all urine samples/day) and 2 (pooling of 3 urine samples/day). Biomarker concentrations were ln-transformed.

		Weekly pools (Protocol 1)[a] (n=8 women, n=24 samples)				Weekly pools (Protocol 2)[b] (n=8 women, n=24 samples)				Agreement between Protocols 1 and 2			
			Percentiles (μg/L)				Percentiles (μg/L)			Pearson	Spearman	Kappa	
Phenolic compound	LOD (μg/L)	% > LOD	5th	50th	95th	% > LOD	5th	50th	95th	(r)	(ρ)	(K)	p-value[c]
2,4-dichlorophenol	0.1	92	<LOD	0.30	2.20	100	0.20	0.30	2.10	0.91	0.80	0.50	0.49
2,5-dichlorophenol	0.1	100	0.30	0.60	73.00	100	0.30	0.75	72.40	0.99	0.97	0.94	0.04
Butyl paraben	0.1	58	<LOD	0.25	24.00	63	<LOD	0.40	32.00	0.95	0.88	0.81	0.15
Ethyl paraben	1.0	79	<LOD	11.40	56.50	88	<LOD	9.20	56.80	0.98	0.96	0.88	0.48
Methyl paraben	1.0	100	3.90	44.40	1673.70	100	8.00	51.05	1117.90	0.98	0.95	0.75	0.11
Propyl paraben	0.1	96	0.20	4.80	173.60	100	0.30	6.05	122.50	0.98	0.95	1.00	0.01
Benzophenone-3	0.2	38	<LOD	<LOD	28.50	42	<LOD	<LOD	36.70	0.91	0.89	0.76	0.37
Bisphenol A	0.1	100	0.50	1.90	5.70	100	0.80	2.00	6.30	0.95	0.95	0.75	0.02
Bisphenol S	0.1	92	<LOD	0.30	14.40	96	0.20	0.40	18.00	0.97	0.93	0.63	0.07
Triclosan	1.0	79	<LOD	2.50	83.70	88	<LOD	2.35	96.10	0.81	0.83	0.44	0.93

LOD, limit of detection.
r indicates Pearson correlation coefficient, ρ indicates Spearman correlation coefficient, K indicates Kappa coefficient (based on biomarker concentration categorized into tertiles).
[a] All individual urine specimens of a day were within-subject pooled in equal volumes for daily pools. Daily pools were within-subject pooled in equal volumes to create weekly pools.
[b] 3 individual urine specimens of a day were within-subject pooled in equal volumes for daily pools. Daily pools were within-subject pooled in equal volumes to create weekly pools.
[c] p-value of Student's t-test comparing biomarker ln-transformed concentrations from Protocol 1 and Protocol 2 weekly pools.

Table 6.3 – Pregnancy exposure window – Descriptive statistics of the biomarker concentrations for the entire pregnancy exposure window estimated by various exposure models considered and agreement between estimates from Protocol 1 (pooling of all urine samples/day) and Protocols 2 (pooling of 3 urine samples/day), 3 (one random spot sample), 4 (mean of 3 random spot samples) and 5 (mean of 8 random spot samples). Biomarker concentrations were ln-transformed.

					Percentiles (μg/L)			Agreement between estimates from Protocol 1 and from the other protocols			
								Pearson	Spearman	Kappa	
Phenolic compound	Protocol	N	LOD (μg/L)	%>LOD	5th	50th	95th	(r)	(ρ)	(K)	p-value^{c}
2,4-dichlorophenol											
	Pregnancy pool, Protocol 1^{a}	16	0.1	88	0.20	0.30	2.70	ref			
	Pregnancy pool, Protocol 2^{b}	16		94	<LOD	0.30	2.40	0.86	0.88	0.89	0.43
	Average of 8 random spot samples, Protocol 5	8		NA	0.15	0.36	1.80	0.92	0.86	0.43	0.44
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	0.35	1.82	0.85	0.80	0.24	0.32
	Single random spot sample, Protocol 3	8		75	<LOD	0.40	0.50	0.17	0.15	-0.14	0.31
2,5-dichlorophenol											
	Pregnancy pool, Protocol 1^{a}	16	0.1	100	0.40	0.55	117.00	ref			
	Pregnancy pool, Protocol 2^{b}	16		100	0.30	1.00	103.20	0.99	0.97	1.00	0.52
	Average of 8 random spot samples, Protocol 5	8		NA	0.20	0.65	65.47	0.98	0.88	0.62	0.09
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	1.05	67.76	0.92	0.88	0.62	0.23
	Single random spot sample, Protocol 3	8		88	<LOD	0.70	13.60	0.75	0.39	0.05	0.19
Butyl paraben											
	Pregnancy pool, Protocol 1^{a}	16	0.1	75	<LOD	0.50	25.80	ref			
	Pregnancy pool, Protocol 2^{b}	16		75	<LOD	0.50	32.00	1.00	0.99	0.81	0.75
	Average of 8 random spot samples, Protocol 5	8		NA	<LOD	0.20	23.43	0.95	0.92	1.00	0.04
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	0.30	17.56	0.91	0.86	0.62	0.52
	Single random spot sample, Protocol 3	8		38	<LOD	<LOD	40.50	0.73	0.44	0.24	0.42
Ethyl paraben											
	Pregnancy pool, Protocol 1^{a}	16	1.0	81	<LOD	9.35	55.20	ref			
	Pregnancy pool, Protocol 2^{b}	16		88	<LOD	9.35	124.40	0.99	0.97	1.00	0.81
	Average of 8 random spot samples, Protocol 5	8		NA	<LOD	1.79	55.08	0.80	0.57	0.43	0.00
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	1.94	40.12	0.84	0.57	0.43	0.00

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Table 6.3 – Continued

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Phenolic compound	Protocol	N	LOD (μg/L)	%>LOD	Percentiles (μg/L)			Agreement between estimates from Protocol 1 and from the other protocols			
								Pearson	Spearman	Kappa	
					5th	50th	95th	(r)	(ρ)	(K)	p-value[c]
	Single random spot sample, Protocol 3	8		50	<LOD	<LOD	154.10	0.54	0.40	0.05	0.03
Methyl paraben											
	Pregnancy pool, Protocol 1[a]	16	1.0	100	5.20	56.05	2595.00	ref			
	Pregnancy pool, Protocol 2[b]	16		100	12.30	55.35	2949.20	0.98	0.99	0.81	0.08
	Average of 8 random spot samples, Protocol 5	8		NA	3.82	18.16	763.76	0.95	0.98	1.00	0.01
	Average of 3 random spot samples, Protocol 4	8		NA	2.57	11.36	330.76	0.86	1.00	1.00	0.03
	Single random spot sample, Protocol 3	8		88	<LOD	12.65	7850.00	0.84	0.79	0.24	0.17
Propyl paraben											
	Pregnancy pool, Protocol 1[a]	16	0.1	100	0.20	7.30	288.70	ref			
	Pregnancy pool, Protocol 2[b]	16		100	0.30	9.45	324.00	0.99	0.98	0.62	0.01
	Average of 8 random spot samples, Protocol 5	8		NA	0.18	2.66	98.72	0.93	0.92	0.62	0.05
	Average of 3 random spot samples, Protocol 4	8		NA	0.13	5.37	51.39	0.90	0.90	0.62	0.21
	Single random spot sample, Protocol 3	8		50	<LOD	0.70	1176.10	0.71	0.65	0.62	0.10
Benzophenone-3											
	Pregnancy pool, Protocol 1[a]	16	0.2	31	<LOD	<LOD	98.80	ref			
	Pregnancy pool, Protocol 2[b]	16		31	<LOD	<LOD	172.90	1.00	1.00	1.00	0.29
	Average of 8 random spot samples, Protocol 5	8		NA	0.22	0.31	15.07	0.96	0.85	0.62	1.00
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	0.56	11.79	0.89	0.85	0.62	0.95
	Single random spot sample, Protocol 3	8		63	<LOD	1.50	9.00	0.71	0.70	0.43	0.85
Bisphenol A											
	Pregnancy pool, Protocol 1[a]	16	0.1	100	0.70	2.45	4.50	ref			
	Pregnancy pool, Protocol 2[b]	16		100	0.80	3.05	6.10	0.88	0.85	0.62	0.10
	Average of 8 random spot samples, Protocol 5	8		NA	0.56	1.71	2.57	0.85	0.79	0.62	0.01
	Average of 3 random spot samples, Protocol 4	8		NA	0.11	1.27	3.05	0.74	0.52	0.43	0.02
	Single random spot sample, Protocol 3	8		88	<LOD	1.20	10.70	-0.67	-0.49	-0.33	0.41

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Table 6.3 – Continued

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Phenolic compound	Protocol	N	LOD (μg/L)	%>LOD	Percentiles (μg/L)			Agreement between estimates from Protocol 1 and from the other protocols			
								Pearson	Spearman	Kappa	
					5th	50th	95th	(r)	(ρ)	(K)	p-value[c]
Bisphenol S											
	Pregnancy pool, Protocol 1[a]	16	0.1	94	0.20	0.45	7.30	ref			
	Pregnancy pool, Protocol 2[b]	16		94	<LOD	0.45	8.60	0.99	0.91	0.61	0.19
	Average of 8 random spot samples, Protocol 5	8		NA	0.16	0.31	4.72	0.68	0.62	0.24	0.10
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	0.27	3.28	0.60	0.59	0.24	0.09
	Single random spot sample, Protocol 3	8		50	<LOD	0.15	0.90	0.17	0.10	0.05	0.05
Triclosan											
	Pregnancy pool, Protocol 1[a]	16	1.0	100	1.70	5.00	248.00	ref			
	Pregnancy pool, Protocol 2[b]	16		100	<LOD	4.50	258.80	0.97	0.86	0.62	0.56
	Average of 8 random spot samples, Protocol 5	8		NA	<LOD	1.62	10.42	0.75	0.60	0.24	0.00
	Average of 3 random spot samples, Protocol 4	8		NA	<LOD	1.51	4.91	0.65	0.48	0.24	0.00
	Single random spot sample, Protocol 3	8		75	<LOD	1.20	2.00	-0.08	-0.19	-0.17	0.01

LOD, limit of detection; NA, not applicable.
r indicates Pearson correlation coefficient, ρ indicates Spearman correlation coefficient, K indicates Kappa coefficient (based on biomarker concentration categorized into tertiles).
[a] All individual urine specimens of a day were within-subject pooled in equal volumes for daily pools. Daily pools were within-subject pooled in equal volumes to create weekly pools and within-subject pregnancy pool was created by pooling equal volumes of weekly pools.
[b] 3 individual urine specimens of a day were within-subject pooled in equal volumes for daily pools. Daily pools were within-subject pooled in equal volumes to create weekly pools and within-subject pregnancy pool was created by pooling equal volumes of weekly pools.
[c] p-value of Student's t-test comparing biomarker ln-transformed concentrations from Protocol 1 and the other protocols considered.

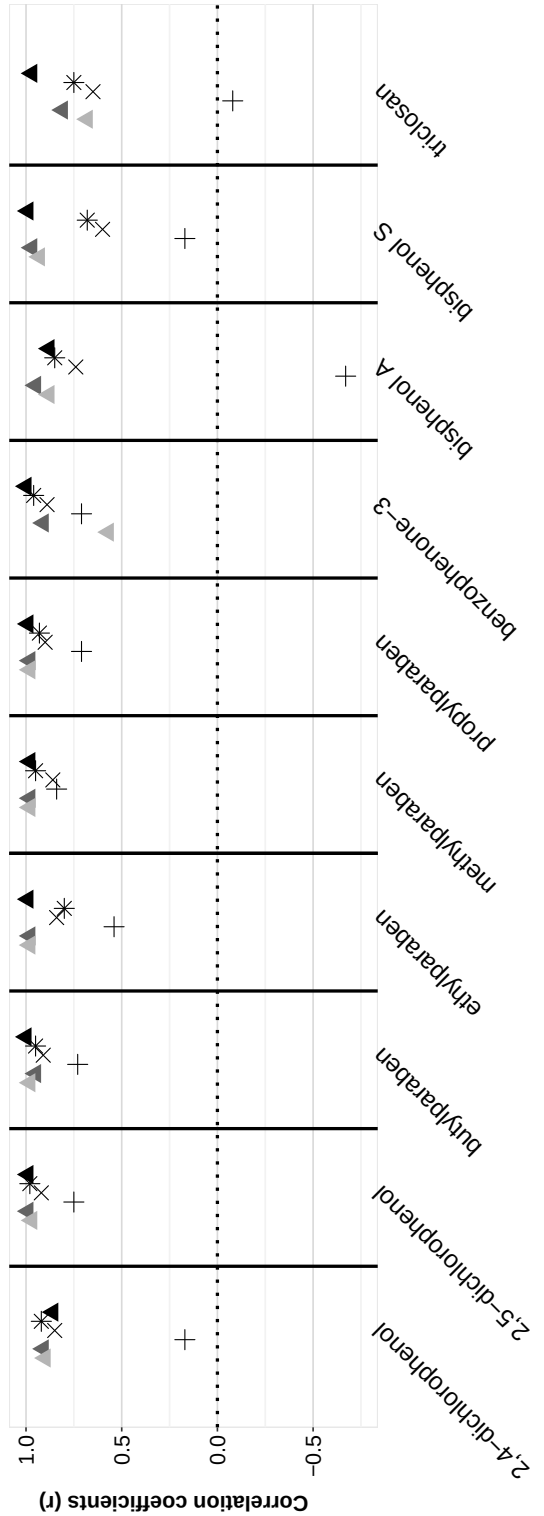


Figure 6.4 – All exposure windows – Pearson correlation coefficients (r) between Protocols 1 (equal volumes of all urine voids were within-subject pooled) and 2 (equal volumes of three urine voids were within-subject pooled for daily pools, triangle marks) for all time windows: exposure estimates over a day (n = 8 women, N = 20 daily averages), a week (n = 8 women, N = 24 weekly averages) and the whole pregnancy (based on three measurement weeks, n = 16 women, N = 16 pregnancy averages). For the whole pregnancy, estimates from Protocols 3 (+ symbol), 4 ($\times$ symbol), and 5 ($*$ symbol) are also given (n = 8 women, N = 8 pregnancy averages).

6.5.3 Impact of the within-subject biomarker variability on dose-response functions

Relying on one biospecimen for exposure assessment

Our simulation study, assuming that the (unmeasured) real exposure variable was associated with a 100g decrease in the health outcome, showed that, when using one random spot urine sample per subject to assess exposure, the average of the effect estimate for methylparaben was -71g (95% confidence interval [CI]: -101, -40) which, compared to the true effect (beta = -100g), corresponds to an attenuation bias of 29% (Table 6.4). The statistical power of a study of 3,000 subjects was 99%.

For bisphenol A, relying on a single spot sample led to an average effect estimate of -31g (95% CI: -76, 16), corresponding to an attenuation bias of 69%. The statistical power was 27% (Table 6.5).

A posteriori disattenuation (i.e., dividing the effect estimate by the compound-specific ICC) did not improve power, as expected, but reduced the attenuation bias to 16% and 19% respectively for methylparaben and bisphenol A (Tables 6.4 and 6.5). The improvement of the bias was milder when ICCs based on external literature (as opposed to the study-specific ICCs) were used. Actually, disattenuation applied with an average value of the biomarker-specific ICC (ICC_2, 0.45 and 0.20 for methylparaben and bisphenol A, respectively) from external studies,[4,5,18,21,27,34] overcorrected the effect estimate for both chemicals (see Tables 6.4 and 6.5).

For both compounds, type I error rate was not increased (5%) when no effect of the true exposure was assumed (i.e. beta = 0g; data not shown).

Increasing the number of urine specimens

Bias in the effect estimate was reduced when the number of biospecimens averaged to assess exposure increased (Figure 6.5, Tables 6.4 and 6.5). Four (for methylparaben) and 18 urine samples (for bisphenol A), were required to limit bias to 10% or less. If disattenuation was applied, the number of samples required was two for methylparaben and three for bisphenol A (Tables 6.4 and 6.5).

Table 6.4 – Effect estimates and statistical power in detecting associations between biomarker-based exposure to methylparaben and a continuous outcome, depending on the number of biospecimens collected per subject to assess exposure (1,000 simulation runs with 3,000 subjects each; true effect, β_{true} = -100g change in the outcome for each unit increase in the true (unmeasured) exposure).

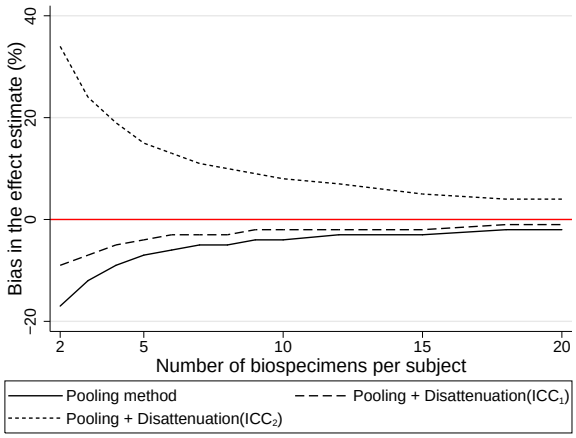
Number of biospecimens per subject	True Effect	Within-subject pooling			Within-subject pooling + *A posteriori* disattenuation using ICC_1[f]			Within-subject pooling + *A posteriori* disattenuation using ICC_2[g]		
		Effect Estimate[a] (95% CI[b])	Power[c]	Bias (%)[d]	Effect Estimate[a] (95% CI[b])	Power[c]	Bias (%)[d]	Effect Estimate[a] (95% CI[b])	Power[c]	Bias (%)[d]
1	-100	-71 (-101, -40)[e]	0.99	-29	-84 (-119, -47)[e]	0.99	-16	-158 (-225, -89)[e]	0.99	58
2	-100	-83 (-116, -49)	1	-17	-91 (-127, -53)	1	-9	-134 (-188, -79)	1	34
3	-101	-89 (-122, -53)	1	-12	-94 (-129, -56)	1	-7	-125 (-172, -75)	1	24
4	-99	-91 (-127, -54)	1	-9	-95 (-132, -56)	1	-5	-118 (-166, -70)	1	19
5	-100	-93 (-126, -58)	1	-7	-96 (-131, -60)	1	-4	-116 (-157, -72)	1	15
6	-101	-94 (-129, -60)	1	-6	-97 (-133, -62)	1	-3	-113 (-155, -72)	1	13
7	-99	-94 (-129, -57)	1	-5	-96 (-132, -58)	1	-3	-110 (-151, -67)	1	11
8	-99	-95 (-130, -59)	1	-5	-97 (-133, -61)	1	-3	-109 (-150, -68)	1	10
9	-102	-96 (-134, -58)	1	-4	-98 (-136, -59)	1	-2	-109 (-152, -65)	1	9
10	-100	-96 (-133, -60)	1	-4	-98 (-135, -61)	1	-2	-108 (-149, -67)	1	8
12	-101	-97 (-134, -61)	1	-3	-99 (-136, -62)	1	-2	-107 (-148, -68)	1	7
15	-100	-97 (-135, -61)	1	-3	-98 (-136, -61)	1	-2	-105 (-145, -65)	1	5
18	-100	-98 (-135, -61)	1	-2	-99 (-137, -61)	1	-1	-105 (-144, -65)	1	4
20	-99	-97 (-132, -60)	1	-2	-98 (-133, -61)	1	-1	-103 (-140, -64)	1	4

[a] Mean of effect estimates over 1,000 simulated studies.
[b] Empirical confidence interval, corresponding to the empirical 2.5 and 97.5 percentiles of the health effect estimates over the 1,000 simulation runs.
[c] Statistical power, estimated as the proportion of studies in which the *P*- value of the parameter characterizing the association between the error-prone exposure variables ($\bar{W}_{ij}$) and the continuous outcome was below 0.05.
[d] Difference between the true effect and the effect estimate, divided by the true effect.
[e] Corresponds to a situation without pooling.
[f] ICC_1 = 0.85 and corresponds to the value estimated in our study population of eight women.[35]
[g] ICC_2 = 0.45 and corresponds to the average from previously published studies in pregnant women.[4,5,18,21,27,34]

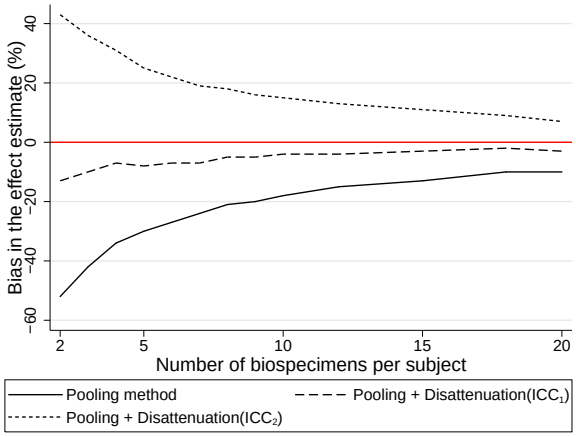
Table 6.5 – Effect estimates and statistical power in detecting associations between biomarker-based exposure to bisphenol A and a continuous outcome, depending on the number of biospecimens collected per subject to assess exposure (1,000 simulation runs with 3,000 subjects each; true effect, β_{true} = -100g change in the outcome for each unit increase in the true (unmeasured) exposure).

