



HAL
open science

Prenatal exposure to phenols and phthalates, child neurodevelopment and the role of the thyroid hormones

Dorothy Nakiwala

► **To cite this version:**

Dorothy Nakiwala. Prenatal exposure to phenols and phthalates, child neurodevelopment and the role of the thyroid hormones. Biotechnology. Université Grenoble Alpes [2020-..], 2020. English. NNT : 2020GRALS003 . tel-03088367

HAL Id: tel-03088367

<https://theses.hal.science/tel-03088367v1>

Submitted on 26 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



THÈSE

Pour obtenir le grade de

DOCTEUR DE L'UNIVERSITÉ GRENOBLE ALPES

Spécialité : MBS - Modèles, méthodes et algorithmes en biologie, santé et environnement

Arrêté ministériel : 25 mai 2016

Présentée par

Dorothy NAKIWALA

Thèse dirigée par **Rémy SLAMA**, Université Grenoble Alpes
et co-encadrée par **Claire PHILIPPAT**

préparée au sein du **Laboratoire CRI IAB - Centre de Recherche Epigenetics, Chronic Diseases, Cancer - Institute for Advanced Biosciences**
dans l'**École Doctorale Ingénierie pour la Santé la Cognition et l'Environnement**

Exposition prénatale aux phénols et aux phtalates, développement neurologique de l'enfant et rôle des hormones thyroïdiennes

Prenatal exposure to phenols and phthalates, child neurodevelopment and the role of the thyroid hormones

Thèse soutenue publiquement le **14 février 2020**,
devant le jury composé de :

Monsieur REMY SLAMA

DIRECTEUR DE RECHERCHE, INSERM DELEGATION ALPES,
Directeur de thèse

Madame ANNE EGO

DOCTEUR EN SCIENCES, CHU GRENOBLE ALPES, Examinatrice

Madame CECILE CHEVRIER

DIRECTRICE DE RECHERCHE, INSERM DELEGATION GRAND-OUEST, Rapportrice

Madame JULIE HERBSTMAN

PROFESSEUR ASSOCIE, UNIVERSITE COLUMBIA A NEW YORK - USA, Rapportrice

Madame BARBARA DEMENEIX

DIRECTRICE DE RECHERCHE, CNRS DELEGATION PARIS-CENTRE, Examinatrice

Madame VALERIE SIROUX

DIRECTRICE DE RECHERCHE, INSERM DELEGATION ALPES, Présidente

Acknowledgements

I will start by expressing my sincere gratitude to my supervisor, **Claire PHILIPPAT**, for the unwavering support and expertise afforded to me throughout my time in the doctoral program. She believed in me right from the masters internship to the end. And on a personal level, thank you for also sharing your motherhood experiences and baby essentials.

In the same spirit, I am grateful to my thesis director **Rémy SLAMA**, the team leader of the Team of Environmental Epidemiology at IAB -UGA – INSERM U1209 (Equip 12) and Willa's biggest fan! Thank you for imparting your knowledge and expertise in to this study. I also thank the entire team 12 for all the support, specifically the Bureau "*cent trois*", and most especially Solène Cadiou for her friendship and support.

I am also grateful to the members of my CSI committee; Prof. Marc Wiesskhof, Dr. Francois Berger and Dr. Monica Baciu.

I am thankful to all my funders, the UGA doctoral school and funders of the research projects under taken during the PhD; Fondation pour la Recherche Médicale, l'INSERM, l'Institut de Recherche en Santé Publique, Nestlé, the French Ministry of Health, l'Agence Nationale de la Recherche (ANR), l'Université Paris-Sud, l'Institut de veille sanitaire (InVS), the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) and la Mutuelle Générale de l'Education Nationale.

I appreciate the managements of the different cohort studies, from which these analyses were realized, EDEN and SEPAGES, specifically Sarah Lyon-Caen. Special thanks to the volunteers and participants in these studies without them this work would have been all but an idea.

To my dearest parents, especially dad, Mr. Joseph Kimbowa, thank you for always believing in me. Siblings and friends; my sister Clare and Jackson, Kim, Peter, Matthew, Liz, Paul, Patricia, Cleo, Arnold, Patrick friends; Lyna Adhiambo, Celia Tarinyebwa, Paola and Francios Rocca, Elizabeth Gabriele, Sarah, Dior, Kola, Olivia and Blaine, Stella Nalukwago, Rose Adjei-Bempah and Brian Matsiko, thank you for the moral, spiritual and emotional support throughout my studies.

Above all, I would like to thank God almighty who has gotten me this far.

Dedication

*I dedicate this work to my Little princess **Willa Valérie Matsiko***

Abstract

Context: The increase in the prevalence of neurodevelopmental disorders, at least in some areas, warrants identification of the possible underlying modifiable risk factors. Industrial chemicals such as phenols and phthalates, which are endocrine disrupting chemicals (EDs), are frequently detected in biospecimens from pregnant women. Several of them have been linked to various adverse neurodevelopmental outcomes, at least from toxicological studies. One of the suggested mechanisms of neurotoxicity of these compounds is disruption of the thyroid hormone pathway, which plays a major role in brain development in early life. Previous research on prenatal exposure to phenolic and phthalate compounds falls short of providing: 1) an understanding of the impact of specific sources of exposure to these compounds, such as personal care products, which is necessary for interventions to reduce exposure; 2) evidence of how early life exposure to ED active phenols other than bisphenol A, such as benzophenone-3, triclosan, dichlorophenols, parabens, could influence child neurodevelopment and 3) documenting possible effects of phthalates and phenols on the thyroid hormone levels, a mechanism hypothesized to mediate neurotoxicity of these compounds.

Aims: The main aims of this thesis were 1) to document if the use of personal care products (PCP) could influence phenols biomarkers concentrations in pregnant women; 2) to assess associations between prenatal exposure to phenols and phthalates and child neurodevelopment at 3 and 5 years (cognition and behavior); and 3) to assess associations between prenatal exposure to phenols and phthalates on the thyroid hormone levels of pregnant women and their newborns.

Methods: We relied on three complementary cohorts. To characterize phenol levels following use of PCPs, our analysis depended on a panel study design of 8 women of the SEPAGES-feasibility study with multiple urine samples (total, 178) and detailed, time-resolved information on PCP use. Associations between phenols, phthalates and neurodevelopment (the cognitive function and behavior) were studied among 546 mother-son pairs of the French prospective EDEN cohort. We then relied on the SEPAGES cohort to study the effects of gestational exposure to phenols and phthalates on thyroid hormone levels among 418 and 421 women and newborns, respectively. In this study, intensive exposure assessment (2 pools of 21 spot urine samples per individual) allowed to limit exposure measurement error.

Results: *PCP as sources of exposure to phenols:* The total number of PCP applications (without distinction) was positively associated with the parabens' and bisphenol S urinary concentrations during pregnancy. These associations varied by time window. The total number of PCP applications was not associated with any of the other phenols assessed.

Phenols, phthalates and neurodevelopment: No phenol or phthalate metabolite pregnancy concentration was negatively associated with child verbal or performance IQ at 5 years. Some associations with child

behavior were observed at 3 and 5 years: Bisphenol A was positively associated with the relationship problems subscale at 3 years, with an incidence rate ratio (IRR) of 1.11; (95% confidence interval (CI): 1.03, 1.20) for each unit increase in Ln bisphenol A concentration, and the hyperactivity–inattention subscale scores at 5 years (IRR: 1.08; 95% CI: 1.01, 1.14). Mono-*n*-butyl phthalate (MnBP) was positively associated with internalizing behavior, relationship problems, and emotional symptom scores at 3 years. Monobenzyl phthalate (MBzP) was positively associated with internalizing behavior and relationship problems scores at 3 years. After dichotomizing behavioral scores, triclosan tended to be positively associated with emotional symptom subscales at both 3 and 5 years.

Phenols, phthalates and thyroid hormones levels: Regarding the associations with maternal hormones, bisphenol A was negatively associated with the Ln (TSH) z-score ($\beta = -0.05$, 95% CI = -0.15, 0.06); MBzP, a metabolite of benzylbutyl phthalate (BBP), was positively associated with the TT4 z-score ($\beta = 0.12$, 95% CI = -0.01, 0.25) and triclosan was negatively associated with the TT3/TT4 ratio z-score ($\beta = -0.05$, 95% CI = -0.11, 0.00). When biomarker concentrations were categorized in tertiles, we observed non-monotonic associations between TSH and triclosan (U-shape) and MnBP, a metabolite of di-*n*-butyl phthalate (DBP) (inverse U-shape). In newborns, only sex-specific effects were observed: bisphenol A was positively associated with the TT4 z-score in male newborns while triclosan was negatively associated with the TT4 z-score in females.

Conclusion: Findings in this study concur with previous literature that PCPs use may contribute to exposure to parabens. Our study was the first to report associations between PCP use and bisphenol S, a substitute of bisphenol A. In line with previous studies, bisphenol A and various phthalates, including DBP and BBP exposure during pregnancy, were associated adverse behavioral symptoms among boys. Our study was the first to report adverse neurobehavioral effects in relation to triclosan exposure. These results have since been replicated in one study from the USA, while another from Canada reported null effects. The four compounds associated with adverse behavioral effects in EDEN were also associated with one or more thyroid hormone levels of mothers or newborns in the SEPAGES cohort, suggesting possible disruption of thyroid hormones homeostasis.

Keywords: endocrine disruptors, phenols and phthalates, prenatal exposure, personal care products, neurodevelopment, intelligence quotient, behavior, thyroid hormones, thyroid stimulating hormone.

Résumé

Contexte : L'augmentation rapide de la prévalence de certains troubles du neurodéveloppement nécessite une meilleure compréhension des facteurs de risque modifiables de ces troubles. Les produits chimiques industriels tels que les phénols et les phtalates sont des perturbateurs endocriniens dont l'exposition est prévalente en population générale. Plusieurs études toxicologiques suggèrent des effets neurotoxiques pour ces composés. Chez l'Homme, les expositions à certains de ces composés durant la vie précoce ont été associées aux comportements et / ou aux fonctions cognitives de l'enfant. L'un des mécanismes sous-jacents à ces effets pourrait être une action de ces composés sur l'axe thyroïdien qui joue un rôle déterminant dans le développement du système nerveux central pendant la grossesse. Lorsque ces travaux de thèse ont été initiés, les travaux publiés portaient majoritairement sur les phtalates et le bisphénol A et aucune étude chez l'Homme n'avait considéré les autres composés de la famille des phénols comme le triclosan ou les parabènes. De plus, les associations avec la fonction thyroïdienne pendant la grossesse avaient été peu documentées. Enfin, une meilleure compréhension de l'impact des sources d'exposition spécifiques à ces composés, telles que l'utilisation de produits de soin personnels, était nécessaire. Ce type d'information est en effet essentiel pour la mise en place d'interventions visant à réduire les expositions.

Objectifs : Les objectifs principaux de ce travail étaient 1) de documenter si l'utilisation de produits de soin personnel pourrait influencer les concentrations urinaires de biomarqueurs d'exposition aux phénols chez les femmes enceintes ; 2) d'évaluer les associations entre l'exposition prénatale aux phénols et aux phtalates et le neurodéveloppement (comportement et fonction cognitive) des enfants à 3 et 5 ans ; et 3) d'évaluer les associations entre l'exposition prénatale aux phénols et aux phtalates et les concentrations d'hormones thyroïdiennes chez la femme enceinte et le nouveau-né, en tant que possible voie sous-jacente des effets sur le neurodéveloppement.

Méthodes : Ce travail s'appuie sur trois cohortes françaises complémentaires. Les associations entre utilisation de produits de soin personnels et concentrations urinaires de phénols ont été étudiées chez huit femmes de l'étude SEPAGES-faisabilité pour lesquelles nous disposons de multiples échantillons d'urine (au total, 178) et d'informations détaillées sur leurs utilisations de produits de soin. Le lien entre, d'une part, les phénols et phtalates et, d'autre part, le développement neurologique (fonctions cognitives et comportement) a été étudié parmi 546 paires mère / fils de la cohorte EDEN. Nous nous sommes ensuite basés sur la cohorte SEPAGES afin d'étudier les effets de l'exposition aux phénols et phtalates sur les niveaux d'hormones thyroïdiennes pendant la grossesse (N = 418 femmes) et à la naissance (N = 421 nouveau-nés). Dans cette étude, un recueil intensif d'échantillons d'urine (42 échantillons d'urine par grossesse) a permis d'évaluer finement les expositions.

Résultats : Les produits de soin personnels comme source d'exposition aux phénols : le nombre total d'utilisation de produits de soin était associé à une augmentation des concentrations urinaires de bisphénol S et des parabènes mais pas des autres phénols (bisphénol A, benzophenone-3, triclosan).

Phénols, phtalates et neurodéveloppement : aucun des composés étudiés n'était associé négativement aux scores de quotient intellectuel à 5 ans. Pour ce qui est du comportement, les concentrations urinaires maternelles de bisphénol A étaient associées à plus de symptômes de type troubles relationnels à 3 ans (IRR = 1.11 (intervalle confiance (IC) 95% = 1.03, 1.20) pour une augmentation de un de la concentration ln-transformée) et de comportements de type hyperactif à 5 ans (IRR = 1.08; IC95% = 1.01, 1.14). Le mono-n-butyl phtalate (MnBP) était associé à une augmentation des scores liés aux troubles émotionnels et relationnels à 3 ans mais pas à 5 ans, tandis que le monobenzyl phthalate (MBzP) était lui associé à une augmentation des scores liés aux troubles internalisés et notamment les troubles relationnels. Enfin lorsque les scores de comportement étaient étudiés en tant que variables binaires, une association entre le triclosan et les scores liés aux troubles émotionnels était observée à 3 et 5 ans.

Phénols, phtalates et concentrations d'hormones thyroïdiennes : les concentrations urinaires de bisphénol A étaient négativement associées aux concentrations de thyrostimuline (TSH) maternelles (ln(TSH)-z-score, $\beta = -0.05$, IC 95% = -0.15, 0.06) tandis que le MBzP, était lui positivement associé aux Z-scores de thyroxine totale (TT4, $\beta = 0.12$, IC95% = -0.01, 0.25) et le triclosan négativement associé au ratio TT3/TT4 ($\beta = -0.05$, IC95% = -0.11, 0.00). Lorsque les expositions étaient étudiées en terciles des relations non linéaires ont aussi été observées entre le triclosan et la TSH (relation en U) et le MnBP et la TSH (U inversé). Les concentrations urinaires de phénols et phtalates n'étaient pas associées aux hormones thyroïdiennes chez le nouveau-né quand les filles et les garçons étaient étudiés ensemble. Après stratification sur le sexe, les concentrations urinaires maternelles de bisphénol A étaient positivement associées au niveau de TT4 chez les garçons tandis que le triclosan était négativement associé à cette hormone chez les filles.

Conclusion : *Les associations positives entre l'utilisation de produits de soin et les concentrations urinaires de parabènes étaient en accord avec les études précédentes sur le sujet. Notre étude était la première à mettre en évidence de telles associations avec le bisphénol S. Les associations observées entre les expositions prénatales au bisphénol A et au DBP et le comportement avaient déjà été mis en évidence dans des études précédentes chez de jeunes garçons. Pour ce qui est du triclosan notre étude était la première à s'intéresser aux associations avec le comportement. Nos résultats ont depuis été répliqués dans une étude américaine tandis qu'une étude au Canada ne montrait pas d'effet. Les composés associés au comportement dans la cohorte EDEN ont tous été associés à au moins une hormone thyroïdienne dans la cohorte SEPAGES, suggérant une possible perturbation de l'homéostasie des hormones thyroïdiennes.*

Mots-clés: *perturbateurs endocriniens, phénols et phtalates, exposition prénatale, produits de soin personnels, neurodéveloppement, quotient intellectuel, comportement, hormones thyroïdiennes, hormone stimulante de la thyroïde.*

Table of contents

1 Introduction.....	2
1.1 Neurodevelopment	2
1.1.1 Assessment of neurodevelopment in epidemiological studies	3
1.1.2 Risk factors for neurodevelopmental disorders.....	6
1.2 Phenols and phthalates	7
1.2.1. Regulation of use of phenols and phthalates in the European Union (EU).....	9
1.2.3 Routes of exposure and metabolism.....	11
1.2.4 Exposure to phenols and phthalates	11
1.2.5 Exposure to phenols and phthalates, role of personal care products.....	13
1.3 Prenatal exposure to phenols, phthalates and neurodevelopment	18
1.3.1 Association between exposure to phenols or phthalates and child cognition	18
1.3.2 Association between prenatal exposure to phenols or phthalates and behavior...	22
1.4 Biological pathways of neurotoxicity of phenols and phthalates.....	27
1.4.1 Role of the thyroid axis in brain development	27
1.4.2 Synthesis and homeostasis of thyroid hormones.....	28
1.4.3 Factors that influence thyroid hormone levels during pregnancy and at birth.....	28
1.4.4 Disruption of the thyroid pathway by phenols and phthalates	29
1.5 Limitations of the current epidemiology studies and perspectives	37
1.6 Thesis aim and objectives	39
2 Use of personal care products and phenol urinary concentrations during pregnancy	42
2.1 French summary	43
2.2 Abstract	44
2.3 Methodology	45
2.4 Results	49
2.5 Discussion	57
2.6 Supplemental material.....	61
3. Phenols, phthalates and child neurodevelopment.....	75
3.1. Phenols, phthalates and cognition of boys in the EDEN Cohort	76
3.1.1 French summary	77
3.1.2 Abstract	78
3.1.3 Methodology	79
3.1.4 Results	85
3.1.5 Discussion	91
3.1.6 Supplementary Materials.....	95
3.2 Phenols, phthalates and behavior of boys in the EDEN Cohort.....	105
3.2.1 French summary	106
3.2.2 Abstract	107
3.2.3 Methodology	108
3.2.4 Results	111
3.2.5 Discussion	120
3.2.6 Conclusion.....	124

4 Phenols and phthalates and thyroid hormone levels during pregnancy and at birth	126
4.2 French summary	127
4.3 Abstract	128
4.5 Results	137
4.6 Discussion	152
4.7 Supplemental Materials	156
5 General discussion	163
5.1 Summary of findings, consistency with the literature and relevance to public health	163
5.1.1 Use of personal care products and phenol urinary concentrations during pregnancy	163
5.1.2 In-utero exposure to phenols, phthalates and child neurodevelopment and disruption of the thyroid axis as a mechanism of neurotoxicity	165
5.2 Methodological considerations	169
5.2.1 Multiple testing and type I error	169
5.2.2 Selection bias and generalizability	170
5.2.3 Confounding bias	170
5.2.4 Measurement error	173
5.3 Conclusion and perspectives	174
6 References	177
7 Annex.....	198
7.2 Regulations of use of phenols and phthalates in France and in the European Union	198
7.3 Publications and oral communication	199

List of abbreviations

ADHD: Attention deficit hyperactivity disorder
AOP: Adverse outcome pathways
ASD: Autism spectrum disorder
BASC: Behavior Assessment System for Children
BBP: Benzylbutyl phthalate
BRIEF: Behavior Rating Inventory of Executive Function
BSID: Bayley Scales of Infant Development
CBCL: Child Behavior Checklist
CCTIRS: Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé
CESD: Center for Epidemiologic Studies Depression Scale Revised
CNIL: Commission Nationale de l'Informatique et des Libertés
CPP: Comité de Protection des Personnes Sud-Est
DAS: Deletion-Substitution-Addition algorithm
DBP: Di--butyl phthalate
DDT: Diclrodiphenyltrichloroethane
DEHP: Di (2-ethylhexyl) phthalate
DEP: Diethyl phthalate
DiBP: Di-iso-butyl phthalate
DIDP: Di-isodecylPhthalate
DINP: Di-isononyl phthalate
DNOP: Di-n-octyl phthalate
ECHA: European Chemicals Agency
ECPA: Editions of the Centre in Applied Psychology
ED: endocrine disrupting compound
ENET: Elastic net
EU: European Union
ExWAS: Environment-wide association study
FCM: Food contact materials
FT4: Free thyroxine
HOME: Home Observation for the Measurement of the Environment Scale questionnaire
IQ: Intellectual quotient
KE: Key events
MnBP: Mono-n-butyl phthalate
MBzP: Monobenzyl phthalate
MCNP: Monocarboxy-isononyl phthalate
MCOP: Monocarboxy-isoocetyl phthalate
MCPP: Mono(3-carboxypropyl) phthalate
MECPP: Mono(2-ethyl-5-carboxypentyl) phthalate
MECPP: Mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHP: Mono(2-ethylhexyl) phthalate
MEOHP: Mono(2-ethyl-5-oxohexyl) phthalate
MEP: Monoethyl phthalate
MiBP: Mono-n-butyl phthalate
MIE: molecular initiating event
NDD: Nedevelopmental disorders
PBDE: Polybrominated diphenyl ethers
PCB: Polychlorinated biphenyls
PCP: Personal care products
REACH: Registration, Evaluation, Authorization and Restriction of chemicals
SDQ: Strength and Difficulties Questionnaire
STAI: State-Trait Anxiety Inventory
SVHC: Substance of very high concern
T3: triiodothyronine
T4: thyroxine
TPO: thyroid peroxidase
TRH: thyrotropin releasing hormone
TSH: Thyroid stimulating hormone / thyrotropin
UIC: median urinary iodine concentration
USA: United States of America
WPPSI-III: Wechsler Preschool and Primary Scale of Intelligence-Third Edition

List of figures

Figure 1.1: Illustration of the timeline of development of the human central nervous system ³	2
Figure 1.2: Illustration of neuropsychological functional domains and clinical phenotypes ¹⁹	4
Figure 1.3: Distribution of IQ in two populations of one million individuals each. The grey line signifies the threshold for IQ scores consistent with intellectual disabilities (IQ < 70). In the unexposed population (blue line), the mean IQ is 100 (standard deviation=15), while in the exposed population (red line) the mean IQ is 95 (standard deviation=15) ¹⁸	5
Figure 1.4: Comparison of median phenol maternal urinary concentrations across several French mother-child cohorts (Adapted from Rolland et al., Submitted ⁴⁸)	12
Figure 1.5: Concentrations of environmental compounds, including phenols and phthalates (left) in conventional consumer products in USA. Extracted from Dodson <i>et al</i> ⁵²	14
Figure 1.6: Schematic representation of potential action sites of phenols and phthalates along the thyroid hormone axis (adapted from Noyes <i>et al</i> ¹⁴⁶)	31
Figure 1.7: Urinary concentrations ($\mu\text{g/L}$) of two phenols (triclosan and bisphenol A) in urine samples collected over a week in two pregnant women from the SEPAGES-feasibility cohort ¹⁶⁶	37
Figure 1.8: Distribution of the effect estimate characterizing the association between exposure and birth weight using the average pregnancy exposure (in red) or one spot urine sample (in green) of exposure to chemical A (ICC of 0.6) and chemical B (ICC of 0.15) (1000 simulations were performed, each with 500 subjects, real effect $\beta_1 = -100 \text{ g}$) ¹⁶⁸	38
Figure 1.9: Schematic illustration of study aims	40
Figure 2.1: Adjusted percent change in phenol urinary concentrations with specific PCP (yes / no) and the total number of PCP applications in the past 0 to 6, 6 to 12 and 12 to 24 hours.	54
Figure 2.2: Adjusted associations between the time elapsed since last use of PCP, modeled using restricted cubic splines with 5 knots, and phenol urinary concentrations	55
Figure 2.3: Variability of urinary concentrations of 10 phenols ($\mu\text{g/L}$) in spot urine samples for one woman collected over a week of follow up (N of woman 1 =72)	63
Figure 2.4: Adjusted percent change in phenol urinary concentrations with specific PCP (yes / no) and the total number of PCP applications in the past 0 to 6, 6 to 12 and 12 to 24 hours, additionally adjusted for time since last meal	65
Figure 2.5: Adjusted percent change in phenol urinary concentrations with total number of PCP applications or single PCP used in the previous 0 to 24 hours	67
Figure 3.1: Path diagram illustrating associations between phenols, phthalate metabolites and IQ of boys.	84
Figure 3.2: Path diagram illustrating associations between phenols, phthalate metabolites and FSIQ of boys in the EDEN Cohort	96
Figure 4.1 : Illustration of final sample size included in the study.	130
Figure 4.2: Adverse outcome pathway (AOP) network for chemically induced thyroid activity showing the integration of multiple individual AOPs under development and proposed. Adapted from Noyes et al ¹⁴⁶	133
Figure 4.3: Distributions of the average (mid- and late pregnancy) phenol and phthalate urinary concentrations in weekly pools (average of 2 within-subject pool of 21 spot samples) of pregnant women in the SEPAGES cohort (N =418)	142
Figure 4.4: TSH concentrations as a function of urinary triclosan and MnBP (ln-transformed) concentrations assessed in a pool of urine samples collected in early pregnancy, coded as restricted cubic splines (3 knots). The predicted curves were adjusted for maternal age, body mass index, gestational age at serum sampling, maternal education level and parity. Shading indicate 95% confidence intervals.	146
Figure 4.5: Adjusted associations between newborn thyroid hormone levels and maternal urinary bisphenol A and triclosan concentrations, stratified by sex.	151
Figure 5.1: Potential toxicities of bisphenol S compared with bisphenol A (Adapted from Qiu <i>et al.</i> , ¹⁹⁴)	165
Figure 5.2 Multivariate models exploring various coding of maternal age in association with TSH pregnancy levels (N=416 women from SEPAGES cohort).	171
Figure 5.3: Correlations across environmental exposures in the Human Early-Life Exposome (HELIX) project, in which 87 environmental exposures during pregnancy (grouped in 19 exposure groups) were assessed in 1301 pregnant mothers in six European birth cohorts.	172

List of tables

Table 1.1: Non-exhaustive list of sources of the phenols and phthalates considered in this study	8
Table 1.2: Regulations of use of phenols and phthalates in France and in the European Union.....	10
Table 1.3: Summary of the results of previous studies that assessed associations of PCP use in the previous 24 to 48 hours with urinary concentrations of phenols and phthalates during pregnancy and PCP survey studies.	16
Table 1.4: Mother-child cohort studies on the associations between maternal phenol and phthalate urinary concentrations with child cognition	20
Table 1.5: Mother-child cohort studies on the association between maternal phenol urinary concentrations and child behavior.....	23
Table 1.6: Mother-child cohort studies on the association between maternal phthalate and urinary concentrations with child behavior.....	25
Table 1.7: Mother-child cohort studies on the associations between maternal phenol urinary concentrations and thyroid hormone levels during pregnancy and at birth.....	33
Table 1.8: Mother-child cohort studies on the associations between maternal phthalate urinary concentrations with thyroid hormone levels during pregnancy and at birth.....	35
Table 1.9: Description of cohorts included in this thesis	40
Table 2.1: Characteristics of the eight women of the SEPAGES feasibility study for whom spot urine samples were analysed for phenol concentrations	49
Table 2.2: Distribution of urinary phenol concentrations ($\mu\text{g/l}$) in 178 urine samples collected from eight women of the SEPAGES feasibility study.....	50
Table 2.3: Number of urine samples for which specific PCPs were used in the 0 to 24 hours preceding urine collection.....	51
Table 2.4: Adjusted percent change (β) in the phenol urinary concentrations in relation to the total number of times PCPs were used in the last 0 to 6, 6 to 12 and 12 to 24 hours (N= 178 urine samples of 8 women)	53
Table 2.5: Spearman's correlation coefficients between phenol concentrations and specific gravity (178 urine samples of 8 pregnant women)	62
Table 2.6: Adjusted percent change (β) in the ln-transformed phenol urinary concentrations in relation to the total number of times PCPs were used in last 0 to 24 (N= 178 urine samples of 8 women); adjusted for time since last meal.....	64
Table 2.7: Adjusted percent change (β) in the ln-transformed phenol urinary concentrations in relation to the total number of times PCPs were used in last 0 to 24 (N= 178 urine samples of 8 women); adjusted for gestational age.	66
Table 2.8: Adjusted percent change (β) in phenol urinary concentrations with use of specific PCP (yes / no) in the past 0 to 6, 6 to 12 and 12 to 24 hours (corresponding to Figure 1).....	68
Table 2.9: Medians ^a of phenol urinary concentrations in previous studies of epidemiological studies of pregnant women that assessed between PCP used in the previous 24 to 48 hours and urinary concentrations of phenols during pregnancy and PCP survey studies	73
Table 3.1: Characteristics of the study population and the mother-son pairs of the French EDEN cohort.....	86
Table 3.2: Concentrations ^a of phenols and phthalate metabolites in maternal urine (N = 452 ^b mother-son pair of the EDEN mother child cohort)	87
Table 3.3: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years (N= 452) ...	89
Table 3.4: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years in the EDEN cohort (N= 419 mother-son pairs, complete case analysis).....	90
Table 3.5: Associations between the covariates included in our final model and boys' IQ (n =452)	97
Table 3.6: Spearman correlation coefficients between biomarker concentrations measured in maternal urine concentrations (n = 452 ^a)	98
Table 3.7: Estimated standardized factor loadings of outcome and exposure indicator variables	99
Table 3.8: Adjusted associations between phenols, phthalate metabolites and Full Scale IQ of boys at 5 years using SEM (N= 452).....	100
Table 3.9: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years in the EDEN cohort, stratified for center of recruitment	101
Table 3.10: Adjusted associations between phenols, phthalate metabolites and IQ (Manual based scales) of boys at 5 years in the EDEN cohort, using multiple linear regression (N= 452).....	102
Table 3.11: Adjusted associations between phenols, phthalate metabolites (concentrations categorized in tertiles) and IQ (Manual based scales) of boys at 5 years in the EDEN cohort, using multiple linear regression (N= 452)	103
Table 3.12: Scoring of the Strengths and Difficulties Questionnaire	109
Table 3.13: Spearman correlation coefficients between SDQ scores assessed at 3 and 5 years among boys of the EDEN mother child cohort.....	111

Table 3.14: Adjusted associations between phenols, phthalate metabolites and behavior at 3 years among boys of the EDEN mother-child cohort (N = 518 to 520 mother-son pairs, depending of the subscale).....	113
Table 3.15: Adjusted associations between phenols, phthalate metabolites and behavior at 5 years among boys of the EDEN mother-child cohort (N = 457 or 458 mother-son pairs, depending of the subscale).....	114
Table 3.16: Adjusted associations between phenols, phthalate metabolites and dichotomized SDQ scores at 3 years (N = 518 to 520 mother-son pairs, depending of the subscale).	116
Table 3.17: Adjusted associations between phenols, phthalate metabolites and dichotomized SDQ scores at 5 years (N = 457 or 458 mother-son pairs, depending of the subscale).	117
Table 3.18: Adjusted associations between phenols, phthalate metabolites and behavior at 3 years among boys of the EDEN mother child cohort (N = 518 to 520 mother-son pairs, depending of the subscale). Analysis simultaneously adjusted for all phenols and phthalate metabolites assessed in our study population	118
Table 3.19: Adjusted associations between phenols, phthalate metabolites and behavior at 5 years among boys of the EDEN mother child cohort (N = 457 to 458 mother-son pairs, depending of the subscale). Analysis simultaneously adjusted for all phenols and phthalate metabolites assessed in our study population	119
Table 4.1: Summary of the molecular initiating events that could be affected by the compounds assessed in SEPAGES and the expected resulting effect on the thyroid hormone levels	134
Table 4.2: Characteristics of pregnant women (N= 415) and their newborns (N = 421) included in this study; SEPAGES cohort, Grenoble.	138
Table 4.3: Distributions of serum thyroid hormone concentrations of the SEPAGES cohort pregnant women and their newborns.....	140
Table 4.4: Spearman correlation coefficients between maternal and newborn thyroid hormones	141
Table 4.5: Multivariate analysis: potential predictors of thyroid hormone levels during pregnancy in our study population (N =416).....	144
Table 4.6: Multivariate analysis: potential predictors of thyroid hormone levels in SEPAGES newborns (N = 421).	145
Table 4.7 : Adjusted associations of maternal thyroid hormone levels with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior to blood collection (N= 416, SEPAGES cohort).	148
Table 4.8: Adjusted associations between maternal urinary phenol and phthalate metabolite concentrations and newborn thyroid hormone levels, SEPAGES cohort.	150
Table 4.9: Adjusted associations of the maternal TT3/TT4 ratio with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior blood collection.	157
Table 4.10: Sensitivity analysis: Adjusted associations of maternal thyroid hormone levels with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior blood collection. Analysis restricted to women for whom blood samples were collected at the end (within 2 days) of the urine collection week, N =339; SEPAGES cohort.	158
Table 4.11: Complete cases analysis*: Association between maternal thyroid hormone levels and urinary phenol and phthalate metabolite concentrations additionally adjusted for smoking (N=335, SEPAGES cohort).	159
Table 4.12: Complete cases analysis*: Association between maternal thyroid hormone levels and urinary phenol and phthalate metabolite concentrations not adjusted for smoking but adjusted for other potential confounders (N=335).....	160
Table 4.13: Complete case analysis*: Association between newborn thyroid hormone levels and maternal urinary phenol and phthalate metabolite concentrations additionally adjusted or not for smoking and alcohol consumption during pregnancy (N=350, SEPAGES cohort).....	161

CHAPTER 1: INTRODUCTION

1 Introduction

1.1 Neurodevelopment

Development of the central nervous system originates in the early prenatal stages to late childhood. This process is characterized by sequential mechanisms including; neurulation, cell proliferation and migration, apoptosis, synaptogenesis, myelination and synaptic pruning (Figure 1.1) ¹. This developmental period is so critical that minor alterations in the normal occurrence of the developmental events could lead to functional abnormalities ranging from mild retardations to complex neurodevelopmental disorders (NDDs) with life-long endurance ².