Number of biospecimens per subject	True Effect	Within-subject pooling			Within-subject pooling + *A posteriori* disattenuation using ICC_1[f]			Within-subject pooling + *A posteriori* disattenuation using ICC_2[g]		
		Effect Estimate[a] (95% CI[b])	Power[c]	Bias (%)[d]	Effect Estimate[a] (95% CI[b])	Power[c]	Bias (%)[d]	Effect Estimate[a] (95% CI[b])	Power[c]	Bias (%)[d]
1	-100	-31 (-76, 16)[e]	0.27	-69	-81 (-199, 43)[e]	0.27	-19	-153 (-378, 82)[e]	0.27	54
2	-100	-48 (-98, 4)	0.40	-52	-86 (-178, 7)	0.40	-13	-143 (-294, 12)	0.40	43
3	-101	-59 (-121, -0)	0.48	-42	-91 (-187, -0)	0.48	-10	-137 (-283, -0)	0.48	36
4	-99	-65 (-129, 1)	0.53	-34	-92 (-182, 2)	0.53	-7	-130 (-259, 2)	0.53	31
5	-100	-70 (-132, -5)	0.54	-30	-93 (-175, -6)	0.54	-8	-126 (-238, -8)	0.54	25
6	-101	-74 (-140, -8)	0.60	-27	-94 (-178, -10)	0.60	-7	-123 (-233, -13)	0.60	22
7	-99	-75 (-142, 1)	0.58	-24	-92 (-175, 2)	0.58	-7	-118 (-223, 2)	0.58	19
8	-99	-78 (-145, -5)	0.60	-21	-94 (-175, -7)	0.60	-5	-117 (-218, -8)	0.60	18
9	-102	-81 (-152, -7)	0.64	-20	-96 (-179, -8)	0.64	-5	-118 (-219, -10)	0.64	16
10	-100	-82 (-152, -5)	0.62	-18	-96 (-177, -6)	0.62	-4	-115 (-213, -7)	0.62	15
12	-101	-86 (-157, -16)	0.65	-15	-97 (-179, -18)	0.65	-4	-114 (-210, -22)	0.65	13
15	-100	-87 (-159, -13)	0.64	-13	-97 (-177, -15)	0.64	-3	-110 (-202, -17)	0.64	11
18	-100	-90 (-166, -16)	0.66	-10	-98 (-181, -18)	0.66	-2	-110 (-202, -20)	0.66	9
20	-99	-89 (-162, -14)	0.63	-10	-96 (-175, -15)	0.63	-3	-106 (-194, -17)	0.63	7

[a] Mean of effect estimates over 1,000 simulated studies.
[b] Empirical confidence interval, corresponding to the empirical 2.5 and 97.5 percentiles of the health effect estimates over the 1,000 simulation runs.
[c] Statistical power, estimated as the proportion of studies in which the *P*- value of the parameter characterizing the association between the error-prone exposure variables ($\bar{W_{ij}}$) and the continuous outcome was below 0.05.
[d] Difference between the true effect and the effect estimate, divided by the true effect.
[e] Corresponds to a situation without pooling.
[f] ICC_1 = 0.38 and corresponds to the value estimated in our study population of eight women.[35]
[g] ICC_2 = 0.20 and corresponds to the average from previously published studies in pregnant women.[4,5,18,21,27,34]



(a) Methylparaben (ICC_1 of 0.85 and ICC_2 of 0.45)



(b) Bisphenol A (ICC_1 of 0.38 and ICC_2 of 0.2)

Figure 6.5 – Bias in the health effect estimate (in %) depending on the number of biospecimens pooled per subject to assess exposure (1,000 simulation runs with 3,000 subjects each; continuous health outcome, true effect β_{true} = -100g), (A), Methylparaben (ICC_1 of 0.85 and ICC_2 of 0.45). (B), Bisphenol A (ICC_1 of 0.38 and ICC_2 of 0.2).

Disattenuation relied either on study-specific ICCs (ICC_1) or on ICCs based on previously published studies (ICC_2).[4,5,18,21,27,34]

6.6 Discussion

Degraded within-subject pooling of three repeated samples per day was an efficient strategy to assess exposures over short to long time periods for chemicals with short biological half-lives such as phenols, expect for benzophenone-3 and triclosan for daily averages of exposure. For the entire pregnancy exposure average, this approach had better statistical performances than an approach relying on few (typically one to three) biospecimens.

We provided an empirical estimation of the amplitude of the attenuation bias existing in epidemiological studies relying on a few spot biospecimens to estimate exposure to chemicals with strong temporal variations during the exposure window of interest. Bias was strong for bisphenol A, which is a compound with a high within-subject variability compared to methylparaben, which displays a lower within-subject variability. Increasing the number of biospecimen reduced the attenuation bias and increased the statistical power to detect associations. Applying the ICC-based *a posteriori* disattenuation method[26] was able to correct part of the attenuation bias, when relied on ICCs derived from our study population. However, applying the same approach using ICCs derived from the literature increased the bias instead of attenuating it, showing the sensitivity of this method to the validity of the ICCs used for the study population.

6.6.1 Study assumptions and limitations

Our comparative analyses relied on a small number of samples (between eight and 24), which might result in a loss of precision in the parameters characterizing agreement (e.g. correlation or kappa coefficients, t-test p-values). However, if we exclude the role of random fluctuations, this loss of precision is not expected to increase agreement between the two approaches. Also, by comparing several daily or weekly exposure averages among the same women (n = 8), we may have reduced the variability of the results, possibly increasing measured correlations. However, an adequate optimal average exposure was required for accurate comparison. This could only be achieved in the reduced number of women with very few missed voids. Missed voids may have artificially increased correlations between the two within-subject pooling approaches, by lowering the number of specimens in the ideal pooling approach. We limited such an issue by selecting women with the lowest rate of missed voids (less than 5%, except for the eight additional women included in the pregnancy exposure window comparison, which had between 5 and 15% of missed voids). We created equal-volume ideal and degraded pools, i.e., volumes of urinary biospecimens to be pooled were not weighted by taking into account total volumes of voids (unknown in our study), nor urine dilution.

Since both pooling approaches are similarly affected, this is not expected to improve or reduce agreement between the two pooling protocols.

We artificially enlarged the study population with a bootstrap method using a limited dataset of true biomarker measurements (eight women, each with eight samples) to characterize bias and power in exposure-response epidemiological studies that could limit the between-subject variance. Consequently, we chose to draw the true exposure averages from a normal distribution. We addressed issues related to exposure misclassification but, in doing so, ignored other issues, such as confounding and selection bias, which may well occur in practice and can complexify the *a posteriori* disattenuation correction.[10] We assumed that measurement error was classical, which is reasonable when biomarkers are used to assess exposure to chemicals.[10,15] We also assumed that the biomarker was a good exposure proxy, but from pharmacokinetic studies, phenols have short biological half-lives, and hence are briefly and almost entirely excreted in the urine after exposure.[17,29,36]

6.6.2 Assessing exposure over time windows of various lengths

For an exposure window of several weeks (typically the whole pregnancy), biomarker concentrations from a single random spot sample (Protocol 3) were in poor agreement (Table 6.3) with pregnancy exposure averages in Protocol 1 (based on the within-subject pooling of all daily biospecimens), showing that relying on a single random spot sample does not accurately represent the pregnancy average. This is in line with our results from the simulation section (Section 6.5.3) and from Perrier et al.[26]. Increasing the number of biospecimens improved the agreement for all of the studied chemicals, with fair agreement with Protocol 1 pregnancy exposure averages when relying on three to eight random biospecimens (Protocols 4 and 5, Table 6.3, r above 0.8), except for triclosan and bisphenols with correlation below 0.7 between Protocol 1 and 4 concentrations. This suggests that relying on half a dozen biospecimens may lead to a reasonable estimate of pregnancy exposure average for some compounds, such as dichlorophenols, benzophenone-3 and parabens but ethylparaben. However, although the exposure ranking was preserved (correlation coefficients), the pregnancy average concentration was not perfectly estimated (p-value for t-test with Protocol 1 pregnancy average below 0.05). For compounds such as ethylparaben, triclosan and bisphenols, which were phenols with a high within-subject variability observed in a previous study on the same population,[35] relying on eight random spot samples to assess pregnancy exposure may not be enough (r below 0.85 with Protocol 1 pregnancy exposure averages). This is consistent with the simulation section (Section 6.5.3), in which we empirically showed that the chemicals with a stronger variability required a larger number of biospecimens to correctly estimate dose-response functions. In contrast to

protocols relying on random spot sample, agreement with the ideal approach was much higher when collecting three repeated daily samples, i.e. the degraded within-subject pooling approach (r=0.86 and 0.89, for 2,4-dichlorophenol and bisphenol A, respectively, and above 0.97 for the other compounds), which confirmed that this approach is efficient to assess exposure over quite long exposure windows, even for highly variable chemicals.

For exposure assessment over a shorter time window, collecting three daily biospecimens was also efficient to characterize the average exposure over a week (linear correlations between the ideal and degraded approaches were above 0.9, except for triclosan, r=0.81, Table 6.2). When it comes to characterizing exposure over a day (Table 6.1), the approach was still efficient, but in a limited manner for benzophenone-3 and triclosan (correlations in the 0.5-0.7 range). However, we suggest caution in interpreting those results, as these compounds had the lowest detection rates. For a few compounds (e.g, bisphenol A and triclosan for the daily window and 2,5-dichlorophenol, propylparaben and benzophenone-3 for the weekly window), the exposure averages differed between Protocol 1 and 2 (p-value for t-test below 0.05), but exposure rankings were preserved for all but triclosan in the daily window. Results for triclosan are quite consistent across the exposure windows; be it for exposure ranking or dose-response functions, collecting half a dozen of biospecimens in the exposure window of interest may not be sufficient to assess exposure.

6.6.3 Empirical characterization of bias and power

Attenuation in regression analyses is a well-known issue in the context of classical-type error.[10,15,26] Using real data, the present study provided an estimate of the attenuation bias occurring when a single error-prone biomarker measurement is used as surrogate of the true underlying exposure to investigate exposure-response relationships. Attenuation bias was strong for bisphenol A (almost 70%), compound with a high variability in our initial population of eight women (ICC = 0.38). This attenuation bias still existed for a compound with low variability (ICC = 0.85), but was moderate (29%). This result confirms the findings from Perrier et al.[26]. With simulated exposure data, they previously reported an attenuation bias of 80% for high variable compounds such as bisphenol A (ICC = 0.2), and of 40% for less variable compounds such as parabens (ICC = 0.6). Increasing the number of specimens used to estimate the average exposure during toxicologically relevant time window reduced bias and increased statistical power. Without *a posteriori* disattenuation, four samples were required for methylparaben (i.e. the compound with limited within-subject variations) and 18 for bisphenol A (compound highly variable within-subject) to limit bias to 10% or less, compared to six and 35 samples in Perrier et al.[26]. Hence, we observed an attenua-

tion bias of lower magnitude and a smaller number of biospecimens required to reduce efficiently bias. This may likely be due to higher ICCs values we had in our SEPAGES-feasibility study population.[28] However, our empirical results are quite consistent with theoretical results from Perrier et al.[26], by showing that a few biospecimens are required to have a reasonable estimation of exposure over a specific time window for compounds with a low within-subject variability, while for highly variable compounds, a few dozen or more are needed.

A posteriori disattenuation using ICCs observed in our population[35] only partly reduced the attenuation bias, which differed from the perfect correction in Perrier et al.[26]. This might be explained by data, simulated using a predefined ICC in Perrier et al.[26], while we calculated ICCs from a small sample size (n = 8 women) that may have reduced the precision of the ICCs values. Using ICCs extracted from the literature (i.e., external studies)[4,5,18,21,27,34] did not efficiently correct for the attenuation bias. Discrepancies in the temporality of urine collection between studies may partly explain the non-validity of external ICCs, since ICCs depend of the considered time window.[35] This underlines the relevance of trying to estimate variability internally, e.g. by collecting and assaying repeated biospecimens from a subsample of the study population so as to correct bias using *a posteriori* disattenuation. When ICCs are not available in the study population, the transfer of ICCs between populations to correct estimates should be cautious.[9]

6.6.4 Within-subject pooling approach

This degraded within-subject pooling approach allows the investigation of short (days, weeks) or long (trimesters of pregnancy) exposure windows for all investigated short half-lived phenols, despite limited efficiency for benzophenone-3 and triclosan in the shortest time windows (day/week). Such an approach permits to combine the information of many samples without increasing assay costs, since a single pooled sample is assayed per woman for a given exposure window.[26,31] We assumed that pooling samples did not entail any error. Pooling error may however exist due to technical process (e.g. technician variability, precision of instruments); physical conditions (e.g. ambient temperature, thawing duration, reaction between compounds from different samples);[32] or an equal-volume pooling strategy which does not take urinary dilution into account. However, in our study, a single technician mainly pooled samples, limiting error due to biospecimens manipulation. Additional studies would be needed to further explore these aspects.

Collecting and pooling three daily urine specimens over toxicologically relevant exposure windows has the advantage of being less cumbersome than a design requiring all urine voids. When using degraded within-subject pooling compared to collecting a

spot biospecimen in each subject, the logistic burden and the overall costs of the study are increased, which may limit the sample size and induce selection bias due to high withdrawal or low participation rates. However, there is no reason to exclude subjects with a single biospecimen, as unbalanced designs were found to give acceptable estimates of dose-response functions, despite a slightly higher bias in effect estimates.[26] Overall, the degraded within-subject pooling allows estimation of exposure averages at lower analytical costs in predefined short and long time windows for most of the studied compounds, as well as the estimation of dose-response functions by reducing attenuation bias in effect estimates. Pooled samples may also limit issue related to limit of detection.[31] However, within-subject pooling must be used jointly with repeated unpooled samples (hybrid pooled-unpooled designs) for estimating ICC and distributional parameters of exposure biomarkers.[20,30]

6.7 Conclusion

A degraded sampling approach relying on the repeated within-subject pooling of three daily samples appeared to be an efficient strategy to increase the number of samples and resulted in accurate exposure average estimates over time windows of various length (days, weeks, the whole pregnancy), without increasing assay costs and being excessively cumbersome. We provided an empirical confirmation that large within-subject variability in a biomarker of exposure (e.g. for bisphenol A) can strongly bias the exposure-effect association and reduce statistical power in epidemiological studies, mostly when relying on a few biospecimens. Bias was also observed for chemicals with low to moderate variability (methylparaben). Increasing the number of biospecimens collected within-subject reduced bias in dose-response function and improved statistical power.

6.8 References

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6.9 Supplemental material

List of Figures

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6.9.1 Appendix 1

6.9.1.1 Impact of the within-subject biomarker variability on dose-response functions: Supplemental methods

We chose two phenols, methylparaben and bisphenol A, because of their contrasted ICCs, with respectively low and high within-subject variability in the studied population of eight women (pregnancy-specific ICC was 0.85 for methylparaben and 0.38 for bisphenol A).[10]

Simulation of exposures

We simulated a population of 3,000 subjects with one to 20 spot urine samples using a bootstrap method based on the eight women from SEPAGES-feasibility study with the lowest rate of missed urine voids (groups A1 and A2). Each of these women had eight random spot samples assayed for phenol biomarkers among all spot samples collected at three occasions of pregnancy (see Figure 6.1).

We assigned for each subject i ($i = 1, ..., 3,000$) one pregnant woman out of the eight from the SEPAGES-feasibility study, with, for each biomarker j ($j = 1, 2$) the average (namely $C_{ij_{mean}}$) of biomarker ln-concentrations in the eight random spot samples. To obtain unclustered subjects (clusters corresponding to the eight pregnant women), for each participant i ($i = 1, ..., 3,000$), and each biomarker j ($j = 1, 2$), we generated X_{ij}, the true but unobserved (ln-transformed) exposure from a normal distribution, using a mean (standard deviation, SD) concentration of 4.64 (1.59) μg/L for ln(methylparaben), and 0.91 (0.75) μg/L for ln(bisphenol A). Concentrations values (means and SD) were extracted from the EDEN French mother-child cohort with approximately log-normal distributions,[11] and not from the SEPAGES cohort, as phenol biomarker were not yet assessed in the SEPAGES cohort biospecimens when this study was conducted.

Then, for each subject i, we generated k ($k = 1, \ldots, 20$) biospecimens with a bootstrap method, by randomly assigning one out of the eight real random spot samples available (for each subject) to each biospecimen k.

For each biospecimen k, the biomarker-specific ln-concentration C_{ijk} was centered around X_{ij} using the formula $W_{ijk} = C_{ijk} + X_{ij} - C_{ij_{mean}}$, where W_{ijk} corresponded to the resulting X_{ij}-centered biospecimen and biomarker-specific concentration. We presented examples of X_{ij}, and W_{ijk} distributions in Figures 6.10 and 6.11.

Simulation of health outcomes

For each subject i and each biomarker j, a continuous outcome Y_{ij} was simulated as $Y_{ij} = \beta_1 X_{ij} + \alpha + \epsilon_{ij}$, with β_1 the true effect assumed to be -100g by one-unit increase

in X_{ij} and ϵ_{ij} the independent normally distributed random error with mean zero. The values for the parameters α (14,900g) and ϵ_{ij} (SD, 1,650g) were selected so as to match the distribution of the offspring weight at age 3 years in the French EDEN mother-child cohort,[6] to reproduce what was previously done by Perrier et al.[5]; and because SEPAGES-feasibility stopped at birth and therefore data on offspring weight at 3 years of age were not collected.

We additionally simulated Y_{ij} considering a null effect of the biomarker (i.e, β_1=0) to explore how the risk of type I error was affected.

Bias and power characterization

For each chemical j, we fitted a linear regression model in the population of 3,000 subjects between the simulated continuous health outcome and a biomarker measurement in one random spot sample (W_{ijk}) and within-subject pools of an increasing number of biospecimens, represented by the average ($\overline{W_{ij}}$) of ln-transformed biomarker concentrations from two to 20 randomly collected biospecimens, as in the previous theoretical study from Perrier et al.[5].

Bias was estimated in percent as the difference between the mean effect estimate (β) over 1,000 studies for the surrogates of exposure (W_{ijk} and $\overline{W_{ij}}$) and the true effect (β_{true}) divided by β_{true}. Negative values of bias correspond to a situation where β is lower in absolute value than the true effect β_{true} (i.e. attenuation) and positive values to a situation where β is greater in absolute value than the true effect β_{true}.

Power was calculated as the fraction of the 1,000 studies with a p-value for the association below 0.05.

***A posteriori* disattenuation**

We additionally reported a posteriori disattenuated effect estimates.[5,8] These estimates were obtained by dividing the estimated regression coefficients by the compound-specific ICC. We used two possible values of the pregnancy-specific ICC: ICC_1, corresponding to the value estimated in our study population of eight women (ICC_1 was 0.85 and 0.38 for methylparaben and bisphenol A, respectively);[10] and ICC_2, corresponding to the averaged ICC from previously published studies in pregnant women (0.45 and 0.20 for methylparaben and bisphenol A, respectively).[1,2,3,4,7,9] ICC_1 was assumed to correspond to the ideal value, but we also used ICC_2 because without repeated assays one cannot estimate ICCs internal to the study population.

6.9.1.2 Appendix 1 references

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Table 6.6 – Characteristics of the 16 pregnant women from the SEPAGES-feasibility cohort included in the current study.

Characteristic	Groups A1 and A2 (n = 8) No. (%)	Group B (n = 8) No. (%)
Civil status		
Married	5 (62.5)	3 (37.5)
Cohabitating	3 (37.5)	5 (62.5)
Maternal education		
High school or less	0	0
Up to 3 years of college	4 (50)	6 (75)
> 3 years of college	4 (50)	1 (12.5)
Missing		1 (12.5)
Smoking history during pregnancy		
Yes	1 (12.5)	3 (37.5)
No	7 (87.5)	5 (62.5)
Parity		
0	5 (62.5)	4 (50)
1	2 (25)	3 (37.5)
≥ 2	1 (12.5)	1 (12.5)
	Median (25^{th}, 75^{th})	Median (25^{th}, 75^{th})
Maternal age at enrolment (years)	28.5 (27.0, 31.5)	30.0 (27.5, 33.0)
Gestational age (weeks)		
Week 1 of urine collection	14.9 (13.6, 16.2)	13.1 (11.6, 14.9)
Week 2 of urine collection	23.9 (22.9, 25.2)	22.5 (21.6, 23.4)
Week 3 of urine collection	32.4 (31.7, 32.9)	31.9 (31.7, 32.6)
Time between two successive weeks of urine collection (weeks)		
Week 1 – Week 2	8.9 (8.1, 10.0)	9.9 (8.6, 10.8)
Week 2 – Week 3	8.4 (7.1, 9.3)	9.4 (7.4, 11.1)
Number of collected urine voids per day	8 (7, 10)	6 (5, 8)

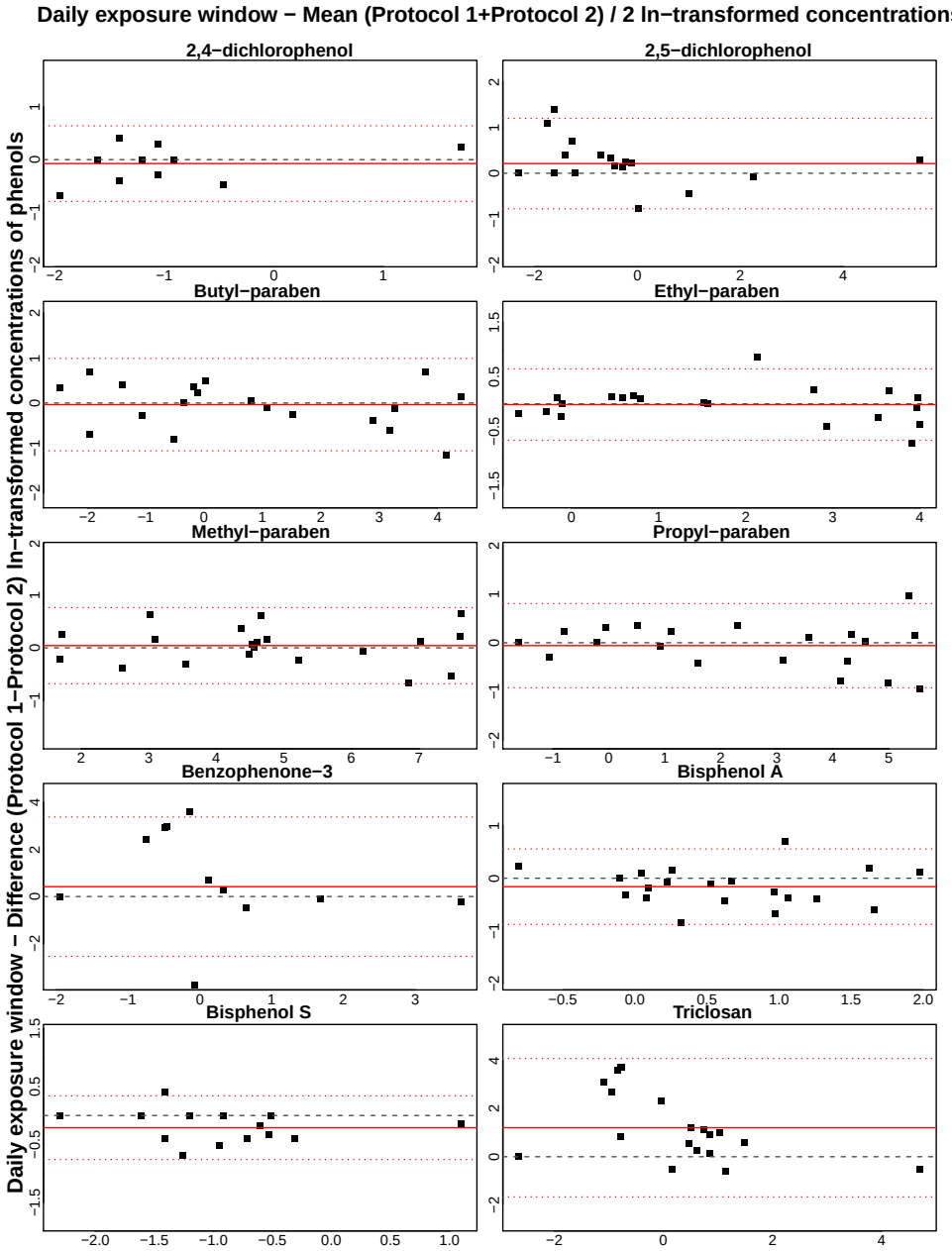


Figure 6.6 – Daily exposure window – Bland-Altman plots for Protocols 1 (equal volumes of all urine voids were within-subject pooled) and 2 (equal volumes of three urine voids were within-subject pooled) daily averages of ln-transformed biomarkers.

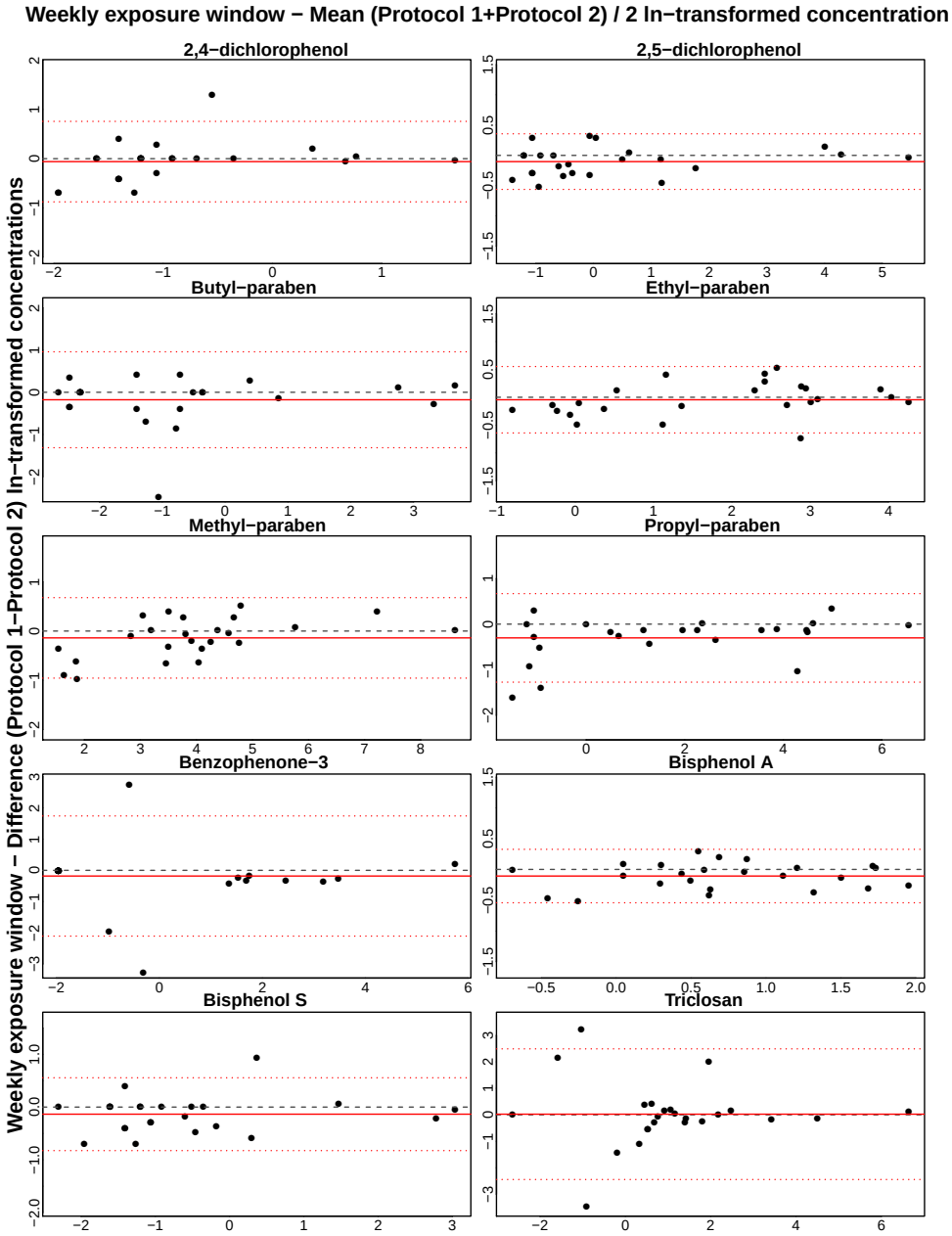


Figure 6.7 – Weekly exposure window – Bland-Altman plots for Protocols 1 (equal volumes of all urine voids were within-subject pooled) and 2 (equal volumes of three urine voids were within-subject pooled) weekly averages of ln-transformed biomarkers.

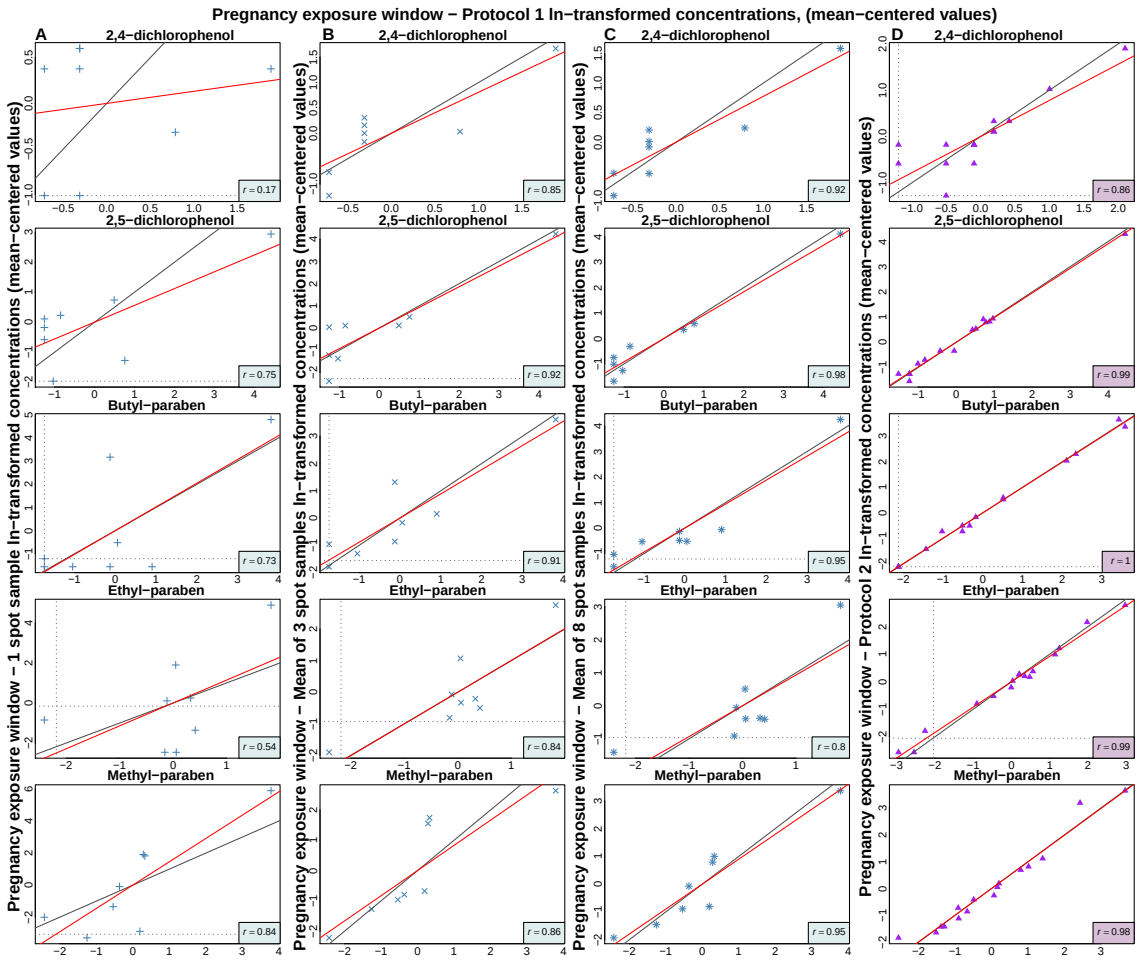


Figure 6.8 – Pregnancy exposure window – Scatter plots of exposure estimates from Protocols 2-5 against Protocol 1 (pooling of all urine samples/day).

Protocol 2 corresponds to within-subject pooling of 3 urine samples/day over three weeks, Protocol 3 to pregnancy exposure relying on one random spot sample, Protocol 4 on the average of three random spot samples, and Protocol 5 on the average of eight random spot samples. Each point corresponds to pregnancy exposure estimate after mean-centering (ln-transformed concentrations, n = 16 women, N = 16 samples for comparison between Protocols 1 and 2; n = 8 women, N = 8 samples for comparison between Protocol 1 and Protocols 3-5). The filled red line represents the regression line and the filled black line the identity line. Horizontal and vertical dotted lines showed the compound-specific limit of detection.

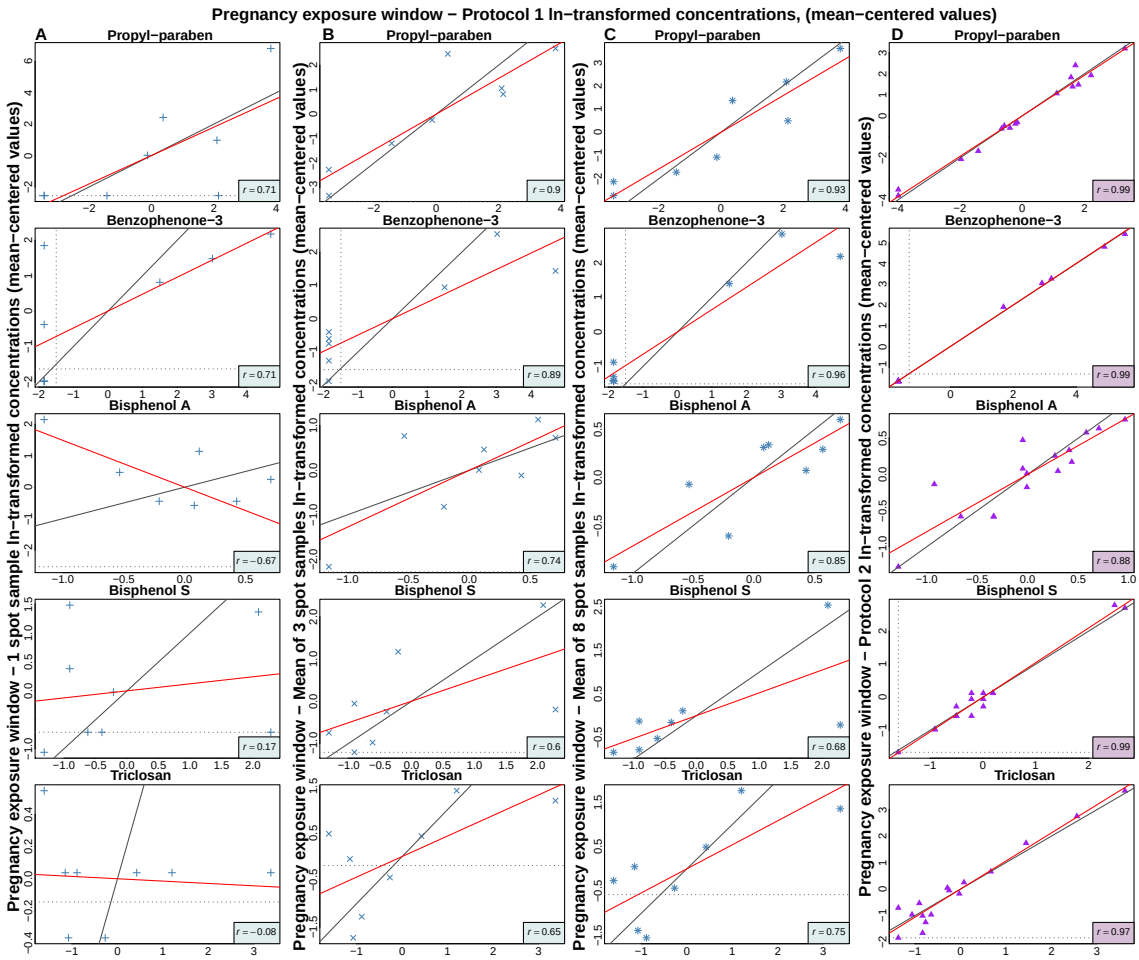


Figure 6.8 – Continued

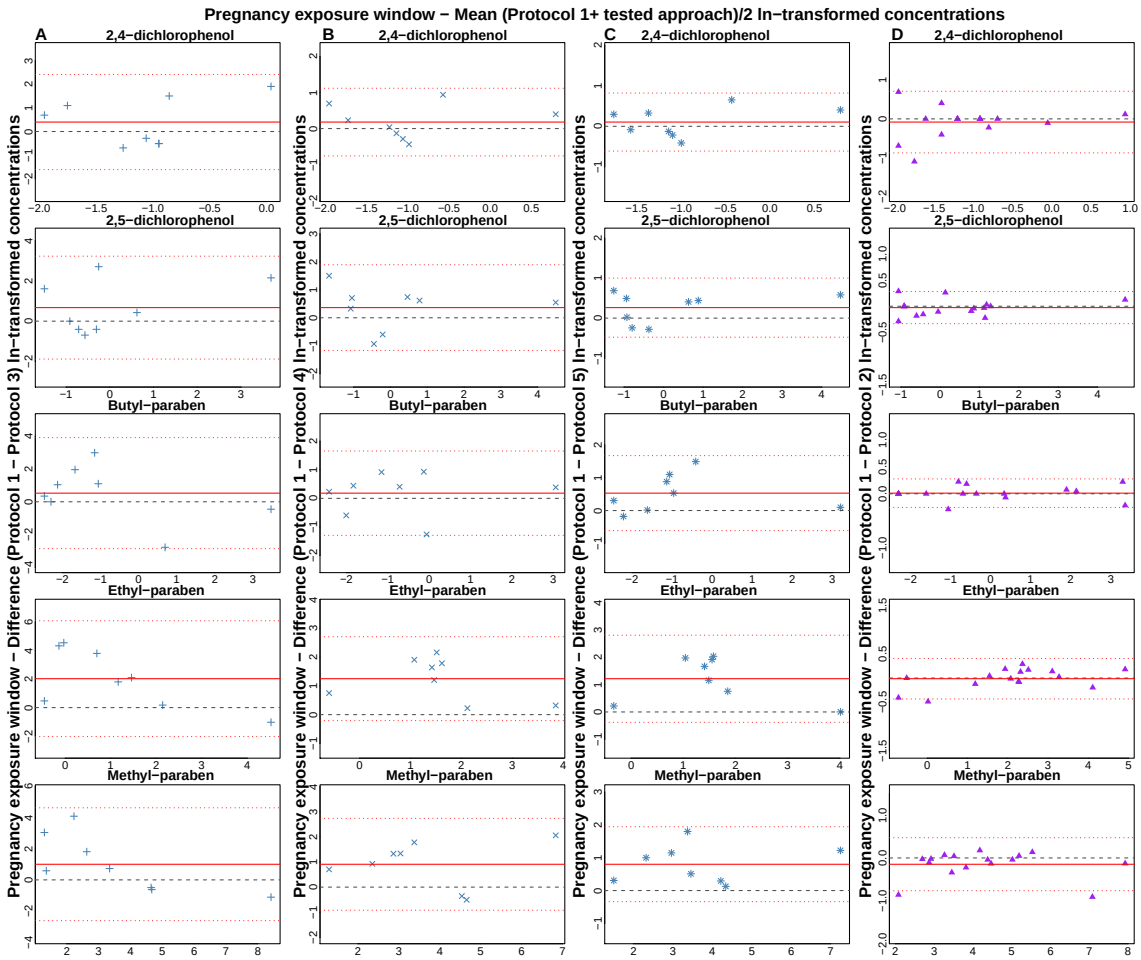


Figure 6.9 – Pregnancy exposure window – Bland-Altman plots for Protocols 3 (A), 4 (B), 5 (C) and 2 (D) against Protocol 1 (pooling of all urine samples/day) pregnancy averages of ln-transformed biomarkers.

Protocol 2 corresponds to within-subject pooling of 3 urine samples/day over three weeks, Protocol 3 to pregnancy exposure relying on one random spot sample, Protocol 4 on the average of three random spot samples, and Protocol 5 on the average of eight random spot samples.