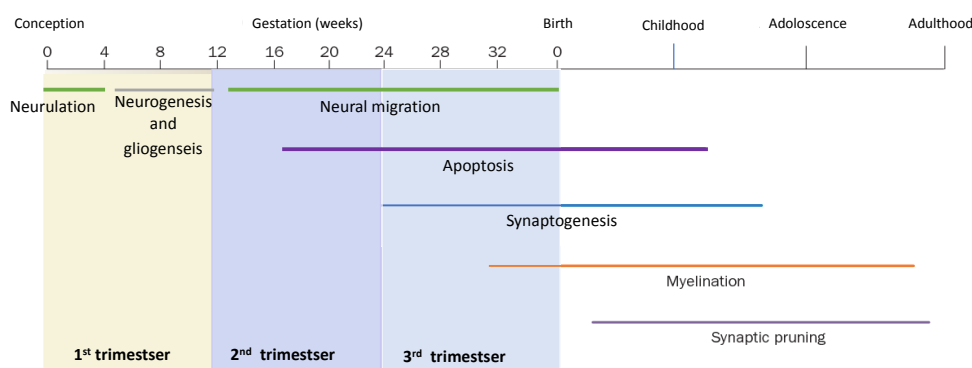


Figure 1.1: Illustration of the timeline of development of the human central nervous system ³

NDDs are defined as a group of conditions with onset in the developmental period leading to long term deficits that produce impairments of personal, social, academic, or occupational functioning ⁴. In the USA, neurodevelopmental disorders such as autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), and mild learning disabilities, are reported to occur in 16.9% of children between 3 to 17 years ⁵. In France, national wide statistics on NDDs are rare. A study carried out between 1997 and 2003, relying on population-based registers covering four French counties, estimated the ASD prevalence at 0.36% among children in these counties ⁶. Although low, this prevalence was exceptionally on the rise during the study period. This trend is similar to that observed in the USA between 2009 - 2011 and 2015 - 2017, with an increase in these disorders from 16.2% to 17.8% ⁷. Globally, a significant increase in the prevalence of ASD and ADHD has also been reported ^{8,9}, with prevalences estimated to be 0.7% ¹⁰ and 7.2% ¹¹ of children worldwide, respectively.

Some studies suggest that, the currently observed increase in prevalence of some disorders such as ASD may not be due to etiological factors. That factors such as changes in diagnostic criteria across the world and awareness of the health professionals could play a part in this rise. In the USA, Bearman and King reported that 26% of the ASD prevalence increase between 1992 and 2005 was attributable to diagnostic accretions and substitutions ¹², while earlier age at diagnosis was reported to explain a 12% of the ASD prevalence increase between 1990 through 2006 ¹³.

Another study from Denmark reported that 60% of the increase in ASD prevalence among children born between 1980 and 1991 was attributable to two changes in reporting practices; change in diagnostic criteria in 1994 and the inclusion of outpatient data to the Danish Psychiatric Register in 1995 ¹⁴. In Australia Nassar et al., reported an increase in ASD prevalence in relation to increased funding for ASD ¹⁵.

Although a considerable proportion of this increase is attributable to these changes, a certain percentage still remains unexplained ¹³. This necessitates a clear understanding of the modifiable underlying risk factors of these disorders, particularly due to the decreased quality of life and societal cost attributable to these disorders. As an example, in Europe, these disorders were estimated to cost about 798 billion euros in 2010 ¹⁶.

1.1.1 Assessment of neurodevelopment in epidemiological studies

In epidemiology, only few studies relied on clinical diagnosis of neurodevelopmental disorders. This is due to the relatively low frequency of these conditions and the fact that in some instances, clinical diagnosis is only confirmed later in childhood ¹⁷.

For this matter, continuous measures of functional domains (illustrated Figure 1.2) related to clinical phenotypes, are commonly considered as proxies for clinical diagnoses ¹⁸ in epidemiological studies.

According to Forns *et al.*, functional domains may include the skills, abilities, capacities and knowledge acquired during maturation of the brain as a result of the development of neural networks. These functional domains are usually classified into cognitive, psychomotor and social-emotional development subdomains (Figure 1.2) ¹⁸.

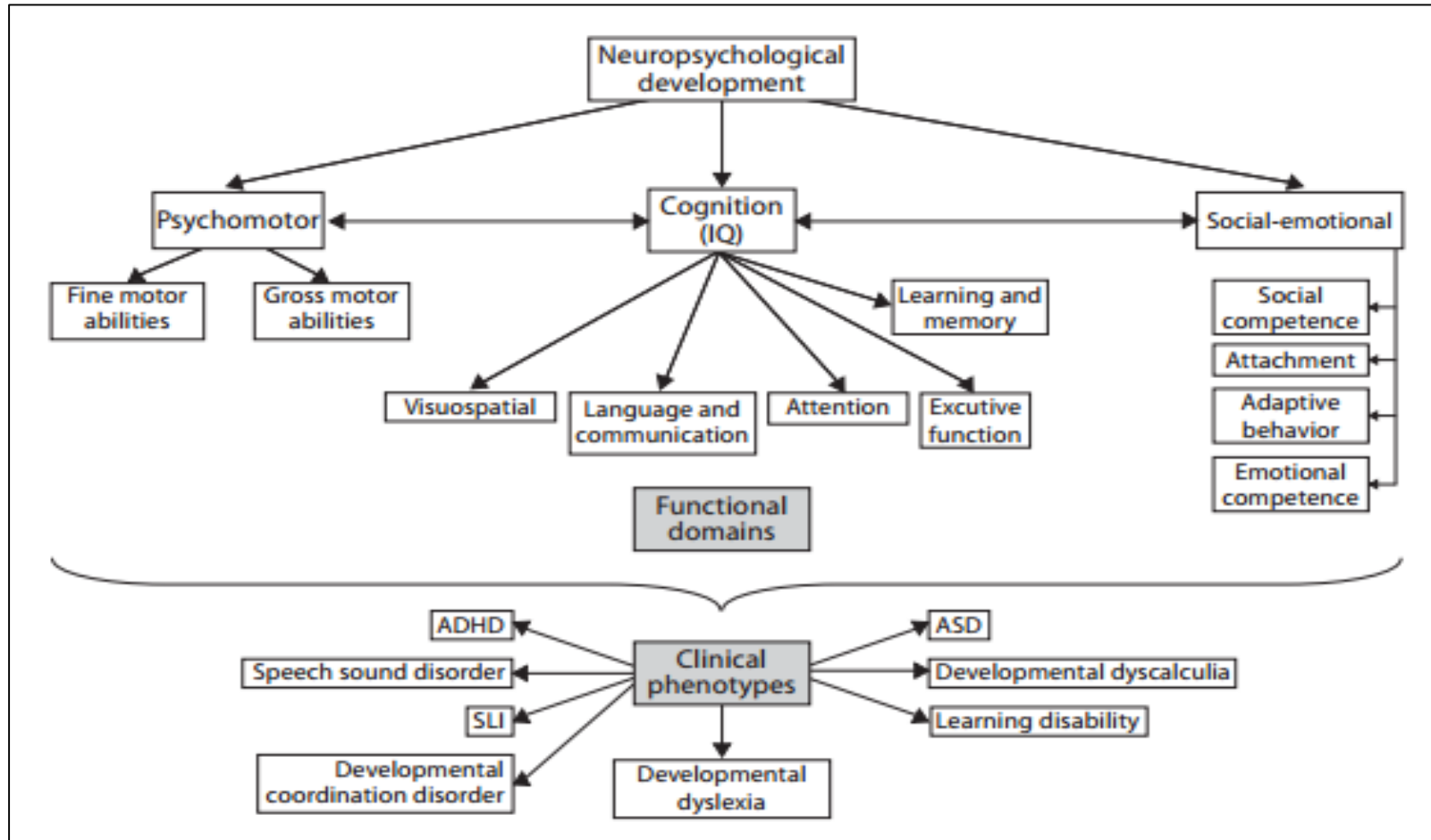


Figure 1.2: Illustration of neuropsychological functional domains and clinical phenotypes¹⁸

Abbreviations IQ: Intelligence quotient, SLI: Specific language impairment, ADHD: attention deficit hyperactivity disorder, ASD: autistic spectrum disorder

As an example, in this thesis we relied on intellectual quotient (IQ) score to assess the cognitive subdomain and on the Strengths and Difficulties scores to assess certain aspects of the socio-emotional subdomain.

In term of results interpretation, as detailed in Braun *et al.*, at the individual level, a loss of a few IQ points in relation to an exposure may not present substantial change in intellectual functioning. Whereas at population level, a shift of the IQ distribution to the left monumentally increases the number of people with intellectual disabilities in a given population (a score below 70 denotes intellectual disability). This is illustrated in Figure 1.3 adapted from Braun *et al.*, that shows that a 5-point shift in IQ scores nearly doubles the proportion of people with IQ scores consistent with intellectual disabilities (IQ <70) in an exposed population (4.48%) compared to an unexposed population (2.27%) (illustrated in Figure 1.3) ¹⁷.

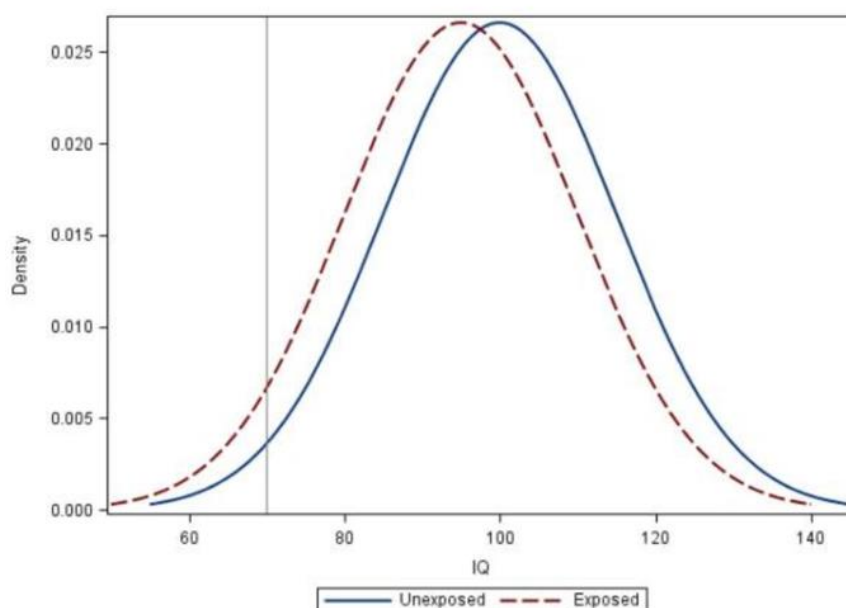


Figure 1.3: Distribution of IQ in two populations of one million individuals each. The grey line signifies the threshold for IQ scores consistent with intellectual disabilities (IQ < 70). In the unexposed population (blue line), the mean IQ is 100 (standard deviation=15), while in the exposed population (red line) the mean IQ is 95 (standard deviation=15) ¹⁷.

1.1.2 Risk factors for neurodevelopmental disorders

Root causes of neurodevelopmental disorders are multifactorial and only partly understood. These disorders were originally considered heritable, with underlying genetic aetiologies such as abnormal number of chromosomes, chromosomal deletion and single gene defects^{19,20}. For example, some disorders such as Down's syndrome (trisomy of chromosome 21) and Fragile X syndrome (CCG repeat expansion of the FMR1 gene) have well-known genetic aetiologies^{21,22}.

However, familial studies have shown that depending on the disorders, heritability could account for 30 to 90% of risk^{19,20} and that various environmental factors such as maternal nutrition, substance use, viral infections, psychological stress and exposure to industrial chemicals could also adversely affect neurodevelopment²³.

Regarding industrial chemicals, sufficient knowledge exists that pinpoints certain metals such as lead and mercury and "legacy" persistent compounds like organochloride pesticides (diclorodiphenyltrichloroethane (DDT)) and polychlorinated biphenyls (PCB) as neurotoxic. As such, these compounds are currently strictly controlled or prohibited²⁴.

Conversely, there has also been an increase in the production of newer, less studied chemicals that are also currently suspected to affect brain development²⁵ such as polybrominated diphenyl ethers (PBDEs), phenols and phthalates²⁶. In this PhD research, we focused on phenols and phthalates, chemicals of high production volume and widespread exposure in the general population, including pregnant women and young children^{27,28}.

1.2 Phenols and phthalates

Several synthetic phenols and phthalates are categorized as endocrine disrupting compounds (EDs). Endocrine disruptors are exogenous substances with the ability to cause adverse effects to an organism and/or its progeny by altering functions of its endocrine system²⁹.

Phenols are chemical substances, which possess an aromatic ring bearing one hydroxyl group or more. Some phenols exist naturally while others can be artificially manufactured for industrial applications. Synthetic phenols are used to manufacture polymers (e.g., polycarbonate plastics) and epoxy resins (bisphenols), as ultraviolet light filters (benzophenone-3), antimicrobial (triclosan) and preservative (parabens) agents, and in herbicides (2,5 dichlorophenol) (see Table 1.1 for specific sources).

Phthalates or phthalic acid esters are artificially manufactured compounds found in a wide variety of products (see Table 1.1 for examples of sources). The usage of phthalates depends on the molecular weight. They are widely used as plasticizers in the production of polyvinyl chloride base plastics such as polyvinyl tiles, rubber, cellulose film, adhesives, coatings (mainly high molecular weight phthalates such as diethyl phthalate (DEP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DBP)) and also as solvents in personal care products such as perfumes and cosmetics (mainly low-molecular weight phthalates, such as benzylbutyl phthalate (BBP), di (2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate, (DiNP) di-n-octyl phthalate (DNOP), di-isodecylPhthalate (DIDP), di-isononyl phthalate (DINP))³⁰ (see Table 1.1 for specific sources).

Table 1.1: Non-exhaustive list of sources of the phenols and phthalates considered in this study

Compounds	Biomarkers assessed		Sources
Low molecular weight phthalates			
Di-ethyl phthalate (DEP)	Monoethyl phthalate	MEP	Personal care products (PCPs), pharmaceutical products, dyes
Di-iso-butyl phthalate (DiBP)	Mono-iso-butyl phthalate	MiBP	Pharmaceutical products
Di-n-butyl phthalate (DBP)	Mono-n-butyl phthalate	MnBP	Personal care products (PCPs), lacquers, vanishes, pharmaceutical products
High molecular weight phthalates			
Benzylbutyl phthalate (BBP)	Mono benzyl phthalate	MBzP	Vinyl flooring, adhesives and sealants, PCPs and food packaging, industrial solvents
Di (2-ethylhexyl) phthalate (DEHP)	Mono-2-ethylhexyl phthalate	MEHP	Polyvinyl chloride plastics, household products (floor tiles, furniture), food packaging and medical devices
	Mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP	
	Mono-2-ethyl-5-oxohexyl phthalate	MEOHP	
	Mono-2-ethyl 5-carboxypentyl phthalate	MECPP	
Di-isononyl phthalate (DiNP)	Mono-4-methyl-7-hydroxyoctyl phthalate	oh-MiNP	Sealants, lacquers, paints
	Mono-4-methyl-7-oxooctyl phthalate	oxo-MiNP	
	Mono-4-methyl-7-carboxyoctyl phthalate	cx-MiNP	
Di-n-octyl phthalate (DNOP)	Mono-(3-carboxypropyl) phthalate	MCPP	Medical devices, PCPs and food packaging
Di-isodecyl Phthalate (DIDP)	Mono(carboxyoctyl) Phthalate	MCOP	Anticorrosive paints
Di-isononyl phthalate (DINP)	Mono-(carboxynonyl) Phthalate	MCNP	Sealants, lacquers, paints
Phenols			
Dichlorophenoacetic acid	2,4 dichlorophenol	2,4-DCP	Herbicides and pesticides
Dichlorobenzene	2,5 dichlorophenol	2,5-DCP	Toilet-deodorizer and moth balls
	Methyl paraben	MP	
	Ethyl paraben	EP	
	Propyl paraben	PP	
	Butyl paraben	BP	Pre-packed foods , PCPs and pharmaceuticals
	Bisphenol A	BPA	
	Bisphenol S	BPS	
	Benzophenone-3	BP3	Personal care products, food and personal care products' packaging
	Triclosan	TCS	Personal care products and textiles

1.2.1. Regulation of use of phenols and phthalates in the European Union (EU)

In the EU various laws regulate the use of some phenols and phthalates in several consumer products to ensure public safety (detailed in Table 1.2 and Annex 7.2).

The cosmetics regulation No 1223/2009 prohibits the use of three phthalates BBP, DEHP, DBP in personal care products and further restricts the use of phenols such as methylparaben, ethylparaben, butylparaben and propylparaben to 0.4% or 0.8% of product weight when used as mixtures or as a single compound, respectively ³¹ (Table 1.2). This law was amended in 2014 to restrict triclosan use to a concentration of 0.3% in products (regulation No 358/2014) and in 2017, to restrict the use of benzophenone-3 to 0.6% of product weight (Regulation 2017/238).

Compound specific laws also exist; in 2011, bisphenol A was banned from baby bottles in the EU. Since then, laws have followed limiting its use in food contact materials and toys. As of 2020, a maximum concentration of bisphenol A of 0.02% by weight shall be authorized in thermal papers. Bisphenol A is recognized as a substance of very high concern (SVHC) for its endocrine disrupting properties by European Chemicals Agency (ECHA), it has not yet been annexed in Registration, Evaluation, Authorization and Restriction of chemicals (REACH) (REACH) ³² (Annex 7.2). REACH is a regulation of the European Union (EU), adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals (detail on this regulation in Annex 7.2).

Phthalate compounds including BBP, DEHP, DIBP and DBP are listed in the REACH annex XIV and require authorization before use ³². Other regulations on use of phthalates in the EU and France include 1) being banned toys, child care products, food contact materials (FCMs) and pediatric / neonatal / maternity ward medical tubes and 2), restricted use in electrical and electronic equipment (Table 1.2).

Table 1.2: Regulations of use of phenols and phthalates in France and in the European Union

Compounds	Legislation number	Jurisdiction	Year of effect	Regulation	
				Ban	Restriction
BBP, DINP, DEHP, DBP and DNOP	Directive 2005/84/EC	EU	2005	Toys and child care products	
BBP, DEHP, DBP and DIBP	Directive (EU) 2015/863	EU	2015		0.1% in electrical and electronic equipment (excluding medical devices)
DIBP	Directive 2007/19/EC	EU	2007	Materials in contact with foodstuffs (FCMs)	
BBP, DEHP, DBP, DIDP and DINP					Specific migration limits (SML) in FCMs
DEHP	Law NO 2012-1442	France	2015	Medical tubes in pediatrics, neonatology and maternity wards in hospitals	
BBP, DEHP, DBP	*EC Regulation 1223/2009)	EU	2009	Cosmetics	
2,4 dichlorophenol	French Law NO 2010-729	France	2010	Moth balls	
Bisphenol A	French Law NO 2010-729	France	2010	Baby bottles	
	EU Regulation 321/2011	EU	2011	Baby bottles	
	Law NO 2012-1442	France	2015	FCMs	
	EU Regulation 10/2011	EU	2018		SML= 0.05 mg/kg from varnishes and coatings in FCMs with food
	Directive 2009/48/EC Regulation (EU) 2016/2235			2018 2020	SML= 0.04 mg/kg in toys 0.02% by weight in thermal papers
Triclosan	Directive 2002/72/EC	EU	2002	FMC plastics	
	*EU Regulation NO 358/2014	EU	2014		0.3% w/w in cosmetics and personal hygiene products 0,2% in mouthwash
	Decision 2016/110/EU		2017	Biocidal products	
Benzophenone-3	*EU Regulation 2017/238	EU	2017		6% w/w in cosmetic sunscreen 0.5% in all types of cosmetics
Methyl, ethyl butyl, and propylparaben	*EC Regulation 1223/2009	EU	2009		0.4% w/w for one paraben and 0.8% for a mixture of parabens in cosmetics
Butyl and propyl paraben	*EU regulation 1004/2014	EU	2014		0.14% w/w for one paraben leave-on cosmetics for application on the nappy area of children below the age of 3 years

Abbreviations: EU: European Union, EC : European Commission, FCM : food contact materials, w/w: weight per weight of product, SML: specific migration limits.

*Regulations pertaining to application of phenols and phthalates in personal care products

1.2.3 Routes of exposure and metabolism

Phenols and phthalates are not chemically bound in products and as a result, these chemicals migrate from product to product (as in the case of food contact materials and personal care product (PCP) containers) or into the ambient environment³⁰. Human exposure occurs via three major routes: 1) ingestion of contaminated foods, drinks and PCPs, 2) inhalation of dust particles or use of aerosol products and 3) dermal absorption following direct contact with contaminated surfaces, household products or PCPs use. Following exposure, phthalates and phenols are absorbed into the body, and are rapidly metabolized and excreted at a half-life less than 24 hours, depending on route of absorption³³⁻³⁹.

Phenols are usually excreted as the original parent compound or in conjugated form, a form that increases water solubility and thereafter excretion in urine.

Phthalate biotransformation is according to their molecular weight: low molecular weight phthalates are changed into their hydrolytic monoesters, while higher molecular weight phthalates are also metabolized to hydrolytic monoesters, and then undergo enzymatic oxidation of the alkyl chain, to produce oxidative metabolites²⁸.

In epidemiological studies, concentrations of metabolites and parent compounds (mainly for phenols) are measured from various biological matrices (in urine, as in the context of this study) to constitute the individual internal dose.

1.2.4 Exposure to phenols and phthalates

Phenols and phthalates are ubiquitous environmental contaminants and are nearly always present at various levels in virtually all people, including sensitive populations of children and expectant mothers. Indeed, biomarkers of these compounds (metabolites or parent compounds) are constantly detected in biological matrices of mothers and their children including urine samples^{27,40}, amniotic fluid^{41,42} and umbilical cord blood⁴³.

In France, several cohort studies have assessed phenol and phthalate urinary concentrations among pregnant women (EDEN (2003-2006)⁴⁴, ELFE (2011)⁴⁵, SEPAGES (2014-2017)^{46,47}), children and adults (ESTEBAN, 2014-2016)⁴⁸). Although the median urinary concentrations tended to be lower in the most recent cohort (SEPAGES) compared to the older (EDEN) cohorts (see Figure 1.4 for the phenols⁴⁷), frequencies of detection are still high. As an example, bisphenol A was detected in 98% of the urine samples from pregnant

women in SEPAGES (2014-2017) and 100% of the urine samples from children aged 6 to 17 in ESTEBAN (2014-2016).

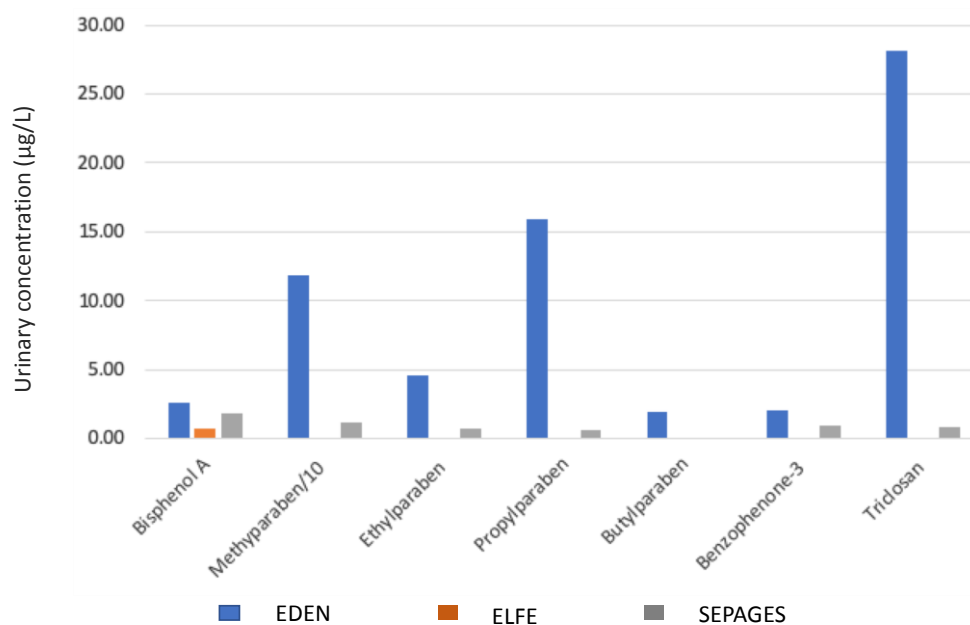


Figure 1.4: Comparison of median phenol maternal urinary concentrations across several French mother-child cohorts (Adapted from Rolland *et al.*, Submitted⁴⁷)
Median concentration of methylparaben is divided by 10

In the wake of stricter regulations on use of bisphenol A and certain phthalates (detailed in section 1.2.1), substitute compounds have become widely used in various consumer products including diisononylcyclohexane-1, 2-dicarboxylate (DiNCH) a plasticizer and various analogues of bisphenol A such as bisphenol S, bisphenol F and bisphenol AF. However, these substitutes may not be safer alternatives with some previously reported to have endocrine disrupting capabilities^{49,50}, hence human studies are needed to understand their exposure sources and possible associated health effects.

1.2.5 Exposure to phenols and phthalates, role of personal care products

A number of phenol and phthalate metabolites have been detected in mixtures of numerous PCPs on Chinese and American markets including soaps and shampoos (parabens, di-ethyl phthalate, bisphenol A and S), lotions (parabens and di-ethyl phthalate), toothpaste (triclosan, bisphenol A and S), facial cleansers (bisphenol A and S), sunscreen (methylparaben and benzophenone-3) and colored cosmetics (di-isobutyl phthalate, di-ethyl phthalate, bisphenol A and parabens⁵¹⁻⁵⁵). Detection rates of some compounds in mixtures of different types of products from the USA are detailed in Figure 1.5.

Owing to their daily use specifically among women, PCPs are potential sources of exposure to phenols and phthalates. It is therefore necessary to characterize how use of various PCPs could influence exposure to these compounds especially during critical periods such as pregnancy.

Previous epidemiological studies assessing the associations of use of PCPs with the urinary concentrations of phenols or phthalates in populations of adults, children ⁵⁶⁻⁶⁰ and pregnant women (results summarized in Table 1.3) ⁶¹⁻⁶⁶ reported that use of many PCPs or single PCPs such as deodorant, shampoo, hair care products, shaving cream, cologne/perfume, soaps, lotions, cosmetics, sunscreen, and nail polish in the past 24 or 48 hours before urine sample collection was associated with higher urinary concentrations of phenols such as bisphenol A, triclosan, parabens, benzophenone-3 and some phthalates (Table 1.3).

With a few exceptions ^{58,60,64,67}, most previous studies characterized use of PCPs as a binary variable (Yes / No) or as the frequency of use in the 24 to 48 hours prior to urine collection, without assessing the exact time of PCP use. These may not be the relevant time windows since experimental studies of non-pregnant humans reported excretion of various phenols to peak between 2 to 11 hours after dermal or oral exposure ^{33,37,38,68,69}.

This suggests that it would be relevant to look at shorter time windows than those considered in most previous studies. Shorter time windows might help in reduction of misclassification of PCP use which might partly be the reason as to why previous studies do not usually report associations with compounds that might be present in the PCPs. For example, bisphenol A and S were frequently detected in PCPs in America ⁷⁰ and China ⁷¹ but previous studies did not always report associations with use PCPs in relation to this compound ^{59,66}. So one of our hypotheses was that for such compounds, that peak within few hours of exposure ³⁸, use of longer time windows might dilute this effect.

Table 1.3: Summary of the results of previous studies that assessed associations of PCP use in the previous 24 to 48 hours with urinary concentrations of phenols and phthalates during pregnancy and PCP survey studies.

	Epidemiological studies ^a							PCP survey studies		
Phenols										
Author	Meeker et al., 2013	Buckerly et al., 2012	Braun et al., 2014	Larsson et al., 2014	Parlett et al., 2014	Fisher et al., 2017	(Ashrap et al., 2018)	Dodson et al., 2012	Liao and Kannan, 2014	Lu et al., 2018
N	105 women, Puerto Rico	50 Women, RFT multistate study, USA	170 women, USA	98 women, Sweden	337 women, SFF multistate study, USA	31 women, Canada	1003 Women, Puerto Rico	USA	China and USA	China
BPA	Mouthwash	N/A	N/A	Cosmetics Creams Mouthwash	NA	N/A		Cleanser Hair products Creams Nail polish Shower gel Sunscreen	Cosmetics Hair products Liquid soap Creams Shower gel Toothpaste	Cleaner Creams Hair products Masks Shower gel Sunscreen
BPS		N/A	N/A	N/A	NA	N/A	N/A	N/A	Cosmetics Liquid soap Creams Shower gel Toothpaste	Cleanser Hair products Toothpaste
TCS	Liquid soap Hair products	N/A	N/A	N/A	NA	N/A	Creams Liquid soap	Hand soap Toothpaste	Cosmetics Hair products Liquid soap Creams Shower gel Toothpaste	Cleaner Creams Hair products Masks Shower gel Sunscreen Toothpaste
BP-3	Creams Mouthwash Sunscreen	N/A	N/A	N/A	NA	N/A	Cosmetics Sunscreen Creams	Sunscreen	N/A	N/A
MP	Cosmetics Creams	N/A	Cosmetics Hair products Perfume Creams,	Cosmetics Creams Mouthwash	NA	Creams Bar soap	Lotion, cosmetics, Hair products	Cosmetics Hair products Liquid soap Creams Sunscreen	N/A	N/A
EP	NA	N/A	N/A	Sunscreen	NA	Creams	Creams	Creams	N/A	N/A

						Cleanser	Cosmetics Hair products Nail polish	Cleanser Cosmetics			
PP	Cosmetics Creams	N/A	Creams Cosmetics	Creams Cosmetics, Mouthwash	NA	Creams Bar soap	Creams Cosmetics Hair products	N/A	N/A	N/A	
BP	Creams Cosmetics	N/A	Cosmetics Hair products Creams	N/A		Creams Cosmetics Deodorant Bar soap Toothpaste	Creams Cosmetics Nail polish	Creams Cosmetics	N/A	N/A	
Phthalates											
DBP	Creams Nailpolish	Cosmetics		Sunscreen	Perfume			Cosmetics			
DEP	Creams Nailpolish Cosmetics, Perfume			Cosmetics	Hair products Bar soap, Deodorant, Perfume			Bar soap Liquid soap Creams Hair products Cleanser Toothpaste			
DEHP	N/A	Cosmetics Perfume						Deodorant Cosmetics Nail polish Sunscreen			
BBP	N/A	Perfume									

Abbreviations PCP: personal care products, BP3: benzophenone-3, BPA: bisphenol A, BPS: bisphenol S, MP: methylparaben, EP: ethylparaben, PP: propylparaben, BP: butylparaben, TCS: triclosan, DBP: Di-n-butyl phthalate, DEP: Diethyl phthalate, DEHP: Di (2-ethylhexyl) phthalate, BBP: Benzylbutyl phthalate

N: sample size and location of study

PCP groups: hair products (shampoo and conditioner), cosmetics (Mascara, eyeliner, lipstick, foundation, contour, eye shadow), perfume (cologne and perfume), Creams (Body, face, hand creams)

N/A: Compound not assessed in the study

^aOnly the PCPs positively associated with urinary concentrations are displayed in the table.

1.3 Prenatal exposure to phenols, phthalates and neurodevelopment

In rodent studies, prenatal exposure to bisphenol A, 2,4- dichlorophenoxyacetic acid the parent compound of 2,4 dichlorophenol, butylparaben and some phthalates were linked to adverse behavior and memory effects ^{72–76}. While gestational exposure to triclosan, benzophenone-3 and some phthalates was reported to disrupt mechanisms involved in fetal brain development. For example, benzophenone-3 altered epigenetic statuses of brain neurons in embryos ⁷⁷, triclosan was reported to significantly decrease thyroxine levels while the phthalates metabolites interfered with calcium signaling pathways ^{78–81}.

Several human studies on the effects of prenatal maternal levels of these substances on neurodevelopment have been carried out ^{82,83}. Of specific interest to our study, are prospective cohort studies of mother-child dyads with the inherent practicability to consider long-term neurodevelopmental outcomes when assessing such relationships. In these studies, continuously distributed measures of functional domains were usually the major outcomes considered (cognition and behavior, illustrated Figure 1.2), and these were assessed using standardized tools administered by professional psychologists or in the form of questionnaires filled by care takers.

1.3.1 Association between exposure to phenols or phthalates and child cognition

Among other tools, cognition was usually assessed with either the mental development index of the Bayley Scales of Infant Development (BSID) or IQ with the Wechsler Scales of Intelligence (Table 1.4). We mainly discuss findings related to IQ as we also assessed IQ in our study. The mental developmental index is sometimes discussed for less-studied compounds.

Epidemiological studies assessing the associations between in-utero exposure to phenols and child intellectual functioning are as recent as 2017. Bisphenol A remains the most highly studied phenol followed by triclosan. Findings in these studies were not in favor of any association between pregnancy levels of bisphenol A, triclosan, benzophenone-3 or parabens with IQ or the mental development index. Among all seven studies of children between 1 and 8 years, none reported a statistically significant association ^{44,84–88}, except one that suggested a negative association between parabens and the mental development index of girls at 2 years ⁸⁹ (Table 1.4).

Associations between gestational levels of phthalates and child IQ were first studied by Factor-Litvak et al, in 2014. This study was based off a distinctive social-economic mother-child cohort from New York inner cities. They found that increased MnBP and MiBP levels were associated with decreasing full scale IQ (FSIQ), while MnBP, MiBP and MBzP were negatively associated with various subscales of the WISC. This study also reported sex specific effects in relation to various subscales, the strengths of the associations varied by sex ⁹⁰. Proceeding longitudinal studies considering IQ of children between 6 and 12 year, found no statistically significant associations ^{44,91,92} (Table 1.4).

In other studies, where cognitive development was assessed with the mental development index, maternal urinary concentrations of several phthalate metabolites were associated with decreased MDI scores at 6 months ⁹³, 2 years ⁹⁴⁻⁹⁶ and 3 years ^{96,97}. Depending on the study, some of the phthalates metabolites incriminated were MnBP ⁹³⁻⁹⁵, DEHP metabolites ⁹³⁻⁹⁵, MBzP and MCPP ⁹⁴

Some of these studies also highlighted sex specific trends (detailed in Table 1.4) ^{91,93,94,96,97}.

Table 1.4: Mother-child cohort studies on the associations between maternal phenol and phthalate urinary concentrations with child cognition

Reference	Cohort	N	Compounds	Age	Tool	Trends observed
Phenols						
^a Nakiwala et al, 2018	EDEN	452	BPA	5-6 years	WPPSI-III	<u>In boys:</u> No association with IQ
Stacy et al., 2017	HOME	438	BPA	5 and 8 years	WISC-IV	<u>In boys & girls:</u> No association observed
Braun et al., 2017	MIREC	896	BPA	3 years	WPPSI-III	<u>In boys & girls:</u> No association observed
Etzel et al., 2018	MIREC	528	TCS	3 years	WPPSI-III	<u>In boys & girls:</u> No association observed
Jackson-Browne et al., 2018	HOME	198	TCS	5 years	WISC-IV	<u>In boys & girls:</u> No association observed
Casas et al., 2015	INMA-Sabadell	438	BPA	1, 4, 7 years	BSID	<u>In boys & girls:</u> No association observed
Jiang et al., 2019	WWCMCC	476	BP3, MP, EP, PP, BP, TCS	2 years	BSID	<u>In boys & girls:</u> No association observed <u>In girls:</u> ↓ MDI with MP and molar sum of parabens
Phthalates						
^a Nakiwala et al., 2018	EDEN	452	MEP, MnBP, MiBP, MECPP, DEHP metabolites, MBzP, MCOP, MCP, MCNP	5-6 years	WPPSI	<u>In boys:</u> No association with IQ
Kim et al., 2017	MOCEH	174	DEHP metabolites, MnBP	6 years	WISC	<u>In boys & girls:</u> No association with IQ
Huang et al., 2015	Taiwan Maternal and Infant Study	110-73	MMP, MEP, MnBP, MBzP, DEHP metabolites	3, 6, 9,12 years	BSID-II & WSCI	<u>In boys & girls:</u> No association with IQ or MDI
Factor-Litvak et al., 2014	CCCEH	328	MnBP, MBzP, DEHP metabolites, MEP, MiBP	7 years	WISC	<u>In boys & girls:</u> ↓ full scale IQ with MBnP and MiBP.

						<p><u>In girls:</u> ↓ working memory with MnBP ↓ perceptual reasoning with MnBP and MiBP <u>In Boys:</u> ↓ perceptual reasoning with MBzP, processing speed with MnBP, ↓ comprehension with MiBP</p>
Doherty et al., 2017	Children's Environmental Health Study	258	DEHP metabolites, MnBP, MiBP, MEP, MBzP, MCPP	2 years	BSID-III	<p><u>In boys:</u> ↑ MDI score with MnBP, MBzP. <u>In girls:</u> ↓ MDI score with MnBP, MiBP, MCPP.</p>
Tellez-Rojo et al., 2013	ELEMENT	135	MEP, MnBP, MiBP, MBzP, MCPP, DEHP metabolites	24, 30, 36 months	BSID-II	<u>In girls:</u> ↓ MDI with MEHP, MEHHP, MEOH, MECPP and ΣDEHP
Kim et al., 2011	MOCEH	460	DEHP metabolites, MnBP	6 months	BSID-II	<u>In boys & girls:</u> ↓ MDI with MEHHP. ↓ MDI with MEOHP

^a See chapter 3 for details about this study.

Abbreviations: BPA: bisphenol A, TCS: triclosan, BP3: benzophenone-3, MP: methylparaben, EP: ethylparaben, PP: propylparaben, BP: butylparaben, 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, BSID: Bayley Scales of Infant Development, WISC: Wechsler Scales of Intelligence, WPPSI: Wechsler Preschool and Primary Scale of Intelligence, IQ: Intellectual Quotient, MDI: mental development index.

1.3.2 Association between prenatal exposure to phenols or phthalates and behavior

Regarding child behavior, various behavioral rating scales have been previously used in epidemiological studies to assess two broad-band behavioral symptoms: externalizing (comprising of symptoms such as hyperactivity / impulsivity / inattention, conduct problems, aggression problems, delinquent behavior, rule-breaking behavior) and internalizing (comprising of symptoms such as emotion, peer relationships, anxiety / depression, somatization, withdrawal) that characterize children's behaviors based on their reactions to stressors. The most commonly used scales in these studies were the Child Behavior Checklist (CBCL), Behavior Assessment System for Children (BASC), Strength and Difficulties Questionnaire (SDQ), Behavior Rating Inventory of Executive Function (BRIEF).

Previous epidemiological studies of phenols are largely focused on bisphenol A. Up until 2015, in a review of existing publications, Mustieles *et al.*, found that only eight prospective birth cohort studies had been carried out in relation to this compound. Six of these studies reported adverse behavioral problems of internalizing or externalizing nature among children between one to nine years⁹⁸⁻¹⁰³. None of these studies reported statistically significant associations in the global sample, however varying findings were observed by sex, with majority occurring among boys than in girls (results summarized in Table 1.5).

Since 2015, several other publications have been added to this research, including studies of other phenols. These studies further confirm that prenatal exposure to bisphenol A could negatively impact child behavior and these effects could be different by sex^{84,85,88,104} (Table 1.5).

Triclosan was also recently reported to be associated with increased externalizing behaviors among boys at 3¹⁰⁴ and 8 years⁸⁷. No statistically significant associations were reported for other phenols¹⁰⁴ (Table 1.5).

Table 1.5: Mother-child cohort studies on the association between maternal phenol urinary concentrations and child behavior

Aurthor	Cohort	N	Compounds	Age (years)	Behavioral assessments	Trends observed
Jackson-Browne et al, 2019	HOME	202	TCS	8	BASC-2	Boys and girls : No significant associations Boys: ↑ behavioral symptom index, externalizing problems, conduct, attention , hyperactivity, somatization problems
Etzel et al, 2018	MIREC	794	TCS	3	BASC	Boys & girls: ↓ externalizing behaviors, hyperactivity
^a Philippat et al, 2017	EDEN	529	BPA, TCS,MP, EP, PP, BP 2,4- and 2,5 DCP, BP3	3 and 5	SDQ	In boys only: ↑ hyperactivity-inattention at 5 yrs and relationship problems at 3 yrs with BPA ↑ emotional symptoms (3 and 5 yrs), conduct problems (3 yrs) with TCS
Stacy et al, 2017	HOME	288	BPA	5 to 10	BASC-S and BRIEF-P	In Boys: ↑ Externalizing problems, internalizing, problems, behavioral regulation index In Girls: ↑ Externalizing behaviors, ↓ behavioral symptoms index,
Braun et al, 2017	HOME	346	BPA	1, 2, 3, 4, 5 and 8	BASC-2	In Girls: ↑ Externalizing with prenatal BPA
Casas et al, 2015	IMNA	438	BPA	4 and 7	SDQ	In boys: ↑ Hyperactivity and inattention scores (4 yrs) In girls: ↓ Inattention scores (4 yrs)
Roen et al, 2015	CCCEH	250	BPA	7 to 9	CBCL	In boys: ↑ Internalizing, externalizing, anxious/depressed, withdrawn/depressed, somatic complaints, rule-breaking and aggressive behavior scores. In girls: ↓ Internalizing problems
Harley et al, 2013	CHAMACOS	292	BPA	7 to 9	BASC-S and CBCL	In boys: ↑ Internalizing, aggressive, depression, anxiety scores. In girls: No clear association
Evans et al, 2014	SFFII	153	BPA	6 to 10	CBCL	In boys : ↑ Internalizing, externalizing, somatic, oppositional/defiant problem scores In girls : No clear association
Perera et al, 2012	CCCEH	198	BPA	3 to 5	CBCL	In boys: ↑ Emotionally reactive, aggressive behavior scores In girls: ↓ Anxious-depressed, aggressive behavior scores
Braun et al, 2011	HOME	244	BPA	2	BRIEF-P and BASC-2	In boys: No clear association In girls: ↑ Externalizing behavior
Braun et al, 2009	HOME	249	BPA	2	BASC-2	Boys: ↑ Internalizing and behavioral symptom Girls: ↑ Externalizing and behavioral symptom index

^a See chapter 3 for details about this study. Abbreviations: BPA: bisphenol A, TCS: triclosan, BP3: benzophenone-3, MP: methylparaben, EP: ethylparaben, PP: propylparaben, BP: butylparaben, BASC: Behavioral assessment for children, BRIEF: Behavior Rating Inventory of Executive Function-Preschool, CBCL: Child Behavior Checklist, SDQ: Strength and Difficulties Questionnaire.

Effects of prenatal exposure to several phthalates including DEHP, BBP, DEP, DBP, DMP, DiNP, DiDP, and DNOP on neurobehavior of children between one to twelve years has been previously investigated in various birth cohorts. An up to date account of these studies is summarized in Zhang et al ⁸², suggesting that exposure to some of these compounds during pregnancy interfered with both internalizing and externalizing behavior (Table 1.6). The incriminated compounds were metabolites of DBP ^{97,104–106}, DEHP ^{104–106}, DEP ^{104,107,108} and BBP ^{97,105} (See Table 1.6).

Sex-specific effects were also reported for some phthalates. In relation to DBP, three studies found internalizing or externalizing behavior problems in boys but not in girls studies ^{97,104,105}. MBzP, the metabolite of BBP was reported to be associated with internalizing behavior problems in girls only in one study ⁹⁷ while two studies reported associations with internalizing ¹⁰⁴ and externalizing ¹⁰⁵ behaviors only in boys.

To sum up, the body of literature presented above clearly shows that epidemiological studies of prenatal exposure to these non-persistent endocrine disruptors in relation to neurodevelopment are recent and largely focused on bisphenol A and phthalates. Only 3 studies have considered other phenolic compounds such as triclosan, dichlorophenols, parabens and benzophenone-3, including two that are part of this thesis report ^{44,104}. In addition, whereas the behavioral outcomes are quite well studied, cognitive outcomes were still under studied in relation to phenolic exposures. It is also important to note that various studies reported gender specific findings, this implies that adverse effects could be different by sex following gestational exposure.

In general, several epidemiological studies link bisphenol A, triclosan and phthalates to deleterious neurodevelopmental effects, investigating potential underlying mechanisms through which these compounds disrupt brain development is key to understanding their general extent of neurotoxicity.

Table 1.6: Mother-child cohort studies on the association between maternal phthalate and urinary concentrations with child behavior

Author	Cohort	N	Compounds	Age	Behavioral assessments	Trends observed
Huang et al, 2019	Taiwan Maternal and Infant Cohort Study	153	MMP, MEP, MnBP, MBzP , MEHP, MEHHP, MEOHP	8-9, 11-12 and 14-15	CBCL	Boys & girls: ↑ Anxious/depressed score, social problems, thought problems, attention problems, delinquent behavior, aggressive behavior, internalizing problems, externalizing problems with MEHP. ↑ Delinquent behavior, externalizing problems with ΣMEHP.
Kim et al, 2018	CHECK	140	MiBP, MnBP, MEHP, MEHHP, MEOHP, MEP	1-2	CBCL	Boys & girls: ↑ Internalizing and total behavioral problems with MEP
Philippat et al, 2017 ^a	EDEN	546	MEP, MnBP, MiBP, MCP, MBzP , MCOP, MCNP, ΣDEHP	3 and 5	SDQ	In boys only: ↑ Internalizing, peer relationship problems and emotional problems scores with MnBP. ↓ hyperactivity-inattention scores with MiBP. ↑ relationship problems scores with MBzP
Gascon et al, 2015	INMA	367	MBzP , MEP, MiBP, MnBP, MEHHP, MEHP, MEOHP, MECPP	1, 4, 7	SDQ and CSRS	In boys & girls: ↑ CP-SCS social competence, inattention with prenatal ΣDEHP.
Lien et al, 2015	Taiwan Maternal and Infant Cohort Study	122	MMP, MEP, MnBP, MBzP , MEHP, MEHHP, MEOHP	8	CBCL	In boys & girls: ↑ Delinquent behavior, aggressive behavior, externalizing problems with MnBP. ↑ Social problems, delinquent behavior, aggressive behavior and with prenatal MEOHP. ↑ Externalizing problems delinquent behavior with DEHP metabolites
Kobrosly et al, 2014	SFFII	153	MEHP, MEHHP, MEOHP, MiBP, MnBP , MBzP , MEP	6-10	CBCL	In boys & girls: ↑ Attention problems, aggressive behavior, oppositional/defiant problem scores with MiBP. ↑ Somatic problems with ΣDEHP. ↓ Affective problems with MEP In boys: ↑ rule-breaking behavior, conduct problems with prenatal MiBP. ↑ conduct problems with MBzP.

						<p>↑ conduct problems with prenatal MnBP In girls: ↓ anxious/depression with ΣDEHP. ↓ internalizing behaviors, anxiety problems with MBzP</p>
Whyatt et al, 2012	CCCEH	319	MBzP, MnBP, MiBP, MEHP, MEHHP, MEOHP, MECPP	3	CBCL	<p>In boys & girls: ↑ Somatic complaints, withdrawn behavior and internalizing behaviors with MnBP. ↑ Emotionally reactive behavior with MiBP. ↑ Withdrawn behavior and internalizing behaviors with MBzP. In boys: ↑ Emotionally reactive behavior with prenatal MnBP In girls: ↑ Somatic complaints and internalizing behaviors with prenatal MBzP</p>
Engel et al, 2010	Mount Sinai Children's Environmental Health Study	177	MECPP; MEHHP, MEHP, MEOHP, MBzP, MCPP, MMP, MEP, MnBP, MiBP	5, 6 and 7-9	BRIEF BASC-PRS	<p>In boys & girls: ↑ Attention, depression and adaptability, emotional control and global executive composite problems with low molecular weight phthalates In boys : ↑ Aggression, conduct, externalizing problems BSI score with low molecular weight phthalates In girls: ↑ conduct problems with low molecular weight phthalates</p>

^aSee chapter 3 for details of this study

Abbreviations: 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, BASC: Behavioral assessment for children, BRIEF: Behavior Rating Inventory of Executive Function-Preschool, CBCL: Child Behavior Checklist, SDQ: Strength and Difficulties Questionnaire

1.4 Biological pathways of neurotoxicity of phenols and phthalates

Phenols and phthalates target various endpoints on the endocrine system including those involved in the development of the brain. These compounds could indeed interfere with signaling of thyroid hormones^{109–113} or steroid hormone levels^{79,111,112,114,115} during pregnancy. During pregnancy, thyroid hormones regulate structural and functional organization^{113,116} while sex steroid hormones are crucial for the and for the sexual differentiation of the brain¹¹⁷.

Other mechanisms by which these compounds could disrupt brain development are via epigenetic mechanisms, through alteration of DNA methylation patterns¹¹⁸ and disruption of calcium signaling pathway¹¹¹.

In this thesis, we assessed how phenols and phthalates could affect levels of thyroid hormones during pregnancy and at birth.

1.4.1 Role of the thyroid axis in brain development

During pregnancy, thyroid hormones regulate migration, proliferation and differentiation of neural cells, as well as signaling and myelination¹¹⁹. Notably, uncorrected low levels of thyroid hormones at birth resulting from perturbation of this pathway during pregnancy often result in severe disorders. A common example of such defects is cretinism, a condition of severely stunted physical and mental growth linked to hypothyroidism due to inadequate iodine intake during pregnancy¹²⁰.

In epidemiological studies, pregnancy overt (low FT4, high TSH) and mild/subclinical (high TSH only, for example, levels above the 98th percentile) hypothyroidism has been linked to adverse neurodevelopmental outcomes among children such as decreased IQ^{121–127}, increase behavioral problems¹²⁸, decreased gray matter volume¹²⁷, increased risk for autistic traits¹²⁷ and impaired psychomotor function¹²⁹. On the other hand, although quite under-investigated, gestational hyperthyroidism (high FT4) was associated with decreased IQ and gray matter volume¹²⁵ and increased behavioral problems^{130,131}.

In early pregnancy, the sole source of thyroid hormones to the growing fetus is the mother¹³². When the fetus thyroid axis is matured (after 18 weeks), the placenta remains permeable to maternal thyroid hormones and they play a role in the homeostasis of the thyroid fetal hormones¹³³. It is therefore key that the maternal thyroid function remains devoid of disruptions throughout pregnancy for normal fetal development.

Aside from iodine deficiency and pre-existing thyroid disease, environmental xenobiotics, specifically endocrine disruptors are suspected to be probable sources of thyroid hormone dysregulation during pregnancy ¹³⁴.

1.4.2 Synthesis and homeostasis of thyroid hormones

The hypothalamus, located in the center of the brain releases thyrotropin releasing hormone (TRH) into the blood stream ¹³⁵, which then stimulates the pituitary gland to release TSH (Figure 1.6). Increase in TSH levels activates the synthesis and secretion of tyrosine in the thyroid follicular cells of the thyroid gland. Iodine is then combined with tyrosine to produce thyroid hormones (thyroxine (T4) and triiodothyronine (T3)) or their precursors. T4 and T3 are then released into the blood stream. T4 is the predominantly secreted hormone by the thyroid gland, but need for the more potent T3 leads to peripheral deiodination of T4 to T3 ¹³⁶.

Both T4 and T3 circulate in the blood as dissolved (free form) hormones or bound to circulating proteins (thyroxine-binding globulin (TBG)). The free forms can target tissue cells, such as neurons, where they bind to TH receptors (TR) and cause gene activation or inhibition leading to desired physiological changes.

A surge of T3 and T4 in blood exerts a negative feedback on the pituitary and hypothalamus to stop the release of TRH and TSH respectively (Figure 1.6).

1.4.3 Factors that influence thyroid hormone levels during pregnancy and at birth

Various factors are reported to influence thyroid hormone levels during pregnancy. Among these is gestational age; in the first trimester the increase in human chorionic gonadotrophin (HCG) stimulates the thyroid which leads to increased T4 and T3 and consequently decreases TSH. These levels are expected to stabilize in rest of the pregnancy ¹³⁷. Reinehr *et al.*, also reported that obese people tend to have higher thyroid stimulating hormone (TSH) and lower T3 blood levels ¹³⁸. Thyroid hormone levels were also reported to decrease with age (Demers, 2011). Smoking has been associated with a fall of serum TSH and a rise of serum FT4 and FT3 ¹³⁹. And finally, iodine deficiency (a median urinary iodine concentration less than 50 µg/L in the general population) lowers circulating T4 and raises serum TSH ¹⁴⁰.

Factors that may affect neonatal thyroid hormone levels are discussed in detail in Herbstman *et al.*, these included maternal factors such as ethnicity, hypertension during pregnancy / preeclampsia, gestational diabetes, maternal nutrition including iodine intake and

smoking during pregnancy. Regarding neonatal factors, gestational age at birth, age of the child at blood collection, birthweight and gender of newborn were important predictors of hormone levels. Another important factor described in the literature is the mode of delivery. Vaginal deliveries (assisted or not) and elective caesarean section resulting in higher average TSH cord and blood spot levels than planned caesarean sections ^{141–143}.

1.4.4 Disruption of the thyroid pathway by phenols and phthalates

Evidence from in vitro and in vivo studies

Bisphenol A is suspected to act as an antagonist of the thyroid hormone receptor-beta in vivo ¹⁴⁴ and thyroid hormone receptors alpha-I and beta-2 in vitro ¹⁴⁵. In addition, this compound has been reported to inhibit thyroid peroxidase (TPO) activity ^{146,147} (Figure 1.6, sites 1.a and 3.b).

Toxicological studies on the mechanism by which triclosan might affect thyroid axis suggested that this compound targets activity of various enzymes along the thyroid axis. It indeed inhibits activity of TPO ¹⁴⁶, deiodinase I ^{148,149}, and it increases levels of thyroid hormone metabolizing enzymes ¹⁵⁰ (Figure 1.6, sites 3.b, 5.a and 4).

Toxicological studies on the mechanisms of other synthetic phenols on the thyroid hormone axis are rare. A previous in vitro study suggested that butylparaben is a weak thyroid hormone receptor agonist, butyl paraben increased the proliferation of the GH3 cells ¹⁵¹ (Figure 1.6, site 1.a). Benzophenone-3 was also reported to be an agonist of the thyroid receptor in vitro assays ¹⁵² (Figure 1.6, site 1.a). We did not find any information on the exact mechanisms of action of 2,5 or 2,4 dichlorophenol on the thyroid axis, however, two other dichlorophenols (2,6 and 2,3 dichlorophenol) were found to be competitors for the T4 binding site of Transthyretin, a carrier protein of T4 ¹⁵³ (Figure 1.6, site 6).

Phthalates have been reported to have various end points on the thyroid axis. DBP and DEHP have antagonistic effects at the TR β receptors ^{154–156}; BBP was reported to up regulate iodide uptake into follicular cells ¹⁵⁷ whereas DBP down regulated NIS activity ^{158–160}. DEHP was reported to interact with proteins and enzymes responsible for the thyroid hormone homeostasis including deiodinases (involved in activation and deactivation of thyroid hormone), transthyretin (a T4 transport protein) and hepatic enzymes (involved in the metabolism of thyroid hormones) ^{161,162}. BBP was also reported to inhibit TPO activity ¹⁴⁶ and 5) (Figure 1.6, sites 1.a, 3.c, 4, 5, 6).

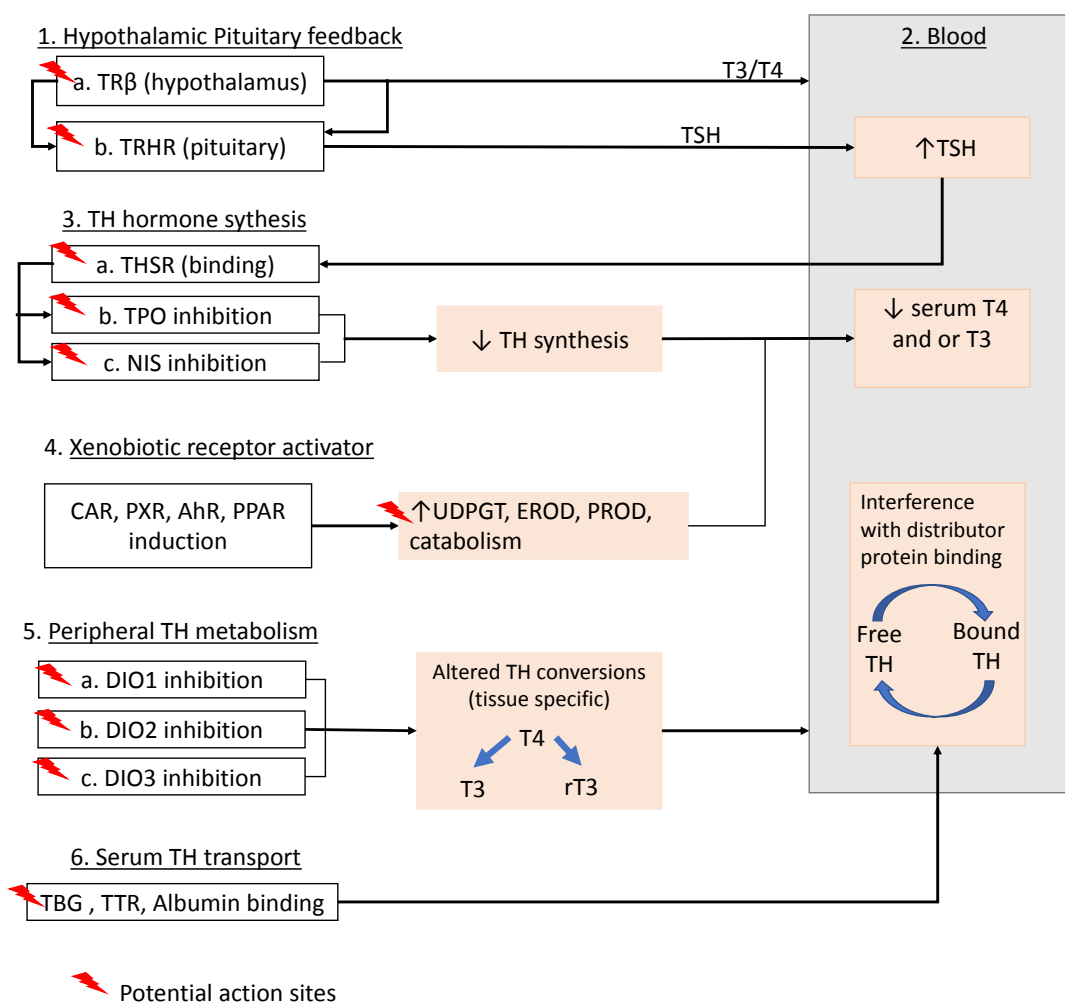


Figure 1.6: Schematic representation of potential action sites of phenols and phthalates along the thyroid hormone axis (adapted from Noyes *et al* ¹⁶³)

In orange: expected measurable key events

Suspected compound action sites; bisphenol A (1.a, and 3.b), parabens (1.a), triclosan (3.b, 5.a and 4) dichlorophenols (6), benzophenone-3 (1.a), phthalates (1.a, 3.c, 4, 5 and 6).

Abbreviations: NIS: sodium / iodine symporter, TH: thyroid hormone, TRH: thyrotropin-releasing hormone, TSH: thyroid stimulating hormone, T4: thyroxine, T3:tri-iodothyronine, DIO1-DIO3: deiodinases 1 to 3, TR β : thyroid hormone receptor beta, TBG: thyroxine binding globulin, TTR: transthyretin, UDPGT: Uridine 5'-diphospho-glucuronosyltransferase, EROD: Ethoxyresorufin-O-deethylation, PROD: Pentosy-resorufin-O-deethylase

Evidence from epidemiological studies

Epidemiological studies of associations between phenols and thyroid hormones of pregnant women and newborns are summarized in Table 1.7.

Only two studies explored the associations between pregnancy concentrations of bisphenol A and levels of thyroid hormones of neonates (in cord or newborn sera). These studies did not find any statistically significant associations in the global sample but reported sex-specific effects. Maternal urinary bisphenol A concentration was inversely associated with newborn TSH assessed in blood spot in males in one study¹⁶⁴ and positively with TT3 in cord blood levels of females¹⁶⁵ in the other study. In relation to maternal thyroid hormone levels, depending of the studies, maternal bisphenol A urinary concentration was either positively^{166,167} or negatively^{164,168} associated with TT4 or FT4 levels. One study also found that maternal urinary bisphenol A concentration was positively associated with TT3¹⁶⁹. Associations with TSH were only reported in one study in relation to this compound, suggesting an inverse association¹⁶⁷.

Regarding the other phenols, *Wang et al.*, found that cord blood FT3 decreased with increased triclosan urinary concentrations during pregnancy¹⁷⁰. Among the five studies that assessed the effects of triclosan exposure on maternal thyroid hormone levels, only two reported statistically significant associations with T4 and TSH. *Berger et al.*, and *Wang et al.*, reported a decrease in TT4¹⁷¹ and FT4¹⁷⁰ levels respectively while *Wang et al.*, showed a decrease in TSH levels¹⁷⁰ in relation to increased urinary concentrations of triclosan.

Effects of paraben exposure during pregnancy on thyroid hormone levels in pregnant women and their newborns were only considered by three and one study(s) respectively. No clear trends emerged in relation to newborn thyroid hormone levels¹⁷¹, while Aker et al reported that butyl paraben was positively associated with FT4¹⁶⁶, while methyl paraben was negatively associated with TSH¹⁶⁹.

Berger et al., found that maternal 2,4-dichlorophenol urinary concentrations were inversely associated with maternal free T4¹⁷¹. Although benzophenone-3 has been reported as thyroid receptor agonistic effects in in vitro assays¹⁵². Epidemiological studies specifically in pregnant women mainly reported null association with THs levels^{171,172}. Only one study suggested that benzophenone-3 was inversely associated with maternal FT3 serum levels¹⁶⁶.

Table 1.7: Mother-child cohort studies on the associations between maternal phenol urinary concentrations and thyroid hormone levels during pregnancy and at birth

Reference	Study population	Cohort	Phenols	N (urine samples) per woman	TH measured	Major findings
Derakhshan et al, 2019	1996 women	SELMA	BPA, BPS, BPF, TCS	1	Women: TSH, FT4, FT3, TT4, TT3	TT4, FT4/FT3 and TT4/TT3 ratios↓ with BPA FT3 ↑ with BPF
Aker et al., 2019	602 women	PROTECT	2,4-DCP, 2,5-DCP, BPA, BPS, BPF, BP3, TCS, TCC, MP, EP, BP, PP	3	Women: TSH, FT4, T3, T4	T3 ↑ with BPA and TCC FT4 ↑ with BPF, TSH ↓ with TCC and MP
Beger et al., 2018	338 women and 364 infants	CHAMACOS	TCS, BP3, 2,4-DC4, 2,5-DC4, MP, PP & BP	2	Newborns: TSH Women: FT3, FT4, TT4, TSH	Newborn blood: No associations Maternal sera: FT4 ↓ with 2,4-DCP, TT4 ↓ with TCS
Wang et al., 2017	398 mother-infant pairs	Prospective cohort from Shanghai, china	TCS	1	Newborns and mothers: FT3, FT4, TSH	Cord blood: FT3 ↓ with TCS Maternal sera: FT4 ↓ and TSH ↓ with TCS in the 3rd trimester
Aung, et al 2017	439 women (116 preterm and 323 controls)	Prospective cohort from Brigham and Women's Hospital	BPA	4	Women: TSH, TT3, FT4 and TT4	↓ TSH ↑ FT4 and TT4
Aker et al., 2016	106 women	PROTECT	BPA, TCS, BP-3, 2,4-DCP, 2,5-DCP, BP, MP, PP	2	Women: FT3, FT4, and TSH	FT4 ↑ with BPA FT3 ↓ with BP3 FT4 ↑ with BP (in samples collected close to each other)
Romano et al., 2015	181 mothers and 249 infants	HOME	BPA	2	Newborns and mothers: TSH, TT4, TT3, FT4, and FT3	Cord blood: No significant association in global sample Girls: TT3 ↑ in girls only Maternal sera: No significant association
Chevrier et al., 2013	335 women and 364 infants	CHAMACOS	BPA	2	Women: TT4, FT4 and TSH Newborns: TSH	Newborn blood: TSH ↓ with BPA in boys Maternal sera: TT4 ↓ with BPA

Abbreviations: BPA: bisphenol A, TCS: triclosan, TCC : triclocarban, BP3: benzophenone-3, MP: methylparaben, EP: ethylparaben, PP: propylparaben, BP: butylparaben, TH: thyroid hormones, T4/TT4 (resp. T4/TT4) : Total thyroxine (triiodthyronine), TSH: thyroid stimulating hormone

As far as we know, only 4 studies have looked at the associations of gestational phthalate exposures on thyroid hormone levels of neonates (summarized Table 1.8). These studies reported that MBzP was negatively associated TSH levels ^{173,174}, MnBP was positively associated with TT3 ¹⁷⁵ and that MEP was negatively associated with TT4 ¹⁷⁴ while one study found null associations ¹⁷⁶.