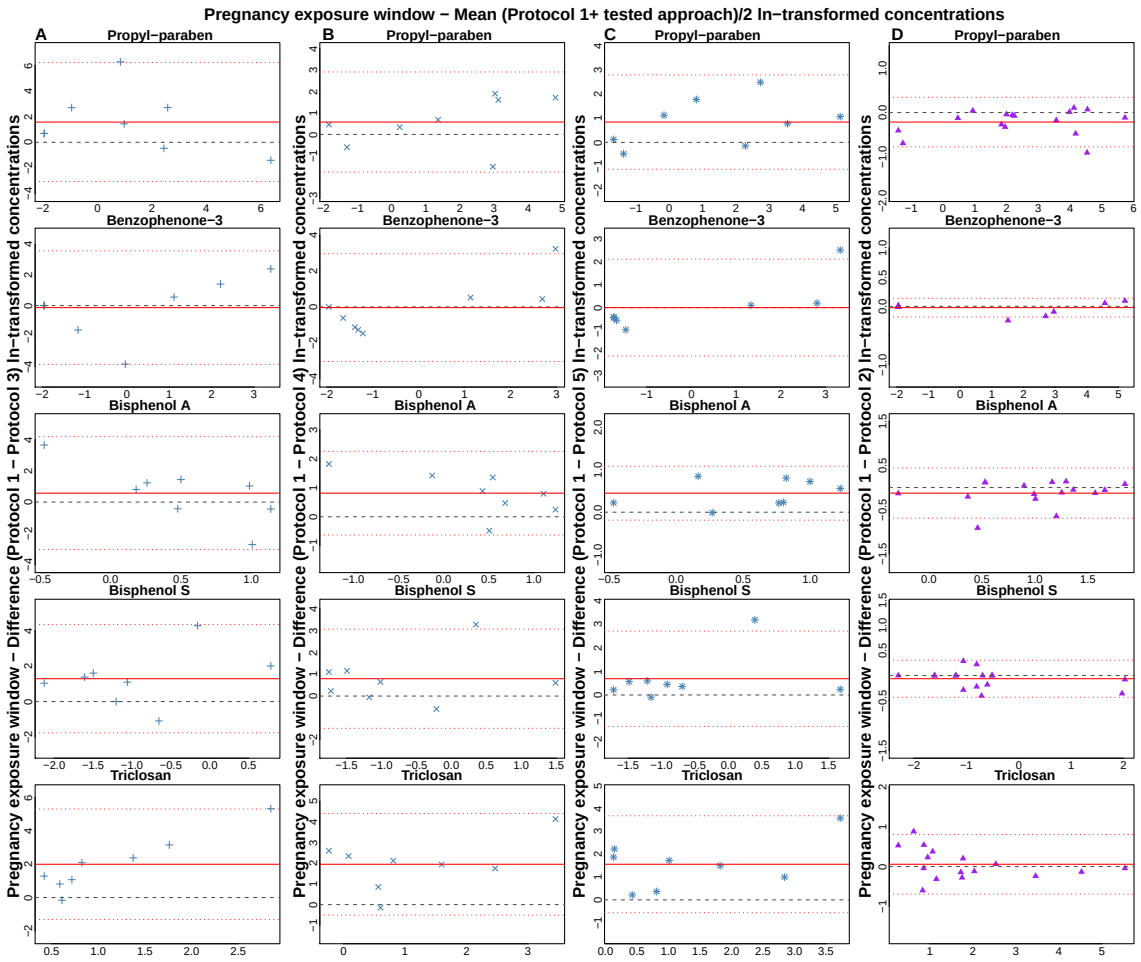
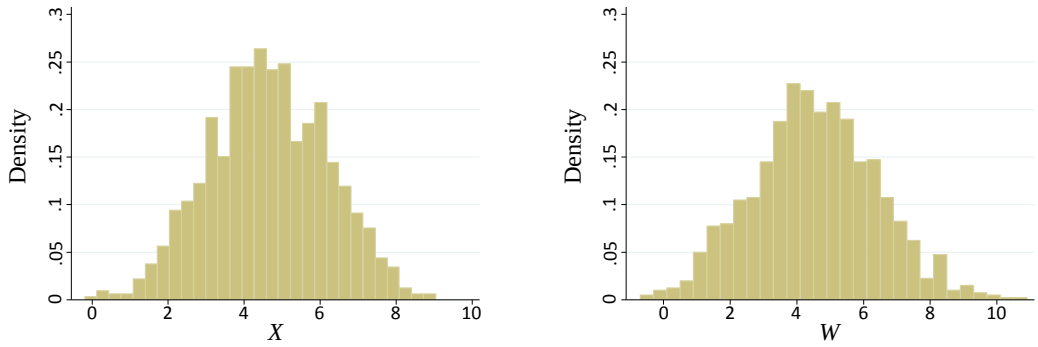


Figure 6.9 – Continued

Methylparaben



Bisphenol A

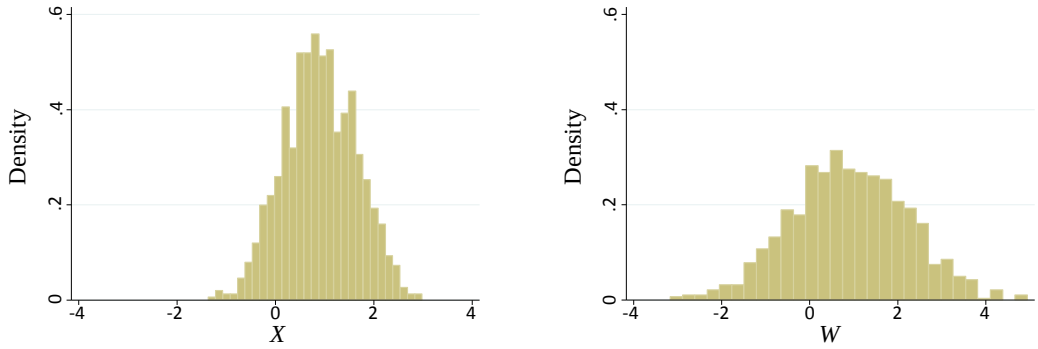


Figure 6.10 – Distribution of the simulated exposures in one of our simulated studies for methylparaben (A, ICC = 0.85) and for bisphenol A (B, ICC = 0.38).

X is the *true* unobserved average exposure and W is the surrogate exposure measured with error (using one spot sample).

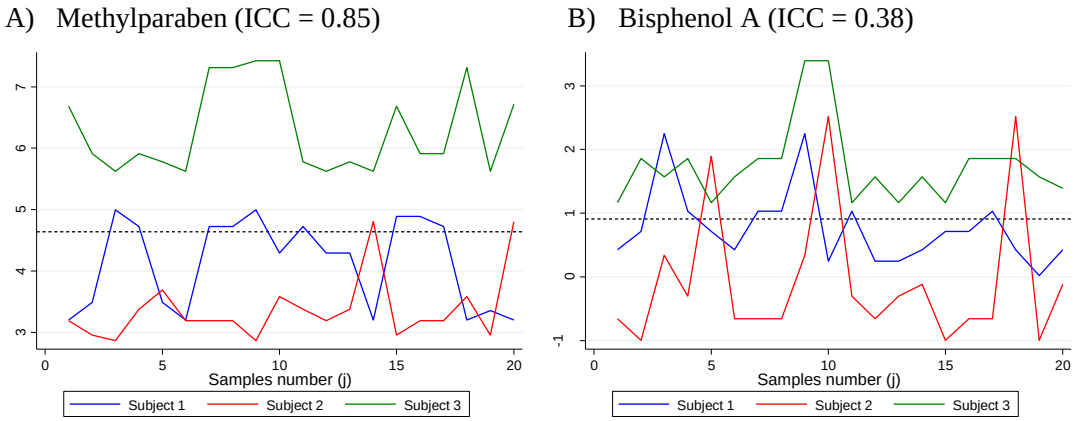


Figure 6.11 – Examples of urinary concentrations of methylparaben (A) and bisphenol A (B) measured with error, for three subjects in one of our simulation runs.

The dashed lines display the true unobserved exposure average of urinary ln-concentrations of the corresponding chemical over a toxicologically relevant exposure window.

Chapter 7

General discussion

In Chapters 4 to 6, results are discussed compared to previous studies, and for each study, strengths and limitations are addressed. This chapter presents a summary of our contributions to epidemiological research on effects of prenatal exposure to phenols and phthalates (Section 7.2). Then, general methodological limitations of our research are discussed (Section 7.3). Finally, multiple avenues for research are proposed for the development of relevant epidemiological studies in the last chapter (Chapter 8).

7.1 French summary

L'objectif de cette thèse est d'étudier l'exposition aux phénols et aux phtalates, sa variabilité chez la femme enceinte et son impact sur la santé respiratoire. Nous observons peu d'associations entre l'exposition prénatale aux phénols et aux phtalates et des effets délétères sur la fonction pulmonaire ou l'apparition de symptômes ou maladies respiratoires chez le garçon dans les cinq premières années de vie. Néanmoins, pour certains composés comme l'éthylparabène et le bisphénol A, être exposé in utero a tendance à être associé à l'apparition de plusieurs symptômes ou maladies respiratoires ou à une diminution de la fonction pulmonaire. Contrairement à notre hypothèse initiale, nous avons aussi observé des associations bénéfiques entre certains composés, notamment le méthylparabène, propylparabène et la benzophénone-3, et les symptômes ou maladies respiratoires. Notre étude étant la première à avoir étudié ces phénols avec un design longitudinal, nos résultats doivent être répliqués dans de futures études. Une des limitations principales de notre étude est probablement l'estimation de l'exposition.

Nos travaux de recherche sur la variabilité intra-individuelle de l'exposition et de son impact sur le biais et la puissance statistique dans les études épidémiologiques reposent sur un nombre restreint de sujets, ce qui limite probablement la généralisabité et la précision de nos résultats. Cependant ils illustrent que la plupart des études explorant les effets des phénols et des phtalates sur la santé humaine, y compris notre étude sur la santé respiratoire, sont limitées par une estimation imparfaite de l'exposition reposant sur des concentrations de biomarqueurs mesurées dans un faible nombre de biospécimens (généralement entre un et trois biospécimens). Ce biais est principalement de l'atténuation sous une hypothèse d'erreur de mesure de type classique. Notre étude sur la variabilité intra-individuelle montre que la variabilité est forte à l'intérieur des jours mais la variabilité des concentrations moyennes journalières est faible entre les jours d'une même semaine. Sur la grossesse entière, la variabilité entre les semaines est importante pour certains composés comme les bisphénols, et assez faible pour d'autres (2,5-dichlorophénol ou parabènes).

Cette variabilité intra-individuelle peut amener, en faisant l'hypothèse d'un type d'erreur de mesure classique, à une mauvaise estimation des niveaux d'exposition sur une fenêtre de temps d'intérêt si le nombre de biospecimens n'est pas suffisant, et ainsi entraîner un biais d'atténuation dans les estimations d'association et un manque de puissance, comme montré dans notre chapitre 6 avec des données réelles. Le pooling intra-sujet d'un petit nombre de biospécimens par jour, répété sur plusieurs semaines, semble une approche efficace et réalisable pour estimer les niveaux moyens d'exposition à des composés non-persistants pour des fenêtres d'exposition courtes (jours) ou assez longues (semaines, grossesse entière). Il pourrait permettre de réduire le risque de

misclassification sans augmenter le coût des analyses car les concentrations de biomarqueurs d'exposition ne sont mesurées que dans les échantillons poolés.

Cette thèse confirme la nécessité de poursuivre la recherche sur les effets possibles des phénols et des phtalates sur la santé humaine, et notamment sur la santé respiratoire, car le niveau actuel de preuve est limité. Trouver le bon compromis entre la taille de l'échantillon et le niveau d'information par sujet est complexe. Cependant, utiliser des designs d'étude adaptés à l'estimation de l'exposition à des composés non-persistants pour limiter le biais et la perte de puissance dans les études est probablement nécessaire (voir la Figure 8.1 basée sur les résultats de cette thèse pour quelques exemples de designs efficaces). Nous montrons ainsi que la voie est ouverte pour les cohortes mères-enfants dites de "troisième génération".[285]

7.2 Summary of the main findings

Associations with respiratory endpoints

In Chapter 4, we investigated the associations between maternal exposure to 11 phthalates and nine phenols during pregnancy assessed via one random spot maternal urine sample on the one side, and several respiratory health endpoints in childhood: asthma diagnosis, wheezing, bronchiolitis/bronchitis, and FEV_1 on the other side. No phenol or phthalate metabolite exhibited clear deleterious associations simultaneously with several respiratory outcomes, but neither is there toxicological evidence for any compound impacting several outcomes simultaneously. For phenols, increased levels of ethyl-paraben, bisphenol A, 2,5-dichlorophenol, tended to be associated with respectively increased asthma rate and reduced FEV_1; increased asthma and bronchiolitis/bronchitis rates; and increased wheezing rate. Only did ethyl-paraben and bisphenol A exhibit some consistency across respiratory outcomes. Contrary to our *a priori* hypothesis, increased exposure to methylparaben, propylparaben and benzophenone-3 were associated with reduced rates of bronchiolitis/bronchitis and wheezing. Regarding phthalates, we observed a trend for an adverse association between MCNP, a metabolite of DIDP, and wheezing, while MCPP, a metabolite of DNOP, DnBP and several HMW phthalates, tended to reduce the rate of bronchiolitis/bronchitis. Conclusions were unchanged when applying *a posteriori* disattenuation to correct estimates for measurement error in exposure relying on a unique random spot biospecimen.

Exposure assessment to nonpersistent EDs during pregnancy

When characterizing the effect of phenols and phthalates pregnancy exposure to health endpoints, the efficiency of relying on a single measure of biomarker concentrations for exposure assessment depends upon the within-subject temporal variability of these concentrations in pregnant women. Time-window specific variability is poorly characterized.

In Chapter 5, we aimed at evaluating the within-subject variability of phenols across several time windows of pregnancy. Most compounds showed a very high variability over the course of a day, while daily averages were more stable over the same week (ICCs above 0.6). This pattern was opposite for bisphenol S. The within-subject variability of the weekly averages considered several weeks apart was low for some compounds (2,5-dichlorophenol, butylparaben, methylparaben) and high for others (ethylparaben, bisphenol S, triclosan). Correcting for urinary dilution via the use of creatinine levels or specific gravity did not greatly impact the observed within-subject variability in phenol biomarkers (Chapter 5).

We chose to focus our work on the phenolic compounds, for which data on urinary biomarker concentration variability during pregnancy for compounds other than

bisphenol A was sparse and more limited compared to phthalates. Moreover, we were supposed to have samples assayed for triclocarban and several bisphenols besides bisphenol A, for which no data on variability was published. Unfortunately, the laboratory could not measure these compounds, except bisphenol S. Having measurements for phthalates would have been valuable, as within-subject variability is compound-specific, but we could not afford the assays for both families of chemicals in the SEPAGES-feasibility study because of budgetary constraint. It is nevertheless interesting to note that a high variability across pregnancy was reported for phthalates in a study by Fisher et al.[103], which sought to characterize the variability of about twenty phthalate metabolites within a day and across several time points of pregnancy (see Table 2.3). They also reported a high within-day variability for some metabolites (MCPP several DEHP metabolites), which was more moderate for others (e.g., MBP,MiBP, MEP, MBzP, and other DEHP metabolites).

These results suggest that relying on a unique biological sample to assess pregnancy exposure to most phenols or other nonpersistent compounds leads to exposure misclassification. In this context, alternative sampling designs are needed in order to limit this exposure misclassification. Consequently, Chapter 6 dealt with our evaluation of the efficiency of four degraded sampling approaches in estimating average exposure to phenols – based on urinary biomarker concentrations – over several time windows (day, week, whole pregnancy), compared to the ideal approach using all urine voids collected over the same time windows. The first degraded approach relied on a within-subject pooling of three daily samples, while the others, more common, respectively relied on one, three and eight biospecimens drawn at random over the whole pregnancy. For long exposure windows such as the entire pregnancy, the degraded within-subject pooling approach gave more reliable average exposure estimates than the three approaches relying on a few biospecimens. However, for many phenols but triclosan and bisphenols A and S, relying on three samples to assess pregnancy exposure preserved exposure ranking although exposure averages could differ from those of the ideal approach. For shorter exposure windows, the degraded within-subject pooling approach also resulted in accurate weekly and daily average estimations of exposure to phenols, but was less suitable for the estimation of benzophenone-3 and triclosan daily exposures, compounds with low detection frequencies in the degraded pools. Assuming a classical-type error structure, we illustrated empirically that the exposure misclassification resulted in attenuation bias in dose-response function estimates when using a single biospecimen to assess exposure over a long-time window, i.e., the whole pregnancy. Bias was strong for bisphenol A (attenuation of 70%), which is a compound with a high within-subject variability over pregnancy (ICC of 0.38 using three random spot samples) compared to methylparaben (around 30%), which displays a lower within-subject variability (ICC

of 0.85 using three random spot samples). This may suggest that the higher the within-subject variability, the stronger the bias. Furthermore, increasing the number of biospecimens used to average the exposure over the time window of interest reduced the bias for both methylparaben and bisphenol A and increased the statistical power to detect associations. We also provided an estimate of a reasonable number of biospecimens required to limit the attenuation bias to 10% or less. Four and 18 samples were needed for methylparaben and bisphenol A, respectively. These results are consistent with those from a theoretical study by Perrier et al.[242], in which 6 and 35 samples were required to reduce bias below the threshold of 10%. The slightly higher ICCs observed in our study (0.38, 0.85) compared to their study (0.2 and 0.6) could explain the somewhat lower number of required samples. This is also in line with results from our comparison of several sampling designs, where an exposure assessment relying on three to eight samples was reasonable for compounds with the highest ICCs (above 0.6 based on between-week ICCs), while the within-subject degraded pooling approach was more suitable for compounds with higher between-week variability. In case where ICCs were internally estimated from a subpart of the population, an *a posteriori* disattenuation method using the ICC values corrected part of the bias in effect estimates for both compounds. On the contrary, using ICCs from external studies increased the bias.

7.3 Methodological considerations

The accuracy of results from our studies depends on both the *validity* of the results, i.e. to which extent the conclusions inferred from the results are correct for the source population (internal validity) and for a target population (external validity); and the *precision*, i.e. how closely repeated studies or measurements lead to similar results. On the one hand, validity is influenced by *systematic* errors also called biases (information, confounding, and selection biases); and on the other hand, precision is affected by *random* errors.[264,301] In this section, our findings from Chapters 4, 5 and 6 are discussed according to possible sources of, firstly, systematic error; secondly, random error; and finally to study designs and statistical methods.

7.3.1 Systematic error

7.3.1.1 Information bias

Information bias, also called misclassification, arises from measurement errors, e.g. in the exposure, the disease status, or the covariates.[264] In this subsection, we will focus

on misclassification related to exposures and outcomes, which is presumably one of the main sources of bias in this thesis.

Information bias on outcomes

With questionnaire-based respiratory outcomes, occurrence of respiratory events may be over- or underestimated due to recall bias.[297] Also, the longer the recall time, the higher the risk of misreporting.[231,195,140] In the EDEN cohort, questionnaires were sent and retrieved each year limiting the risk of incorrect recall. It has been shown that when parents fill ISAAC questionnaires for their children, recall of wheezing and asthma symptoms over a 12-month period is not impacted by the season of responding despite seasonal variations in symptoms,[297] while on the contrary, reports of rhinitis symptoms are more prone to recall bias in favor of recent months. This suggests that recall bias related to wheezing and asthma is probably limited when using ISAAC questionnaires about symptoms in the last 12 months, and smaller than for allergic diseases. Respiratory questionnaires were reported to have a limited sensitivity regarding asthma definition (the ability to adequately identify subjects with asthma disease), but an excellent specificity (the ability to adequately identify subjects without the disease).[349,192] Therefore, cumulative incidences may have been overestimated, which is in line with the high incidence of asthma we observed in our study. However, contrary to studies aiming at estimating prevalence and incidence, and for which this can be an issue and high sensitivity is worth, a high specificity is preferable for studies evaluating the impact of exposures on asthma development.[240] Additionally, using a question referring to *doctor-diagnosed asthma* increases the specificity of the asthma definition,[349] while its sensitivity does not increase because of a difficulty to diagnose the disease.[1,295,133] ISAAC questionnaire is validated in different languages, including French.[19,255] Although there is a limitation because it is only validated for school-aged children, at the time of the EDEN cohort follow-up (recruitment occurred in 2003-2006), there was no standardized questionnaire specifically developed for pre-school children.

For the spirometric test, the mean value of FEV_1 in our population was lower than the predicted value for boys of this age. This could be first related to discrepancies between our population and the population of reference. Indeed, percent predicted values are calculated as the departure from reference values, i.e. values that a normal healthy child with the same age, height, sex, and ethnic group, would have had for the spirometric parameters.[251] Hence, if children in our study were too different from those on which were calculated reference values, using predicted values may bring some error. Our population is mostly Caucasian and since this is the most represented ethnic group in the study providing the equations and reference values,[251] this error is likely limited. The second hypothesis for the low predicted lung function is the difficulty for children around 5 years of age to maintain sufficient pressure during

the test,[38] and for at least one second, which was the time threshold for acceptable measures in our study. As we retained only measures satisfying acceptability criteria, children who are trained to perform a spirometric test (i.e., those with a respiratory disease, which should have a lower pulmonary function in general than those without a respiratory disease) were more likely to perform well compared to more healthy children who performed a spirometric test for the first time. By reducing the variability of the outcomes between subgroups of population (those with and without respiratory diseases), these two points could introduce bias in the effect estimates and yield to an underestimation of the potential effect of phenols and phthalates. This hypothesis is supported by exploratory analyses excluding boys diagnosed with asthma, and in which most trends of deleterious associations with FEV_1 were stronger, especially for MiBP, MCOP, and DEHP metabolites. The measure of spirometric parameters may be prone to random error from the technical instruments used, but, to unknown extent.

Thus, while minimized by the design of our study, information bias related to outcome misclassification may still occur from both the measure of spirometric parameters and questionnaires-based respiratory endpoints.

Information error on exposures

Relying on one spot sample to estimate exposure to nonpersistent chemicals over long time windows such as the whole pregnancy is likely to lead to misclassification error (Chapters 5 and 6). When spot biomarker concentrations vary around the true value – which could be approximated by the mean of many measurements repeated throughout the exposure window of interest – the error corresponds to what is called classical type error.[18,67] Additionally, as suggested by a theoretical approach by Perrier et al.[242] and confirmed in Chapter 6 using real data, the bias was stronger for chemicals with the highest within-subject variability. Classical type error always biases the dose-response estimates towards the null (attenuation bias) when the exposure is considered continuous (or binary) in the statistical analyses. However, when the exposure variable is categorical, the bias may be in either direction,[264,18,104] which limits the relevance of exposure categorization into tertiles (Chapter 4). If one assumes a simple form of classical type error, i.e. an additive classical error, *a posteriori disattenuation* is a simple method that uses the compound-specific ICC to correct the dose-response estimates without improving statistical power.[242,256] In Section 4.9 (Chapter 4), disattenuated estimates were greater in absolute value, especially for chemicals with the lowest ICCs (e.g., bisphenol A, MCNP, MCOP and DEHP metabolites). This was expected from the equation, since the lower the ICC, the higher the attenuation bias. This approach works for simple regression models (no adjustment factors),[242] but may be too simplifying for adjusted models, when a more complex attenuation factor may be used.[67] Additionally, as no ICCs were available in our study, the corrected findings should be

interpreted cautiously. Indeed, as shown in Chapter 6, using ICCs from external studies may lead to estimates biased away from the null, possibly more than when no correction is applied. This is an important result of this thesis, with practical implications for study designs.

In Chapter 4, using a standardization method previously developed in our team,[214] we aimed to reduce the between-subject variability in biomarkers levels arising from biospecimens collection conditions and year of biomarker measurement that could differ between subjects. This undesirable variability was probably limited since correlations between raw and standardized concentrations were high. This was also supported by analyses using raw biomarker concentrations with findings which were close to those obtained after standardization. However, in the absence of repeated exposure data within subject, as was the case in Chapter 5, the ability to standardize measurement conditions may be limited. Moreover, this approach assumes that all subjects have the same daily patterns, which is likely not true. Although going in the right direction, this approach is limited in practice if no information is available on subject's behaviors.

In Chapters 5 and 6, exposure assessment relied on a complete collection of urine voids in participants over the different time windows, limiting misclassification. However, random measurement error could still occur due to analytical error (instrument imprecision, technician error), and individual's pharmacokinetic factors. For an identical exposure, inter-subject pharmacokinetic differences can result in different internal exposure levels assessed through surrogates such as biomarkers.[307,320,144,53]

There is no consensus on how to best handle values below the LOD. We relied on the machine readings, but other methods exist such as likelihood-based estimation and multiple imputation.[302,75,191,26] Using multiple imputation could enlarge confidence intervals by adding uncertainty to values below the LOD, and hence, may reduce the precision of the estimates for chemicals with the highest rate of values below the LOD in the three studies of this thesis.

Total actual volume of urine voids was unknown, and we chose the most conservative approach, which was to take equal volumes of all urine voids to generate the pooled samples. While pools can also be weighted on creatinine, this option was not available at the time of the pooling process, since creatinine concentrations were measured by the CDC laboratory. Currently, the consequences of either approach on bias and efficiency are unknown, and it would be interesting to test this second approach (creatinine-weighted pools) in light of our results. Additionally, standardization for creatinine or specific gravity is likely irrelevant in pooled samples as the mean of concentrations measured in individual samples before pooling and corrected for these factors (i.e., divided by creatinine level or specific gravity) will differ from the concentration measured in the pooled sample and corrected for creatinine or specific gravity from this unique

sample.[242] Indeed, mathematically, the sum of ratios is in general not equal to the ratio of sums. This was a reason why results presented in Chapters 5 and 6 relied on uncorrected concentrations. The results based on creatinine- or specific gravity-corrected concentrations were not too different to those relying on the uncorrected concentrations. This may suggest that, even if it may be incorrect to use creatinine or specific gravity for pooled samples, this does not strongly affect the results and yields to the same conclusions.

Finally, the concentration in pools has been assumed to perfectly represent the mean of concentrations from all spot samples. That is, we assumed no additional error due to the pooling process. Such an error could arise from chemical reactions in mixed urine, and from instrumental imprecision and technician manipulation during the pooling process.[272,274,271] The actual data on phenols could be used to empirically improve knowledge on pooling error, e.g. by comparing the mean of concentrations in unpooled samples and the concentration in the related pooled sample taking into account other sources of error such as the analytical (assay) error. However, this corresponds to another large analysis, which was not done during this thesis. This is an area which requires further investigation.

7.3.1.2 Confounding

Confounding can limit inferences drawn from our study in Chapter 4. Confounding can occur when an extraneous factor – the *confounder* – modifies the association between the exposure and outcome considered.[264] The confounder is not an intermediate factor, i.e., it does not mediate the effect of the exposure on the outcome.[264]

In Chapter 4, efforts were made to limit this bias. In EDEN cohort, a lot of information on potential confounding factors were collected, which limits the risk of unmeasured confounding factors. Potential confounding factors and predictor variables were identified based on the *a priori* knowledge. Some of these covariates were not retained in the final model. This was justified by the fact that the number of covariates (and associated categories) was much higher than the number of events divided by 10 in the case of the survival models or higher than the population size divided by 10 for the linear regression models, which is a "safe" limit for the number of terms to include in regression models.[125] However, when added in the model, these covariates did not influence the estimated measure of association by more than 5%. This suggests that they had no strong confounding effect. Some confounding factors such as a pregestational diabetes were removed thanks to the study design by restricting the EDEN eligible population to non-diabetic subjects. Diabetes is an endocrine disorder, and hence a potential confounder, as it could influence both the maternal exposure to

phenols and phthalates during pregnancy (proxy of fetal exposure in our study), for example via metabolic modifications;[257,62,313] and the respiratory health outcomes, for instance by increasing, in the offspring, the risk of neonatal complications, and the morbidity in general, including an increased pulmonary morbidity and a higher risk of health impairments.[333,277,89,30] Socioeconomic status (SES) is a complex factor and residual confounding may still occur in spite of adjustment using the highest parental educational level as proxy, as we do not know to which degree the use of this factor can address the confounding. However, most women were highly-educated, nonsmoking, and Caucasian, which limits the influence of potential unmeasured SES-related confounding factors or unmeasured co-exposures.

Maternal pre-pregnancy body mass index (BMI) and gestational duration were not controlled for in our analyses. This was done to limit the risk of overadjustment bias,[270] as these factors may be on the biological pathway between phenols and phthalates exposure during pregnancy and respiratory health in the offspring. Indeed, in a few studies, the exposure to several phenols and phthalates has been associated with changes in lipid metabolism, BMI, and with gestational duration.[258,305,182,343,180,247,39,196,184] Results remained unchanged despite any additional adjustment for maternal pre-pregnancy BMI, gestational duration (several codings tested), or exclusion of preterm births. This suggests that the risk of overadjustment would have been limited if we had chosen to control for these factors in the analyses.

In the FEV_1% analysis, we chose to adjust for height and age of boys in the final linear regression models because there was a risk of residual confounding, although that information was taken into account in the equations to calculate the percent predicted values.[251] First, our study population might differ from the reference population used for estimating the reference values and the GLI equations, and adjusting for age and height would avoid any residual effect of these variables in the percent predicted values. Second, the use of FEV_1% values does not mean that all age- or height-related effects are removed. For example, a younger (or smaller) child is more likely to have difficulties to maintain flow during the test and has smaller absolute lung volumes.[38]

Potential residual confounding may also exist through the multicenter nature of EDEN cohort, particularly due to variables that vary spatially, such as socioeconomic factors, smoking, diet, childcare access, and sources of aeroallergens. Levels of several phenols and phthalates (bisphenol A, triclosan, ethylparaben, MiBP and MBP) differed statistically between our two centers, as well as the asthma rate, which was higher in Poitiers than in Nancy. However, in exploratory analyses, there was no effect modification driven by differences between the two centers: the estimates from stratified analyses had the same direction, and there was no statistical evidence that phenols and phthalate associations differed between Nancy and Poitiers (p for interaction above

0.3, analyses not detailed in Chapter 4). In order to limit the potential for residual confounding from (measured or unmeasured) spatially-varying factors, in addition to center, we controlled for the type of area of living (rural/urban/city-center) in our models. In the EDEN population, this additional adjustment has been shown to limit residual confounding when the unmeasured confounding factors were correlated with the type of residential area, and not to bias the results, even without correlation.[239]

We cannot exclude confounding from an unmeasured factor. For instance, we did not have data on maternal or fetal metabolism. However, metabolic disorders, which can result in excessive variations in metabolism, were likely limited in our study; e.g. known diabetes before pregnancy was one of the exclusion criteria at the cohort enrollment. Also, the proportions of included women with gestational diabetes, gestational hypertension and preeclampsia were low, and exploratory analyses excluding these women did not modify the effect estimates.

7.3.1.3 Selection bias

Selection bias is a distortion of the measured association due to the effect of exposures on outcomes that differs between the studied population – *selected subjects* – and the source population – *eligible subjects* –.[264,130] For Hernán et al.[130], selection bias results from conditioning on common effects, contrary to confounding bias resulting from common causes of exposure and outcome. This means that exposure and outcome of interest can be conditionally associated because of a common effect on a third factor, which is used for the selection of the population. This bias can arise from selection criteria (e.g., population selected within strata of the common effect), or from loss to follow up, if the exposure, the health outcome and/or any factor influencing them have an impact on either the selection of subjects, the risk of being lost to follow up, or the risk of having missing data.[264,130]

Phenols, phthalates and respiratory endpoints (Chapter 4)

Our study is nested in EDEN cohort, which has a prospective design. Therefore, factors influencing the enrollment in the cohort are not expected to introduce selection bias, contrary to a retrospective setting.[264] Phenols and phthalates were assayed in maternal urine of boys if the offspring had a complete follow-up until age three.[244] Mothers of offspring included in our study differed from excluded women on several characteristics: for instance, included women were more likely to be from Poitiers, to have a high-school diploma or more, not to smoke during pregnancy. This may introduce bias if factors influencing the selection affect the exposure or the outcome as well. For example, if exposure levels are more strongly associated with respiratory outcomes in excluded women, we are likely to underestimate the associations in our

study sample. A follow-up until 5 years of age and at least an acceptable spirometric test were required for inclusion in the spirometry analysis. Selection bias may occur due to a possible overrepresentation of children well-trained for spirometric tests i.e., those with respiratory disorders. This may attenuate the associations estimated in our study, except if the selected children are more sensitive to endocrine disruptors.

The proportion of missing data was relatively high, with 277 out of 587 included boys (47%) who had at least one missing value in a covariate. Analyses of complete cases would have strongly reduced the sample size causing a substantial loss of statistical power and precision, with enlarged standard errors and confidence intervals.[188] Also, bias may arise because of the selection of a very specific respondent population in complete cases analyses, which can differ from the nonrespondents.[188] This is the reason why we imputed missing values.[117,335] We used a multiple imputation method (chained equations) that allows inclusion of auxiliary variables in the imputation model that are not in the final analyses, and which imputes missing data with actual values.[337] Multiple imputation methods rely on the *missing at random* (MAR) assumption. The missing-data mechanism is considered MAR when the probability of missingness is not completely random, and may depend on the observed data, but not on the unobserved data.[188,155,117] Compared with *missing completely at random* (MCAR), the MAR assumption is more realistic as some patterns of missingness were observed in our data, with the missingness related to some observed variables. Also we maximized the chance that this assumption holds by adding auxiliary variables, used as predictors of missingness for the multiple imputation. We cannot exclude that some data were *not missing at random* (NMAR), which may limit the validity of the multiple imputation approach. However, we chose simpler (binary) coding for most variables, such as postnatal smoking. In this case, this may limit the risk of nonresponse by the heaviest smokers (NMAR mechanism), as smoking and the number of smoked cigarettes were two distinct questions. Additionally, we chose to use the educational level instead of household income as a more reasonable proxy of the SES status, since income is expected not to be missing at random if subjects with high income are less likely to give information on their income.[335] Using simple imputation yielded to very similar results but slightly narrower confidence intervals. The simple imputation method considered imputed values as real observed values, hence, artificially more precise estimates were expected. This suggests also that results would likely have been very close if we had used other existing methods for multiple imputation.

Focusing on one sex was a way to avoid any issue related to bias (selection or confounding biases), at the cost of a loss of information regarding effects in the other sex. Phenols and phthalates assays and analyses of the associations with respiratory outcomes in girls are planned in EDEN projects led by the team of Isabella Annesi-Maesano

(Inserm, UMR-S 1136, Epidemiology of allergic and respiratory diseases Department, Paris, France). Additionally, effects of prenatal exposure to phenols and phthalates on respiratory health in the offspring will be studied in other populations such as those of SEPAGES cohort and the Human Early-Life Exposome (HELIX) project.[327]

Within-subject variability of urinary phenols during pregnancy and empirical validation of a within-subject pooling approach (Chapters 5 and 6) In these studies, the representativeness of the overall French population was not sought (also the case of the EDEN cohort), and is unlikely to be achieved. Little is known on the exposure levels in pregnant women assessed through multiple biospecimens, but having a limited number of included women has the advantage to limit the variability due to inter-subject metabolic differences.

Most included women shared similar sociodemographic characteristics, e.g., a high educational level (undergraduate college degree or above), no smoking during pregnancy, primiparity. This might have reduced between-subject variance in exposure levels if these factors are associated with exposure levels, compared to analyses on a more heterogeneous population. Thus, it is safer to assume that the ICCs and variability patterns that we observed were culture- and population-specific.

7.3.1.4 External validity (generalizability)

External validity relates to how research findings can be generalized to source populations or to other target populations, with other characteristics of studied subjects, or from another time period.[264,263]

The study population in Chapter 4 is likely to be more educated and included more nonsmoking mothers than the source population of the EDEN cohort (Table 4.2). This suggests cautious generalizability of our findings to the source population. Additionally, the EDEN population, relying on two recruitment centers (Poitiers and Nancy) was not meant to represent the entire French population, and was more educated than the national population of pregnant women,[131] which may lead to concerns about the generalizability to all French pregnant women. However, this relates to the statistical inference and it does not mean that our results are not generalizable in terms of scientific inference or biological relationships.[263] Representativeness is not needed to estimate an effect of an exposure on an outcome. On the contrary, having a more homogeneous population can enhance the validity of the study, even though an overall effect estimated on a representative population sample may not apply to every subgroup.[263] Naturally, generalizing our results beyond the first five years of life would be unwarranted, since respiratory symptoms can progress differently across the lifecourse of individuals, with major changes during childhood and adolescence likely

due to genetic and environmental factors.[5,133,114] For example, childhood wheezing and asthma can persist, aggravate later in life, or in other cases, are only transient with a spontaneous regression during childhood or adolescence, but can also reappear later in adulthood. Since nonmonotonic dose-responses are expected with EDs,[315] findings may also depend on exposure levels, which could result in possibly stronger, weaker, or even inverse associations with other ranges of exposure levels. Our focus on male offspring impeded drawing inferences about female offspring. However, in the context of endocrine disruption, there are *a priori* biological reasons to consider boys and girls separately.[136]

Regarding our studies from Chapters 5 and 6, our population was probably not representative of all pregnant women. As pregnancy is a particular physiological stage, our findings are not generalizable to non-pregnant populations, but our aim was precisely to study exposure assessment in this specific population. As seen in Chapter 6, having this population-specific information should be highly valuable for further analyses on SEPAGES cohort.

7.3.2 Sources of random error

One major source of random error influencing the precision of the estimates is sample size.[264]

Our population from the SEPAGES-feasibility study, in Chapters 5 and 6, is limited to a small number of women who collected a sample of all their urine voids (n=30) during three weeks, a study design which was very cumbersome. Indeed, almost half of the population did not give information for all (missed and collected) urine voids. We drew the protocol of analyses so as to answer several key research questions related to exposure assessment to phenols in the most efficient manner. Budget constraints constituted an hindrance for increasing the number of biospecimens; women; or compounds (e.g., including phthalate measurements) in our analyses (we only had the budget to analyze around 330 samples for one family of compounds). Restricting our analyses to one single aim would have improved precision of the estimates by increasing the number of relevant samples per woman or by included additional women. For example, Fisher et al.[103] examined the within-day variability of bisphenol A and phthalate metabolites, by relying on a larger number of women (n=66) who collected a sample of all their urine voids over one single day. This approach is more adapted than ours in terms of precision of ICCs for characterizing the within-day variability of biomarker concentrations, but it does not allow characterizing the temporal variability over one week. On the contrary, our approach was intended to characterize this between-day variability. Including additional women in our study population would have introduced bias since the not-included-women had a higher rate of missing voids. Moreover, 30

women is not a small sample size when thinking in terms of repeated biospecimens, nor are our included populations of eight and 16 women in Chapter 5 and 6. Indeed there were approximately 1,500 and 3,000 collected biospecimens which were used for pools before phenol assays for the eight and 16 women, respectively.

For the study on respiratory health (see Chapter 4), the sample size was limited, first, by the number of pregnant women recruited in 2003-2006; and second, by the number of mother-child pairs included in three previous studies,[245,244,79] since the maternal urine sample was assayed for phenols in this preselected population only (n=604). Despite inevitable losses to follow up or withdrawals, we included a high rate of eligible subjects in our study (n=587, 96% of this population), which is the second largest study in terms of sample size, behind Smit et al.[286] on this research question. In their study, Smit et al.[286] did not investigate the effects of phenols and relied on a single maternal blood sample for exposure assessment, which may be highly subject to measurement error[64,165] due to very short elimination half-lives from the blood compartment.[237,164,160,303,147,148] The sample size was strongly reduced for the FEV_1% analyses, as having spirometric measures depended on the participation in a clinical examination at 5 years of age; and on a spirometric test matching the acceptability criteria defined by the European Respiratory Society (ERS), including an expiration time longer than one second.[38]

In Chapter 4, we chose to report all results without formally testing statistical significance or correcting for multiple comparisons, increasing the risk of chance findings (type I error).[33,135,132,34] However, for certain epidemiologists, strict significance testing and correction for multiple tests should not always be done in the context of epidemiological research.[262,241,261,310,118] For example, for Rothman[262], reporting results corrected for multiple comparisons can be seen as considering that data are only random numbers, implying that no associations are expected to be real. This does not take into account *a priori* (biologic) knowledge, behind the scientific hypothesis. Using a multiple correction method increases also type II error, i.e., the risk of having nonsignificant test results when associations are real.[262,241,261] As a result, publication bias, i.e. when studies with statistically significant or clinically positive results are more likely to be published than others,[283,152] may be increased because of a reduced representativeness if only significant results are published after multiple-testing correction.[118] In addition, relying on significance tests rises the issue of providing a dichotomous response to a research question (e.g., the estimation of a dose-response relationship) which may be better answered in quantitative terms (estimation of the effect size and measurement precision) using confidence intervals.[262,241] Hence, our findings should be considered with caution, as those of an exploratory approach aiming to estimate the effects of *in utero* phenols and phthalates exposure on child's respiratory health in general.

7.3.3 Study design and statistical methods

Phenols, phthalates and respiratory endpoints (Chapter 4)

Contrary to a cross-sectional design, our prospective design respects the temporality, which is relevant in attempting to make valid inferences.

For associations between prenatal exposure to phenols and phthalates and questionnaire-based respiratory outcomes, we chose to use a survival model and not binomial regression. Binomial regression models ignore temporal variations in outcome risk and potential confounders (time-varying covariates), and do not efficiently accommodate subjects lost to follow up in the context of a prospective cohort study. Conversely, survival models are more appropriate to take into account the entire information available in a cohort study, including time-varying covariates and censoring. More specifically, our survival model allows incorporation of time-varying adjustment factors. For example, postnatal passive smoking cannot have an effect on the risk of respiratory disease before the age when it starts, just like exposure to furry pets before the age of pet arrival in the home (the latter variable was not included in the final model but its effect has been tested). Moreover, 136 subjects (for the analysis of wheezing outcome) were lost to follow up before the end of the five years of follow-up. With binomial regression, when one considers a cohort with a long-term follow-up, the information on missing questionnaires (used for the definition of the outcome) or dropout cannot efficiently be taken into account, implying to exclude subjects lost to follow up, resulting in possible reduced statistical power and possible selection bias. Survival models and, in particular, discrete-time models in the case of an interval-censored follow-up, are designed to properly handle censoring, and allow to take all the available information into account without reducing the size of the studied population.[154] In addition to their theoretical superiority, survival models were theoretically and empirically shown to be more efficient with long observation times or when the probability of the event changes over time,[15,116,177] even with identical censored times.

Compared to the other approaches, one issue for our survival model could arise from the complexity of asthma diagnosis in early life that can hide the true onset of the disease.[133] Asthma being an heterogeneous disease, its diagnosis is based on the history of poorly specific and sensitive clinical symptoms, as well as a possible physical examination. This can create a lag between the first occurrence of symptoms and the diagnosis, and thus limit the reliability of regression estimates for the asthma outcome. Although it was investigated in adults and the extent of generalizability to children is unknown, the year of asthma diagnosis (onset) was found to be fairly accurate when reported by subjects in questionnaires, which may limit this issue.[311]

For a follow-up of five years, findings using our survival approach and binomial regression (i.e., logistic regression) tended to be similar, although p-values sometimes

differed. Also, using modified Poisson regressions (log-link), suggested as a more suitable approach than logistic regressions to estimate relative risks in longitudinal studies for outcomes with a high prevalence,[360] led to results which were very close to both those from our survival approach and from a logistic regression model.