Among studies considering pregnancy serum thyroid hormone and TSH levels in relation to phthalate exposure, findings are heterogeneous (See for details Table 1.8). In a recent study of 98 women set to undergo amniocentesis, Huang et al, found that TSH levels decreased significantly with MiBP concentration ¹⁷⁵. This negative association was reported by several other studies in relation to other metabolites including, MBzP ¹⁶⁵, MEHP and MCPP ¹⁷⁷. An increase in TSH has also been reported in association to various DEHP metabolites ¹⁷⁶. Similarly, results of T4 were bipartite, free T4 was negatively associated with DEHP metabolites ^{176,177}, MnBP ¹⁷⁸, MBzP ¹⁷⁶ and positively with MCPP and MiBP ^{177,179}. In relation to total T4, negative associations with reported with exposure to DEHP ¹⁷⁶, MnBP ^{178,180} and MBzP ¹⁷⁶, while for MEHP, a metabolite of DEHP, reports of positive associations with total T4 also exist ¹⁷⁹.

FT3 was found to be negatively associated with MCPP, MCOP ¹⁷⁷ and positively with MEP and MiBP ^{175,179}.

In summary, heterogeneous results were observed in studies of these compounds. These discrepancies could partly be a consequence of differences in study designs such as timing of urine and blood sample collection, differences in exposure levels and as well as due to the multiplicity of associations tested that could have led to chance findings, usually including five hormones * 10 phthalate metabolites or 12 * phenols without correction for multiple comparison, risking chance findings. Null findings were also reported in some studies; this could be a result of relying on a small number of urine samples to assess exposure which limits study power (this is discussed in detail in section 1.5).

In addition, sex specific effects on neonatal thyroid hormone levels have been reported in relation to prenatal exposure to bisphenol A and some phthalates ^{164,165,181}. As a hypothesis, such effects could be due to the fact that some of these compounds have weak estrogenic effects ^{182,183}. In humans, estrogen is reported to elevate serum thyroxine levels ¹⁸⁴.

Table 1.8: Mother-child cohort studies on the associations between maternal phthalate urinary concentrations with thyroid hormone levels during pregnancy and at birth

Reference	Study population	Cohort	Phenols and phthalate metabolites	N (urine samples) per subject	TH measured	Major findings
Huang et al., 2018	98 pregnant women	TBCS	MEP, MnBP, MBzP, MMP, MiNP, MCMHP, MiBP, MEHP, MEOHP, MEHHP and MECPP	2	Mothers and infants: TT4, FT4 and TT3	Cord blood : TT3 ↑ with MnBP Maternal sera: TSH ↓ with MiBP and MEOHP, FT3 ↑ with MiBP TT3 ↓ with MCMHP
Romano et al., 2018	206 women and 276 infants	HOME	MEP, MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MMCHP, OH-MiNP, oxo-MiNP and cx-MiNP	2	Mothers and infants: TSH, TT4, FT4, TT3, FT3	Cord sera: TSH ↓ with MBZP, TT4 ↓ with MEP Maternal sera: TT4 ↓ with MEP
Gao et al., 2017	1,397 women	C-ABC	MEP, MnBP and MEHP	1	T4, FT4 and TSH	FT4 ↓ with MnBP, MEHP , TT4 ↑ with MnBP and MEHP
Yao et al., 2016	2521 mother-infant pairs	MABC	MEP, MMP, MBzP MEHP and MEHHP,	1	TSH, TT4, FT4, and TT3	Cord sera: No association Maternal sera: TSH ↑ with MEHP and MEHHP, FT4 ↓ with MBzP, MEHP and MEHHP, TT4 ↓ with MBzP, MEP, MEHP, and MEHHP
Huang et al., 2016	97 women	Cheng Kung University Hospital	MiNP, MCMHP, MEP, MMP, MBzP, MiBP, MnBP, MEHP, MEOHP, MEHHP and MECPP,	1	Mothers: T4, FT4 and T3	TT4 ↓ with MnBP

Johns et al., 2015	439 women	PROTECT	MEP, MnBP, MBzP, MEHP, MEHHP, MEOHP, MECPP, MIBP and MCPP	4	Mothers: TSH, TT4, FT4, TT3 and FT3	Longitudinal: ↓ FT3 with MCPP Stratified by visit: ↑ FT4 with MiBP (16-20 weeks), ↓ FT4 with ΣDEHP (24–28 weeks), ↓ FT3 with MCPP, MCOP (24-28 weeks)
Johns et al., 2016	106 women	LifeCodes	MEHP, MnBP, MEHHP), MEOHP, MECPP, MCPP, MCOP, MCNP, MBzP, MiBP and MEP.	2	Mothers: FT4, FT3 and TSH	Longitudinal analysis: ↓ TSH with MEHP, MiBP, MCPP, ↑ FT4 with MCPP, ↑ TT4 with MEHP, ↑ FT3 with MEP Stratified by visit: TSH ↓ with ΣDEHP and MCPP (10 weeks), MBzP and MnBP (18 weeks), FT4 ↑ with MBzP (35 weeks) and MCPP (10 and 35 weeks)
Kuo et al., 2015,	148 mother-child pairs	Women from E-Da Hospital, Taiwan	MEHP, MEHHP, MEOHP, MnBP, MiBP, MEP, MBzP, MMP, and MiNP	1	TSH, TT4, FT4 and T3	Cord blood: TSH ↓ with MBzP
Huang et al., 2007	76 women	Cheng Kung University Hospital	MnBP, MBzP, MEHP, MEP, MMP	1	TSH, TT4, FT4, TT3 and FT3	TT4 ↓ with MnBP FT4 ↓ with MnBP

Abbreviations: 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, MMCHP: mono-2-methylcarboxyhexyl phthalate, OH-MiNP: Hydroxy-(mono-methyl-octyl) phthalate, cx-MiNP: Carboxy-(mono-methyl-heptyl) phthalate, oxo-MiNP: Mono-oxo-isononyl phthalate, TH: thyroid hormones, T4/TT4 (resp. T4/TT4): Total thyroxine (triiodothyronine), TSH: thyroid stimulating hormone.

1.5 Limitations of the current epidemiology studies and perspectives

Epidemiological studies investigating associations of prenatal exposure to short lived compounds such as phenols and phthalates^{33–36} with health outcomes, generally rely on one and sometimes up to three spot urine measurements (taken at different study periods) to estimate exposure during pregnancy. High within subject temporal variability has been observed for some these compounds (Figure 1.7)¹⁸⁵. Intraclass correlation coefficients (ICC) for 10 phenols including benzophenone-3, bisphenol A and S, 2,4 dichlorophenol, 2,5 dichlorophenol, methylparaben, ethylparaben, propylparaben, butylparaben and triclosan were previously discussed in detail by Vernet et al¹⁸⁶. This study reported high to moderate within-day variability, with ICCs ranging from 0.03; (95% CI: 0.00, 0.15) for ethylparaben to 0.50 (95% CI: 0.26, 0.73) for bisphenol S¹⁸⁶. Regarding the phthalates, low ICC were previously reported for metabolites of DEHP, as well as MCPP, MCOP and MCNP (ICC ranging from 0.18 to 0.33) and moderate ICCs for MnBP, MBzP and MEP (0.5 to 0.69)¹⁸⁷.

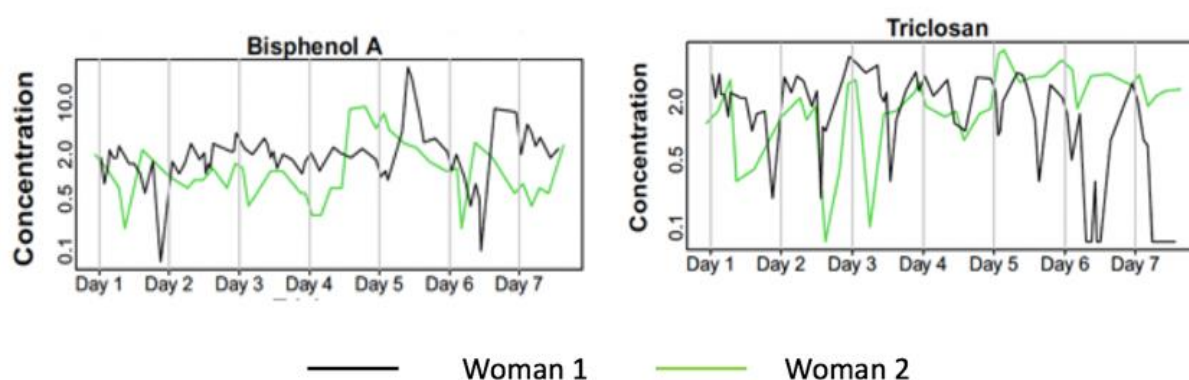


Figure 1.7: Urinary concentrations ($\mu\text{g/L}$) of two phenols (triclosan and bisphenol A) in urine samples collected over a week in two pregnant women from the SEPAGES-feasibility cohort.

Relying on one to 3 spot samples poorly estimates exposure throughout pregnancy and could lead to a measurement error of classical nature. This error is expected to bias dose–response relationships towards the null¹⁸⁸ and limits study power. A simulation study performed in our research team, showed that when a spot urine sample is used to assess exposure, the effect estimate can be biased towards the null by up to 40% or 80%, compared to the real effect, depending on the intra-individual variability of the compounds considered (illustrated in Figure 1.8)¹⁸⁹.

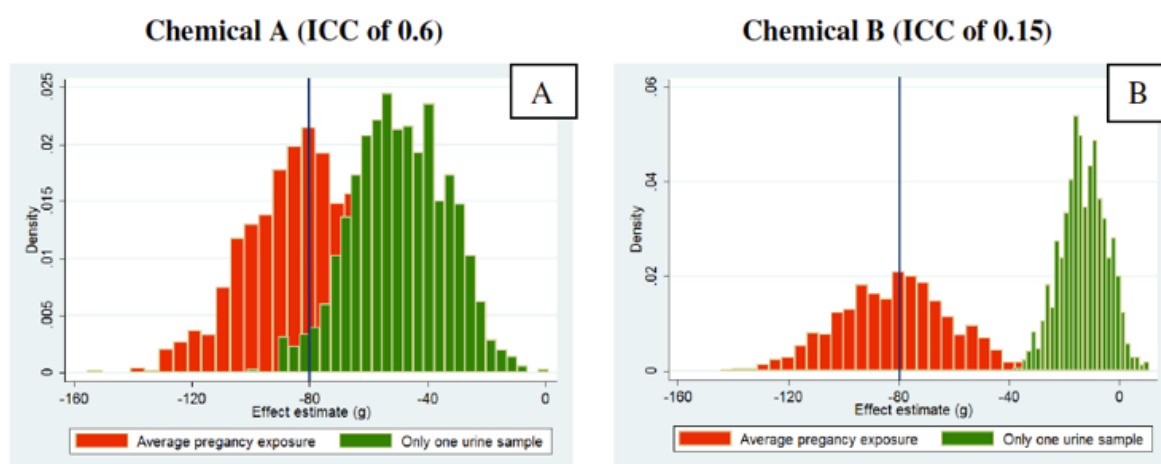


Figure 1.8: Distribution of the effect estimate characterizing the association between exposure and birth weight using the average pregnancy exposure (in red) or one spot urine sample (in green) of exposure to chemical A (ICC of 0.6) and chemical B (ICC of 0.15) (1000 simulations were performed, each with 500 subjects, real effect $\beta_1 = -100$ g) ¹⁸⁹.

A suggested solution to limit this measurement error and the resulting bias is the collection of repeated urine samples so as to estimate average exposure over the relevant exposure time window. However, assaying exposure in many urine samples per individual is costly and could limit sample size. A trade off approach, is to perform within-subject pooling of biospecimens over relevant exposure time windows and assess the chemical of interest in the pools instead of in spot samples.

Two previous studies, a simulation and an empirical study demonstrated that pooling spot samples collected over a period of interest decreased attenuation bias and improved statistical power ^{189,190} compared to the situation where only a spot urine sample was used. Vernet et al., further demonstrated that for compounds with high variability such as bisphenol A, a pool of about 18 samples decreased bias from 61% to 10% and improved study power from 27% to 66% ¹⁹⁰ compared to the situation where only one spot sample was used

1.6 Thesis aim and objectives

Previous research on prenatal exposure to phenolic and phthalate compounds falls short of providing an understanding of the impact of specific sources of exposure to these compounds, such as personal care products, on the internal dose of phenols in pregnant women, which is crucial for interventions to reduce exposure. Previous studies were indeed limited among pregnant women and did not consider potential substitutes of widely-used compounds such as bisphenol A (e.g., bisphenol S). In addition, when this thesis was initiated, no study had explored the associations between phenols other than bisphenol A, such as benzophenone-3, triclosan, dichlorophenols, parabens and child neurodevelopment, some of which were previously linked to adverse behavior and memory in rodent studies^{72,74} or reported to disrupt various mechanisms involved in fetal brain development^{79–81} in rodent studies.

Epidemiological studies documenting possible effects of phthalates and phenols on the thyroid hormone levels, a mechanism hypothesized to mediate neurotoxicity of these compounds, were also rare with heterogeneous results.

Furthermore, majority of the studies assessed phthalate and phenol concentrations in one to three urine samples collected during pregnancy. This was a major limitation, which is evidenced to lead to exposure misclassification with consequential bias towards the null and under estimation of effects (see Figure 1.8 above)^{188,189}.

In this research, we aimed to address these gaps and broaden the literature on neurodevelopmental effects of phthalate and phenol exposure during pregnancy by tracing their paths from the environmental sources, specifically from maternal use of personal care products, to their neuropsychological endpoints (cognition and behavior) and the potential underlying mechanisms of neurotoxicity such as disruption of the thyroid function.

The specific objectives of this thesis were (illustrated in Figure 1.9):

1. To characterize how use of personal care products during pregnancy could influence phenol biomarker concentrations in pregnant women (aim 1, chapter 2).
2. To assess associations between prenatal exposure to phenols and phthalates and child neurodevelopment (cognition and behavior, aim 2, chapter 3).
3. To assess associations between prenatal exposure to phenols and phthalates on the levels of thyroid hormones, of pregnant women and their newborns (aim 3, chapter 4).

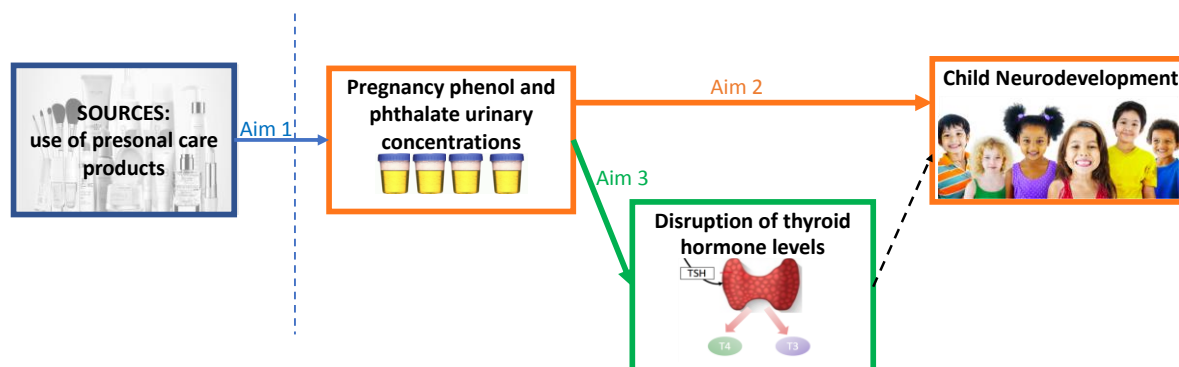


Figure 1.9: Schematic illustration of study aims

These analyses were based on three complementary cohorts (detailed in Table1.9), two of which relied on repeated urine samples (including pooled samples for one study) to estimate phenols and phthalate metabolites urinary concentrations

Table1.9: Description of cohorts included in this thesis

Recruitment and place	Sample size (N included in our analysis)	Compounds assessed	Strengths	Key reference
EDEN: Etude des Déterminants pré et post natals du développement et de la santé de l'Enfant				
February 2003 to January 2006 Nancy and Poitiers, France	2002 mother child pairs (457- 452 mother-son pairs included in our analysis)	Phenols and phthalates	Assessments of neurodevelopmental outcomes Assessments of other phenols than bisphenol A, such as benzophenone-3, triclosan, dichlorophenols, parabens Larger sample size compared to previous studies	Heude et al., 2016 ¹⁹¹
SEPAGES-feasibility study				
July 2012 to July 2013 Grenoble, France	40 pregnant (8 were included in our analysis)	Phenols and substitutes (bisphenol S)	Phenol concentrations assessed in repeated samples with exact time of collection (8-80 samples per woman) Type and exact time of use of personal care products	Vernet, et al., 2018 ¹⁸⁶
SEPAGES : Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effets sur la Santé				
July 2014- July 2017 Grenoble, France	484 mother-child pairs (416 women and 421 newborns)	Phenols, phthalates and substitutes	Recruitment early in pregnancy Exposure assessed in pooled samples based on repeated urine collection (42 samples per woman) Assessmens of serum thyroid hormone levels for both mother and their child	Lyon-Caen, et al., 2019 ⁴⁶

**CHAPTER 2 : USE OF PERSONAL CARE PRODUCTS AND PHENOL URINARY
CONCENTRATIONS DURING PREGNANCY**

2 Use of personal care products and phenol urinary concentrations during pregnancy

The work presented in this chapter is currently under review in the International Journal of Hygiene and Environmental Health.

Title: Use of personal care products during pregnancy in relation to urinary concentrations of select phenols: a longitudinal analysis from the SEPAGES feasibility study

Dorothy Nakiwala^{1*}, Céline Vernet², Sarah Lyon-Caen¹, Anna Lavorel¹, Matthieu Rolland¹, Claire Cracowski³, Isabelle Pin^{1,4}, Antonia M. Calafat⁵, Rémy Slama^{1**}, Claire Philippiat^{1**} and the SEPAGES study group

¹University Grenoble Alpes, Inserm, CNRS, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Institute for Advanced Biosciences (IAB), Grenoble, France

²Center for Environmental Research and Children's Health (CERCH), School of Public Health, University of California, Berkeley, USA

³Unité de Pharmacologie Clinique, CIC de Grenoble, Grenoble, France

⁴Centre Hospitalier Universitaire (CHU), Service de pédiatrie, Grenoble, France

⁵Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The SEPAGES study group includes: Sarah Lyon-Caen, Johanna Lepeule, Valérie Siroux, Claire Philippiat, Joane Quentin, Isabelle Pin and Rémy Slama.

** Co-last authors

*Corresponding author. Address: Institute for Advanced Bioscience (IAB), Site Santé , Allée des Alpes, 38700, La Tronche, France, Phone: +33 4 76 54 94 66, Email:

dorothy.nakiwala@univ-grenoble-alpes.fr

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views 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.

2.1 French summary

Introduction

L'exposition à certains phénols synthétiques fait l'objet d'une inquiétude grandissante, particulièrement chez les femmes enceintes car ce sont des perturbateurs endocriniens et nombreux sont ceux encore autorisés dans les produits de soins personnels (Personal care product - PCP). Notre objectif est d'évaluer le lien entre l'utilisation de ces PCP et les concentrations urinaires de phénols durant la grossesse.

Méthodes

Huit femmes ont consigné durant une semaine dans un journal de bord le moment exact et le type d'utilisation des PCP. Nous avons mesuré les concentrations de phénols (quatre parabènes, les bisphénols A et S, deux dichlorophénols, le triclosan et le benzophénone-3) au sein de 178 échantillons d'urine, collectés sur sept jours consécutifs à trois moments distincts de la grossesse. Nous avons caractérisé l'utilisation des PCPs en considérant le nombre total d'utilisations ou leur utilisation (oui/non) au sein de trois fenêtres de temps distinctes (entre 0 et 6h, 6 et 12h, et 12h et 24 heures avant le recueil d'urine). Les relations entre l'utilisation de PCP et les concentrations urinaires de phénols ont été évaluées par des régressions linéaires et des modèles tobit ajustés.

Résultats

Le nombre total d'utilisation des PCP est positivement associé avec les concentrations d'éthylparabène, popylparabène et butyleparabène. Douze types différents de PCPs sont positivement associés avec la concentration d'au moins un parabène. La concentration du bisphénol S augmentait avec le nombre d'utilisations de PCP ($\beta = 0.12$, 95% intervalle de confiance = 0.06; 0.18) et l'utilisation spécifique de PCP telle que la crème antirides, le baume à lèvres, le fond de teint, les soins du visage et les gels douches. Ces associations étaient variables selon les fenêtres de temps étudiées.

Conclusion

Nos résultats concernant les parabènes sont cohérents avec les résultats d'études précédentes et confirment que l'utilisation des PCPs augmente l'exposition aux parabènes. Cette étude est la première à faire le lien entre utilisation de PCPs et de bisphénol S, un résultat qui appelle à des recherches plus approfondies au sein de cohortes incluant des recueils d'urine répétés et une description précise de l'utilisation de PCPs.

2.2 Abstract

Background: Exposure to certain synthetic phenols is of growing concern, in particular among pregnant women, because of their endocrine disrupting nature. Many phenols are authorized in personal care products (PCP). We aimed to assess if use of PCPs by pregnant women could influence their urinary concentrations of synthetic phenols.

Methods; We used a panel design with intense urine sample collection. Eight women completed a diary with exact time and use of PCPs over three weeks. We measured the concentrations of phenols (four parabens, bisphenol A and S, two dichlorophenols, triclosan, and benzophenone-3) in 178 urine samples, collected during 7 consecutive days at 3 time points during pregnancy. We characterized PCP use as the total number of PCP applications or as a single PCP use (yes / no) in three time windows (0 to 6, 6 to 12 and 12 to 24 hours before each urine sample collection). We used adjusted linear and Tobit regressions to assess associations between PCP use and phenol urinary concentrations.

Results: The total number of PCP applications was positively associated with ethylparaben, propylparaben and butylparaben, but not methylparaben concentrations. We observed a peak in urinary concentration of ethylparaben, butylparaben and propylparaben at 2.86, 2.55 and 2.67 hours since last PCP use, respectively and about twelve different PCPs types were positively associated with at least one of these parabens. The bisphenol S concentration increased by 12.4% (95%CI: confidence interval: 5.9; 19.3) for each additional PCP application in the 12 to 24-hours time window as well as with use of specific PCPs such as anti-stretchmarks cream, facial cleanser and shower gel. Associations with specific PCPs varied by time window.

Conclusion: Our study shows that PCPs' use was associated with a short-term increase in the urinary concentration of ethylparaben, butylparaben and propylparaben, but not methylparaben. This study also reported a positive an association between the use of PCPs and the bisphenol S concentration, a finding that warrants further investigation in cohorts with repeated collection of urine samples and detailed information on PCP use.

2.3 Methodology

Study population

The study population consisted of a sub-sample of the SEPAGES-feasibility (Suivi de l'exposition à la pollution atmosphérique durant la Grossesse et Effets sur la Santé) cohort that recruited 40 pregnant women before the 17th gestational week (GW, calculated from the date of the last menstrual period) from private obstetrical practices (Grenoble, France) between July 2012 and July 2013^{186,192}.

Exclusion criteria were inability to write or speak French, being under 18 years of age, planning to give birth outside of the four maternity hospitals of the Grenoble urban area, and not being enrolled in the French social security system.

This feasibility cohort was approved by the appropriate ethical committees including the Comité de Protection des Personnes Sud-Est, the Commission Nationale de l'Informatique et des Libertés, the Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé and the Agence Nationale de sécurité du Médicament et des produits de santé. All participating women provided written informed consent for biological measurements and data collection. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subject research.

Urine collection during pregnancy, assessment of phenol biomarker concentrations and specific gravity

Pregnant women were followed up for 7 consecutive days at three periods of their pregnancy (median at 13 GW (min–max: 10–18); 23 GW (min–max: 21–26) and 32 GW (min–max: 29–33)) and asked to collect all their urine voids over the three weeks of follow-up and to record the exact date and time of each micturition. Each spot urine sample was collected in a polypropylene container provided by the study team. After collection, urine spot samples were stored at 4°C in the participants' home refrigerators until collection by study staff which occurred two or three times per week. The samples were transported in coolers to the INSERM research center (Institute for Advanced Biosciences (IAB), Grenoble), where specific gravity, a marker of urine dilution was measured using a handheld PAL 10-S refractometer (Atago, Bellevue, WA, USA). Samples were then aliquoted into polypropylene cryovials and frozen at -80°C.

We took advantage of a previous project which aimed to finely characterize variability of phenol urinary biomarker concentrations and relied on the eight women of the SEPAGES-F cohort with the smallest percentage of missing voids (< 5% over the 3 weeks of follow-up)¹⁸⁶. For these women, phenols concentrations were analyzed in a total of 178 samples: 130 (73%) belonged to two women for whom we had no missing voids in week 1 (72 voids from woman 1, 42 voids from woman 2) plus 8 voids each, randomly selected during the second and the third collection weeks; and 48 samples from the remaining six women for whom 8 voids each were randomly selected over the 3 weeks of follow up (Table 1)¹⁸⁶.

Urine samples were sent on dry ice to the CDC (Atlanta, GA, USA) where phenols were quantified using online solid-phase extraction high-performance liquid chromatography-isotope dilution-tandem mass spectrometry¹⁹³. Total urinary concentrations of 2,4- and 2,5-dichlorophenols, benzophenone-3, bisphenol A, bisphenol S, triclosan, butylparaben, methylparaben, ethylparaben, and propylparaben were quantified. 2,4- and 2,5-dichlorophenol are not expected to be found in PCPs and were only included in this study as controls (i.e., no association with PCP was expected for these compounds).

Use of personal care products and confounders

During the three weeks of follow-up, women used diaries to record each PCP usage and time of use. Relying on the date and exact time of each use of PCP reported by the women (the list of PCPs is given in Table 3), we characterized PCP use in relation to each urine void as the total number of PCP applications as well as the use of specific PCPs (yes / no) in the 0 to 6, 6 to 12 or 12 to 24 hours before each urine collection.

In addition, women provided the date and exact time of food and drink consumption during the follow up weeks. At the start of the study, we also collected information about their anthropometry (weight and height), demographic characteristics, and social economic status including age, parity, marital status, education level and profession.

Statistical methods

Phenol concentrations below the limit of detection (LOD) were replaced by specific instrumental reading values; when no signal was detected, we imputed the phenol-specific lowest instrumental reading value divided by the square root of two. All phenol concentrations were ln-transformed prior to analysis to approach normal distribution.

To study the associations between use of PCPs and phenol urinary concentrations, we either used adjusted linear regression (for phenols with less than 10% non-detection frequency) or Tobit models (for compounds with up to 10% non-detection frequency)^{194,195}. We considered the total number of PCP applications and the use of specific PCP in the 0 to 6, 6 to 12 or 12 to 24 hours before each urine collection.

We ran additional analyses looking at the time since last PCP use, with the aim of detecting peaks in phenol concentration following use of any PCP positively associated with that particular phenol. For this analysis, time since last use of PCP was coded as restricted cubic splines (5 knots).

Adjustment factors were identified *a priori* as possibly associated with the use of PCP and/or the phenol urinary concentrations. These included the women (fixed effect), specific gravity, hour (categories: 0000-0600, 0601-1200, 1201-1800, 1801-2359 hours) and day (weekday/weekend) of urine collection as well as the use of PCP in the time windows other than the one studied (e.g., analysis looking at the association with PCP use in the 12 to 24 hour windows was adjusted for PCP use in the 0 to 6 and 6 to 12 time window).

In the models exploring the associations of urinary phenol concentrations with use of a specific PCP (yes / no), for power reasons, PCPs were not considered if the frequency of use was less than five times in any of the time windows. These included mouth wash, nail polish, sunscreen, eye shadow and thermal spring water in all windows, intimate soap in the 0 to 6 and 6 to 12 hour windows, intimate soap in the 6 to 12 hour windows and toothpaste in the 12 to 24-hour window.

Depending on the analysis, effect estimates are reported as the percent change in phenol urinary concentration either 1) in a period in which a PCP had been used, compared to a period without PCP use (analysis relying on specific PCP use); or 2) for an additional PCP application (analysis relying on the total number of PCP used).

Sensitivity analyses

We considered other adjustment factors suspected to be associated with phenol urinary concentrations including gestational age at sampling, and, for the phenols likely to be found in food (parabens and bisphenols¹⁹⁶⁻¹⁹⁸), the time elapsed since the last meal (food or drink). For comparison with previous studies, we also considered use of PCPs 24 hours before urination (Supporting information, Figure S3).

With the exception of the two dichlorophenols considered as negative controls, the studied phenols were selected because they have been previously detected in various PCP ^{52,54,70,71}. In addition, higher urinary concentrations of paraben urinary concentrations in relation to use of multiple or single PCPs have been consistently reported in previous studies ^{48,56–59,61,64,66,199–202}. In this setting of high a priori hypothesis for an association between PCP use and most of the studied phenols, we decided to not correct our analysis for the number of tests performed ²⁰³. However, we were aware that chance findings cannot be rule out. This is why in the discussion section we gave more weight to the associations that were previously described in literature while other associations should be considered as hypotheses generating.

All analyses were carried out using STATA/SE 14 (StataCorp, College Station, TX, USA) and R version 3.4.3 statistical software.

2.4 Results

Study participants

The eight women included in this study were aged between 26.7 to 37.6 years at enrolment; five were nulliparous, two primiparous and one was multiparous. All women had a college degree (Table 2.1).

Table 2.1: Characteristics of the eight women of the SEPAGES feasibility study for whom spot urine samples were analysed for phenol concentrations

	Woman 1	Woman 2	Woman 3	Woman 4	Woman 5	Woman 6	Woman 7	Woman 8
Age (years)	33.5	27.4	26.7	30.8	37.6	30.6	28.9	28.0
Years of education after high school)	6	6	5	7	9	5	5	5
Height (cm)	169	167	162	170	167	169	173	157
Weight before pregnancy (Kg)	58	76	71	69	59	62	60	53
Number of children	1	1	0	0	2	0	0	0
Number of urine samples included in the study	8	8	80	8	50	8	8	8

Urinary phenol concentrations

Of the 178 urine samples collected over the three periods of follow up, 8% (n = 13) were collected between 00 and 6:00, 34% (n = 62) between 6:01 and 12:00, 26% (n = 46) between 12:01 and 18:00 and 32% (n = 57) between 18:01 and 23:59 hours. Most samples (71%) were collected during a weekday.

Phenols were detected in more than 74% of the urine samples except for benzophenone-3 that was only detected in 35% (N=62) of the samples. The highest concentrations were observed for the four parabens, followed by triclosan and bisphenol A (Table 2.2). Within samples spearman correlation coefficients were higher between compounds of similar chemical structures: among the parabens (Spearman's coefficient (ρ) = 0.57 to 0.97) and between the dichlorophenols (ρ = 0.53). We also observed moderate positive correlations between bisphenol A and 2,4 dichlorophenol (ρ = 0.50) and between triclosan and the dichlorophenols (ρ = 0.49 and 0.53 for 2,4- and 2,5- dichlorophenol, respectively). The Spearman's correlation coefficients between the phenols and specific gravity ranged from -0.12 (propylparaben) to 0.79 (2,4-dichlorophenol, supplemental material Table 2.5).

The intraclass correlation coefficients (ICC) for these compounds were previously discussed in detail by Vernet et al ¹⁸⁶. Briefly, we observed high to moderate within-day

variability (supplemental Figure 2.3), with ICCs ranging from 0.03; (95% CI: 0.00, 0.15) for ethylparaben to 0.50 (95% CI: 0.26, 0.73) for bisphenol S ¹⁸⁶.

Table 2.2: Distribution of urinary phenol concentrations ($\mu\text{g/l}$) in 178 urine samples collected from eight women of the SEPAGES feasibility study

Biomarker	LOD	% >LOD	Percentiles			GM	Min	Max
			5	50	95			
Benzophenone-3	0.3	35	<LOD	<LOD	17.2	0.8	0.3	378.5
Bisphenol A	0.1	99	0.2	1.6	8.9	1.5	0.1	30.4
Bisphenol S	0.1	97	0.1	0.3	2.6	0.3	0.1	212
2,4 dichlorophenol	0.1	99	0.1	0.3	1.4	0.3	0.1	6.2
2,5 dichlorophenol	0.1	98	0.1	0.4	9.2	0.5	0.1	278.4
Methylparaben	1.0	99	3.7	128	4080	129.0	0.8	8730
Ethylparaben	1.0	83	<LOD	6.1	1262	6.4	0.1	203.5
Propylparaben	0.1	100	0.2	14.9	4962	12.1	0.1	1253.6
Butylparaben	0.1	90	<LOD	2.0	88.2	1.6	0.1	335.4
Triclosan	1.0	74	<LOD	1.8	28.1	1.6	0.1	1474.8

LOD: Limit of detection, GM: geometric mean, Min: minimum, Max: maximum

Use of personal care products

Women reported use of over 23 different PCPs listed in Table 2.3. The most frequently used PCPs through the day were toothpaste (used in the last 24 hours for 98% of the urine samples), face cream (80%), deodorant (76%) and makeup remover (63%) (Table 2.3). The median number of PCP applications varied by time window and was 1 (min: 0, max:16), 1(min: 0, max:15) and 3 (min:0, max: 12) in the 0 to 6, 6 to 12 and 12 to 24 hour window preceding urination, respectively.

Table 2.3: Number of urine samples for which specific PCPs were used in the 0 to 24 hours preceding urine collection (^aN = 169).

PCPs	0 to 6 hours		6 to 12 hours		12 to 24 hours		0 to 24 hours	
	^a N	(%)	^a N	(%)	^a N	(%)	^a N	(%)
Toothpaste	78	(46.2)	93	(55.0)	136	(80.5)	166	(98.2)
Face cream	59	(34.9)	58	(34.3)	85	(50.3)	136	(80.5)
Deodorant	47	(27.8)	34	(20.1)	57	(33.7)	129	(76.3)
Makeup remover	16	(9.5)	35	(20.7)	58	(34.3)	106	(62.7)
Mascara	41	(24.3)	32	(18.9)	44	(26.0)	112	(66.3)
Foundation	38	(22.5)	31	(18.3)	39	(23.1)	103	(60.9)
Bar soap	25	(14.8)	25	(14.8)	39	(23.1)	85	(50.3)
Shower gel	26	(15.4)	13	(7.7)	33	(19.5)	66	(39.1)
Shampoo	21	(12.4)	18	(10.7)	30	(17.8)	67	(39.6)
Body cream	12	(7.1)	14	(8.3)	28	(16.6)	49	(29.0)
Conditioner	15	(8.9)	15	(8.9)	20	(11.8)	48	(28.4)
Lip/Chapstick	17	(10.1)	17	(10.1)	21	(12.4)	43	(25.4)
Perfume	11	(6.5)	8	(4.7)	15	(8.9)	32	(18.9)
Contour	16	(9.5)	8	(4.7)	16	(9.5)	36	(21.3)
Facial cleanser	11	(6.5)	8	(4.7)	13	(7.7)	26	(15.4)
Anti-stretchmarks' cream	10	(5.9)	6	(3.6)	12	(7.1)	23	(13.6)
Hand cream	6	(3.6)	4	(2.4)	11	(6.5)	20	(11.8)
Intimate soap	4	(2.4)	3	(1.8)	5	(3.0)	12	(7.1)
Thermal spring water	5	(3.0)	2	(1.2)	3	(1.8)	8	(4.7)
Eye shadow	4	(2.4)	1	(0.6)	1	(0.6)	6	(3.6)
Sunscreen cream	1	(0.6)	0	(0)	2	(1.2)	3	(1.8)
Nail polish	1	(0.6)	0	(0)	1	(0.6)	2	(1.2)
Mouth wash	1	(0.6)	0	(0)	1	(0.6)	2	(1.2)

^aN: Including urine samples collected after the first report of PCP use

Associations between use of PCPs and parabens' urinary concentrations

For three of the four parabens assessed, we observed an increase in concentration in relation to the total number of PCP applications, and these associations varied by time window across parabens (Table 2.4). In the 0 to 6 time window, the concentrations of ethylparaben and butylparaben increased by 7.9% (95%CI = 0.9; 15.4) and 14.84% (95%CI = 5.9; 24.5) for each additional PCP application, respectively, while the concentration of propylparaben increased by 11.1% (95%CI = 0.9; 22.3) and 12.9% (95%CI = 3.9; 22.7) for each additional PCP application in the 6 to 12 hours and 12 to 24 hours' time windows respectively. No association was observed between the total number of PCP applications and the methylparaben concentration in all considered time windows (lowest p-value = 0.2) nor for ethylparaben and butylparaben in the longer time windows (6 to 12 and 12 to 24 hours, lowest p-value = 0.24).

Among the 18 different PCPs considered in the specific PCP analysis, 12 were associated with increased urinary concentrations of at least one paraben in at least one time window. These corresponded to 48 positive associations (25%) out of the 192 tests performed in our main analysis for parabens (17 PCPs * 4 parabens in 0 to 6 time window, 16 PCPs * 4 parabens in 6 to 12 time window and 18 PCPs * 4 parabens in 12 to 24 time window, window (Figure 2.1 and supplemental material Table 2.8). These PCPs included toothpaste (ethylparaben, butylparaben), face cream (ethylparaben, propylparaben, butylparaben), hand cream (propylparaben, butylparaben), makeup-remover (all parabens), coloured cosmetics: mascara (all parabens), foundation (methylparaben, propylparaben, butylparaben), contour and lip/chap stick (ethylparaben, propylparaben, butylparaben), bar soap (methylparaben, ethylparaben), shampoo (propylparaben), facial cleanser (ethylparaben) and perfume (propylparaben, butylparaben) (Figure 2.1 and supplemental material Table 2.8). As observed with the total number of PCP applications, the positive associations for ethylparaben and butylparaben were mostly observed in the 0 to 6 hour window. Most of the positive associations for methylparaben were observed in the 12 to 24 hour window.

Relying on the time elapsed since last PCP use, we observed a peak in urinary concentration of ethylparaben, butylparaben and propylparaben at 2.86, 2.55 and 2.67 hours since last use of a PCP respectively. No peak was observed for methylparaben, for which concentrations tended to remain constant with the time elapsed since last PCP use (Figure 2.2).

Table 2.4: Adjusted percent change (β) in the phenol urinary concentrations in relation to the total number of times PCPs were used in the last 0 to 6, 6 to 12 and 12 to 24 hours (N= 178 urine samples of 8 women)

	0 to 6 hours		6 to 12 hours		12 to 24 hours	
	β	95% CI	β	95% CI	β	95% CI
Benzophenone-3	-23.4	[-42.6 ; 2.2]	-16.5	[-37.0 ; 10.7]	7.0	[-14.2 ; 33.4]
Bisphenol A	-5.7	[-10.6 ; -0.5]	-4.1	[-9.4 ; 1.5]	3.7	[-1.3 ; 8.9]
Bisphenol S	1.2	[-5.1 ; 8.0]	4.6	[-2.4 ; 12.1]	12.4	[5.9 ; 19.3]
2,4 dichlorophenol	-2.0	[-4.6 ; 0.6]	-0.2	[-3.0 ; 2.6]	1.6	[-0.8 ; 4.0]
2,5 dichlorophenol	-2.2	[-4.8 ; 0.6]	-0.6	[-3.5 ; 2.4]	2.4	[-0.1 ; 5.0]
Methylparaben	0.5	[-6.1 ; 7.5]	2.7	[-4.4 ; 10.4]	4.1	[-2.1 ; 10.8]
Ethylparaben	7.9	[0.9 ; 15.4]	-0.7	[-7.6 ; 6.6]	-1.2	[-7.1 ; 5.0]
Propylparaben	7.5	[-1.8 ; 17.7]	11.1	[0.9 ; 22.3]	12.9	[3.9 ; 22.7]
Butylparaben	14.8	[5.9 ; 24.5]	5.3	[-3.4 ; 14.7]	-3.7	[-10.5 ; 3.7]
Triclosan	-0.1	[-9.5 ; 10.3]	3.2	[-7.1 ; 14.6]	5.5	[-3.7 ; 15.5]

Effect estimates are reported as the percent change in phenol urinary concentration for an additional PCP application in the studied time window.

Adjustment factors: woman, specific gravity, hour and day of urine sample collection and use of PCP in other time windows than the window considered (i.e. for the 0 to 6 time window, we adjusted for PCP use in the 6 to 12 and 12 to 24 hour windows, for the 6 to 12 hour window, we adjusted for use in the 0 to 6 and 12 to 24 hour windows and for the 12 to 24 hour window, we adjusted for use in 0 to 6 and 6 to 12 hour windows)

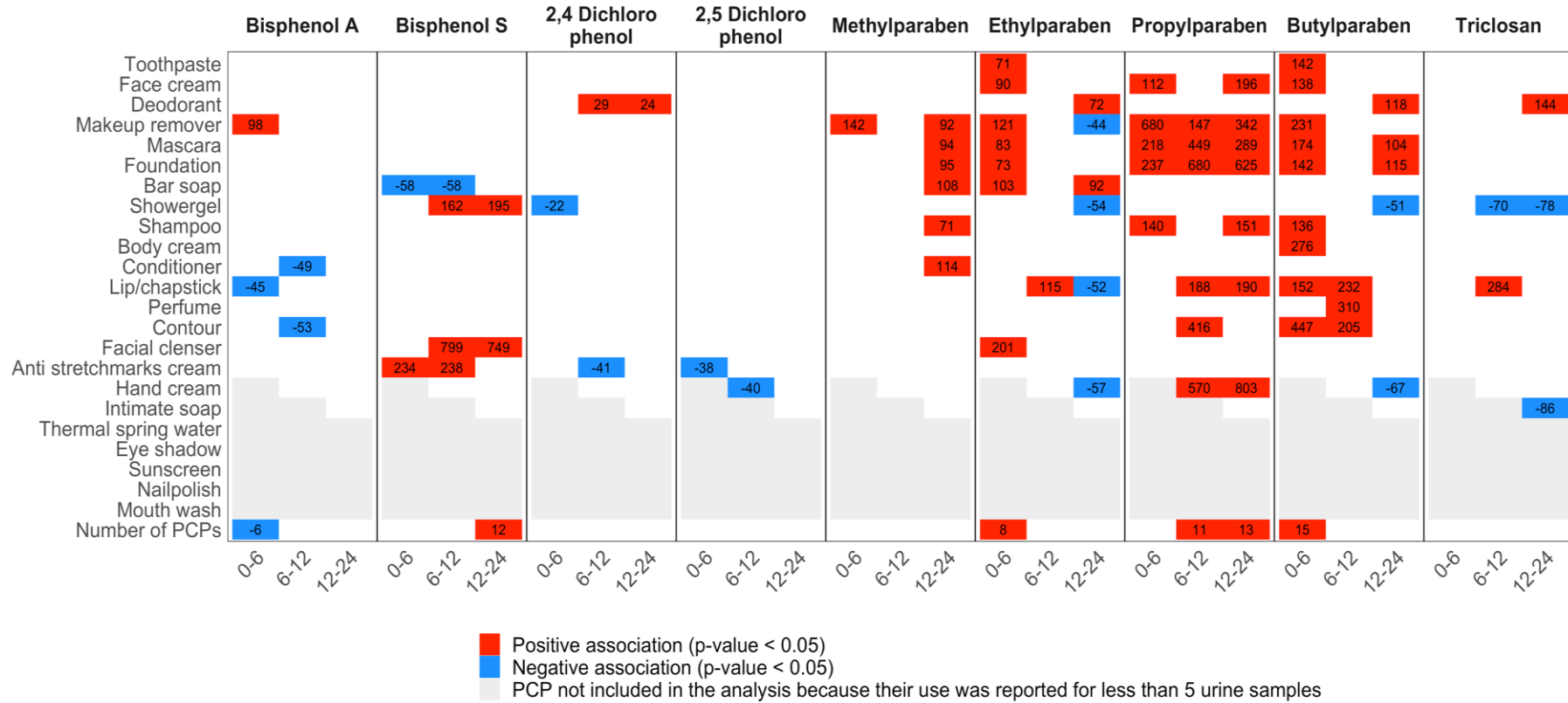


Figure 2.1: Adjusted percent change in phenol urinary concentrations with specific PCP (yes / no) and the total number of PCP applications in the past 0 to 6, 6 to 12 and 12 to 24 hours.

Adjustment factors were woman (fixed effect), specific gravity, day and hour of urine sample collection and use of PCP in other windows than the window considered. Only associations with p-value below 5% are displayed

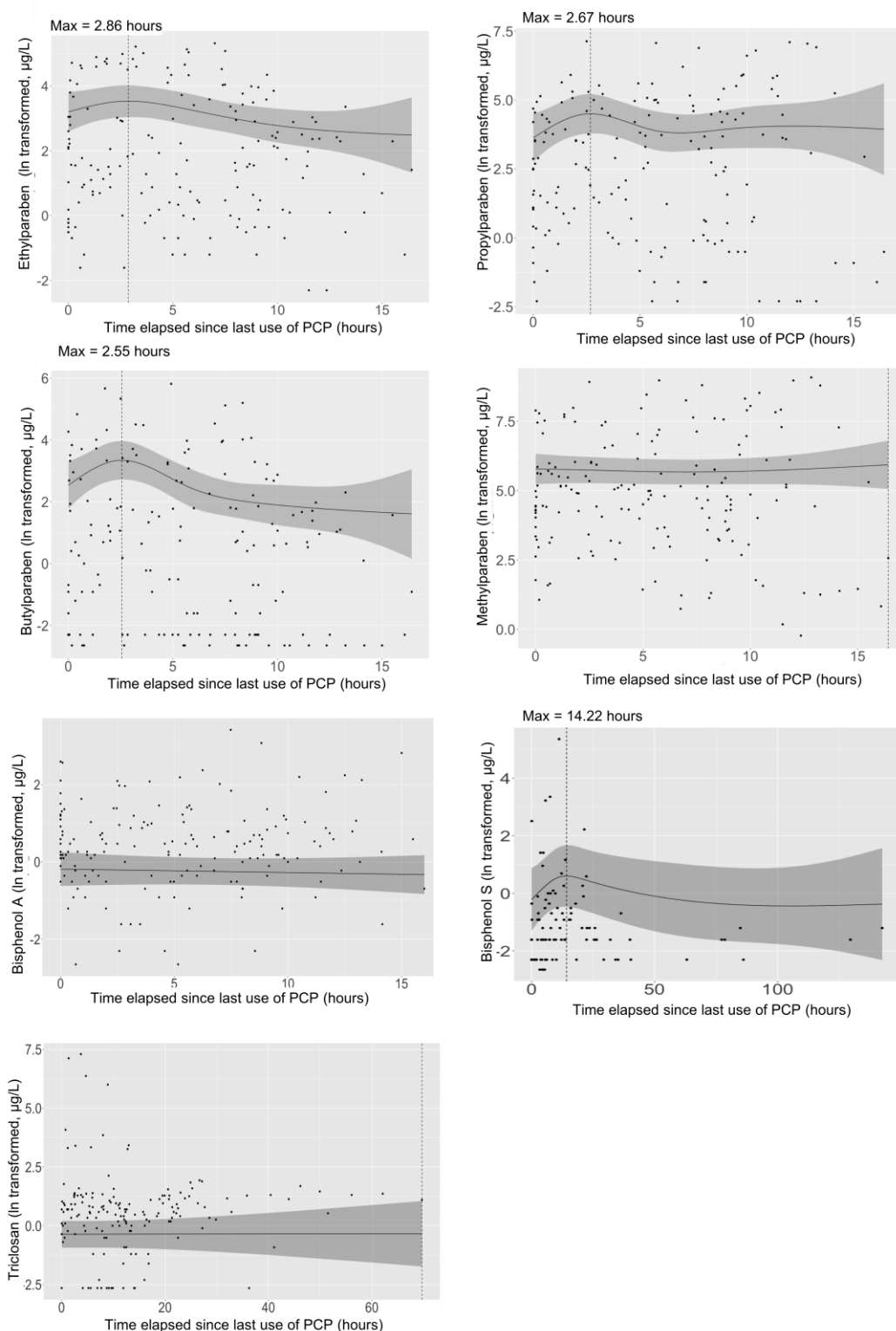


Figure 2.2: Adjusted associations between the time elapsed since last use of PCP, modeled using restricted cubic splines with 5 knots, and phenol urinary concentrations. Max: time since last use of PCP (in hours) at which highest predicted concentration was observed. Adjustment factors were woman (fixed effect), specific gravity, time and day of urine collection.

Other phenols and PCP use

Bisphenol S concentrations increased by 12.4% (95% CI = 5.9 ; 19.3, Table 2.4) for each additional PCP use in the past 12 to 24 hours. No association was seen in the earlier time windows. In line with these results, in the analysis relying on time since last PCP use, we observed a peak in bisphenol S urinary concentration at 14.2 hours since last PCP use (Figure 2.2). Specific PCPs such as anti-stretchmarks cream (in the 0 to 6 and 6 to 12 hour windows), facial cleanser and shower gel (in the 6 to 12 and 12 to 24 hour windows) were positively associated with bisphenol S (Figure 2.1 and supplemental material Table 2.8).

No positive associations were observed with bisphenol A with the number of PCP applications, in all three time windows considered. In the single PCP use models, the only positive association observed for this compounds was with makeup remover use in the 0 to 6 time window (Figure 2.1 and supplemental material Table 2.8). The concentration of bisphenol A tended to remain constant with the time elapsed since last PCP use (Figure 2.2).

Null associations were observed with the number of PCP applications and concentrations of triclosan, benzophenone-3, 2,4 and 2,5 dichlorophenol. Regarding use of specific PCPs, triclosan concentrations increased with use of deodorant in the 12 to 24 hour window and lip/chapstick in the 6 to 12 hour window, but remained constant with time elapsed since last PCP use. Use of deodorant was associated with an increase in the 2,4-dichlorophenol urinary concentration (Figure 2.1 and supplemental material Table 2.8).

Benzophenone-3 was excluded from the specific PCPs use analysis because of the non-convergence of the Tobit model, probably in relation to the relatively high percentage of samples with non-detectable concentrations of this phenol (65%).

Sensitivity analysis

Adjusting for time since last meal and gestational age at urine sampling did not change our main results (supplemental material Table 2.6, Table 2.7 and Figure 2.4). For comparison with previous studies, results for the 0 to 24 hour time window are displayed in the Supplemental Materials Figure 2.5.

2.5 Discussion

This study relying on detailed information on intra-individual variations in PCP use and phenol concentrations of eight women in which 178 urine samples were assayed, suggested that use of many PCPs was positively associated with urinary concentrations of ethylparaben, butylparaben, propylparaben and bisphenol S. These increases in urinary concentrations were observed within 0 to 6 hours for ethylparaben and butylparaben, or 12 to 24 hours for bisphenol S after PCP use. Results were unclear for propylparaben, with the analysis relying on time since last use suggesting that the maximal value was attained at approximately 3 hours after PCP use (Figure 2.2), while the analysis relying on the total number of applications (Table 2.4) suggested associations for the 6 to 12 and 12 to 24 hour time windows.

Strengths and limitations

We relied on repeated urine measurements to quantify urinary phenol concentrations and simultaneously collected information on the type of PCP used and the exact time of use. This allowed us to precisely characterize temporal relations between PCP use and phenol urinary concentrations, and to consider shorter time windows (0 to 6 and 6 to 12 hours prior to urination) than those considered in previous studies which were usually longer than 24 or 48 hours prior to urination^{56,58,59,61,66,199,200,204}. Previous studies relying on these longer time windows may have missed the rise in phenol urinary concentrations reported in toxicokinetic studies within a few hours following PCP use (between 2 to 11 hours depending of the compounds and routes of exposure)^{37,38,205,206}. In addition, for two of the eight women included, we assessed phenol concentrations in all of the urine samples of a week (N = 114). Although restricted to two subjects, this design is ideal if one seeks to detect a rise in urinary concentrations of short lived compounds after a given exposure (e.g., use of PCPs) because all the urine samples produced after the given exposure are available.

Our small sample size (eight pregnant women) is a result of the tradeoff between building on a within-subject (temporal) contrasts than between-subject contrasts. Generally, studies like ours, relying on within-subject contrasts are less prone to confounding bias (for example due to differences between subjects using and those not using specific PCPs). The downside is the limited between-subject variability both in terms of metabolism and pattern of use of PCPs, that limits generalizability of findings. For comparison, to our knowledge, one of the two previous studies with such detailed information on both urinary phenol concentrations

and PCP use, was also restricted to eight participants⁶⁷. Furthermore, we cannot rule out residual confounding arising from other factors not assessed in our study such as use of pharmaceuticals^{207,208} and exposure to thermal receipts' ink²⁰⁹, which are sources of some of the studied phenols.

Associations between use of PCPs and parabens' urinary concentrations

Parabens are commonly used as preservatives in a wide range of PCPs, alone or in combination^{52,70}. The total number of PCP applications was associated with higher urinary concentrations of ethylparaben, propylparaben and butylparaben; these findings are in line with former findings of studies among pregnant women^{64,200} and non-pregnant adults and children^{58,59}. For these three parabens, we observed a peak in the urinary concentration at approximately 3 hours after the last PCP use. This is in between what was reported following oral (about 2 hours for butylparaben and propylparaben among non pregnant individuals^{69,205}) and dermal exposure (8 hours for butylparaben in men³³), suggesting that urinary concentrations may be a result of a combination of exposure through different routes.

Ethylparaben, propylparaben and butylparaben were positively associated with various specific PCPs. Some of these associations were consistent with previous studies; this was in particular the case of associations of these three parabens with creams, colored cosmetics (mascara, foundation and contour) and hair care products (shampoo and conditioner)^{59,61,64,66,199–201}; of ethylparaben with cleansers⁶⁴; and of butylparaben with deodorant^{59,64}, perfume and toothpaste⁶⁴. Other associations were highlighted for the first time by our study; for all three parabens with makeup remover and for ethylparaben with bar soap, deodorant and toothpaste.

Compared to previous studies of pregnant women that assessed associations between PCP use and urinary concentrations, the median concentration of ethylparaben and butylparaben were higher in our study^{61,64,200,202} while the propylparaben median was similar to that reported in Sweden and Puerto Rico^{61,202} but lower than those reported in the USA²⁰⁰ (Table 2.9).

Similar to other parabens, methylparaben has been detected in a wide range of PCPs in the US^{52,70}. In our study this paraben was not clearly associated with the number of PCP applications in all three time window considered, nor with the time elapsed since last PCP use. This could be due to other predominant sources than PCP use, such as diet¹⁹⁸ or pharmaceuticals use^{207,208} that were not extensively considered in this study. When PCPs were

considered separately, methylparaben exhibited positive associations with four, including makeup-remover, coloured cosmetics, conditioner and bar soap. Our results for coloured cosmetics and soap were in line with previous studies of pregnant women^{61,64,66,200}, adolescent girls¹⁹⁹ and non-pregnant adult women^{48,59}. The median concentration of methylparaben in our study was higher than that reported in three of these studies^{61,64,200} and lower than that in Braun *et al.*²⁰⁰ (Table 2.9).

In summary, the associations between the paraben concentrations and PCPs were observed in varying time windows. For a given route of exposure, half-life and excretion time are expected to be relatively similar across the four parabens studied^{33,69,205}. The differences in time windows could therefore be due to difference in routes of exposure across parabens.

Other phenols and PCP use

Studies assessing phenols in PCPs from the USA and China reported presence of bisphenol A and S in lotions, shampoo, conditioner, facial cleansers, sunscreens, lipsticks and toothpaste^{54,70,71}. In our study, the bisphenol S urinary concentration increased with total number of PCP applications in the 12 to 24 hour time window. In line with this result we observed a peak in urinary concentration 14.2 hours after last PCP use.

Specific PCPs attributed to this increase were anti-stretchmarks cream, facial cleanser and shower gel, similar to PCPs in which bisphenol S was previously detected in other regions. Two previous studies among pregnant women⁶¹ and Norwegian female and male adults⁵⁷ did not highlight any associations with PCP use within 24 hours prior to urine collection for this phenol, thus our results should be interpreted with caution. Concentrations of bisphenols S in our study were similar to those reported by Ashrap *et al.*,⁶¹ (Table 2.9).

Bisphenol S is a substitute compound for bisphenol A and may in some instances be used in products that used to contain bisphenol A. We observed no positive association with bisphenol A, a compound that has recently been recognized as substance of very high concern in the EU²¹⁰.

Our results did not strongly support associations between triclosan and the number of PCP applications. In the analysis of specific PCPs analysis, two of the observed associations for triclosan were positive, i.e., increasing triclosan concentration following use of deodorant and lip/chap stick) while the other two negative (i.e. decreasing triclosan concentration in relation to intimate soap and shower gel). Contrary to previous publications^{59,199}, no significant association between toothpaste use and triclosan concentration was observed. In the EU,

triclosan concentrations in toothpaste are limited to a concentration of 0.3%. We were limited in studying previously reported positive associations between triclosan concentrations and liquid soap, hair spray and sunscreen^{61,66,199,204} because of low use of such PCPs in our study population. Concentrations of triclosan in our study were lower than those recorded among pregnant women from Puerto Rico^{61,66}.

We observed no association between aggregated PCP use and benzophenone-3 concentrations. Unfortunately we did not have enough variability for both use of sunscreen (reported less than 5 times) and benzophenone-3 concentrations (only 35% of detection) to study this association.

Conclusion

Among eight French pregnant women, we observed that the use of PCPs was associated with a short-term increase in urinary concentration of ethylparaben, butylparaben and propylparaben and to some extent (for specific PCP use only) with methylparaben. This was consistent with previous studies conducted among pregnant women in and non-pregnant individual in America and Sweden. This study also reported that use of various PCPs was associated with increased bisphenol S concentrations, a possible substitute of bisphenol A with suspected endocrine disrupting capacity²¹¹. This finding needs replication in further studies.

2.6 Supplemental material

List of figures

- Figure 2.3: Variability of urinary concentrations of 10 phenols ($\mu\text{g/L}$) in spot urine samples for one woman collected over a week of follow up (N of woman 1 =72)
- Figure 2.4: Adjusted percent change in phenol urinary concentrations with specific PCP (yes / no) and the total number of PCP applications in the past 0 to 6, 6 to 12 and 12 to 24 hours, additionally adjusted for time since last meal.
- Figure 2.5: Adjusted percent change in phenol urinary concentrations with total number of PCP applications or single PCP used in the previous 0 to 24 hours

List of tables

- Table 2.5: Spearman's correlation coefficients between phenol concentrations and specific gravity (178 urine samples of 8 pregnant women)
- Table 2.6: Adjusted change (β) in the ln-transformed phenol urinary concentrations in relation to the total number of times PCPs were used in last 0 to 24 (N= 178 urine samples of 8 women); adjustment factors include time since last meal
- Table 2.7: Adjusted change (β) in the ln-transformed phenol urinary concentrations in relation to the total number of times PCPs were used in last 0 to 24 (N= 178 urine samples of 8 women); adjustment factors include gestational age
- Table 2.8: Adjusted associations between use of specific PCP (yes / no) in the past 0 to 6, 6 to 12 and 12 to 24 hours (corresponding to Figure 1)
- Table 2.9: Medians^a of phenol urinary concentrations in previous studies of epidemiological studies of pregnant women that assessed between PCP used in the previous 24 to 48 hours and urinary concentrations of phenols during pregnancy and PCP survey studies

Table 2.5: Spearman's correlation coefficients between phenol concentrations and specific gravity (178 urine samples of 8 pregnant women)

	BP3	BPA	BPS	DC4	DC5	MP	EP	PP	BP	TCS	SG
BP3	1.00										
BPA	0.15	1.00									
BPS	0.04	0.23	1.00								
DC4	0.04	0.50	0.42	1.00							
DC5	-0.12	0.10	0.36	0.67	1.00						
MP	0.24	0.33	0.19	0.11	-0.40	1.00					
EP	0.03	0.28	0.09	0.10	-0.36	0.62	1.00				
PP	0.09	0.29	0.04	-0.08	-0.51	0.64	0.80	1.00			
BP	0.19	0.24	0.16	0.08	-0.38	0.94	0.58	0.57	1.00		
TCS	-0.06	0.26	0.31	0.53	0.49	0.15	0.13	-0.02	0.14	1.00	
SG	0.01	0.44	0.32	0.79	0.67	-0.02	0.03	-0.12	-0.01	0.48	1.00

Abbreviations; BP3:benzophenone-3, BPA: bisphenol A, BPS: bisphenol S, DC4; 2,4-dichlorophenol, DC5: 2,5-dichlorophenol, MP: methylparaben, EP: ethylparaben, PP: propylparaben, BP: butylparaben, TCS: triclosan, SG: specific gravity

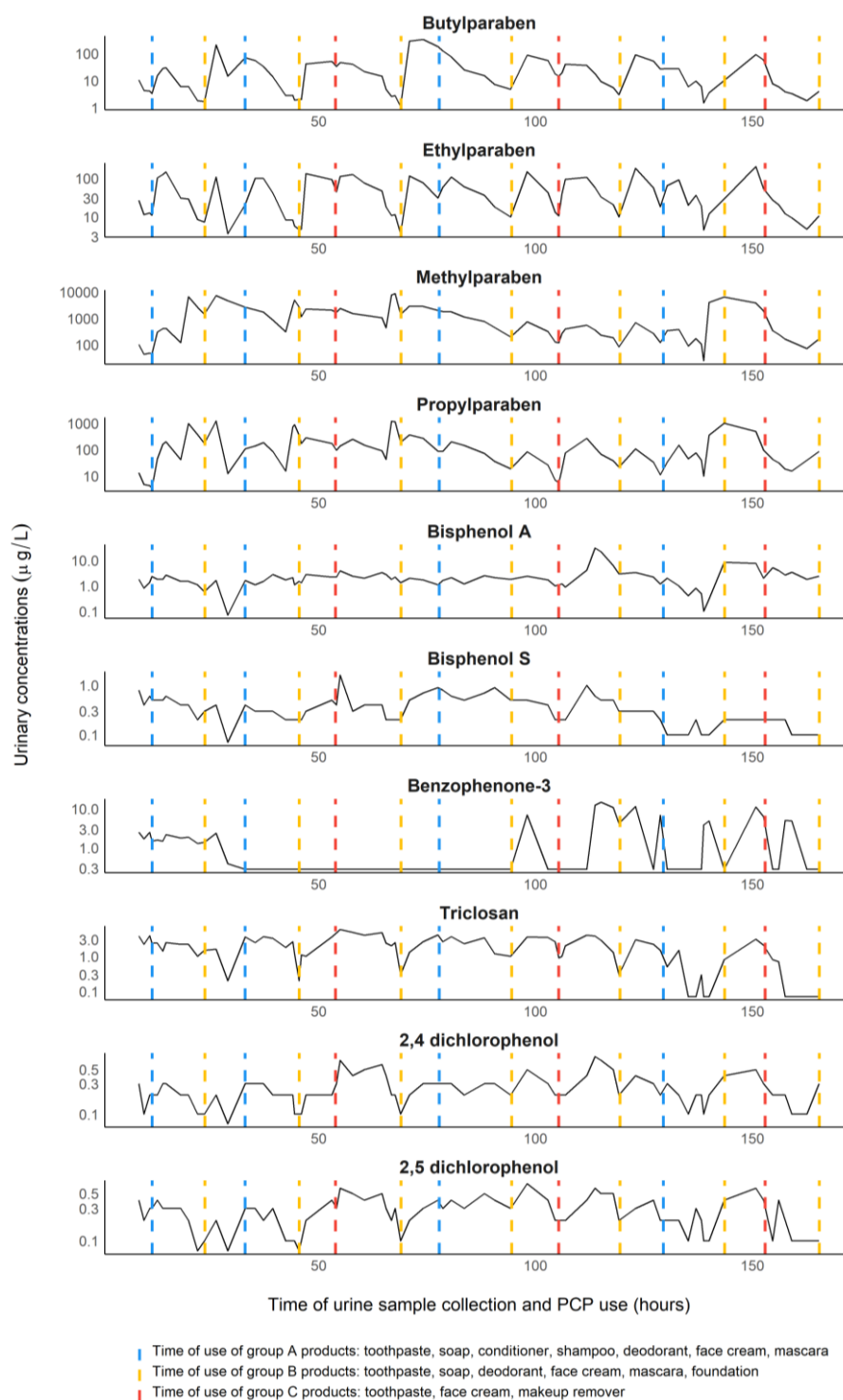


Figure 2.3: Variability of urinary concentrations of 10 phenols ($\mu\text{g/L}$) in spot urine samples for one woman collected over a week of follow up (N of woman 1 =72)

Table 2.6: Adjusted percent change (β) in the ln-transformed phenol urinary concentrations in relation to the total number of times PCPs were used in last 0 to 24 (N= 178 urine samples of 8 women); adjusted for time since last meal.

	0 to 6		6 to 12		12 to 24	
	β	95%CI	β	95%CI	β	95%CI
Bisphenol A	-2.0	[-7.8 ; 4.3]	-6.8	[-12.3 ; -0.9]	3.7	[-0.6 ; 8.1]
Bisphenol S	-3.4	[-10.4 ; 4.2]	-4.6	[-11.5 ; 2.8]	10.0	[4.5 ; 15.7]
Methylparaben	-4.2	[-11.3 ; 3.5]	-0.2	[-7.6 ; 7.8]	2.7	[-2.5 ; 8.3]
Ethylparaben	10.0	[1.8 ; 18.8]	0.2	[-7.2 ; 8.2]	-1.3	[-6.3 ; 4.0]
Propylparaben	-3.1	[-12.9 ; 7.6]	1.0	[-9.1 ; 12.1]	8.7	[1.2 ; 16.8]
Butylparaben	12.9	[2.9 ; 23.8]	9.4	[-0.1 ; 19.8]	-4.4	[-10.2 ; 1.7]

Effect estimates are reported as the percent change in phenol urinary concentration for an additional PCP application in the studied time window.