One strong assumption in our discrete-time survival model, just like for the Cox model,[82] is the proportionality of hazards, i.e., the hazard functions remain proportional over time.[158] To assess the validity of our model, we checked the proportional hazards assumption by including predictor (i.e. independent variable) by time interaction effects for each predictor in the model, and we tested the statistical significance for difference between hazard ratios within each interval.[158] There was no evidence of nonproportional hazards, suggesting that proportionality holds over time for all variables.

Finally, our survival analysis may be more sensitive to confounding than binomial regression. Indeed, a covariate X associated with the outcome only at a given time t may become a confounding factor at time $t+1$ because of a selection phenomena over time, changing the association between X and the exposure. Subjects with a higher risk of developing the outcome are less likely to be in the population at risk at time $t+1$, and hence there may be an association between the factor X and the exposure in the remaining population, turning X into confounder.

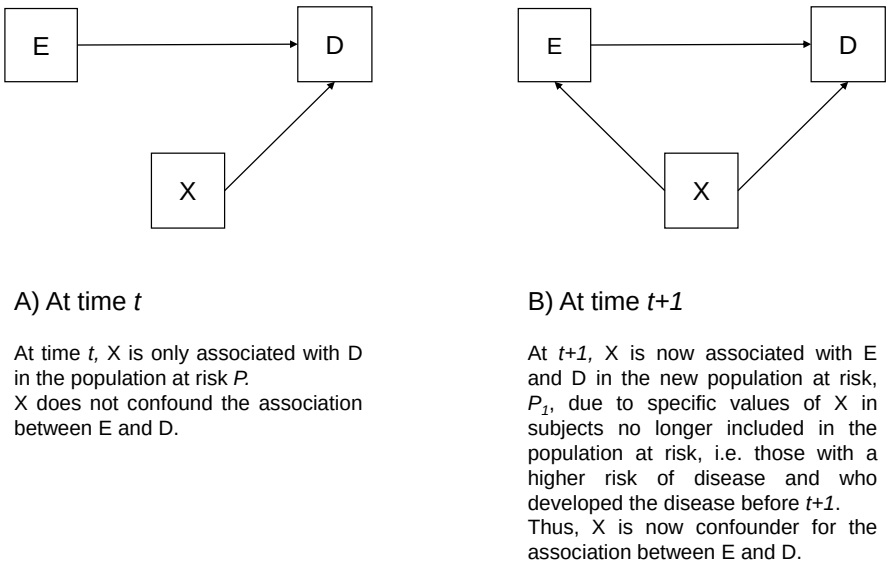


Figure 7.1 – Survival analysis and confounding

Within-subject variability of phenols (Chapter 5)

To estimate the within-day variability of phenols, our analysis relied on the limited number of two subjects. Therefore, while they would have allowed to take into account the subject-specific variability, hierarchical models (including a random effect

for both the subject and day variables) could not be used due to convergence issues and variance components estimates equal to zero. However, we centered phenol concentrations around the woman-specific weekly mean before analysis, which probably limits the subject-specific residual variability. Both one-way random-effect ANOVA and mixed models with a random intercept (maximum likelihood estimations) can be used to calculate ICCs.[253] In the supplementary material (Chapter 5, Tables 5.8-5.9, and 5.11), we showed that estimates using the maximum likelihood models were very close to those from the ANOVA model. Additionally, when a large portion of the measured concentrations was below the LOD (e.g., for benzophenone-3 and triclosan), models were more susceptible to extreme values that would have artificially reduced the ICCs, due to more homogeneity between women, and hence, a proportionally larger within-subject variability. Thus, we are very cautious when interpreting our findings for these two compounds.

Empirical validation of a within-subject pooling approach (Chapter 6)
By a log-transformation, we attempted to approximate a normal distribution of the biomarker concentrations. Unfortunately, the normality of the distribution was likely limited by the small number of samples in each comparison analyses. Therefore, the agreement parameters (correlation coefficients, Kappa coefficients, and t-tests) should be considered with caution. In the empirical replication of the study from Perrier et al.[242], we proposed to limit the effect of data clustering (the study relied on 8 women) by drawing a normal distribution using the EDEN compound-specific means and standard deviations. This might not represent the real distribution of repeated samples if our population had been larger, and the normal distribution does not take into account the absence of concentration information below the LOD. However, the goal was to empirically replicate the study from Perrier et al.[242], which assumed a normal distribution of the concentrations for their two chemicals. Additionally, the number of biospecimens required to reduce the attenuation bias to 10% or below was estimated using the mean of 8 biospecimens as pregnancy average exposure. These numbers are likely to be underestimated, since the pregnancy average exposure should rely on many more urine biospecimens. The underestimation might be stronger for low ICCs, which could explain the great consistency between our results and those from Perrier et al.[242] about methylparaben (four and six biospecimens were required to reduce bias below the 10% threshold in this thesis and in Perrier et al.[242], respectively), in spite of a mild difference in ICC values (0.85 and 0.6, respectively); whereas results were less consistent for bisphenol A (18 required biospecimens in this work versus 35 in the theoretical study of Perrier et al.[242]) for nearly the same difference between ICCs (0.38, versus 0.20 in Perrier et al.[242]).

To conclude, in Chapter 4, our attempts to limit the impact of confounding, selection of subjects and information biases, gave rather confidence in our findings. However, one essential source of error, which we have largely highlighted in Chapters 5 and 6, arises from the exposure assessment. In the case when the hypothesis of classical type error holds, the high within-subject variability of nonpersistent chemicals, may have largely biased the estimates towards the null, for a continuous exposure variable.

The analyses carried out in Chapters 5 and 6 would benefit greatly from a greater sample size. Our population being highly selected, the generalizability is most likely limited, but it does not impact the internal validity.

In the next chapter, we conclude this thesis and we suggest perspectives for future research.

Chapter 8

Conclusion and perspectives

8.1 Conclusions

In this thesis, we were interested in the exposure during pregnancy to two families of nonpersistent EDs, their temporal variability and their effects on childhood respiratory health. Chapter 4 is one of the few prospective studies on this topic with a relatively large sample size compared to previous studies. It is also the first study investigating effects of phenols other than bisphenol A in a longitudinal setting. We provided limited evidence of adverse effects of prenatal exposure to certain phenols and phthalates on respiratory health until 5 years of age in our population of boys. Prenatal exposure to some other phenols and phthalates tended to reduce the risk of respiratory symptoms or illnesses. One of the major limitations of this study was probably exposure assessment, as shown in the other studies of the thesis. This is also a major concern for almost all the entire published research on the topic, since the studies relied on one to three biospecimens. We have confirmed that attenuation bias is likely a consequence of this reliance on a small number of biospecimens (Chapter 6).

Our study, aiming at characterizing the within-subject variability of phenol urinary biomarkers during pregnancy (Chapter 5), had the significant advantage of relying on a comprehensive collection of urine voids over several weeks in pregnant women, while previous studies on variability generally relied on two to three random spot samples.[37,50,51,119,153,204,246,294] To the best of our knowledge, no previous published study had such detailed data. The temporal variability of phenols within a given day was shown to be very high for all studied phenols while the daily average exposures within a week were much less variable. Variability of weekly average exposures was found to depend on the considered chemical. Hence, this variability is expected to result in measurement error that may bias estimates in epidemiological studies on the effects of such chemicals on human health. Therefore, the common approach, which is relying on the collection of few random spot biospecimens over the pregnancy, is

probably inefficient. It is imperative to consider new sampling designs to estimate the average exposure to such chemicals over time windows of interest.

Chapter 6 aimed at evaluating the efficiency of a sampling design relying on the within-subject pooling of a small number of repeated samples. The degraded within-subject pooling of three instead of all daily biospecimens was efficient to estimate exposure to nonpersistent chemicals over time windows of weeks or more and to a lesser extent of days, with very few exceptions. Additionally, when assuming a classical-type error, at least a few dozen of biospecimens were needed to strongly limit the attenuation bias for chemicals with very high temporal variability, confirming the validity of such an approach. Also, using external estimates of ICCs may lead to inefficient correction for measurement error with the *a posteriori* disattenuation method. This suggests that having an internal estimate of the ICC, even from only a subgroup of the population should be preferred.

This thesis provides knowledge on exposure assessment to nonpersistent chemicals, and attempts to propose an achievable solution to disentangle the exposure assessment from measurement error due to within-subject variability. We propose in Figure 8.1 a few sampling designs, which, based on the results of this thesis, may be efficient strategies for exposure assessment to nonpersistent chemicals in future studies, over long (e.g., the whole pregnancy, trimesters of pregnancy) and short (e.g., specific weeks and days of pregnancy) time windows of interest. Based on ICCs and our results from the simulations, sampling designs highlighted in blue may be strategic choices leading to the best result with the minimal number of biospecimens, as they cover a wide range of situations (chemicals and/or exposure windows of interest). Contrary to compounds with high ICCs and for which relying on a reduced number of samples (collected in a short time window) may be enough to have a reasonable exposure assessment, if chemicals have low ICCs, the more information we have, i.e. the more biospecimens are collected, the better the design.

8.2 Perspectives

In this section, avenues for future research are proposed to address the limitations encountered in this thesis, and to make attempts for filling the gaps in this research field.

From a statistical point of view, a prospective design with cohort recruitment at the earliest stage of (or before) pregnancy and long-term follow-up of the offspring is likely the most relevant design to investigate the effects of prenatal exposure to phenols and phthalates and draw valid inferences. It allows the prospective collection of data on various exposures, outcomes, and on numerous potential confounding factors to limit

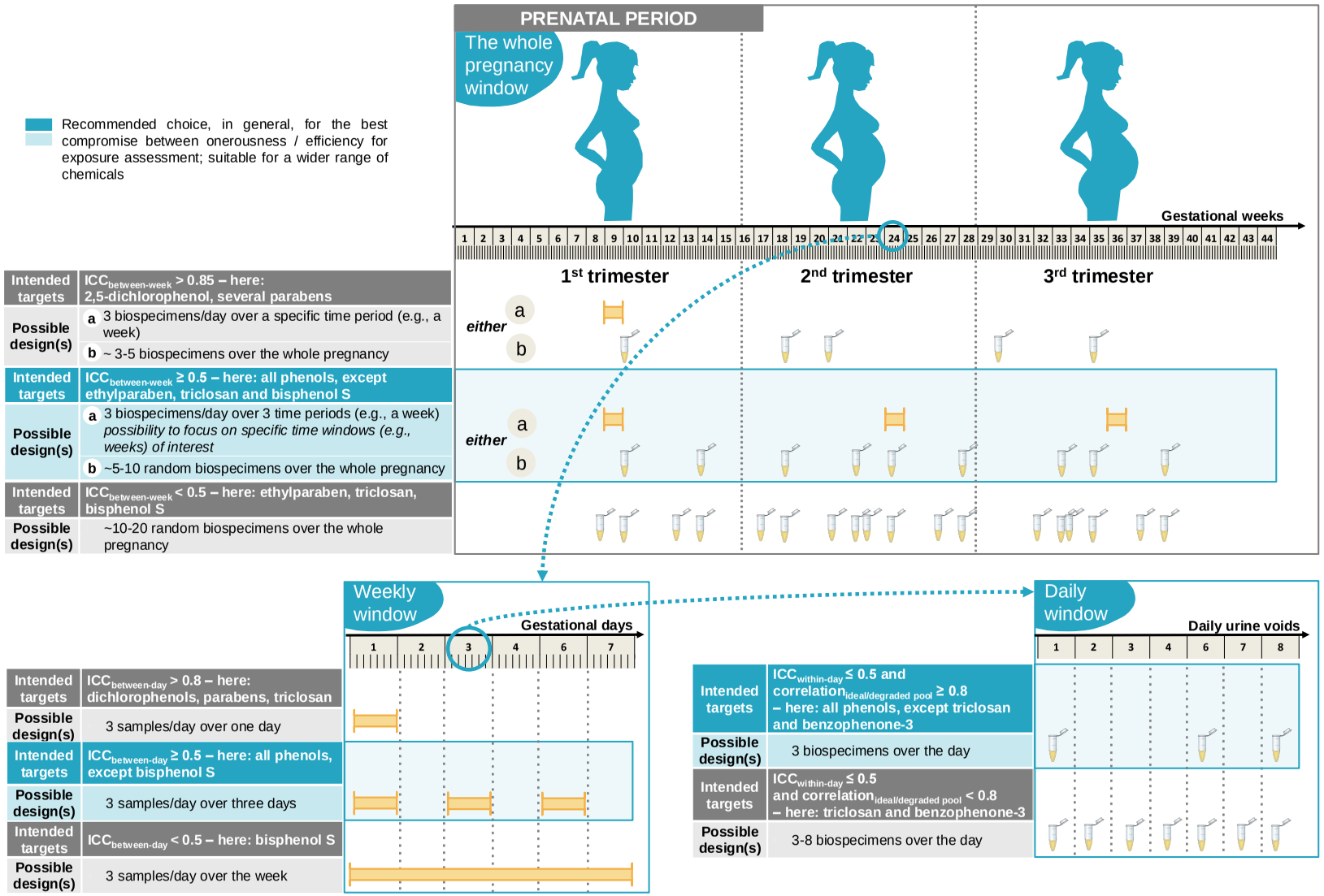


Figure 8.1 – Sampling designs for exposure assessment to nonpersistent chemicals during pregnancy (for exposure windows such as the whole pregnancy, trimesters, specific weeks and days) and drawn according to the results of this thesis.

confounding bias, with respect of the temporality between exposures and outcomes.[57] Studies with very early recruitment, at the beginning or even before pregnancy are particularly relevant to investigate the effects of periconceptional environmental exposures on human reproduction and development. Examples include, e.g., the Longitudinal Investigation of Fertility and the Environment (LIFE) Study[55,56] and the Environment and Reproductive Health (EARTH) Study.[208,128] Web-based periconceptional prospective studies such as the Boston University Pregnancy Study Online (PRESTO) [1] are also particularly relevant in this context.

The accuracy of future studies on the effects of prenatal exposure to nonpersistent EDs on respiratory health may be optimized by improving the exposure assessment. The need is to collect the right number of samples at the right time, i.e., the toxicologically-relevant window of susceptibility. Increasing the number of biospecimens to be assayed for chemicals is a way to improve exposure assessment. For compounds with low temporal variability (e.g., methylparaben), we showed in our last study that collecting at least 4 samples during pregnancy, which is easily achievable, significantly limits the attenuation bias, when a classical measurement error is assumed (see Figure 8.1 for a summary of potential relevant designs for biospecimen collection). However, if one is interested in evaluating the effects of much more variable chemicals (e.g., bisphenol A), a few dozen biospecimens are needed, which is obviously more cumbersome for participants and is likely to increase overall costs of the study and participants' dropout. One interesting approach to reduce assays costs is the within-subject pooling of biospecimens. Even within-subject pooling of a small number (e.g., three daily biospecimens) of repeated biospecimens allowed a reasonably good estimate of the average exposure to nonpersistent chemicals with low and high temporal variations (Chapter 6), and studies could benefit from this relatively light design without increasing analytical costs. Such a design is achievable, and currently used for exposure assessment in SEPAGES cohort including 484 parent-child trios [2]. It would be interesting that further research evaluates the existence and the impact of pooling error which could add valuable knowledge to this recent development, since studies on this topic are based on simulations.[272,274] Further studies should also consider assaying chemicals in some individual repeated biospecimens in a sample of the participants to evaluate the variance of biomarkers in individual samples and to calculate ICCs. Hybrid (unpooled and pooled) designs can combine the advantages of both designs.[272] Other approaches such as regression calibration or SIMEX methods could also be considered in epidemiological studies on the health effects of phenols and phthalates to correct for measurement error when several biospecimens are assessed for exposure biomarkers.[109,29,67,108]

1. http://sites.bu.edu/presto/
2. http://sepages.inserm.fr/en/home/

A large sample is required for the evaluation of multiple outcomes with low expected relative risks. Since statistical power will not necessarily increase monotonously with improved exposure assessment, the optimal trade-off between sample size and precision per subject is difficult to define, which is why further variability studies using multiple and not only three biospecimens, with characterization of the impact on dose-response relationships are warranted.

To investigate the effect on lung function, further studies could benefit from spirometric tests later in life (e.g., around 8 years of age), when most children are able to have acceptable flow-volume curves and therefore measures less prone to error. Characterizing the effects of prenatal exposure on the evolution of the lung function over the lifecourse is also highly relevant. If one is interested in lung function in preschool children, using more suitable parameters (e.g., $FEV_{0.5}$ or $FEV_{0.75}$) should provide more usable information.[38,243,230] Lung functions tests in neonatal period using tidal breath analysis could be considered to explore the effects of phenols and phthalates in very early life, as currently done in SEPAGES cohort. Ongoing (e.g., MeDALL,[16,47] HELIX,[327] EXPOsOMICS,[322] or new international collaborations/projects could be relevant options to greatly increase sample size (and thus the statistical power), and better characterize the impact of environmental chemicals such as phenols and phthalates.

From a scientific point of view, characterizing the effects of prenatal exposure to environmental pollutants on respiratory health is relevant. Longitudinal studies in human population could provide information on possible pathways that remain to be clarified, e.g., inflammation process, immunomodulation, oxidative stress, epigenetic changes, or gene-environment interactions.[284,325,322,327] This would allow the identification of immune or inflammatory markers and gene polymorphisms that could modify or mediate the associations.[248] Additionally, such studies could provide information needed to identify possible susceptible populations, and critical windows of sensitivity (possible links with the different stages of lung development).[207] Such information would be helpful to define more accurately the health effects to be expected in the offspring. Additional studies, investigating metabolic mechanisms (including experimental or animal studies) are needed to understand more deeply the behavior of these nonpersistent chemicals in the human body, and to characterize possible specific (gestational or pre-gestational) conditions that could influence their metabolism during pregnancy. Additionally, biomarkers in non-invasive fetal biological matrices, such as the meconium or neonates' hair are interesting to get close to the fetal exposure.[312,187,141,340,246]

The endocrine system controls physiological functions by hormonal peaks and variations, and feedback mechanisms. Therefore, further studies may also explore, instead of average exposure, the impact of acute exposures, as well as the impact of variations

in exposure levels, showing the relevance of repeated sampling and assays in order to provide some answers to this research question.

Collecting exposure data in the offspring in prospective cohorts would be helpful to disentangle the effects of pre- and postnatal exposure on health in childhood.

Also, more development to identify asthma biomarkers would be highly valuable to improve sensitivity and specificity of the outcome definition in epidemiological studies and physicians could benefit from this progress for diagnosis and management of the disease.

Long-term follow-up cohort studies, i.e. with repetition of outcomes assessment – *lifecourse epidemiology* – should provide information on the progression of symptoms.[174] Puberty and adolescence are accompanied by many physiological (hormonal), physical and behavioral changes, which may modify the natural history of respiratory symptoms and diseases,[107,236] and may modify the effect of environmental exposures (e.g., EDs) on respiratory health. Consequently, a follow-up until this key period, and later, until adulthood, would be of great interest.

Longitudinal studies should also include exposure assessment to emerging chemicals (e.g., bisphenols F, AF used as substitutes for bisphenol A, di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH®) used as phthalate alternative), with hundreds of them being potential EDs.

Contrary to toxicological studies, for which environment and exposures are controlled, in an observational setting, such that of most epidemiological studies, humans are exposed to many environmental agents, which can interact with each other, and with identical or different targets in human organisms. Therefore, understanding the effects of mixtures of pollutants is also warranted, especially in the case of EDs. Indeed, the population is concurrently exposed to compounds that are both agonists and antagonists of same receptors. This may result in complex dose-response relationships (e.g., non-monotonic) for EDs or mixture of pollutants.[315] Advanced statistical methods can be used to take into account multiple exposure, including environment-wide association studies (EWAS) selection methods, multivariate regression–based statistical methods, sparse partial least squares, and elastic net (reviewed in Agier et al.[6]). Further developments are requested for correlated exposures. In this context, tackling the challenge of assessing multiple exposures simultaneously (the concept of *exposome*) and examining their impact on human health is particularly relevant.[325] Several projects have been launched to that purpose. For example, the HELIX project,[327] the EXPOsOMICS project,[322] the HEALS[3] and HERCULES[4] projects aim to develop and provide a better understanding of the effect on the exposome on health.