Adjustment factors were woman (fixed effect), specific gravity, time of urine sample collection, hour of urine sample collection, time elapse since last meal, and use of PCP in other windows other than the window considered.

Effect estimates correspond to the change in the ln concentration of phenols for one additional application of PCPs in the given time windows.

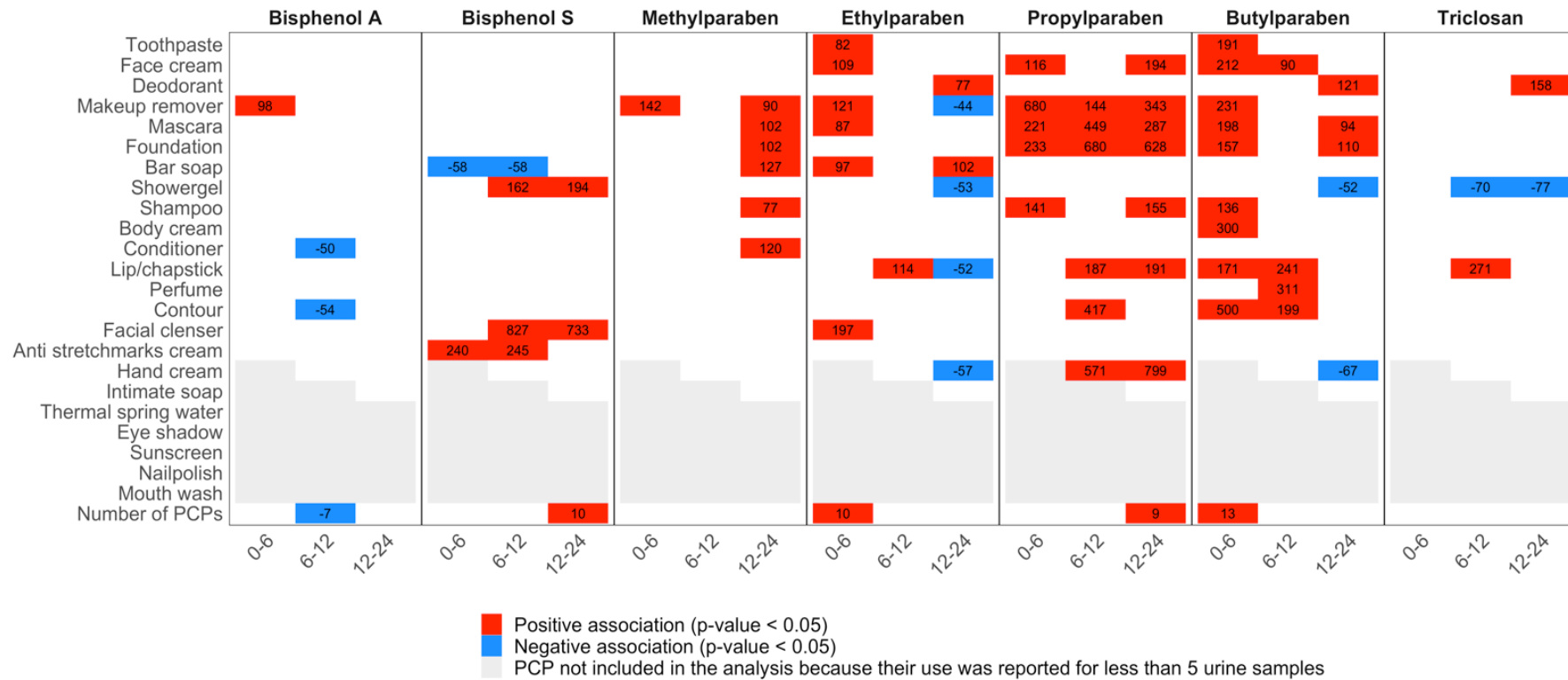


Figure 2.4: Adjusted percent change in phenol urinary concentrations with specific PCP (yes / no) and the total number of PCP applications in the past 0 to 6, 6 to 12 and 12 to 24 hours, additionally adjusted for time since last meal. Adjustment factors were woman (fixed effect), specific gravity, time of urine sample collection, hour of urine sample collection time since last meal and use of PCP in windows other than the window considered.

Table 2.7: Adjusted percent change (β) in the ln-transformed phenol urinary concentrations in relation to the total number of times PCPs were used in last 0 to 24 (N= 178 urine samples of 8 women); adjusted for gestational age.

	0 to 6 -hours		6 to 12-hours		12 to 24-hours	
	β	95%CI	β	95%CI	β	95%CI
Benzophenone-3	-23.9	[-42.4 ; 0.4]	-13.8	[-34.0 ; 12.6]	2.4	[-17.3 ; 26.7]
Bisphenol A	-2.0	[-7.6 ; 4.0]	-7.2	[-12.6 ; -1.4]	3.9	[-0.3 ; 8.3]
Bisphenol S	-3.6	[-10.3 ; 3.5]	-4.3	[-11.0 ; 3.0]	9.8	[4.4 ; 15.5]
2,4 dichlorophenol	-1.8	[-4.6 ; 1.1]	-1.6	[-4.4 ; 1.4]	1.3	[-0.7 ; 3.4]
2,5 dichlorophenol	-1.1	[-4.0 ; 1.8]	-3.1	[-6.0 ; -0.2]	1.9	[-0.2 ; 4.1]
Methylparaben	-2.2	[-9.2 ; 5.3]	0.3	[-7.0 ; 8.2]	2.4	[-2.8 ; 7.9]
Ethylparaben	8.9	[1.1 ; 17.3]	0.1	[-7.2 ; 8.0]	-1.2	[-6.2 ; 4.0]
Propylparaben	-2.3	[-11.6 ; 8.0]	0.2	[-9.6 ; 11.0]	9.1	[1.7 ; 17.1]
Butylparaben	10.7	[1.4 ; 20.9]	6.2	[-2.8 ; 16.0]	-3.0	[-8.8 ; 3.1]
Triclosan	-4.0	[-13.7 ; 6.9]	1.0	[-9.4 ; 12.6]	3.1	[-4.4 ; 11.0]

Effect estimates are reported as the percent change in phenol urinary concentration for an additional PCP application in the studied time window.

Adjustment factors were woman (fixed effect), specific gravity, time of urine sample collection, hour of urine sample collection, gestational age, and use of PCP in other windows other than the window considered.

Effect estimates correspond to the change in the ln concentration of phenols for one additional application of PCPs in the given time windows

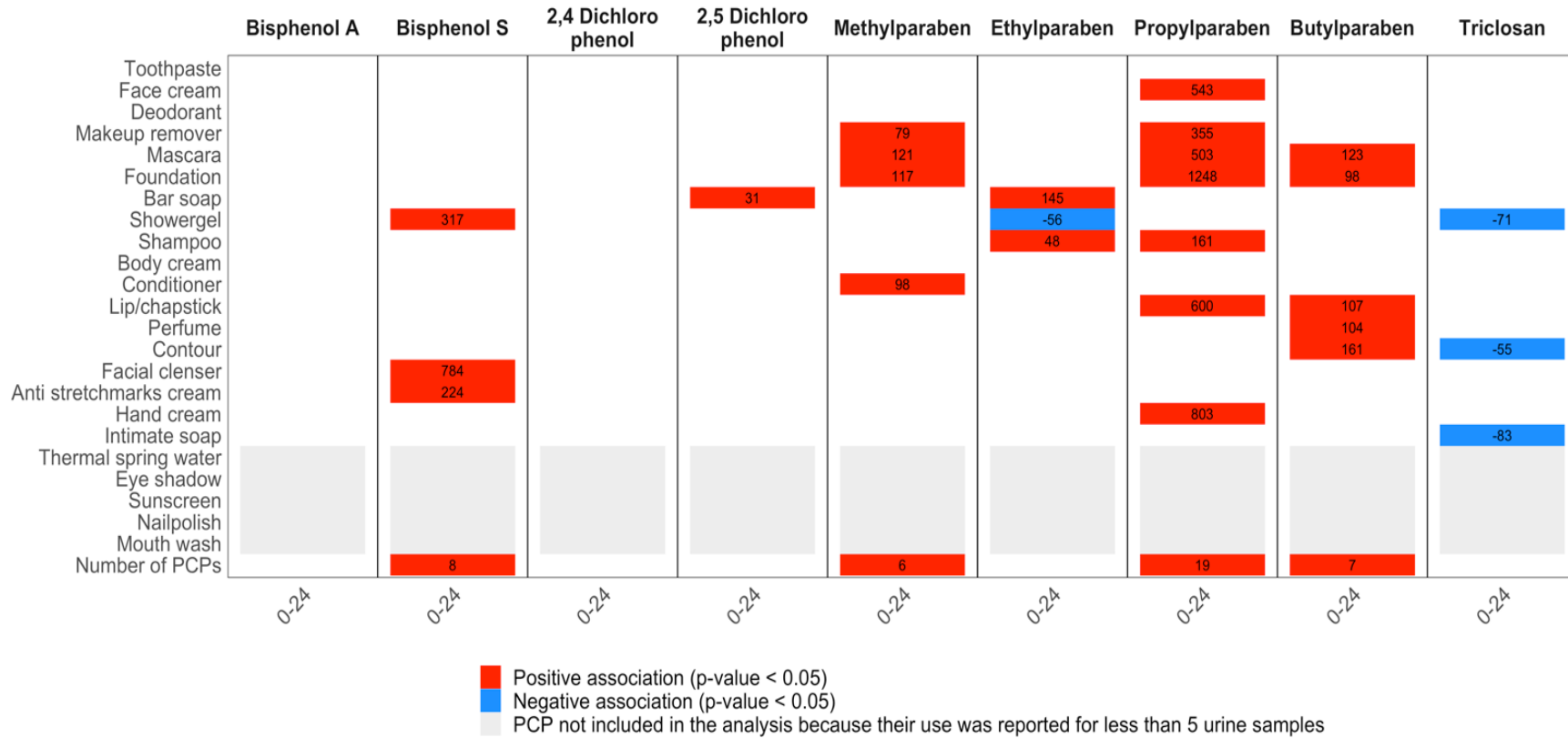


Figure 2.5: Adjusted percent change in phenol urinary concentrations with total number of PCP applications or single PCP used in the previous 0 to 24 hours
 Adjustment factors were woman (fixed effect), specific gravity, time of urine sample collection and hour of urine sample.

Table 2.8: Adjusted percent change (β) in phenol urinary concentrations with use of specific PCP (yes / no) in the past 0 to 6, 6 to 12 and 12 to 24 hours (corresponding to Figure 1)

	Bisphenol A					
	0-6 hours		6-12 hours		12-24 hours	
	β	95%CI	β	95%CI	β	95%CI
Toothpaste	-16.6	[-42.2 ; 20.3]	-11.6	[-39.7 ; 29.4]	-15.8	[-52.2 ; 48.3]
Face cream	-22.3	[-48.4 ; 17.2]	-24.8	[-50.1 ; 13.4]	-10.6	[-41.8 ; 37.4]
Deodorant	-31.2	[-55.1 ; 5.6]	-22.2	[-52.2 ; 26.7]	-11.7	[-42.4 ; 35.1]
Makeup remover	98.1	[5.0 ; 273.8]	12.8	[-33.2 ; 90.4]	-9.8	[-41.7 ; 39.8]
Mascara	-10.5	[-42.2 ; 38.6]	-7.6	[-46.5 ; 59.6]	22.1	[-22.8 ; 93.1]
Foundation	-15.0	[-46.0 ; 33.7]	-21.5	[-55.1 ; 37.1]	0.4	[-38.1 ; 62.9]
Bar soap	-5.5	[-44.1 ; 59.7]	-3.3	[-46.2 ; 73.8]	18.4	[-27.0 ; 92.0]
Shower gel	-35.4	[-59.4 ; 2.7]	-7.4	[-50.3 ; 72.4]	-16.4	[-46.9 ; 31.6]
Shampoo	-37.2	[-61.7 ; 2.8]	-32.3	[-61.5 ; 18.9]	5.8	[-30.3 ; 60.5]
Body cream	-21.9	[-58.6 ; 47.1]	-2.9	[-46.8 ; 77.3]	-5.2	[-39.5 ; 48.5]
Conditioner	-44.1	[-69.6 ; 2.7]	-48.8	[-73.2 ; -2.3]	-15.7	[-50.7 ; 44.1]
Lip/Chapstick	-44.9	[-67.6 ; -6.5]	-41.6	[-66.7 ; 2.6]	8.0	[-32.7 ; 73.4]
Perfume	-35.7	[-64.8 ; 17.5]	33.4	[-32.7 ; 164.6]	50.7	[-14.9 ; 167.0]
Contour	-16.2	[-54.5 ; 54.4]	-53.4	[-77.3 ; -4.5]	-26.5	[-56.8 ; 25.3]
Facial cleanser	-26.9	[-65.3 ; 53.9]	-12.8	[-56.5 ; 75.2]	2.9	[-45.0 ; 92.5]
Anti-stretchmarks' cream	3.4	[-60.5 ; 170.5]	-50.1	[-81.3 ; 32.9]	-46.5	[-73.7 ; 8.7]
Hand cream			0.2	[-59.8 ; 149.9]	44.6	[-21.0 ; 164.4]
Intimate soap					35.9	[-50.9 ; 275.7]
Thermal spring water						

	Bisphenol S					
	0-6 hours		6-12 hours		12-24 hours	
	β	95%CI	β	95%CI	β	95%CI
Toothpaste	-9.2	[-42.0 ; 42.2]	19.2	[-25.3 ; 90.2]	41.3	[-29.4 ; 182.8]
Face cream	-1.7	[-40.9 ; 63.5]	11.7	[-32.8 ; 85.7]	11.7	[-34.4 ; 90.3]
Deodorant	-1.2	[-40.9 ; 65.0]	-7.7	[-48.6 ; 65.7]	46.5	[-12.2 ; 144.2]
Makeup remover	0.8	[-54.8 ; 124.7]	51.9	[-21.6 ; 194.1]	-2.0	[-43.6 ; 70.3]
Mascara	-4.9	[-44.1 ; 61.9]	-6.5	[-51.8 ; 81.5]	64.3	[-5.9 ; 186.9]
Foundation	-0.5	[-42.7 ; 72.9]	-12.0	[-55.4 ; 73.7]	51.5	[-16.0 ; 173.1]
Bar soap	-58.2	[-77.1 ; -23.6]	-58.3	[-78.7 ; -18.1]	-2.3	[-44.0 ; 70.3]
Shower gel	28.1	[-27.0 ; 124.9]	162.4	[23.4 ; 458.2]	195.3	[70.3 ; 412.3]
Shampoo	-33.3	[-63.1 ; 20.5]	-25.6	[-62.2 ; 46.2]	42.2	[-13.8 ; 134.5]
Body cream	61.6	[-27.6 ; 260.4]	40.6	[-34.4 ; 201.5]	21.7	[-31.0 ; 115.0]
Conditioner	-46.8	[-74.4 ; 10.5]	-51.2	[-77.5 ; 6.1]	14.5	[-39.9 ; 118.0]
Lip/Chapstick	80.8	[-7.7 ; 254.2]	-7.5	[-54.7 ; 89.2]	64.8	[-9.7 ; 200.5]
Perfume	-17.9	[-61.6 ; 75.4]	45.2	[-38.6 ; 243.4]	-18.5	[-60.3 ; 67.4]
Contour	70.6	[-21.4 ; 270.1]	100.0	[-19.6 ; 397.8]	83.7	[-6.5 ; 261.1]
Facial cleanser	24.9	[-39.6 ; 158.3]	799.0	[355.0 ; 1676.2]	749.0	[360.3 ; 1466.0]

Anti-stretchmarks' cream	234.2	[2.3 ; 991.7]	238.0	[1.2 ; 1028.9]	119.7	[-8.3 ; 426.4]
Hand cream			-9.2	[-70.9 ; 182.9]	-5.5	[-55.4 ; 100.2]
Intimate soap					6.2	[-70.3 ; 279.5]
Thermal spring water					541.6	[-48.7 ; 7922.8]

2,4 Dichlorophenol

Toothpaste	1.1	[-15.0 ; 20.2]	-2.6	[-18.7 ; 16.7]	8.5	[-17.0 ; 41.8]
Face cream	-9.1	[-25.1 ; 10.3]	1.0	[-16.8 ; 22.5]	-9.7	[-26.3 ; 10.6]
Deodorant	16.8	[-4.2 ; 42.3]	28.7	[2.7 ; 61.2]	24.4	[2.2 ; 51.6]
Makeup remover	11.9	[-17.6 ; 51.8]	0.6	[-21.8 ; 29.4]	-6.5	[-24.3 ; 15.4]
Mascara	-9.5	[-26.4 ; 11.1]	-0.8	[-23.2 ; 28.3]	11.9	[-9.8 ; 38.8]
Foundation	-9.0	[-26.6 ; 12.7]	-1.9	[-24.6 ; 27.7]	5.9	[-15.7 ; 33.0]
Bar soap	-9.9	[-29.4 ; 15.0]	16.3	[-11.5 ; 52.9]	5.8	[-15.5 ; 32.5]
Shower gel	-22.2	[-37.4 ; -3.2]	-18.1	[-38.8 ; 9.7]	-15.6	[-31.8 ; 4.5]
Shampoo	-13.3	[-31.4 ; 9.7]	-1.1	[-24.4 ; 29.3]	4.8	[-14.1 ; 27.8]
Body cream	-0.4	[-26.2 ; 34.2]	24.2	[-6.5 ; 65.0]	4.3	[-15.6 ; 28.9]
Conditioner	-18.9	[-39.4 ; 8.4]	-1.4	[-27.6 ; 34.2]	-2.3	[-24.4 ; 26.2]
Lip/Chapstick	-1.8	[-24.0 ; 26.9]	14.6	[-12.8 ; 50.6]	0.4	[-20.2 ; 26.3]
Perfume	-1.9	[-26.6 ; 31.1]	2.4	[-26.3 ; 42.2]	-8.8	[-30.7 ; 20.0]
Contour	-6.3	[-30.1 ; 25.6]	-23.8	[-46.0 ; 7.5]	-6.4	[-27.5 ; 20.8]
Facial cleanser	-15.2	[-39.9 ; 19.6]	33.0	[-3.7 ; 83.6]	32.2	[-1.1 ; 76.7]
Anti-stretchmarks' cream	-34.5	[-58.3 ; 2.9]	-41.0	[-62.7 ; -6.5]	-17.9	[-41.2 ; 14.5]
Hand cream			-3.7	[-37.7 ; 48.8]	-6.5	[-29.8 ; 24.7]
Intimate soap					-33.6	[-57.2 ; 3.2]
Thermal spring water					44.4	[-45.5 ; 282.6]

2,5 Dichlorophenol

Toothpaste	-4.4	[-20.1 ; 14.4]	0.6	[-16.5 ; 21.2]	23.7	[-6.2 ; 63.1]
Face cream	-12.1	[-28.2 ; 7.5]	-7.1	[-24.1 ; 13.7]	5.8	[-14.4 ; 30.7]
Deodorant	-8.4	[-25.6 ; 12.9]	11.3	[-12.3 ; 41.3]	19.7	[-2.8 ; 47.5]
Makeup remover	6.3	[-23.1 ; 46.9]	16.2	[-11.0 ; 51.7]	15.9	[-7.3 ; 44.8]
Mascara	-14.0	[-30.5 ; 6.4]	-6.7	[-28.5 ; 21.7]	10.6	[-11.5 ; 38.3]
Foundation	-14.1	[-31.1 ; 7.2]	-7.9	[-29.8 ; 21.0]	6.9	[-15.6 ; 35.4]
Bar soap	-5.2	[-26.5 ; 22.4]	3.3	[-22.4 ; 37.3]	22.4	[-3.2 ; 54.9]
Shower gel	-9.7	[-28.6 ; 14.3]	4.9	[-23.5 ; 43.9]	-0.1	[-20.6 ; 25.8]
Shampoo	-3.8	[-24.8 ; 22.9]	-8.2	[-30.7 ; 21.5]	12.0	[-9.0 ; 37.8]
Body cream	-12.6	[-36.4 ; 20.1]	2.2	[-24.5 ; 38.3]	5.3	[-15.9 ; 32.0]
Conditioner	-6.5	[-31.0 ; 26.8]	-5.5	[-31.6 ; 30.6]	15.1	[-12.0 ; 50.6]
Lip/Chapstick	-0.5	[-24.3 ; 30.7]	3.0	[-23.0 ; 37.7]	-5.5	[-25.9 ; 20.7]
Perfume	5.8	[-22.2 ; 44.0]	5.9	[-25.3 ; 50.2]	3.6	[-22.6 ; 38.8]
Contour	10.4	[-19.1 ; 50.6]	23.9	[-14.1 ; 78.7]	4.6	[-20.2 ; 37.2]
Facial cleanser	-14.0	[-40.6 ; 24.5]	14.8	[-18.8 ; 62.4]	26.2	[-7.6 ; 72.4]

Anti-stretchmarks' cream	-38.2	[-61.8 ; -0.1]	-15.4	[-48.2 ; 38.1]	16.5	[-18.3 ; 66.2]
Hand cream			-0.5	[-61.7 ; -5.4]	-12.5	[-35.1 ; 18.1]
Intimate soap					11.4	[-32.9 ; 84.9]
Thermal spring water					40.3	[-50.1 ; 294.6]

Methylparaben

Toothpaste	35.3	[-12.2 ; 108.6]	17.1	[-25.3 ; 83.8]	45.9	[-25.2 ; 184.8]
Face cream	51.9	[-6.5 ; 146.8]	27.3	[-21.6 ; 106.6]	16.4	[-29.9 ; 93.4]
Deodorant	-16.9	[-49.8 ; 37.5]	39.5	[-21.4 ; 147.9]	51.0	[-8.6 ; 149.4]
Makeup remover	142.1	[9.5 ; 435.1]	54.2	[-19.9 ; 196.6]	91.7	[10.9 ; 231.2]
Mascara	45.9	[-12.4 ; 142.9]	44.4	[-23.5 ; 172.9]	93.5	[13.4 ; 230.2]
Foundation	19.5	[-29.6 ; 102.9]	35.6	[-29.2 ; 159.9]	95.3	[11.1 ; 243.2]
Bar soap	12.4	[-38.7 ; 106.2]	17.8	[-40.2 ; 132.2]	108.2	[19.0 ; 264.1]
Shower gel	-26.2	[-58.9 ; 32.6]	-4.6	[-56.6 ; 109.4]	-29.6	[-60.3 ; 25.0]
Shampoo	11.3	[-38.1 ; 99.9]	29.0	[-33.9 ; 151.8]	71.4	[4.5 ; 181.3]
Body cream	16.4	[-47.5 ; 157.9]	15.6	[-45.8 ; 146.3]	28.6	[-26.8 ; 126.0]
Conditioner	6.5	[-48.3 ; 119.5]	10.3	[-48.8 ; 137.9]	113.6	[12.9 ; 304.2]
Lip/Chapstick	21.1	[-38.3 ; 137.7]	71.4	[-16.3 ; 251.2]	-30.8	[-62.1 ; 26.5]
Perfume	-5.1	[-55.9 ; 104.4]	51.9	[-36.4 ; 262.8]	-21.3	[-62.0 ; 62.8]
Contour	49.0	[-31.5 ; 224.1]	-17.7	[-67.0 ; 105.3]	-17.7	[-58.2 ; 62.1]
Facial cleanser	25.5	[-50.6 ; 219.0]	50.5	[-37.2 ; 260.8]	-10.0	[-59.0 ; 97.6]
Anti-stretchmarks' cream	-64.8	[-89.6 ; 18.6]	-44.7	[-84.0 ; 90.9]	-7.3	[-62.2 ; 127.5]
Hand cream			10.0	[-65.1 ; 246.7]	65.1	[-22.7 ; 252.8]
Intimate soap					48.8	[-56.6 ; 410.7]
Thermal spring water					-55.2	[-96.7 ; 509.6]

Ethylparaben

Toothpaste	70.8	[10.5 ; 164.1]	-0.8	[-36.9 ; 56.2]	46.2	[-25.4 ; 186.6]
Face cream	90.3	[16.4 ; 211.1]	23.9	[-24.2 ; 102.4]	-8.5	[-45.3 ; 53.1]
Deodorant	37.1	[-17.8 ; 128.4]	-4.4	[-46.6 ; 71.2]	71.8	[3.3 ; 185.7]
Makeup remover	121.2	[2.3 ; 378.0]	-36.0	[-66.1 ; 20.8]	-44.1	[-67.1 ; -4.9]
Mascara	82.8	[10.1 ; 203.6]	-41.5	[-68.9 ; 10.2]	14.3	[-32.9 ; 94.4]
Foundation	72.8	[2.0 ; 192.6]	-44.4	[-70.9 ; 6.3]	15.6	[-34.1 ; 102.7]
Bar soap	103.5	[9.6 ; 277.7]	64.9	[-17.5 ; 229.3]	91.5	[8.3 ; 238.7]
Shower gel	-38.6	[-65.5 ; 9.1]	-43.0	[-73.6 ; 23.5]	-53.8	[-73.7 ; -18.8]
Shampoo	75.4	[-3.6 ; 219.2]	10.5	[-44.2 ; 119.0]	42.3	[-14.2 ; 136.1]
Body cream	114.5	[-2.7 ; 372.5]	30.0	[-38.6 ; 175.5]	-9.9	[-48.5 ; 57.7]
Conditioner	42.2	[-32.8 ; 200.8]	8.1	[-51.2 ; 139.4]	44.7	[-25.3 ; 180.1]
Lip/Chapstick	41.1	[-27.2 ; 173.3]	114.5	[6.2 ; 333.6]	-52.1	[-73.5 ; -13.5]
Perfume	72.5	[-19.5 ; 269.8]	-3.1	[-59.2 ; 130.2]	8.0	[-47.6 ; 122.5]
Contour	86.6	[-13.1 ; 300.8]	-0.8	[-59.6 ; 143.9]	-37.9	[-68.1 ; 20.9]
Facial cleanser	201.4	[21.8 ; 646.1]	-49.1	[-78.2 ; 19.0]	-32.0	[-68.3 ; 45.8]

Anti-stretchmarks' cream	-66.3	[-89.9 ; 12.5]	-15.1	[-75.1 ; 189.9]	26.4	[-48.1 ; 207.7]
Hand cream			68.5	[-44.4 ; 410.5]	-57.2	[-79.4 ; -10.9]
Intimate soap					169.2	[-23.2 ; 844.0]
Thermal spring water					363.4	[-62.9 ; 5684.6]

Propylparaben

Toothpaste	11.5	[-39.4 ; 104.9]	6.7	[-43.4 ; 101.0]	143.6	[-4.9 ; 524.1]
Face cream	112.3	[9.4 ; 312.0]	52.0	[-21.6 ; 194.9]	196.1	[47.9 ; 492.7]
Deodorant	-21.8	[-61.9 ; 60.5]	46.2	[-35.5 ; 231.6]	44.6	[-29.3 ; 195.7]
Makeup remover	679.6	[173.0 ; 2126.4]	146.9	[3.9 ; 486.7]	341.5	[114.1 ; 810.3]
Mascara	217.8	[60.6 ; 528.9]	449.3	[134.3 ; 1187.6]	288.8	[90.1 ; 695.0]
Foundation	236.6	[71.6 ; 560.0]	680.2	[240.8 ; 1686.2]	625.4	[253.8 ; 1387.6]
Bar soap	-16.4	[-65.2 ; 101.1]	-41.8	[-78.2 ; 55.3]	-3.3	[-56.9 ; 117.0]
Shower gel	51.1	[-33.4 ; 242.8]	82.7	[-39.1 ; 448.3]	-8.4	[-58.9 ; 104.2]
Shampoo	139.9	[6.6 ; 440.1]	102.3	[-19.9 ; 411.1]	150.9	[26.4 ; 398.2]
Body cream	11.2	[-63.4 ; 237.4]	87.2	[-34.8 ; 437.6]	15.8	[-47.3 ; 154.2]
Conditioner	37.7	[-51.2 ; 288.4]	1.6	[-66.2 ; 205.6]	80.0	[-27.9 ; 349.1]
Lip/Chapstick	-1.7	[-60.5 ; 144.9]	187.9	[9.0 ; 660.3]	190.2	[28.3 ; 556.2]
Perfume	-39.5	[-79.3 ; 77.0]	15.5	[-65.8 ; 290.1]	59.8	[-42.2 ; 342.0]
Contour	117.2	[-24.9 ; 528.3]	416.5	[48.0 ; 1702.8]	69.8	[-32.8 ; 328.8]
Facial cleanser	125.6	[-38.2 ; 723.3]	74.9	[-48.0 ; 488.1]	-22.5	[-74.0 ; 130.6]
Anti-stretchmarks' cream	-23.5	[-86.2 ; 325.5]	57.5	[-72.6 ; 804.2]	14.9	[-67.6 ; 307.9]
Hand cream			569.8	[61.7 ; 2674.0]	802.7	[252.8 ; 2209.6]
Intimate soap					-21.7	[-86.5 ; 354.9]
Thermal spring water					47.6	[-96.1 ; 5431.1]

Butylparaben

Toothpaste	142.0	[42.6 ; 310.6]	22.4	[-29.4 ; 112.2]	32.6	[-41.6 ; 201.2]
Face cream	138.3	[33.0 ; 327.0]	73.0	[-3.5 ; 210.0]	-17.2	[-55.0 ; 52.5]
Deodorant	30.6	[-28.5 ; 138.6]	12.9	[-43.5 ; 125.7]	117.6	[18.6 ; 299.2]
Makeup remover	230.8	[31.2 ; 734.1]	7.2	[-50.3 ; 131.4]	-41.6	[-69.3 ; 10.9]
Mascara	174.3	[50.6 ; 399.5]	19.5	[-43.7 ; 153.6]	103.8	[8.9 ; 281.5]
Foundation	141.7	[29.9 ; 349.7]	-0.5	[-54.0 ; 115.1]	114.8	[10.8 ; 316.2]
Bar soap	71.9	[-17.4 ; 257.7]	-5.4	[-58.6 ; 115.8]	83.8	[-7.5 ; 265.4]
Shower gel	-14.8	[-58.3 ; 74.2]	54.8	[-41.6 ; 309.8]	-51.1	[-75.6 ; -2.0]
Shampoo	135.6	[16.6 ; 376.0]	4.8	[-53.4 ; 135.7]	41.5	[-21.6 ; 155.6]
Body cream	275.9	[43.5 ; 884.7]	-3.9	[-62.5 ; 146.1]	-14.0	[-56.5 ; 69.9]
Conditioner	56.3	[-35.0 ; 275.6]	-48.7	[-79.6 ; 28.6]	44.1	[-32.9 ; 209.4]
Lip/Chapstick	151.8	[10.7 ; 472.8]	231.6	[39.9 ; 686.2]	-33.5	[-67.8 ; 37.3]
Perfume	102.8	[-18.0 ; 401.4]	310.2	[46.2 ; 1050.4]	36.4	[-42.7 ; 224.7]
Contour	447.2	[126.5 ; 1222.2]	205.1	[8.2 ; 760.3]	-27.0	[-66.2 ; 57.5]
Facial cleanser	100.6	[-34.1 ; 510.2]	-47.5	[-81.4 ; 48.4]	-5.3	[-64.0 ; 149.0]

Anti-stretchmarks' cream	-63.3	[-91.4 ; 57.2]	-51.4	[-89.7 ; 129.3]	-64.6	[-88.5 ; 8.8]
Hand cream			31.6	[-64.2 ; 384.3]	-67.2	[-86.8 ; -18.5]
Intimate soap					22.7	[-77.9 ; 582.3]
Thermal spring water					41.4	[-93.3 ; 2885.1]

Triclosan

Toothpaste	45.8	[-23.3 ; 177.0]	26.5	[-35.3 ; 147.4]	89.6	[-29.7 ; 411.4]
Face cream	34.9	[-34.2 ; 176.7]	36.8	[-33.4 ; 180.9]	-26.8	[-65.5 ; 55.2]
Deodorant	63.5	[-22.3 ; 243.8]	57.7	[-32.8 ; 270.4]	144.3	[16.6 ; 412.2]
Makeup remover	-3.6	[-70.9 ; 219.8]	-31.2	[-74.4 ; 85.2]	-25.8	[-67.6 ; 69.9]
Mascara	-25.7	[-65.5 ; 60.1]	2.7	[-60.8 ; 168.7]	5.4	[-52.9 ; 135.9]
Foundation	-29.1	[-67.8 ; 56.5]	14.7	[-56.8 ; 204.9]	38.8	[-40.2 ; 222.3]
Bar soap	18.1	[-52.3 ; 192.6]	146.4	[-11.3 ; 584.2]	94.5	[-15.6 ; 348.4]
Shower gel	-24.5	[-67.1 ; 73.2]	-69.6	[-90.2 ; -5.9]	-77.8	[-90.1 ; -49.9]
Shampoo	34.9	[-43.9 ; 224.5]	51.6	[-45.2 ; 319.1]	27.6	[-39.0 ; 167.2]
Body cream	-9.7	[-72.2 ; 193.9]	-1.7	[-68.1 ; 202.8]	-15.8	[-63.5 ; 94.5]
Conditioner	-40.1	[-79.9 ; 78.5]	6.7	[-67.2 ; 247.8]	10.4	[-57.6 ; 187.1]
Lip/Chapstick	109.8	[-21.7 ; 461.9]	283.9	[34.6 ; 995.0]	2.4	[-57.5 ; 146.9]
Perfume	23.7	[-60.3 ; 285.2]	59.7	[-55.9 ; 479.0]	-13.9	[-71.0 ; 155.7]
Contour	-14.7	[-72.7 ; 166.8]	-21.1	[-79.4 ; 201.5]	-57.5	[-84.3 ; 15.0]
Facial cleanser	6.9	[-73.3 ; 327.3]	126.1	[-37.6 ; 718.5]	53.9	[-51.6 ; 390.0]
Anti-stretchmarks' cream	-65.4	[-94.2 ; 106.5]	-82.1	[-97.1 ; 11.9]	-38.2	[-83.5 ; 131.4]
Hand cream			114.2	[-60.3 ; 1056.5]	20.4	[-60.5 ; 266.9]
Intimate soap					-86.0	[-97.8 ; -9.0]
Thermal spring water					186.5	[-93.8 ; 13210.6]

Adjustment factors were woman (fixed effect), specific gravity, day and hour of urine sample collection and use of PCP in other windows than the window considered

Table 2.9: Medians^a of phenol urinary concentrations in previous studies of epidemiological studies of pregnant women that assessed between PCP used in the previous 24 to 48 hours and urinary concentrations of phenols during pregnancy and PCP survey studies

Reference	Country	N	Benzophenone-3	Bisphenol A	Bisphenol S	Methylparaben	Ethylparaben	Propylparaben	Butylparaben	Triclosan
Current study	France	8	<LOD (<LOD- 2.4)	1.6 (0.2-2.8)	0.3 (0.1-0.5)	128 (3.7- 445.5)	6.1 (<LOD-27)	14.9 (0.2- 94.2)	2.0 (<LOD-10)	1.8 (<LOD-3.4)
Meeker et al., 2013	Puerto Rico	105	31.3(11.5-172)	2.5(1.3-4.4)	NA	153 (57.6-381)	NA	36.7(10.1-130)	0.4 (<0.2-5.5)	26.2(1.3 -121)
Braun et al., 2014	USA	170	NA	NA	NA	146 (52.5-538)	NA	39.9 (8.2-149)	NA	
Larsson, 2014	Sweden	98	NA	1.29 (0.80-5.02)	NA	11(40-680)	2.4 (0.89-84)	18 (3.5-280)	<LOD (<LOD-11)	<LOD (<LOD-7.3)
Fisher, 2017	Canada	31	NA	NA	NA	27.21 (9.73-102.5)	1.87 (0.14- 10.72)	4.46 (1.06-18.6)	0.35 (0.5-1.83)	
Ashrap, 2018	Puerto Rico	1003	27.3(11.2-119.8)	2.0 (1.3-3.5)	0.4 (0.2-0.9)	92.8 (25.70-245.4)	1.50 (0.70-8.9)	17.8 (3.30-75.4)	0.20 (0.10-1.8)	15.4(3.6 ; 867.1)

Abbreviations: LOD: Limit of detection, NA: not analyzed

^aMedians with 5th -75th percentiles in parenthesis

CHAPTER 3 : PHENOLS, PHTHALATES AND CHILD NEURODEVELOPMENT

3. Phenols, phthalates and child neurodevelopment

This chapter constitutes of two studies carried out among mother-son pairs of the EDEN cohort. One on the associations between prenatal exposure to phenols and phthalates on cognition (section 3.1) is discussed in detailed since I am the first author of the corresponding manuscript published in the Environment health journal ⁴⁴ and was in charge of both statistical analysis and manuscript writing. The second study (section 3.2) explored the effect of phenols and phthalates on child behavior. This study was published in the Environmental health perspectives journal ¹⁰⁴, I was mainly involved in the statistical analysis and am second author.