3. http://www.heals-eu.eu/
4. https://emoryhercules.com/

Finally, from a public health point of view, quantification of exposure levels and identification of the different exposure sources in various susceptible populations (e.g., pregnant women, infants, children) are required for risk assessment and regulatory decisions. The SEPAGES feasibility study design is particularly dedicated to identify main exposure sources in pregnant women, as it relies on the comprehensive collection of urine voids in participants as well as an intensive data collection on the exposure sources (e.g., use of personal care products, dietary lifestyle and food intake, pharmaceuticals) for each study day. A study about subject's behaviors and the associations with biomarker concentrations in urines is currently under way in our group. Reproducing such studies in other populations (e.g., non French pregnant women, men and children) may improve knowledge on exposure levels and sources in the different populations.[221,49]

From such studies and animal pharmacokinetic research, physiological based pharmacokinetic (PBPK) modeling, which integrates the physical and biological characteristics of a chemical with the body physiological functions to predict internal dose (or exposure through reverse dosimetry) in target tissues/organs,[217] could be developed and adapted in the context of pregnancy.[2] Some models already exist for a few phenols or phthalates (e.g., bisphenol A, parabens, DEHP),[278,3] however they are poorly used in the epidemiological setting. Some PBPK models have been adapted to the context of pregnancy[200] and this approach is planned to be used in the HELIX project for some compounds (e.g. DEHP)[327] but PBPK models are lacking for many phenols and phthalates in the context of pregnancy. Further research in this area could help to fill the gaps, which pose a challenge for regulatory decision making, as (i) most decisions are based on tolerable daily intakes (TDIs), minimal risk levels estimated from uncertainty-corrected (interspecies extrapolation) no observed adverse effect levels (NOAELs) or lowest observed adverse effect levels (LOAELs) and because (ii) low dose effects and non-monotonic dose responses are expected for EDs.[193,315]

To conclude, we have contributed to show that the road for human cohorts of a new type (so called *third generation birth cohorts*[285]), with strongly improved assessment is now open.

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Appendix A

Sources of phenols and phthalates

Their uses in the manufacture of daily-life products is widespread.

A.1 Phenols

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane) is a high production volume chemical employed in polymer plastics (polycarbonate) and epoxy resins. The main uses of bisphenol A include food and beverage containers (plastic packaging, can coating), printed thermal papers, CDs and DVDs, toys, medical equipment, water pipes coating, and dental resins and sealants.[113,11,54,104,20,41]

Bisphenols F, S and AF are other bisphenols with uses mainly similar to those of bisphenol A. Bisphenol F is used in epoxy resins and thermal printer paper, while bisphenol S is produced for polycarbonate and polyesthersulphone materials, as well as for epoxy and polyester resins.[17,42,82]

Phenolic compounds include also benzophenone, parabens, triclosan and dichlorophenols. Parabens are esters of p-hydroxybenzoic acid which are antifungal and antimicrobial preservatives. They are present for these properties in cosmetics, personal care products, pharmaceuticals and food.[66,12,18,13]

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is another phenolic compound used as an antimicrobial agent in personal care products, including consumer antiseptic wash products (antibacterial soaps, body and mouth washes), toothpastes, deodorants, as well as in household cleaners, clothing, kitchenware and toys.[64,78,9,110]

Benzophenones include several derivatives (e.g., benzophenone-2, and oxybenzone, also called benzophenone-3) of benzophenone, a high production chemical. They are used as flavor additive for diet in beverages, soft candy, and in industrialized baked goods.[43] They are also used for other products such as plastics, coating, insecticides, agricultural chemicals and pharmaceuticals. In addition, benzophenones are present as ultraviolet (UV)-blockers in clear plastic or glass packaging, sunglasses, and in sunscreeens.[64] They can be found in inks, paints, varnishes and personal care products (e.g. soaps, nail polishes, lotions, fragrances) or household and laundry cleaners to prevent damaging of colors and scents.[81,77]

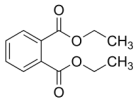
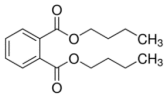
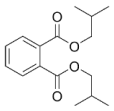
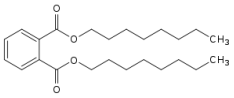
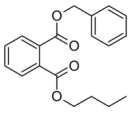
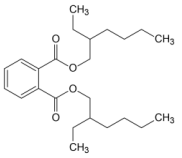
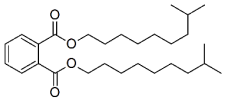
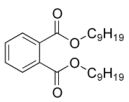
Dichlorophenols are intermediates in the industrialized synthesis of chlorinated chemicals, such as pesticides and herbicides. Hence, dichlorophenols can be present in the environment (drinking water, ambient air) as byproducts of the chlorinated disinfection, and as degradation products of some pesticides. Additionally, 2,4-dichlorophenol can be formed from triclosan transformation in the environment or the organism. 2,5-dichlorophenol is also a derived compound of paradichlorobenzene employed in moth balls (banned from this use in European Union) and room deodorizers.[77,125]

A.2 Phthalates

Phthalates, also known as esters of phthalic acid (see Table A.1), include many industrial chemicals produced in high volume. These compounds are mainly used as plasticizers, i.e. used to soften plastics, to increase their transparency, their flexibility and durability. These plastics have a wide range of usages, which include polyvinyl chloride (PVC) for consumer products such as shoes, clothing, toys, packagings; for household and car building materials (e.g., wire and cable, floor and wall coverings, synthetic leathers and fabrics), and in medical devices. Additionally, non-plastic uses exist, with some phthalates being components of solvents (for inks and paints), adhesives, personal care products (e.g. soap, nail polish, lotion, fragrances) and excipients in pharmaceuticals.[63,26,122]

They can be classified on the basis of their molecular weight, as high and low molecular weight phthalates (HMW and LMW phthalates). Some of the most common HMW phthalates (ester side-chain lengths of five or more carbons) include di-2-ethylhexyl phthalate (DEHP), di-isodecyl phthalate (DIDP) and di-isononyl phthalate (DINP); and LMW phthalates (one to four carbon atoms on the ester side-chain) include diethyl phthalate (DEP), dibutyl phthalate (DBP), and di-isobutyl phthalate (DiBP).[79]

Table A.1 – Main phthalates studied in this thesis, with their chemical formula and uncomprehensive list of exposure sources.

Phthalate (abbreviation)	Molecular formula	2D Structure	Main uses and products
Diethyl phthalate (DEP)	$C_{12}H_{14}O_4$		- Plasticizer for food packaging, automobile products, toys, artificial turf, toothbrushes, rubber - Solvent for fragrances and colors in personal care products, adhesives, detergents, sealants, and pharmaceuticals.
Di-n-butyl phthalate (DnBP)	$C_{16}H_{22}O_4$		-Plasticizers for PVC products, shoes, rain coats, shower curtains, coating manufacturing, toys, nail polishes, fingernail elongators -Solvents for adhesives, paints, printing inks, sealants, binding agents, personal care products, pharmaceuticals and food supplements.
Di-isobutyl phthalate (DiBP)	$C_{16}H_{22}O_4$		
Di-n-octyl phthalate (DNOP)	$C_{24}H_{38}O_4$		- Plasticizer for soft and flexible plastics: medical devices, wire, cables, floor tiles, art supply - Solvent for personal care products, laundry detergent, baking soda, pesticides
Butyl-benzyl phthalate (BBzP)	$C_{19}H_{20}O_4$		- Plasticizer for PVC floor and wall covering, food conveyor belts, automobile products - Solvent for paint binders, glues and adhesives, mouldable sealants, art supply
Di-2-ethylhexyl phthalate (DEHP) or Dioctyl phthalate (DOP)	$C_{24}H_{38}O_4$		- Plasticizer for soft and flexible PVC floor and wall covering, toys, electronics, plumbing, shoes, plastic packaging material, medical devices, automobile products, art supply - Solvent for paints, inks, lacquers, adhesives, sealants, paper
Di-isodecyl phthalate (DIDP)	$C_{28}H_{46}O_4$		- Plasticizer for soft and flexible PVC for polymer-related uses: and non-PVC uses (e.g., rubbers, toys, plastic packaging material (including food packaging), and other material as substitute for DEHP - Solvent for paints, inks, lacquers, adhesives, sealants
Di-isononyl phthalate (DINP)	$C_{26}H_{42}O_4$		- Plasticizer for soft and flexible PVC for polymer-related uses: and non-PVC uses (e.g., rubbers, toys, plastic packaging material (including food packaging), and other material as substitute for DEHP - Solvent for paints, inks, lacquers, adhesives, sealants

Appendix B

Toxicokinetics

B.1 Phthalates

B.1.1 HMW phthalates

In human toxicological studies, following oral and dermal exposure, DEHP, DnBP DEP are rapidly absorbed and their metabolites reportedly appear in blood within 0.5-1 h. Major metabolites are primary metabolites (simple monoesters) with peak concentrations around 2 h post exposure, except for the monoester of DBP, monobutyl phthalate (MBP) which reaches its maximum level in blood 4 h after dermal exposure.[87,60,61,58,1,56,48,46] Plasma elimination half-lives of simple monoesters are very short (below or around 2 h) in most studies.[87,60,61,56] Secondary metabolites tend to reach their maximum levels later, with a longer elimination half-life from plasma.[61] Metabolites are mainly in their unconjugated form.[60,56]

Primary and secondary metabolites are excreted rapidly in the urines, mostly within 24 hours post exposure. For DEHP, around 50% of the administered dose is excreted in urine within 24-48 h via its monoester MEHP and up to four secondary metabolites, with 90% of the metabolites excreted within 24 h.[87,96,60,61,56,3,4,73] In all studies, compared to secondary metabolites, MEHP only represents a small fraction of the excreted dose (6-7%), with a shorter terminal elimination half-life (4-6 h). Elimination half-lives for secondary metabolites tends to be longer, between 6 and 24 h depending of the metabolites, the longest being for carboxy-metabolites.[60,61,56,4,65] Majority of metabolites are in their conjugated forms, predominantly the glucuronidated conjugate.[87,4,65,73] This is expected since the phase II conjugation facilitates urinary excretion of the metabolites by increasing water solubility. Carboxy secondary metabolites are the least conjugated species with almost 50% in the free form.[73]

Regarding other HMW phthalates, DINP roughly follows similar metabolic behavior than DEHP. The monoester MINP is the fastest to appear in urine with a peak concentration around 2 h post dose;[4,59,65] has an excretion half-life between 3-8 h; and only represents up to 3% of the excreted dose. On the contrary, secondary metabolites are in majority in the urine, with excretion half-lives probably longer since there are still detected after 24h (not the monoester), especially the carboxy metabolites. Around 32.9-43.6% of the administered amount is excreted within 48 h.[4,59,65] Glucuronide conjugates is the major form for metabolites.[4,65]

B.1.2 LMW phthalates

For LMW phthalates, the elimination is quite different. Due to their shorter alkylchains, their monoester are more hydrophilic and require less metabolic biotransformations before being excreted in urines. Thus, after oral or dermal administration of DBP, DEP, DnBP and DiBP in human volunteers, the excretion of metabolites is faster than for HMW phthalates and, simple monoesters are the predominant forms.[3,48,46,97,58,73] Additionally, MEP, with a shorter side-chain, is mostly excreted in its free form while MBP needs to be conjugated and its excretion is slower.[48,46,73] Excretion half-lives for monoesters are between 1.9 and 6 h, and between 2.9 and 6.9 h for the secondary metabolites.[97,58,73] The longest half-life is for the carboxy-metabolites similarly to HMW phthalates.[73] While almost all an oral dose is excreted via urines within 24 h after the exposure, only a small fraction of a topic application is excreted in urine within 24 h (6-13%).[58,73,46]

Difference observed in excreted fractions of total administered amount between compounds and routes of exposure (oral / dermal) might be due to discrepancies in metabolism and elimination. The lipophilicity of phthalates is varying with the alkylchain length. High lipophilic compounds such as DINP might undergo more complex oxidation pathways with the addition of several functional groups, generating metabolites not measured in the reported studies.[62,99,98] Also, distribution in other compartments needs to be further studied in humans since it can exist. For example, some phthalate metabolites, MEHP MEP and MiBP have been found in sweat samples of several humans. The excretion may have occur through sweat pathway from the systemic circulation, or the presence is sweat may indicate a release of these phthalates from storage sites such as adipose tissue.[29] Excretion via feces might also be non-negligible for some phthalates.[23,71]

B.2 Phenols

B.2.1 Parabens

Following oral or topical administration, parabens are readily absorbed through skin and the gastrointestinal tract to undergo first-pass metabolism in the liver, in the skin or in subcutaneous fat tissue.[111,123] Parabens are hydrolyzed via esterases, to their main but nonspecific metabolite, *p*-hydroxybenzoic acid (PHBA). Another fraction can be absorbed without hydrolysis (parent parabens) and will be mostly conjugated to glycine, glucuronide and sulfate to be excreted in urines.[111,123,19] The nonspecific metabolites also undergo conjugation, with glucuronide, sulfate and predominantly with glycine (*p*-hydroxyhippuric acid, PHHA). Parent parabens can additionally undergo additional oxidation reactions, which generate oxidized new metabolites, as for phthalates.[119,74] Minor excretion can also occur via bile and feces.[111] Additionally, it has been reported that the metabolic profile of parabens may depend upon the exposure route.[103,123]

For dermal exposure, the absorption is partial, and skin penetration decreases with increased ester chain length of parabens.[48,111] Additionally, permeation of the skin and therefore absorption of parabens is influenced by the formulation of personal care products,[70,57,88] the repetition of dermal applications[45,19] or a damaged skin.[86]

Based on *in vitro* and *in vivo* studies, between 30 and 50% of the administered dose is absorbed.[6,88,5,80] However, real life behaviors such as repeating topical application, increasing the time of contact, have been shown to increase permeation.[22] Also human skin properties differs from rat skin (slower hydrolysis).[34,86] Very few human studies investigated the toxicokinetics of parabens in humans. In 26 male Caucasians, dermally applied butylparaben readily penetrated the skin and was detectable in blood within 1 h, reached a peak concentration at 3 h post dose and decreased thereafter but did not reach baseline level after 24 h.[48] Urine metabolites were detected within 8-12 h, mostly as the glucuronidated conjugate and 1.5-2.1% as the free parent butylparaben. Only 0.9% of the administered dose was recovered in urines but nonspecific PHBA and PHHA, nor the sulfate conjugate were measured in the study, that could explain the low excreted fraction.[46]

After oral exposure, it is slightly different with quick and almost complete absorption and excretion (> 80% of the dose, predominantly in the first 24 h) in humans[74] as in animals.[5,111] Methylparaben, *iso*-butylparaben, and *n*-butylparaben metabolites, appear with a peak concentrations within 2 h post dose.[74] PHHA represents 60% of the metabolites, and PHBA only 3-7% for all parabens. Metabolic profiles differ between methylparaben and the two butylparabens, presumably due to difference in the ester chain length as for phthalates.[48,46,13,58] Parent methylparaben, more hydrophilic than butylparaben, requires less biotransformation to be excreted, and represents 17.4% of the administered dose (7.1% of free methylaparaben). On the contrary, parent *iso*-butylparaben and *n*-butylparaben, more lipophilic, represent less than 7% (below 1% for the free form). Additionally, unhydrolyzed methylparaben preferentially undergoes sulfate conjugation (64%), contrary to glucuronide conjugation for *iso*- and *n*-butylparabens (almost 90%). Finally, unlike for methylparaben, oxidized metabolites are non-negligible for the two butylparabens with a more complex ester side chain, as suggested for some phthalates.[58] Terminal elimination half-lives are brief, between 2.5 and 6.9 h for methylparaben, with the longest for the parent compound. For the other parabens, half-lives are relatively similar between the parent compound and the metabolites (3.6-3.7 h for *iso*- and *n*-butylparabens). Similar fractions of glucuronide and sulfate conjugates have been reported in biomonitoring studies,[123] suggesting that saturation or inhibition of the enzymes responsible of the conjugation may not occur at environmental exposure levels.[47]

Regarding other parabens the human literature is lacking. Based on the experimental evidence ethyl and propylparabens are also rapidly absorbed and excreted in urines, mainly as their nonspecific metabolites conjugated with glycine. Their ester chain length are between those from methyl and butylparabens.

B.2.2 Benzophenone-3

Toxicokinetics data are limited, especially in humans. From existing literature, benzophenone-3 partially penetrates the skin readily after a dermal exposure, in proportion of up to 10% of the applied dose, based on experimental data and human studies.[51,37,36,47,49,31,30,95] Benzophenone-3 can be found in the stratum corneum 30 minutes after a topical application, and systemic absorption occurs within 1-2 h after exposure.[47,49,95] Also, concomitant uses of an insect repellent with a benzophenone-3 containing sunscreen can enhance the percutaneous absorption of the chemical.[55] Benzophenone-3 undergoes phase I and phase II metabolic reactions in the body, as

the other phenols and phthalates. Major phase I reactions in rats and humans are hydroxylation and demethylation,[84,83,121,120,124,76] which generates several specific metabolites such as 2,4-dihydroxylbenzophenone (namely benzophenone-1) a major metabolite, 2,2'-dihydroxy-4-methoxybenzophenone (namely benzophenone-8), and 2,3,4-trihydroxybenzophenone (in trace amounts).[95,121,53,50] All metabolites and the unchanged benzophenone-3 can be excreted in urine in various forms such as glucuronide and sulphate conjugates after phase II glucuronidation or sulfation.[120,124] Conjugates are predominant.[84,37,30] Urine is the main excretion route followed by feces,[52,84,83] with terminal excretion half-life of 15.9 hours in rats.[52] Elimination half-life is unknown in humans.

Proportion of free form has been found to be related to the exposure levels, with the smallest fraction of the free form for the highest exposure levels.[120] Additionally, discrepancies were reported between proportions of benzophenone-3 and its derivatives in urines of Chinese and U.S. individuals.[120] Benzophenone-3 parent form was predominant in the U.S. urine samples (almost entirely as glucuronide conjugate) while in Chinese urines, derivatives were found in majority. It might be due to population-related differences in metabolism or to different sources and routes of exposure in the two populations since direct sources of exposure to benzophenone-3 derivatives exist via cosmetics or food packaging.[15,2,14]

B.2.3 Triclosan

Orally administered triclosan was found to be rapidly and relatively completely absorbed in the gastrointestinal tract in humans as in other species (rodents, monkeys, dogs), with maximum concentrations in plasma within 6 h after exposure (see Rodricks et al.[92], The 2010 Cosmetic Ingredient Review Expert Panel (CRI)[110] for review). Absorption is similar whether it be by oral dose ingestion or by swallowing with use of triclosan-containing toothpaste,[33,32] but it is more limited (up to 10%) when using mouthwash or toothpaste without swallowing.[67,68] Triclosan chronic exposure (daily use of triclosan-containing mouth hygiene products) does not seem to induce triclosan accumulation.[94,8,7,25,68] Percutaneous absorption of triclosan is more limited with only up to 10% of the applied dose recovered in the body in plasma or urine within 24 h after application (Queckenberg et al.[91], and several studies reviewed in Rodricks et al.[92], The 2010 Cosmetic Ingredient Review Expert Panel (CRI)[110]). Phase I metabolism is minor for triclosan (e.g. generation of 2,4-dichlorophenol in small amount), which is almost completely metabolized as glucuronide and sulfate conjugates trough first-pass metabolism in the liver. Contrary to other species (dogs, mice) the glucuronide conjugate is predominant (77-90%) in plasma and urine in humans (reviewed in Rodricks et al.[92]). Following dermal exposure, triclosan metabolism can occur in the skin.[75] The excretion of triclosan metabolites occurs mostly via the urine within 72 h (up to 87% of the administered dose) and to a lesser extent via feces.[69,106,16,112,105] The elimination half-life in humans is of 10-20 h after oral or dermal exposure[92,91] and can rise up to 1.4 days when repeating dermal application. All metabolites return to baseline levels within 7 days following single or chronic exposure, suggesting no accumulation of triclosan in humans.