3.1. Phenols, phthalates and cognition of boys in the EDEN Cohort

In-utero exposure to phenols and phthalates and the intelligence quotient of boys at 5 years

Dorothy Nakiwala¹, Hugo Peyre^{2,3}, Barbara Heude^{4,5}, Jonathan Y. Bernard^{4,5,6}, Rémi Béranger⁷, Rémy Slama¹, Claire Philippat¹, and The EDEN mother-child study group

¹ Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Institute for Advanced Biosciences (IAB), Inserm U1209, CNRS UMR 5309, Université Grenoble Alpes, 38000 Grenoble, France

²Assistance Publique-Hôpitaux de Paris, Robert Debré Hospital, Child and Adolescent Psychiatry Department, Paris, France

³Cognitive Sciences and Psycholinguistic Laboratory, Ecole Normale Supérieure, Paris, France.

⁴U1153 Epidemiology and Biostatistics Sorbonne Paris Cité Research Centre (CRESS), Early Origin of the Child's Health and Development (ORCHAD) Team, Inserm, Villejuif, France

⁵Université Paris Descartes, Villejuif, France

⁶Singapore Institute for Clinical Sciences (SICS), Agency for Science, Technology and Research (A*STAR), Singapore.

⁷Inserm U1085–IRSET, Université Rennes 1, Rennes France

Published in the Environmental Health journal

<https://doi.org/10.1186/s12940-018-0359-0>

3.1.1 French summary

Introduction

Les effets d'une exposition précoce aux perturbateurs endocriniens, tels que les phénols ou phtalates, sur le développement des fonctions cognitives de l'enfant suscitent des inquiétudes. Les études épidémiologiques sur le sujet se sont principalement concentrées sur les métabolites des phtalates et le bisphénol A. Notre étude avait pour but d'investiguer les relations entre l'exposition in-utero aux phtalates, bisphénol A, d'autres phénols (parabènes, triclosan, dichlorophénols et benzophénone-3) et le Quotient Intellectuel (QI) chez des garçons de 5-6 ans.

Méthodes

Nous avons sélectionné 452 couples mère/fils issus de la cohorte française EDEN et mesuré les concentrations de 11 métabolites des phtalates et 9 métabolites des phénols (4 parabènes, le benzophénone-3, bisphénol A, 2 dichlorophénols et le triclosan) à partir d'échantillons d'urine maternelle collectés entre les 22^e et 29^e semaines de grossesse. Les QI verbal et de performance ont été évalués à l'âge de 5-6 ans par un psychologue via l'échelle « Wechsler Preschool and Primary Scale of Intelligence (WPPSI) ». Les modèles d'équations structurelles et la correction de Benjamini and Hochberg (correction « False Discovery Rate ») ont été utilisés afin d'investiguer les associations entre l'exposition maternelle aux phénols, phtalates et le QI des garçons.

Résultats

Les concentrations des métabolites des phénols et phtalates n'étaient pas négativement associées avec les QI verbal et de performance des enfants (p -valeurs ≥ 0.09). Le mono(3-carboxypropyl) phtalate tendait à être associé à une augmentation du QI verbal ($\beta = 0,136$, intervalle de confiance à 95%, 0,01; 0,27) mais cette relation disparaissait après correction pour les comparaisons multiples (p -valeur corrigée, 0,71).

Conclusion

A notre connaissance, cette étude est la première à examiner les relations entre une exposition aux parabènes, benzophénone-3, dichlorophénols, triclosan et le développement cognitif de l'enfant. Les résultats ne suggèrent pas d'association négative entre une exposition in-utero aux phénols, phtalates et les QI Verbal et de Performance.

3.1.2 Abstract

Background

There are concerns that developmental exposure to endocrine disrupting chemicals such as phenolic compounds and phthalates could affect child cognitive functions. Epidemiological studies assessing such associations have mainly focused on phthalate metabolites and bisphenol A but not on the other phenolic compounds. Our study aimed to assess the relationship between in-utero exposure to phthalates, bisphenol A and other phenolic compounds (parabens, triclosan, dichlorophenols and benzophenone-3) and the Intelligence Quotient (IQ) of boys at 5-6 years.

Methods

In 452 mother-son dyads from the French EDEN cohort, we measured 11 phthalate metabolites and 9 phenolic compounds (4 parabens, benzophenone-3, bisphenol A, 2 dichlorophenols and triclosan) in spot urine samples collected between 22 and 29 gestational weeks. Verbal and performance IQ of children was assessed at 5-6 years by a psychologist using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI). We used adjusted Structural Equation Models (SEM) and the Benjamini and Hochberg false discovery rate (FDR) correction to assess the associations between maternal urine phenol and phthalate metabolite concentrations considered simultaneously and the boys' IQ.

Results

No phenol or phthalate metabolite concentration was negatively associated with child verbal or performance IQ (p-values ≥ 0.09). Mono(3-carboxypropyl) phthalate tended to be associated with increased verbal IQ ($\beta = 0.136$, 95% confidence interval, 0.01; 0.27) but this association disappeared after correction for multiple comparison (corrected p-value, 0.71).

Conclusion

To our knowledge, our study is the first to consider developmental exposure to parabens, dichlorophenols, triclosan and benzophenone-3 in relation to child cognitive development. Our findings did not suggest an inverse association between in-utero phenols and phthalates exposure and child verbal and performance IQ.

3.1.3 Methodology

Study population

The study population was a sample of mother-son pairs of the EDEN (*Etude des Déterminants pré et post natalis du développement et de la santé de l'Enfant*) cohort ²¹². The EDEN cohort consists of 2,002 pregnant women recruited before the end of the 28th gestational week from February 2003 through January 2006 in the obstetric departments of Nancy and Poitiers university hospitals, France. The cohort received approval from the ethics committee of Kremlin-Bicêtre University hospital. To be included in the cohort the participants gave informed written consent for themselves and for their children.

Among the 998 boys of the EDEN cohort born alive, 452 had IQ assessments at 5 years and urinary concentrations of phenols and phthalate metabolites assessed in maternal urine. Urinary concentrations of phenols and phthalate metabolites were assessed in a framework of previous projects that evaluated associations between these compounds and male genital malformation, explaining why we focused on boys ²¹³ and growth ^{214–216}. Inclusion criteria for biomarker assessments was being a boy, having at least one urine sample available during pregnancy and having data on growth during the pre and postnatal period (up to 3 years).

While this prevents generalizability of our findings to females, focusing on one sex is not a source of bias. Moreover, in the context where sex specific effects are plausible ^{217–220}, a study restricted to one sex will indeed have higher statistical power than a study of similar sample size including both sexes in which two sex-specific analyses are conducted.

Assessment of IQ

IQ was assessed at an average age of 5.7 (Standard Deviation (SD): 0.1) years using the French version of the Wechsler Preschool and Primary Scale of Intelligence-Third Edition (WPPSI-III), ²²¹ that was translated, culturally adapted to French norms, and calibrated in a sample of 999 children representative of the French youth aged 2.5 to 7.25 years by the ECPA (Editions of the Centre in Applied Psychology) ²²².

Assessments were carried out by two trained psychologists using the seven core subtests to assess the verbal and performance IQ scales. The seven subtests include: information (number of correct answers to questions that address a broad range of general knowledge topics, 34 items), vocabulary (number of words correctly defined, 25 items), word reasoning (number of concepts correctly identified from a series of clues, 28 items), block design (number of

correct designs recreated using blocks, 20 items), matrix reasoning (number of matrices correctly completed, 29 items) and the picture concepts subtest (number of correct selections of 2 or 3 pictures with common characteristics, 28 items) and coding (number of correct symbols copied corresponding to geometric shapes, 59 items) ²²³.

Exposure assessment

We measured concentrations of bisphenol A, benzophenone-3, triclosan, 2,4 and 2,5 dichlorophenol, methyl, ethyl, propyl and butylparaben along with 11 phthalate metabolites (monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethylhexyl) phthalate (MEHP), monobenzyl phthalate (MBzP), monocarboxy-isooctyl phthalate (MCOP), mono(3-carboxypropyl) phthalate (MCP) and monocarboxy-isononyl phthalate (MCNP)) from maternal urine samples collected during pregnancy between 22 and 29 gestational weeks using online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry ^{224,225}. Creatinine, a proxy of urine dilution, was also measured. Assessments were performed at the National Center for Environmental Health laboratory at the CDC in Atlanta, Georgia, USA

Confounding

Confounders were identified a priori from literature and included variables associated with both exposure and the outcome as well as variables associated with the outcomes only; center of recruitment (Poitiers / Nancy), maternal age (continuous), child age at assessment (continuous), parity (nulliparous / ≥ 1 child), maternal body mass index (continuous), parental education level (average number of years spent in school by mother and father), breastfeeding duration (never / breastfed), household income (≤ 1500 , 1500 – 3000 and ≥ 3000 Euros), smoking during pregnancy (yes / no), maternal psychological difficulties during pregnancy (yes / no) and child cognitive stimulation (continuous).

Child cognitive stimulation was assessed using items from the short form of the Home Observation for the Measurement of the Environment Scale questionnaire (HOME), at 5 years. The maternal psychological difficulties score during pregnancy was constructed by combining scores of the Center for Epidemiologic Studies Depression Scale Revised (CESD) and of the

State-Trait Anxiety Inventory (STAI) two questionnaires designed to assess depression and anxiety respectively.

Statistical analysis

For concentrations below the limit of detection we used the instrumental reading values. To allow ln-transformation, instrumental reading values equal to 0 (i.e. indicative of no signal) were replaced by the lowest instrumental reading value divided by the square root of two ²¹⁵.

To limit the impact of between subject variations in urine sampling conditions, concentrations were standardized for collection conditions such as hour of sampling, gestational age at collection, duration of storage at room temperature before freezing, day of sampling, year of biomarker assessments at CDC (2008 or 2011) and creatinine levels. This was done using a 2 steps statistical approach ^{215,226}. We first studied the influence of each sampling condition and creatinine levels on the measured biomarker concentrations using adjusted linear regression and then we predicted standardized concentrations using the effect estimates for each sampling condition that was associated with the biomarker concentrations (p-value < 0.2) and the measured biomarker concentrations ²²⁶. The standardized concentrations assumed that all samples were collected under similar conditions and were used in all of our analyses. All biomarker concentrations were ln-transformed before analysis.

We used multiple imputation using the *mi impute chained* command in stata 14 with 5 imputed datasets ²²⁷ to handle the missing data for the HOME score (N = 25, 5.5%), BMI (N = 4, 0.9%), parity, income and maternal depression and psychological difficulties (N = 1, 0.2%). We performed additional sensitivity analysis using only complete cases (no imputation of the missing covariate).

To study the associations between the urinary biomarker concentrations and the children's IQ scores, we used Structural Equation Models (SEM). SEMs simultaneously assess relationships between one or more independent variables and one or more dependent variables. They comprise of two parts, 1) a measurement model based on factor analysis that relates observed variables to latent constructs and observed variables. 2) a structural model, based on ordinary regression which describes the relationships between the generated latent exposure and outcome constructs.

Latent constructs underlying the IQ outcomes included 2 factors: the verbal and performance factors, that were determined a priori from previous literature ^{228,229}. The

Confirmatory Factor Analysis we performed on our data also supported these two dimensional structure. The performance IQ factor was constructed using the matrix reasoning, block design and picture concept scores while verbal IQ factor was constructed using the information, vocabulary and word reasoning scores of the WPPSI (Figure 3.1).

For the exposures, we first considered 3 latent variables based on factorial analysis, one predicted by the 2 dichlorophenols, one by the 4 parabens and one by the 4 DEHP metabolites. Because the latent variable based on the two dichlorophenols caused the model to be unidentified, we decided not to include a latent variable for the dichlorophenols and instead included only one of the dichlorophenols (2,5 dichlorophenol) as an observed variable.

The final model consisted of 2 latent exposure variables representing paraben and DEHP exposures and all other biomarker concentrations were added as observed variables (Figure 3.1).

We allowed the error covariance between, methyl and propyl paraben, butyl and ethyl paraben and the latent variable of parabens with MEP to be freely estimated. Estimating covariance between the DEHP metabolites freely did not improve the fit of the model and was not included in our final model.

We reported standardized association effect estimates, expressed as the change in SD of IQ scores associated with a 1-SD increase in the log-transformed biomarker concentrations.

Sensitivity analysis

In our main analysis, we used crude scores and adjusted for child age because we observed negative associations between the age standardized scores with child age at IQ assessment. Addition analysis with age standardized scores in the main model yielded similar results to those observed with crude scores (results not shown).

We tested 26 associations (2 outcomes * 13 exposure) and cannot exclude the part of associations observed due to chance. For this reason, in addition to the regular p-value we presented in the results section the p-values corrected for multiple testing using the Benjamini and Hochberg false discovery rate (FDR) method ²³⁰.

In sensitivity analysis, we also run a SEM model using only one construct for IQ (full scale IQ constructed using all six subscales of the WPPSI-III, Supplemental Material, Figure 3.2) and performed analysis using only complete cases (no imputation of the missing covariates). Because exposure levels and IQ scores differed by recruitment centers, we performed additional analysis stratified by center.

While used in psychology and sociology, SEM have so far had limited use in environmental epidemiology^{231,232}. For this reason, we also conducted more classic analyses using the manual based IQ scores instead of latent constructs and with adjusted multivariate linear regression models (one model per outcome and exposure). We explored the shape of the associations by studying exposures categorized as tertiles.

Analysis was carried out using MPLUS 6 (Muthen & Muthen, Los Angeles, CA, USA) and STATA/SE 14 (StataCorp, College Station, TX, USA) statistical softwares.

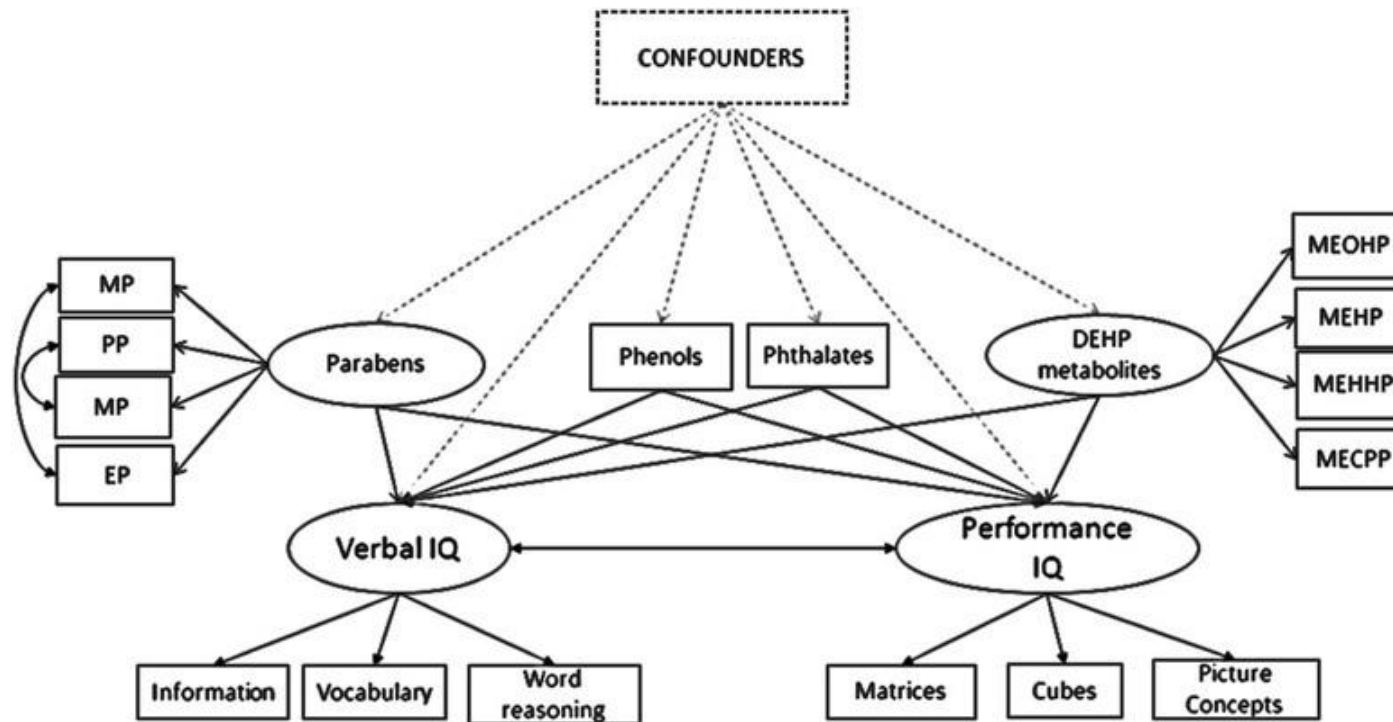


Figure 3.1: Path diagram illustrating associations between phenols, phthalate metabolites and IQ of boys.

Shapes representations: circles: latent variables, rectangles: observed variables, dashed and solid arrows: linear relationships, double headed arrows: correlations.

Abbreviations: MP: methyl paraben, EP: ethyl paraben, PP: propyl paraben, BP: butyl paraben, DEHP: di(2-ethylhexyl) phthalate metabolites, MECPP: mono(2-ethyl-5-carboxypentyl) phthalate, MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate, MEOHP: mono(2-ethyl-5-oxohexyl) phthalate, MEHP: mono(2-ethylhexyl) phthalate.

Phenols represents 2,5-dichlorophenol, benzophenone 3, bisphenol A and triclosan.

Phthalates represents mono-n-butyl phthalate, monobenzyl phthalate, monocarboxy-isononyl phthalate, monocarboxy-isooctyl phthalate, mono(3-carboxypropyl) phthalate, monoethyl phthalate and mono-isobutyl phthalate.

Confounders include maternal age, age of child at IQ assessment, HOME stimulation score, maternal body mass index before pregnancy, parental education level, parity, monthly revenue, breast feeding duration and maternal psychological

3.1.4 Results

Population characteristics

Majority of mothers in the study population (63%) were recruited from Poitiers. On average, mothers were 29.9 (SD: 4.7) years old and 46% were nulliparous. Fifty-five percent of the mothers and 44% of the fathers had more than 2 years of education after high school while 28% of the mothers reported not breast-feeding at all and 23% smoked during pregnancy (Table 3.1). Compared to mother son-pairs of the EDEN cohort not included in our analysis, pairs from our study population were more likely to be from Poitiers, from households that earned > 1500 euros, and less likely to have smoked during pregnancy (Table 3.1). Associations between maternal characteristics and child IQ are shown in Supplementary Material, Table 3.5.

Table 3.1: Characteristics of the study population and the mother-son pairs of the French EDEN cohort

Characteristics	Study population (N =452) ^a		All boys in EDEN cohort (N =998) ^b	
	N	(%)	N	(%)
Recruitment center				
Poitiers	285	(63)	534	(53)
Nancy	167	(37)	466	(47)
Parity				
Nulliparous	208	(46)	437	(44)
≥1	243	(53)	562	(56)
Missing	1	(0.2)	1	(0.1)
Maternal education				
≤ 2 years after high school	197	(44)	460	(46)
High school +2 years	105	(23)	220	(22)
High school +3years	145	(32)	290	(30)
Missing	5	(1)	23	(2)
Paternal education				
≤ 2 years after high school	224	(50)	484	(48)
High school +2 years	97	(21)	188	(19)
High school +3years	104	(23)	216	(22)
Missing	27	(6)	112	(11)
Household monthly revenue (euros)				
≤ 1500	63	(14)	170	(17)
1500 - 3000	275	(61)	573	(57)
≥ 3000	113	(25)	248	(25)
Missing	1	(0.2)	9	(1)
Smoking during pregnancy				
No	348	(77)	722	(72)
Yes	102	(23)	263	(26)
Missing	2	(0.4)	15	(2)
Maternal psychological difficulties ^c				
No	321	(71)	678	(68)
Yes	130	(29)	317	(31)
Missing	1	(0.2)	5	(1)
Was breastfeed				
Never	127	(28)	270	(27)
Yes	325	(72)	721	(72)
Missing			9	(1)
	Mean	(SD)	Mean	(SD)
Maternal age at pregnancy	29.9	(4.7)	29.3	(4.9)
Total HOME score	17.3	(2.3)	17.2	(2.3)
Body Mass Index (kg/m ²)	23.5	(4.6)	23.1	(4.6)
Child age at IQ assessment ^d	5.7	(0.1)	5.7	(0.1)
Verbal IQ scores ^e	106.4	(15.1)	106.3	(14.6)
Performance IQ scores ^e	98.6	(14.6)	99.4	(14.6)

^aIncludes mother-child pairs that have biomarker assessment and IQ scores at 5-6 years.

^bIncludes boys of the EDEN cohort born live N = 998.

^cMaternal depression or anxiety during pregnancy assessing using the CESD and the STAI respectively.

^dIncludes only boys for whom IQ was assessed.

^eIQ in standard scale (mean=100 and SD =15) and standardized for age.

Most of the compounds assessed were detected in more than 93% of the urine samples except for triclosan, ethyl and butyl paraben that were detected in 73% to 84% of the samples (Table 3.2). As expected, correlations between the 4 parabens (spearman's coefficients; 0.46 to 0.82), the 2 dichlorophenols (spearman's coefficients: 0.69) and the DEHP metabolites (spearman's coefficients; 0.78 to 0.98) were high (Supplementary material, Table 3.6). The other coefficients of correlation were in the -0.11 to 0.61 range.

Table 3.2: Concentrations^a of phenols and phthalate metabolites in maternal urine (N = 452^b mother-son pair of the EDEN mother child cohort)

Compounds (µg/L)	LOD (µg/L)	% < LOD	Percentiles		
			5th	50th	95th
Phenols					
2,4-dichlorophenol	0.2	2	0.3	1.0	10.0
2,5-dichlorophenol	0.2	0	1.7	9.2	305
Bisphenol A	0.4	0.4	0.9	2.4	9.3
Benzophenone-3	0.4	7	<LOD	2.2	63.7
Triclosan	2.3	18	<LOD	25.6	691
Methyl paraben	1	0	8.0	101.5	1529
Ethyl paraben	1	27	<LOD	3.4	689
Propyl paraben	0.2	1	0.5	13.4	255
Butyl paraben	0.2	14	<LOD	2	57.7
Phthalates					
Monoethyl phthalate (MEP)	0.6	0	21.9	100.8	591
Mono-n-butyl phthalate (MnBP)	0.2	0	11.6	44.6	444
Mono-isobutyl phthalate (MiBP)	0.2	0	11.8	38.8	168
Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	0.2	0	12.5	39.2	176
Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	0.2	0	6.9	29.0	106
Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	0.2	0	5.8	23.3	87
Mono(2-ethylhexyl) phthalate (MEHP)	0.5	3	1.5	7.6	37
Monobenzyl phthalate (MBzP)	0.3	0	4.7	18.9	114
Monocarboxy-isooctyl phthalate (MCOP)	0.2	0	1.1	4.0	19
Mono(3-carboxypropyl) phthalate (MCPP)	0.2	0	0.8	2.0	9.4
Monocarboxy-isononyl phthalate (MCNP)	0.2	1	0.4	1.3	9.7
Creatinine			0.4	1.0	1.8

^aConcentrations were standardized for creatinine concentrations and sampling conditions including hour of sampling, gestational age at collection and duration of storage at room temperature before freezing, day of sampling, year of biomarkers assessment.

^bRestricted to mother-son pairs with biomarker concentrations and IQ scores at 5-6 years available.

Associations between biomarker concentrations and boys' IQ scores

After adjustment and imputation of the missing covariates, SEM with two latent constructs underlying the IQ outcomes converged normally and fit indices suggested a statistically good fit: Root Mean Square Error of Approximation (RMSEA) = 0.048, Comparative Fit Index (CFI) = 0.930, Tucker-Lewis fit index (TLI) = 0.921. The standardized factor loadings for indicators of both outcomes and exposure latent factors were uniformly high (standardized factor loadings > 0.53) and significant (p-values < 0.001), suggesting convergent validity²³³ (Supplementary material, Table 3.7).

The only significant (uncorrected p-value below 0.05) association we observed was a positive association between MCP and verbal IQ ($\beta = 0.136$ SD of IQ scores for a 1-SD increase in the log-transformed biomarker concentrations; 95% CI: 0.006; 0.266, (Table 3.3). No other phenol or phthalate was associated with performance or verbal IQ (uncorrected p-values ≥ 0.09 , Table 3.3).

Sensitivity analysis

When we restricted our analysis to mother-child pairs with no missing values on the covariates (N = 419), the positive association observed between MCP and verbal IQ was slightly attenuated (Table 3.4); β was 0.109 (95% CI: -0.024; 0.243) compared to 0.136 (95% CI: 0.006; 0.266) in our main analysis and a positive association between triclosan and verbal IQ emerged ($\beta = 0.101$, 95% CI: 0.008; 0.194 p-value = 0.034).

Running a SEM model with only one construct for IQ led to similar results and the only significant (uncorrected p-value below 0.05) association observed was a positive association between MCP and Full Scale IQ (Supplemental Material, Table 3.8).

In the stratified analysis, we observed some heterogeneity in associations of IQ with benzophenone-3 and triclosan exposure by center (Supplementary material, Table 3.9).

Using the manual based IQ scores and running multivariate linear regression models (one model per outcome and exposure) instead of SEM suggested no significant associations between phenol, phthalate metabolites and the IQ (all p-values were > 0.13) Supplementary material, Table 3.10). Similarly, no significant association was observed after categorizing exposure in tertiles (p-value for heterogeneity > 0.07, Supplementary Material, Table 3.11), except for triclosan (p-value for heterogeneity = 0.05) The results suggested a protective effect

with verbal IQ, boys in the second tertile had a higher verbal IQ compared to those in first tertile ($\beta = 3.82$, 95% CI: 0.70; 6.93, Supplemental Material, Table 3.11).

It should be noted that after correction for multiple comparisons using FDR correction, none of the associations mentioned above remained significant (Table 3.3 and Supplemental material Table 3.9). As an example, after correction the p-value for the association between MCPP and verbal IQ observed in our main analysis was 0.71.

Table 3.3: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years (N= 452)

	Verbal IQ				Performance IQ			
	β	95% CI	P-value	Corrected P-value ^c	β	95% CI	P-value	Corrected P-value ^c
Phenols								
Parabens ^a	-0.031	[-0.138; 0.076]	0.57	0.81	0.037	[-0.096; 0.170]	0.58	0.81
2,5 Dichlorophenol	0.015	[-0.073; 0.102]	0.74	0.88	0.007	[-0.101; 0.115]	0.90	0.96
Benzophenone-3	0.066	[-0.025; 0.156]	0.16	0.71	0.006	[-0.105; 0.118]	0.91	0.96
Bisphenol A	-0.045	[-0.138; 0.047]	0.33	0.71	-0.059	[-0.173; 0.054]	0.31	0.71
Triclosan	0.078	[-0.012; 0.168]	0.09	0.71	0.0001	[-0.112; 0.112]	1.00	1.00
Phthalates								
MEP	-0.055	[-0.153; 0.042]	0.27	0.71	-0.023	[-0.144; 0.097]	0.70	0.88
MnBP	-0.094	[-0.229; 0.041]	0.17	0.71	-0.078	[-0.243; 0.088]	0.36	0.71
MIBP	0.045	[-0.057; 0.147]	0.39	0.71	0.042	[-0.084; 0.168]	0.51	0.81
MCPP	0.136	[0.006; 0.266]	0.04	0.71	0.094	[-0.036; 0.254]	0.25	0.71
MBZP	0.005	[-0.098; 0.108]	0.92	0.96	0.089	[-0.037; 0.216]	0.17	0.71
MCOP	-0.076	[-0.178; 0.026]	0.15	0.71	-0.055	[-0.181; 0.070]	0.39	0.71
MCNP	0.019	[-0.083; 0.121]	0.72	0.88	0.060	[-0.066; 0.186]	0.35	0.71
DEHP ^b	-0.042	[-0.143; 0.059]	0.41	0.71	-0.034	[-0.158; 0.090]	0.59	0.81

Root Mean Square Error of Approximation (RMSEA): 0.048 (95% Confidence Interval (CI): 0.043; 0.053), Comparative Fit Index (CFI) = 0.930, Tucker-Lewis fit index (TLI) = 0.921

Abbreviations: CI: confidence interval, MnBP: mono-n-butyl phthalate, MBZP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoocetyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate and MIBP: mono-isobutyl phthalate.

^aLatent variable of methyl, ethyl, propyl and butyl parabens

^bLatent variable of di(2-ethylhexyl) phthalate metabolites: Mono(2-ethyl-5-carboxypentyl) phthalate, Mono(2-ethyl-5-hydroxyhexyl) phthalate, Mono(2-ethyl-5-oxohexyl) phthalate and Mono(2-ethylhexyl) phthalate.