It is noteworthy that the proportion of the sulfate conjugate in the plasma compared to the proportion of the glucuronide conjugate tends to increase with increasing the administrated levels of triclosan while it never occurs in urines.[68,69] On the one hand, this may suggest either saturation of the glucuronide conjugation or induction

of sulfation; and on the other hand this may also suggest that prior excretion there is conversion to the glucuronidated conjugate in the kidney or increased reabsorption of the sulfate conjugate in renal tubules to get back to the initial ratio.[92]

B.2.4 Dichlorophenols

To our knowledge, there are very few toxicokinetics data of dichlorophenols in humans. 2,5-dichlorophenol is the major metabolite of the paradichlorobenzene[38,39,85] and 2,4-dichlorophenol is a minor metabolite of triclosan.[92] Based on *in vitro* and animal experimentations, dichlorophenols or their precursors are readily absorbed from the gastrointestinal tract, the skin or the respiratory tract.[44,77] Once in the body, dichlorophenols are rapidly metabolized by phase I and mainly phase II reactions and are found preferentially in a conjugated form.[39,102,124,125,77] In humans the glucuronide is presumably the main metabolite, with 89% against 8% as sulfate conjugate and only 3% in the free form in urine samples of adult volunteers.[124] Other metabolites include dichloromethoxyphenols,[102] and based on an *in vitro* study on human cytochrome P450 metabolism, other metabolites such as 2-chloro-1,4-hydroxyquinone, 2-chloro-1,4-benzoquinone and 1,2,4-hydroxybenzene can also be detected.[72] In rats, metabolites are rapidly distributed in tissues (within 15 minutes after dosing); mainly in liver and kidney and also in brain, muscle, fat and spleen and blood.[102,35,89] In mammals, no accumulation was reported. Rapid decreases of concentrations in tissues are followed by the excretion of metabolites, primarily through the urine (80-90%), and to a lesser extent, in feces, within 24 h to several days.[102,101,39]

B.2.5 Bisphenols

After an oral exposure, bisphenol A is readily absorbed from the gastro-intestinal tract, and undergoes intensive first-pass metabolism through the liver (see Figure B.2.5), which results in almost entire conjugation of the chemical in glucuronide and sulfate conjugates within few hours.[117,116,118,108,109] In serum, parent bisphenol A and its conjugates reach their maximum concentrations within 1-2 h following ingestion of the dose, and all the species are shortly eliminated from blood, with serum elimination half-lives of 4-7 h.[117,108,109] Although bisphenol A may accumulate in adipose tissue[28,24] and low concentrations in liver, brain, and sweat have been reported,[28,29] bisphenol A species are rapidly and primarily cleared from the blood by the kidney into the urine in humans and non-human primates, with almost 100% of the administered dose recovered in the urines within 24 h as total (free and conjugated) bisphenol A.[107,93,115,108,109] Terminal elimination half-life in urine is very short (1-5 h).[117,116,108,109] Only a small fraction of the administered dose is excreted as free bisphenol A (<2%) while between 3-15% of the dose is recovered as sulfate conjugate, and the major metabolite is the glucuronide form (85-87%). Other minor metabolites (e.g. bisphenol A-bis-sulfate) can be found in blood and urine samples.[109,108]

The fraction of free bisphenol A in serum and urine, as well as its time course in the body may be influenced by the route of exposure which can bypass, for a certain amount of the dose, the hepatic phase II conjugations.[10,90,114] In dogs, sublingual exposure with transmucosal absorption in the oral cavity was found to lead to higher internal levels of free bisphenol A than absorption through the gastro-intestinal tract after gavage.[27] Similarly, slow chewing of diet compared to brief swallowing appear to result

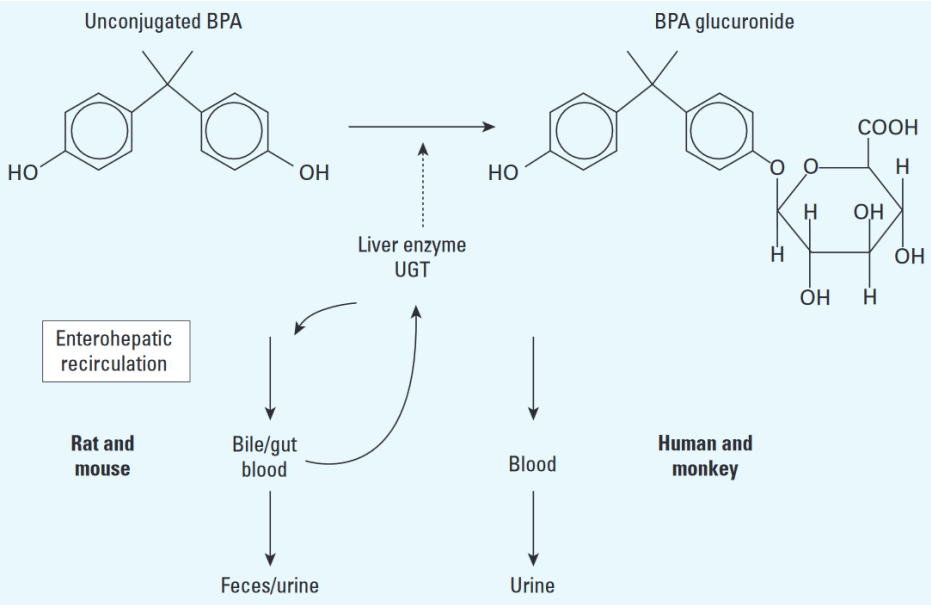


Figure B.1 – Schematic diagram depicting the glucuronidation of bisphenol A in the liver and the route of elimination of unconjugated bisphenol A from serum in rodents and primates after initial absorption from the gut and transport to the liver. Extracted from Taylor et al.[107].

in more unconjugated bisphenol A in serum and urine in non-human primates[93,107] and humans.[117,116,109,108] Also, dermal exposure and percutaneous absorption of bisphenol A, e.g. from the handling of thermal receipt paper may increase unconjugated internal bisphenol A levels in serum or urine.[40,21]

Toxicokinetics data on bisphenol A alternatives are scant, but an *in vitro* study suggests that bisphenol A alternative, bisphenol S has a metabolism similar to that of bisphenol A. Glucuronidation may be the primary metabolic pathway for bisphenols.[100]

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Appendix C

Attenuation factor in linear regression and ICC

Effects of classical measurement error are the loss of statistical power and bias in regression estimates.

C.1 Simple linear regression

The classical additive measurement error model states that $W = X+U$, where X is the true exposure, and W the surrogate of exposure measured with error $U \sim \mathcal{N}(0, \sigma_u^2)$, U being independent of X.

The simple linear regression of an outcome Y (e.g., health parameter) on the true exposure X is given by:

$$Y = \beta_0 + \beta_1 X + \epsilon, \tag{C.1}$$

with the Ordinary Least Squares (OLS) estimate of β_1

$$\beta_1 = \frac{\mathrm{Cov}(X, Y)}{\mathrm{Var}(X)} \tag{C.2}$$

When exposure is measured with error, the linear regression model is now

$$Y = \beta_0 + {\beta_1}^* W + \epsilon^*, \tag{C.3}$$

with the OLS estimate

$${\beta_1}^* = \frac{\mathrm{Cov}(W, Y)}{\mathrm{Var}(W)} = \lambda\beta_1, \tag{C.4}$$

where λ is the attenuation factor. Because $W = X + U$,

$$\begin{aligned} {\beta_1}^* &= \frac{\mathrm{Cov}(X+U, Y)}{\mathrm{Var}(X+U)} \\ &= \frac{\mathrm{Cov}(X, Y) + \mathrm{Cov}(U, Y)}{\mathrm{Var}(X) + \mathrm{Var}(U)} \\ &= \frac{\mathrm{Cov}(X, Y)}{\mathrm{Var}(X) + \mathrm{Var}(U)} \end{aligned} \tag{C.5}$$

when X is independent of U, and U independent of Y.

Hence the attenuation factor is,

$$\begin{aligned}\lambda = \frac{{\beta_1}^*}{\beta_1} &= \frac{\frac{\mathrm{Cov}(X,Y)}{\mathrm{Var}(X)+\mathrm{Var}(U)}}{\frac{\mathrm{Cov}(X,Y)}{\mathrm{Var}(X)}} \\ &= \frac{\mathrm{Var}(X)}{\mathrm{Var}(X)+\mathrm{Var}(U)} \\ &= ICC\end{aligned} \tag{C.6}$$

C.2 Multiple linear regression

Considering the case of additional covariates Z in the model and measured without error, the model for X is:

$$Y = \beta_0 + \beta_1 X + \beta_z Z + \epsilon \tag{C.7}$$

and the model using exposure measured with error is now:

$$Y = \beta_0 + {\beta_1}^* W + {\beta_z}^* Z + \epsilon^* \tag{C.8}$$

From Carroll et al.[1], ${\beta_1}^* = \lambda_1 \beta_1$, with

$$\lambda_1 = \frac{\sigma^2_{x|z}}{\sigma^2_{w|z}} = \frac{\sigma^2_{x|z}}{\sigma^2_{x|z} + \sigma^2_u}, \tag{C.9}$$

with $\sigma_{x|z}$ the residual variance of the regression of X on Z, and $\sigma_{w|z}$, the residual variance of the regression of W on Z.

$\lambda_1 = \lambda = ICC$, only when X and Z are not correlated.

Otherwise, considering the regression of X on Z given by:

$$X = \gamma_0 + \gamma_1 Z + \nu \tag{C.10}$$

we have the OLS estimate for the regression coefficient of one covariate Z

$$\begin{aligned}{\gamma_1}^* &= \frac{\mathrm{Cov}(Z,X)}{\mathrm{Var}(Z)} \\ &= \frac{\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)}{\mathrm{E}(Z^2) - \mathrm{E}(Z)^2},\end{aligned} \tag{C.11}$$

with E the expectations.
Hence,

$$\begin{aligned}\mathrm{Var}(\nu) = \sigma^2_{x|z} &= \mathrm{Var}(X) - \gamma_1^2 \mathrm{Var}(Z) \\ &= \mathrm{Var}(X) - \left[\frac{\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)}{\mathrm{E}(Z^2) - \mathrm{E}(Z)^2}\right]^2 \mathrm{Var}(Z) \\ &= \mathrm{E}(X^2) - \mathrm{E}(X)^2 - \left[\frac{\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)}{\mathrm{E}(Z^2) - \mathrm{E}(Z)^2}\right]^2 (\mathrm{E}(Z^2) - \mathrm{E}(Z)^2) \\ &= \frac{[\mathrm{E}(X^2) - \mathrm{E}(X)^2][\mathrm{E}(Z^2) - \mathrm{E}(Z)^2] - [\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)]^2}{\mathrm{E}(Z^2) - \mathrm{E}(Z)^2}\end{aligned} \tag{C.12}$$

and so,

$$\begin{aligned}\lambda_1 &= \frac{\sigma^2_{x|z}}{\sigma^2_{w|z}} \\ &= \frac{\sigma^2_{x|z}}{\sigma^2_{x|z} + \sigma^2_u} \\ &= \frac{[\mathrm{E}(X^2) - \mathrm{E}(X)^2][\mathrm{E}(Z^2) - \mathrm{E}(Z)^2] - [\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)]^2}{[\mathrm{E}(X^2) - \mathrm{E}(X)^2 + \mathrm{E}(U^2)][\mathrm{E}(Z^2) - \mathrm{E}(Z)^2] - [\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)]^2}\end{aligned} \tag{C.13}$$

Since

$$\begin{aligned}ICC &= \frac{\mathrm{Var}(X)}{\mathrm{Var}(X) + \mathrm{Var}(U)} \\ &= \frac{\mathrm{E}(X^2) - \mathrm{E}(X)^2}{\mathrm{E}(X^2) - \mathrm{E}(X)^2 + \mathrm{E}(U^2)}\end{aligned} \tag{C.14}$$

the relation between ICC and λ_1, in the presence of a covariate Z, is

$$\begin{aligned}\lambda_1 &= \frac{{\beta_1}^*}{\beta_1} \\ &= \frac{[\mathrm{E}(X^2) - \mathrm{E}(X)^2][\mathrm{E}(Z^2) - \mathrm{E}(Z)^2] - [\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)]^2}{\frac{[\mathrm{E}(X^2) - \mathrm{E}(X)^2]}{ICC}[\mathrm{E}(Z^2) - \mathrm{E}(Z)^2] - [\mathrm{E}(XZ) - \mathrm{E}(X)\mathrm{E}(Z)]^2}\end{aligned} \tag{C.15}$$

As a result, the relation between λ and ICC is more complicated when considering multiple linear regression.

In general, the bias due to measurement error is not restricted to X regression estimate; the regression estimate for Z is also biased, except when Z and X are independent.[1]

C.3 Appendix references

[1] Carroll, R. J., Ruppert, D., Stefanski, L. A., and Crainiceanu, C. M. *Measurement Error in Nonlinear Models: A Modern Perspective*. 2 edition. Boca Raton: Chapman and Hall/CRC, June 21, 2006. 484 pp.

Appendix D

Publications and communications

D.1 Publications and communications related to the thesis

D.1.1 Accepted articles

Vernet C, Pin I, Giorgis-Allemand L, Philippat P, Benmerad M, Quentin J, Calafat AM, Ye X, Annesi-Maesano I, Siroux V*, Slama R*, and the EDEN mother-child cohort study group. In utero exposure to select phenols and phthalates and respiratory health in five-year-old boys: a prospective study. Environ Health Perspect 2017;125:9. Available on: https://ehp.niehs.nih.gov/wp-content/uploads/2017/09/EHP1015.alt_.pdf.

*Co-last authorship

Vernet C, Philippat P, Calafat AM, Ye X, Lyon-Caen S, Siroux V, Schisterman E, Slama R. Within-day, between-day and between-week variability of urinary concentrations of phenol biomarkers in pregnant women. Environ Health Perspect 2018;126:3. Available on: https://ehp.niehs.nih.gov/wp-content/uploads/2018/03/EHP1994.alt_.pdf.

D.1.2 Article in preparation

Vernet C, Philippat P, Agier L, Calafat AM, Ye X, Lyon-Caen S, Siroux V, Schisterman E, Slama R. An empirical validation of the biospecimens within-subject pooling approach. [*In preparation*]

D.1.3 Oral communications

Vernet C, Philippat P, Agier L, Lyon-Caen S, Siroux V, Slama R. An Empirical Validation of the Biospecimens Within-subject Pooling Approach. 3rd Early Career Researchers Conference on Environmental Epidemiology, Freising, Mar. 2018.

Vernet C, Pin I, Giorgis-Allemand L, Philippat P, Benmerad M, Quentin J, Calafat AM, Ye X, Annesi-Maesano I, Siroux V*, Slama R*, and the EDEN mother-child cohort study group. Prenatal Exposure To Select Phenols And Phthalates And Pulmonary Function In Five-Year Old Male Offspring. 2nd Paris Workshop on Endocrine Disruptors Effects on Wildlife and Human Health, Paris, Jan. 2016.

Vernet C, Pin I, Giorgis-Allemand L, Philippat P, Benmerad M, Quentin J, Calafat AM, Ye X, Annesi-Maesano I, Siroux V*, Slama R*, and the EDEN mother-child cohort study group. Prenatal Exposure To Select Phenols And Phthalates And Pulmonary Function In Five-Year Old Male Offspring. 2nd ISEE Early Career Researchers Conference on Environmental Epidemiology, Utrecht, Nov. 2015.

Vernet C, Pin I, Giorgis-Allemand L, Philippat P, Benmerad M, Quentin J, Calafat AM, Ye X, Annesi-Maesano I, Siroux V*, Slama R*, and the EDEN mother-child cohort study group. Prenatal Exposure To Select Phenols And Phthalates And Pulmonary Function In Five-Year Old Male Offspring. Congress of the International Society for Environmental Epidemiology, Saõ Paulo, Aug-Sep. 2015.

D.1.4 Poster communication

Vernet C, Philippat C, Siroux V, Lyon-Caen S, Pin I, Lorimier P, Calafat AM, Ye X, Schisterman E, Slama R. Empirical validation of a within-subject pooling approach to improve accuracy of estimation of exposure to biomarkers with strong temporal variations. Congress of the International Society for Environmental Epidemiology, Rome, Sep. 2016.

D.2 Other publications

Slama R, Vernet C, Nassan FL, Hauser R, Philippat C. Characterizing the effect of endocrine disruptors on human health: The role of epidemiological cohorts. Comptes Rendus Biologiques 2017.

Soomro MH, Baiz N, Philippat C, Slama R, Siroux V, Vernet C, Bornehag CG, Annesi-Maesano I, and the EDEN Mother-Child Cohort Study Group. Prenatal exposure to phthalates and the development of eczema phenotypes in male children: Results from the EDEN mother-child Cohort study. Environ Health Perspect 2018. Available on: https://doi.org/10.1289/EHP1829.

Exposure to nonpersistent endocrine disruptors during pregnancy using biomarkers of exposure: Within-subject variability and effects on respiratory health in the offspring.

ABSTRACT:
Phenols and phthalates include chemicals widely used in daily-life products, resulting in ubiquitous exposure of the general population. There is growing concern regarding the effects on human health of these compounds, suspected to be endocrine disruptors, particularly during early life. Epidemiological research on the health effects of phenols and phthalates in offspring generally rely on a few biospecimens to assess exposure. These studies are limited by the possibly strong within-subject variability, which may result in exposure misclassification. The within-subject variability in the context of pregnancy and its possible impact on dose-response functions are poorly characterized.
The aim of this thesis was to study the exposure to several phenols and phthalates during pregnancy by: 1) investigating the possible associations between this exposure and respiratory outcomes in childhood; 2) characterizing the temporal within-subject variability of these compounds during pregnancy; and finally 3) studying the efficiency of a within-subject pooling approach using a small number of daily biospecimens for exposure assessment.
Associations between exposure to phenols and phthalates and respiratory health relied on n = 587 mother-child pairs from the French EDEN prospective cohort. Developments about the assessment of exposure during pregnancy relied on n = 16 pregnant participants of the SEPAGES-feasibility study who had collected all their urine samples for three weeks.
This work quantified the within-subject variability of phenol and phthalate biomarker concentrations during pregnancy over various time scales (day to months), and confirmed empirically that this variability is likely to strongly bias the dose-response functions in human-based epidemiological studies exploring the effects of gestational exposure to these chemicals.
This thesis adds to the emerging literature on respiratory health impacts of early-life exposure to several phenols and phthalates. However, as for most studies on the human health effects of phenol and phthalate exposure, it is potentially challenged by this exposure assessment issue. Thus, this work emphasizes the relevance of more elaborate sampling strategies for exposure biomarkers in future epidemiological studies. These results have relevance for studies outside the context of pregnancy, and also for other nonpersistent compounds. New designs, such as the within-subject pooling of biospecimens validated in this study, are needed so as to efficiently characterize the health impact of nonpersistent chemicals.

Keywords: *endocrine disruptors; phenols and phthalates; childhood respiratory health; prenatal exposure, within-subject temporal variability; exposure measurement error.*

Ph.D carried out at the Institute for Advances Biosciences: research center Inserm U 1209, CNRS UMR 5309, University Grenoble Alpes; F-38700 La Tronche, France.

Estimation de l'exposition à des perturbateurs endocriniens non persistants pendant la grossesse : Variabilité intra-individuelle et effets sur la santé respiratoire de l'enfant.

RESUME :
Les phénols et les phtalates incluent des composés très largement utilisés dans des produits de la vie quotidienne. Une grande partie de la population générale y est donc largement exposée. Ces composés sont suspectés d'être des perturbateurs endocriniens et des effets sur la santé chez l'Homme ont été rapportés, notamment après une exposition périnatale. Les études épidémiologiques sur les effets sur la santé humaine reposent généralement sur un faible nombre de biospécimens pour estimer l'exposition. Cependant, la variabilité intra-individuelle des phénols et des phtalates est potentiellement forte, ce qui peut entraîner une mauvaise classification de l'exposition dans les études sur les effets des phénols et des phtalates et limite leurs conclusions. La variabilité intra-individuelle des phénols et des phtalates au cours de la grossesse n'est pas très bien caractérisée à l'heure actuelle.
L'objectif de cette thèse est d'explorer l'exposition aux phénols et aux phtalates et plus précisément : 1) d'étudier les associations entre une telle exposition pendant la grossesse et la santé respiratoire de l'enfant au cours de ses premières années de vie ; 2) de caractériser la variabilité temporelle intra-individuelle de ces composés au cours de la grossesse ; et 3) d'évaluer l'efficacité d'une approche basée sur le pooling intra-sujet d'un nombre réduit d'échantillons journaliers pour estimer l'exposition.
Les associations entre l'exposition aux phénols et phtalates et la santé respiratoire reposent sur n = 587 couples mères-enfants de la cohorte prospective française EDEN. Les développements sur l'estimation de l'exposition au cours de la grossesse s'appuient sur n = 16 femmes enceintes ayant participé à l'étude de faisabilité de la cohorte SEPAGES.
Les travaux de cette thèse quantifient la variabilité intra-individuelle des concentrations urinaires des biomarqueurs d'exposition aux phénols et des phtalates au cours de la grossesse pour des échelles de temps variées (du jour à plusieurs mois). Ils confirment empiriquement que cette variabilité peut biaiser fortement les fonctions doses-réponses dans les études épidémiologiques explorant les effets de l'exposition fœtale à ces composés chez l'Homme. Les résultats de cette thèse enrichissent la littérature émergente sur les effets des expositions précoces aux phénols et phtalates sur la santé respiratoire de l'Homme. Cependant, notre étude ainsi que la plupart des recherches précédentes sont potentiellement limitées par les problématiques liées à la mesure de l'exposition. Ce travail souligne l'importance de stratégies d'échantillonnage des biomarqueurs d'exposition plus élaborées pour l'étude de ces composés dans de futures études épidémiologiques. Ces résultats sont aussi pertinents en dehors du contexte de la grossesse et pour d'autres composés non-persistants. De nouvelles approches, telles que le pooling répété pour chaque sujet d'un petit nombre de biospécimens journaliers, validé dans cette thèse, sont nécessaires pour caractériser efficacement l'impact des composés non-persistants sur la santé de l'Homme.

Mots-clés : *perturbateurs endocriniens ; phénols et phtalates santé respiratoire de l'enfant ; exposition prénatale ; variabilité temporelle intra-individuelle ; erreur de mesure de l'exposition.*

Thèse réalisée au sein du l'Institut pour l'Avancée des Biosciences: centre de recherche Inserm U 1209, CNRS UMR 5309, Université Grenoble Alpes; F-38700 La Tronche, France.