^cP-values corrected for multiple comparisons using the Benjamini and Hochberg false discovery rate method

Table 3.4: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years in the EDEN cohort (N= 419 mother-son pairs, complete case analysis)

	Verbal IQ				Performance IQ			
	β	95% CI	Pvalue	Corrected Pvalue ^c	β	95% CI	Pvalue	Corrected Pvalue ^c
Phenols								
Parabens ^a 2,5	-0.060	[-0.170; 0.050]	0.28	0.76	0.033	[-0.105; 0.171]	0.64	0.76
dichlorophenol	0.017	[-0.072; 0.107]	0.70	0.79	0.027	[-0.084; 0.138]	0.64	0.76
Benzophenone-3	0.044	[-0.049; 0.138]	0.35	0.76	-0.048	[-0.164; 0.068]	0.42	0.76
Bisphenol A	-0.032	[-0.126; 0.063]	0.51	0.76	-0.067	[-0.184; 0.050]	0.26	0.76
Triclosan	0.101	[0.008; 0.194]	0.03	0.76	0.007	[-0.111; 0.125]	0.91	0.91
Phthalates								
MEP	-0.039	[-0.140; 0.062]	0.45	0.76	0.012	[-0.113; 0.137]	0.85	0.88
MnBP	-0.103	[-0.244; 0.037]	0.15	0.76	-0.096	[-0.271; 0.078]	0.28	0.76
MIBP	0.046	[-0.060; 0.152]	0.40	0.76	0.035	[-0.096; 0.166]	0.60	0.76
MCPP	0.109	[-0.024; 0.243]	0.11	0.76	0.058	[-0.107; 0.223]	0.49	0.76
MBZP	0.028	[-0.078; 0.133]	0.61	0.76	0.112	[-0.018; 0.242]	0.09	0.76
MCOP	-0.062	[-0.170; 0.045]	0.26	0.76	-0.022	[-0.155; 0.111]	0.74	0.81
MCNP	0.027	[-0.077; 0.131]	0.61	0.76	0.062	[-0.068; 0.191]	0.35	0.76
DEHP ^b	-0.070	[-0.174; 0.033]	0.18	0.76	-0.062	[-0.191; 0.066]	0.34	0.76

Model fit indices; RMSEA=0.049(95% CI= 0.043-0.054), CFI=0.930, TFI=0.920

Abbreviations: CI: confidence interval, MnBP: mono-n-butyl phthalate, MBZP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isooctyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate and MIBP: mono-isobutyl phthalate.

^aLatent variable of methyl, ethyl, propyl and butyl parabens

^bLatent variable of di(2-ethylhexyl) phthalate metabolites: Mono(2-ethyl-5-carboxypentyl) phthalate, Mono(2-phthalate and Mono(2-ethylhexyl) phthalate.

^cP-values corrected for multiple comparisons using the Benjamini and Hobergg false discovery rate method

3.1.5 Discussion

Results from our main analysis were suggestive of a positive association between maternal urinary concentrations of MCPP and verbal IQ of boys at 5-6 years that did not persist after correction for multiple comparisons. No other phenols or phthalate metabolites were associated with boy's IQ in our main analysis. In the center stratified analyses, there was some evidence of heterogeneity in associations of IQ outcomes with benzophenone-3 and triclosan exposure by center.

Phthalates and child IQ

MCPP is a metabolite of di-n-octyl phthalate, used as a plasticizer in the production of plastics to impact flexibility, it is found in products such as carpet back coating, wire, cables, and adhesives²³⁴. Among the few studies that explored the associations between prenatal exposure to phthalates and cognitive function, only 3 studies examined child IQ⁹⁰⁻⁹² and none of these studies measured MCPP in urine, limiting comparison. Two studies looked at phthalate metabolites in maternal and childhood urine and found no significant associations between child IQ at 6 years (N=182)⁹² or 11 years (N = 110)⁹¹ and prenatal exposure while they reported negative associations with childhood exposure to DEHP metabolites and MnBP.

Factor-Litvak et al., found that when boys and girls were studied altogether MnBP, MBZP and MIBP were inversely associated with the full scale IQ of the Wechsler Intelligence Scale for Children (WISC). After stratifying for sex, they reported inverse association between MIBP and the full-scale IQ of boys at 7 years (N=155)⁹⁰. This study included Hispanics and African Americans women of inner city New York which could limit comparability with our results that relied on a population that mostly included highly educated Caucasian European women.

Both aforementioned studies had smaller population sizes than this current study (n = 452 boys): 110 boys and girls⁹¹, 182 boys and girls⁹² and 155 boys⁹⁰. The positive association we observed between MCPP and verbal IQ did not persist after correction for multiple comparisons and the effect direction was opposite to what was reported for other phthalate metabolites in a previous study on Full scale IQ.

All together, these findings did not provide strong support for an effect of MCPP on child IQ. We used IQ to evaluate cognitive function of children while other assessments such as the Bailey Scales of Infant Development (BSID) could be used. Among studies relying on

the BSID, two reported that DEHP metabolites and MEP were negatively associated with the mental index of boys at 6 months and 2 years^{217,235} while another reported positive association between MCPP along with metabolites of di(n-butyl) phthalate (MnBP and MBzP) and their mental index in boys at 2 years²¹⁹. Report of null associations also exist^{236,237}.

Phenols and child IQ

In our study population prenatal exposure to bisphenol A was not associated with boy's IQ at 5 years. In line with our results, two human studies did not report associations between maternal urinary concentrations of bisphenol A and child IQ at 5 and 8 years^{238,239} while another study reported decreased Full Scale and verbal IQ scores at 7 years in association with higher levels of bisphenol A in cord blood²⁴⁰. Frequency of detection in cord blood was low (56%) compared to levels assessed in maternal urine (frequency of detection in this study was 99% and 90% or 86% in two previous studies by Stacy et al. and Braun et al., respectively^{238,239}).

Two other studies performed among younger children (1, 3 and 4 years versus 5-6 years in our study) and relying on the BSID, the McCarthy Scales of Children's Abilities (MSCA) or the Behaviour Rating Inventory of Executive Function-Preschool (BRIEF-P) to assess cognitive function did not report any association with prenatal exposure to bisphenol A^{220,241} except among girls that had lower scores on the emotional control and inhibition scale of the BRIEF-P with increased prenatal exposure to bisphenol A²²⁰.

None of the phenols assessed was associated with child IQ in our main analysis while after stratification for recruitment centers, benzophenone-3 and triclosan were associated with some IQ scores (the direction of the association varied according to IQ scale (performance versus verbal IQ) and recruitment centers).

As far as we know, our study is the first study assessing the associations of prenatal exposure to dichlorophenol, benzophenone-3, triclosan and parabens with child cognition in humans. In the animal literature, only one rodent study attempted to assess the effects of butyl paraben on spatial memory of rats. This study reported that high exposure of a pregnant rat to butyl-paraben (200 mg/kg/day) could negatively affect spatial memory in offspring²⁴². Although animal studies suggest deleterious effects on cognitive development of offspring exposed prenatally butyl paraben, we did not observe such effects on the IQ of boys at 5-6 years.

Strengths and limitations

Strengths of this study included the larger sample size ($n = 452$ boys) compared to previous studies that examined prenatal exposure to bisphenol A and phthalates and child cognitive development among boys and girls separately (N ranged between 64 to 266 boys ^{90,91,220,238,239,243,244}).

While most previous studies used multiple linear regression, we relied on SEM to study the association with cognitive function. SEM permitted grouping of compound esters (parabens) and metabolites from similar parent compounds (DEHP metabolites) through latent variables. Use of latent variables limited the number of associations tested compared to the classical approaches that relied on the use of one regression model per outcome and exposure.

Furthermore, in sensitivity analysis we used the Benjamini and Hochberg FDR method to control for multiple testing and limit chance findings. One limitation of the FDR approach is that it does not take into account correlation across exposure and outcome variables.

As in most of the previous studies, we used one single urine measurement to assess exposure. For compounds with a short half-life such as phenols and phthalates that may not accurately depict exposure throughout pregnancy. The resulting measurement error is likely to be of classical type and to lead to attenuation bias in the effect estimate and to power loss ^{245,246}, which may explain our null findings.

We only assessed exposure for boys, if this is not a source of bias, it impedes drawing conclusion for female offspring, for which a previous study has reported deleterious effect of bisphenol A on the executive function ²²⁰.

We only considered in-utero exposure while previous studies that assessed both pre and post-natal exposure have suggested adverse IQ associations with phthalates and bisphenol A exposure during childhood and early adolescence ^{91,92,238}.

We adjusted for many potential confounders, but residual confounding cannot be ruled out, for example; we did not adjust for nutritional habits and maternal IQ, which could affect either the exposure and/or IQ of the children. We only assessed exposure to phenols and phthalates while other chemicals might affect child neurodevelopment ²⁶. A previous study that assessed exposure to 81 chemicals among Spanish women did not report a strong correlation for bisphenol A while phthalates were negatively correlated with some metals such as cadmium and copper (ρ ranged between -0.3 and -0.5 ²⁴⁷), suggesting that associations with health outcome for bisphenol A and phthalate are unlikely to be confounded by co-exposures.

Conclusion

Our study did not provide evidence of an association between any of the phenol and phthalate biomarkers assessed in maternal urine and boy's IQ at 5-6 years. While we relied on a larger sample size (N = 452 boys) than previous studies, the use of a spot sample to assess exposure limited our statistical power. Since phenols and phthalates may have sex-specific effects, our null findings among boys cannot be generalized to girls. Future studies should consider use of multiple urine samples per individual to assess exposure during pre and post-natal life among boys and girls.

3.1.6 Supplementary Materials

List of figures

Figure 3.2: Path diagram illustrating associations between phenols, phthalate metabolites and FSIQ of boys in the EDEN Cohort

List of tables

Table 3.5: Associations between the covariates included in our final model and boys' IQ (n =452)

Table 3.6: Spearman correlation coefficients between biomarker concentrations measured in maternal urine concentrations (n = 452^a)

Table 3.7: Estimated standardized factor loadings of outcome and exposure indicator variables

Table 3.8: Adjusted associations between phenols, phthalate metabolites and Full Scale IQ of boys at 5 years using SEM (N= 452)

Table 3.9: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years in the EDEN cohort, stratified for center of recruitment

Table 3.10: Adjusted associations between phenols, phthalate metabolites and IQ (Manual based scales) of boys at 5 years in the EDEN cohort, using multiple linear regression (N= 452)

Table 3.11: Adjusted associations between phenols, phthalate metabolites (concentrations categorized in tertiles) and IQ (Manual based scales) of boys at 5 years in the EDEN cohort, using multiple linear regression (N= 452)

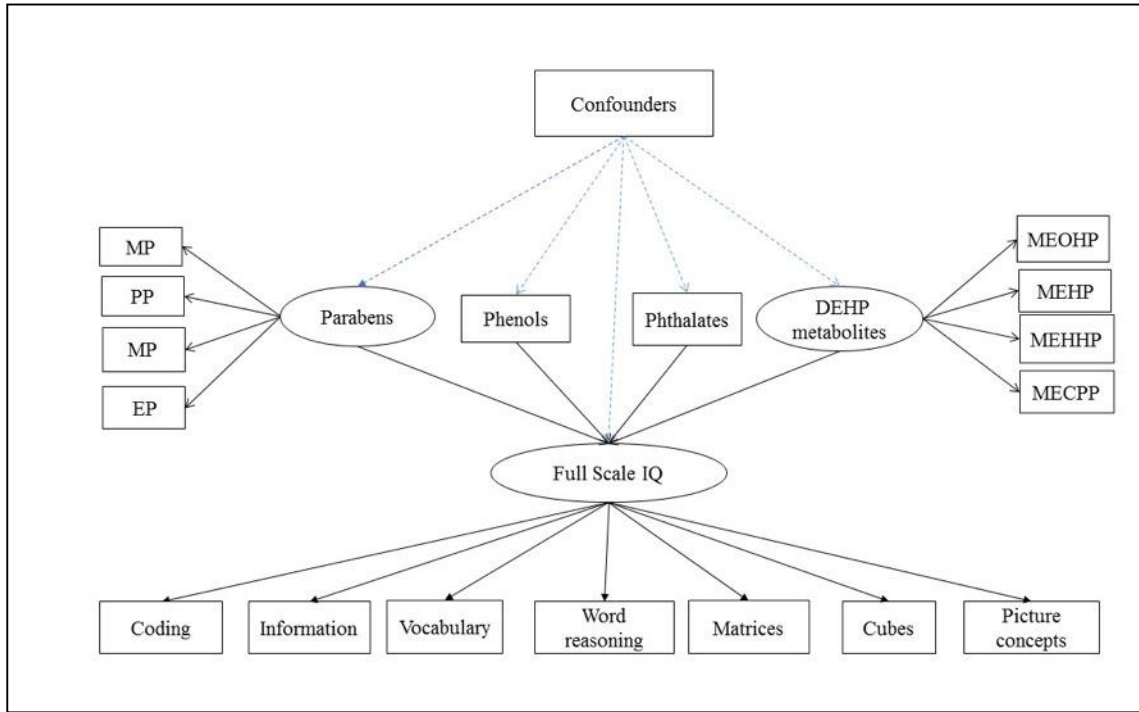


Figure 3.2: Path diagram illustrating associations between phenols, phthalate metabolites and FSIQ of boys in the EDEN Cohort

Table 3.5: Associations between the covariates included in our final model and boys' IQ (n =452)

Characteristics	VERBAL IQ				PERFORMANCE IQ			
	Crude		Multivariate ^a		Crude		Multivariate	
	β	P-value	β	P-value	β	P-value	β	P-value
Recruitment center								
Poitiers	ref				ref			
Nancy	0.12	0.01	0.10	0.09	0.06	0.28	0.02	0.81
Parity								
Nulliparous	ref				ref			
≥ 1 child	-0.17	0.07	-0.13	0.01	-0.19	0.10	-0.10	0.10
Household monthly revenue (euros)								
≤ 1500	ref				ref			
1500 - 3000	0.29	<0.001	0.17	0.01	0.16	0.05	0.02	0.76
≥ 3000	0.45	<0.001	0.20	0.01	0.27	0.001	-0.01	0.91
Smoking during pregnancy								
No	ref				ref			
Yes	0.004	0.9	0.09	0.05	-0.07	0.17	-0.02	0.66
Maternal psychological difficulties								
No	ref				ref			
Yes	-0.12	0.01	-0.06	0.21	-0.13	0.02	-0.07	0.18
Was breastfeed								
Never	ref				ref			
Yes	0.12	<0.001	0.10	0.06	0.20	<0.001	0.11	0.06
Maternal age at pregnancy	0.14	0.004	0.11	0.03	0.06	0.26	0.05	0.42
Total HOME score at 5 years	0.19	0.003	0.10	0.09	0.17	0.06	0.04	0.58
Maternal Body Mass Index (kg/m ²)	-0.14	0.004	-0.06	0.21	-0.12	0.03	-0.26	0.26
Child age at IQ assessment	-0.02	0.62	-0.01	0.81	0.03	0.61	0.04	0.49
Parental education ^b	0.41	<0.001	0.27	<0.001	0.34	<0.001	0.27	<0.001

^a Model simultaneously adjusted for all the covariates mentioned in the table

^b Number of years spent in school (Mean of maternal and paternal education duration in years)

β = Change in SD of IQ scores associated with a 1-SD increase in the covariates

abbreviations: ref: reference category

Table 3.6: Spearman correlation coefficients between biomarker concentrations measured in maternal urine concentrations (n = 452^a)

	DC4	DC5	BPA	BP3	TCS	MP	EP	PP	BP	MEP	MnBP	MiBP	MCPP	MBzP	MEHP	MEOHP	MEHHP	MECPP	MCOP	MCNP	
DC4	1.00																				
DC5	0.69	1.00																			
BPA	0.01	0.02	1.00																		
BP3	0.11	0.06	0.05	1.00																	
TCS	0.37	0.03	-0.05	0.14	1.00																
MP	0.06	0.00	0.01	0.19	0.12	1.00															
EP	0.14	0.01	-0.03	0.17	0.14	0.58	1.00														
PP	0.08	-0.01	-0.05	0.17	0.16	0.82	0.48	1.00													
BP	0.10	-0.02	0.00	0.23	0.17	0.55	0.72	0.46	1.00												
MEP	0.15	0.06	0.05	0.18	0.20	0.30	0.15	0.31	0.22	1.00											
MnBP	0.01	0.08	0.14	0.12	-0.05	-0.01	0.08	0.03	0.04	0.10	1.00										
MiBP	-0.05	0.05	0.13	0.07	-0.06	0.08	0.03	0.05	0.00	0.04	0.42	1.00									
MCPP	0.06	0.08	0.25	0.02	-0.06	0.00	0.03	0.00	-0.02	0.00	0.61	0.26	1.00	0.26							
MBzP	-0.03	-0.02	0.19	0.09	-0.05	0.01	0.01	0.01	-0.05	0.07	0.43	0.38	0.26	1.00							
MEHP	0.07	0.10	0.24	0.03	0.00	-0.03	-0.01	-0.03	-0.05	0.07	0.22	0.25	0.24	0.27	1.00						
MEOHP	0.09	0.11	0.24	0.03	0.02	-0.04	0.00	-0.07	-0.08	0.05	0.31	0.28	0.32	0.35	0.84	1.00					
MEHHP	0.09	0.12	0.25	0.02	0.00	-0.04	0.00	-0.08	-0.07	0.05	0.29	0.27	0.31	0.33	0.82	0.98	1.00				
MECPP	0.10	0.11	0.21	0.00	-0.01	-0.07	-0.03	-0.09	-0.11	0.01	0.24	0.25	0.26	0.30	0.78	0.93	0.90	1.00			
MCOP	0.06	0.03	0.24	0.04	0.01	-0.01	-0.02	0.00	-0.02	0.02	0.12	0.21	0.26	0.21	0.30	0.34	0.34	0.38	1.00		
MCNP	0.15	0.08	0.20	0.01	0.03	0.01	0.04	0.03	0.01	-0.01	0.12	0.11	0.32	0.16	0.21	0.26	0.26	0.26	0.42	1.00	

Abbreviations: BP: butylparaben, BPA: bisphenol A, BP3: benzophenone-3, DCP: dichlorophenol, EP: ethylparaben, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoocetyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MECPP: mono(2-ethyl-5-carboxypentyl) phthalate, MEHHP: mono(2-ethyl-5-hydroxyhexyl) phthalate, MEHP: mono(2-ethylhexyl) phthalate, MEOHP: mono(2-ethyl-5-oxohexyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, MP: methylparaben, PP: propylparaben, TCS: triclosan.

^aMother-son pairs with IQ scores and biomarker concentration assessment.

Table 3.7: Estimated standardized factor loadings of outcome and exposure indicator variables

Latent variables	Predictors	Factor loading	S.E	P-Value	Variance explained by latent variable
Verbal IQ	Information	0.866	0.017	<0.001	0.749
	Vocabulary	0.794	0.022	<0.001	0.630
	Word reasoning	0.864	0.017	<0.001	0.747
Performance IQ	Cubes	0.743	0.042	<0.001	0.553
	Matrices	0.562	0.044	<0.001	0.316
	Picture concepts	0.564	0.044	<0.001	0.319
Parabens	Methylparaben	0.892	0.079	<0.001	0.553
	Propylparaben	0.819	0.079	<0.001	0.316
	Butylparaben	0.565	0.063	<0.001	0.319
	Ehtylparaben	0.584	0.058	<0.001	0.553
DEHP	MEHP	0.846	0.013	<0.001	0.716
	MEOHP	0.999	0.001	<0.001	0.999
	MEHHP	0.984	0.002	<0.001	0.969
	MECPP	0.941	0.006	<0.001	0.885

Root Mean Square Error of Approximation (RMSEA): 0.048 (95% Confidence Interval (CI): 0.043; 0.053), Comparative Fit Index (CFI) = 0.930, Tucker-Lewis fit index (TLI) = 0.921

Abbreviations: SE: standard error, DEHP: di(2-ethylhexyl) phthalate metabolites, MECPP: Mono(2-ethyl-5-carboxypentyl) phthalate, MEHHP: Mono(2-ethyl-5-hydroxyhexyl) phthalate, MEOHP: Mono(2-ethyl-5-oxohexyl) phthalate, MEHP: Mono(2-ethylhexyl) phthalate

Table 3.8: Adjusted associations between phenols, phthalate metabolites and Full Scale IQ of boys at 5 years using SEM (N= 452)

Phenols	β	95% CI	P-value	Corrected P-value ^c
Parabens ^a	-0.022	[-0.130; 0.084]	0.68	0.50
2.5 Dichlorophenol	0.018	[-0.070; 0.104]	0.69	0.74
Benzophenone-3	0.060	[-0.030; 0.149]	0.19	0.50
Bisphenol A	-0.049	[-0.140; 0.043]	0.30	0.55
Triclosan	0.070	[-0.020; 0.159]	0.12	0.50
Phthalates				
MEP	-0.055	[-0.150; 0.044]	0.27	0.55
MnBP	-0.093	[-0.230; 0.038]	0.17	0.50
MIBP	0.047	[-0.053; 0.150]	0.37	0.56
M CPP	0.135	[0.009; 0.268]	0.04	0.50
MBZP	0.015	[-0.086; 0.118]	0.77	0.77
MCOP	-0.076	[-0.179; 0.024]	0.14	0.50
MCNP	0.025	[-0.079; 0.124]	0.63	0.74
DEHP ^b	-0.044	[-0.144 0.056]	0.39	0.55

Root Mean Square Error of Approximation (RMSEA): 0.05 (95% Confidence Interval (CI): 0.046; 0.055), Comparative Fit Index (CFI) = 0.912, Tucker-Lewis fit index (TLI) = 0.907. Abbreviations: CI: confidence interval, MnBP: mono-n-butyl phthalate, MBZP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoctyl phthalate, M CPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate and MIBP: mono-isobutyl phthalate.

^aLatent variable of methyl, ethyl, propyl and butyl parabens

Table 3.9: Adjusted associations between phenols, phthalate metabolites and IQ of boys at 5 years in the EDEN cohort, stratified for center of recruitment

	POITIERS (N= 285)								NANCY (N=168)							
	VERBAL IQ				PERFORMANCE IQ				VERBAL IQ				PERFORMANCE IQ			
	β	95% CI	P-value	P-value ^c	β	95% CI	P-value	P-value ^c	β	95% CI	P-value	P-value ^c	β	95% CI	P-value	P-value ^c
Phenols																
PBM^a	-0.07	[-0.199; 0.061]	0.30	0.78	-0.04	[-0.195; 0.126]	0.67	0.95	-0.02	[-0.187; 0.150]	0.83	0.94	0.08	[-0.136; 0.288]	0.48	0.94
DC5	0.01	[-0.097; 0.120]	0.84	0.95	0.00	[-0.133; 0.130]	0.99	0.99	0.02	[-0.134; 0.164]	0.84	0.94	-0.01	[-0.180; 0.154]	0.88	0.94
BP3	0.12	[0.012; 0.228]	0.03	0.48	0.12	[-0.011; 0.252]	0.07	0.62	-0.01	[-0.172; 0.152]	0.90	0.94	-0.26	[-0.433; -0.077]	0.01	0.07
BPA	-0.01	[-0.123; 0.101]	0.84	0.95	-0.05	[-0.189; 0.084]	0.45	0.95	-0.09	[-0.238; 0.063]	0.25	0.58	-0.02	[-0.190; 0.148]	0.81	0.94
TCS	-0.01	[-0.121; 0.105]	0.89	0.95	-0.11	[-0.246; 0.028]	0.12	0.66	0.22	[0.067; 0.363]	<0.001	0.07	0.18	[0.008; 0.344]	0.04	0.35
Phthalates																
MEP	0.02	[-0.108; 0.139]	0.81	0.95	0.04	[-0.109; 0.190]	0.60	0.95	-0.09	[-0.248; 0.069]	0.27	0.58	-0.03	[-0.209; 0.150]	0.75	0.94
MnBP	-0.17	[-0.332; -0.011]	0.04	0.48	-0.13	[-0.323; 0.067]	0.20	0.73	-0.01	[-0.246; 0.232]	0.96	0.96	-0.07	[-0.343; 0.198]	0.60	0.94
MIBP	0.02	[-0.105; 0.148]	0.74	0.95	0.04	[-0.118; 0.189]	0.65	0.95	0.15	[-0.016; 0.314]	0.08	0.49	0.05	[-0.134; 0.237]	0.59	0.94
MCPP	0.11	[-0.043; 0.271]	0.16	0.68	0.08	[-0.108; 0.272]	0.40	0.94	0.14	[-0.091; 0.367]	0.24	0.58	0.05	[-0.213; 0.303]	0.73	0.94
MBZP	-0.01	[-0.137; 0.123]	0.92	0.95	0.09	[-0.071; 0.244]	0.28	0.78	-0.02	[-0.193; 0.150]	0.81	0.94	0.11	[-0.082; 0.301]	0.26	0.58
MCOP	0.01	[-0.120; 0.140]	0.88	0.95	-0.12	[-0.279; 0.034]	0.13	0.66	-0.12	[-0.296; 0.048]	0.16	0.58	0.15	[-0.041; 0.340]	0.13	0.54
MCNP	-0.01	[-0.146; 0.126]	0.88	0.95	0.09	[-0.071; 0.259]	0.27	0.78	0.02	[-0.133; 0.165]	0.84	0.94	0.03	[-0.141; 0.193]	0.76	0.94
DEHP^b	0.02	[-0.115; 0.150]	0.80	0.95	0.03	[-0.134; 0.190]	0.74	0.95	-0.13	[-0.282; 0.027]	0.11	0.54	-0.10	[-0.277; 0.073]	0.25	0.58

Model fit indices for Poitiers; RMSEA=0.046(95% CI= 0.043-0.054), CFI=0.937, TFI=0.929

Model fit indices for Nancy; RMSEA=0.047(95% CI= 0.037-0.057), CFI=0.935, TFI=0.926

Abbreviations; DC5: 2,5 dichlorophenol, BP3: benzophenone-3, BPA: bisphenol A, TCS: triclosan, MEP: monoethyl phthalate, MnBP: mono-n-butyl phthalate, MIBP: mono-isobutyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MBZP: monobenzyl phthalate, MCOP: monocarboxy-isoctyl phthalate, MCNP: monocarboxy-isononyl phthalate

^aLatent variable of methyl, ethyl, propyl and butyl parabens

^bLatent variable of di(2-ethylhexyl) phthalate metabolites: Mono(2-ethyl-5-carboxypentyl) phthalate, Mono(2-ethyl-5-hydroxyhexyl) phthalate, Mono(2-ethyl-5-oxohexyl) phthalate and Mono(2-ethylhexyl) phthalate

Table 3.10: Adjusted associations between phenols, phthalate metabolites and IQ (Manual based scales) of boys at 5 years in the EDEN cohort, using multiple linear regression (N= 452)

	Full Scale IQ			Verbal IQ			Performance IQ		
	β	95% CI	P-value	β	95% CI	P-value	β	95% CI	P-value
Phenols									
Parabens ^a	0.14	[-0.65; 0.93]	0.73	-0.02	[-0.83; 0.79]	0.96	0.50	[-0.33; 1.33]	0.24
Dichlorophenol ^b	0.45	[-0.38; 1.27]	0.29	0.21	[-0.63; 1.05]	0.62	0.20	[-0.66; 1.06]	0.65
Benzophenone-3	0.01	[-0.73; 0.76]	0.97	0.21	[-0.55; 0.97]	0.59	0.01	[-0.77; 0.80]	0.98
Bisphenol A	-0.65	[-2.31; 1.02]	0.45	-0.88	[-2.58; 0.82]	0.31	-0.46	[-2.22; 1.29]	0.60
Triclosan	0.04	[-0.46; 0.55]	0.87	0.32	[-0.19; 0.84]	0.22	-0.28	[-0.82; 0.25]	0.30
Phthalates									
MEP	-0.56	[-1.77; 0.65]	0.37	-0.72	[-1.95; 0.51]	0.25	-0.07	[-1.33; 1.20]	0.92
MnBP	-0.09	[-1.30; 1.13]	0.89	-0.28	[-1.51; 0.95]	0.66	-0.10	[-1.37; 1.17]	0.87
MIBP	0.38	[-1.14; 1.91]	0.62	0.15	[-1.40; 1.71]	0.85	0.89	[-0.71; 2.50]	0.27
MCPP	1.05	[-0.55; 2.65]	0.20	0.73	[-0.90; 2.36]	0.38	0.95	[-0.73; 2.63]	0.27
MBZP	0.14	[-1.12; 1.40]	0.83	-0.31	[-1.60; 0.98]	0.64	0.76	[-0.57; 2.08]	0.26
MCOP	-0.57	[-2.03; 0.90]	0.45	-1.06	[-2.55; 0.42]	0.16	-0.02	[-1.55; 1.52]	0.98
MCNP	0.63	[-0.65; 1.91]	0.33	0.10	[-1.21; 1.41]	0.88	1.02	[-0.32; 2.37]	0.13
DEHP ^c	-0.42	[-1.92; 1.08]	0.58	-0.75	[-2.27; 0.78]	0.34	-0.05	[-1.63; 1.52]	0.95

Abbreviations: CI: confidence interval, MnBP: mono-n-butyl phthalate, MBZP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-iso-octyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate and MIBP: mono-isobutyl phthalate.

^aMolar sum of methyl, ethyl, propyl and butyl parabens

^bMolar sum of 2,4 and 2,5 Dichlorophenol

^cMolar sum of di(2-ethylhexyl) phthalate metabolites: Mono(2-ethyl-5-carboxypentyl) phthalate, Mono(2-ethyl-5-hydroxyhexyl) phthalate, Mono(2-ethyl-5-oxohexyl) phthalate and Mono(2-ethylhexyl) phthalate.

Table 3.11: Adjusted associations between phenols, phthalate metabolites (concentrations categorized in tertiles) and IQ (Manual based scales) of boys at 5 years in the EDEN cohort, using multiple linear regression (N= 452)

	Full Scale IQ				Verbal IQ				Performance IQ			
	β	95% CI	Phet	ptrend	β	95% CI	Phet	ptrend	β	95% CI	Phet	ptrend
Σ Parabens												
T2	2.35	[-0.66; 5.37]	0.31	0.48	1.50	[-1.57; 4.58]	0.27	0.56	2.70	[-0.47; 5.87]	0.10	0.04
T3	0.98	[-2.12; 4.08]			-1.09	[-4.24; 2.06]			3.28	[0.04; 6.53]		
Σ Dichlorophenol												
T2	0.32	[-2.72; 3.36]	0.77	0.47	0.53	[-2.57; 3.63]	0.94	0.90	-0.14	[-3.34; 3.06]	0.94	0.77
T3	1.06	[-1.94; 4.06]			0.22	[-2.84; 3.29]			0.42	[-2.75; 3.58]		
Benzophenone-3												
T2	-0.23	[-3.25; 2.80]	0.96	0.87	-0.24	[-3.32; 2.84]	0.65	0.43	0.33	[-2.86; 3.51]	0.81	0.51
T3	0.19	[-2.89; 3.28]			1.12	[-2.02; 4.26]			1.05	[-2.19; 4.30]		
Bisphenol A												
T2	0.32	[-2.80; 3.44]	0.95	0.89	-0.36	[-3.52; 2.81]	0.48	0.25	0.56	[-2.70; 3.83]	0.83	0.55
T3	-0.20	[-3.23; 2.83]			-1.80	[-4.89; 1.28]			0.97	[-2.22; 4.16]		
Triclosan												
T2	3.02	[-0.03; 6.07]	0.13	0.59	3.82	[0.70; 6.93]	0.05	0.14	1.90	[-1.31; 5.11]	0.18	0.53
T3	0.81	[-2.24; 3.86]			2.31	[-0.79; 5.40]			-1.04	[-4.25; 2.16]		
Phthalates												
MEP												
T2	-1.89	[-4.88; 1.10]	0.46	0.50	-1.71	[-4.75; 1.33]	0.42	0.42	-1.85	[-4.99; 1.30]	0.30	0.30
T3	-1.09	[-4.18; 2.00]			-1.89	[-5.03; 1.26]			0.49	[-2.75; 3.74]		
MnBP												
T2	1.35	[-1.67; 4.38]	0.67	0.81	1.38	[-1.70; 4.46]	0.44	0.24	1.32	[-1.87; 4.50]	0.63	0.76
T3	0.50	[-2.62; 3.62]			-0.52	[-3.69; 2.65]			1.43	[-1.85; 4.71]		
MIBP												
T2	-1.50	[-4.47; 1.47]	0.13	0.27	-0.51	[-3.56; 2.54]	0.82	0.66	-2.45	[-5.55; 0.65]	0.12	0.41
T3	1.72	[-1.35; 4.78]			0.52	[-2.62; 3.65]			2.76	[-0.44; 5.96]		
MCPP												
T2	2.83	[-0.14; 5.80]	0.15	0.20	3.07	[0.04; 6.10]	0.14	0.74	2.53	[-0.60; 5.66]	0.21	0.09
T3	2.18	[-0.85; 5.21]			1.67	[-1.42; 4.75]			2.37	[-0.82; 5.56]		

MBZP												
T2	0.19	[-2.85; 3.23]	0.98	0.85	-2.03	[-5.13; 1.06]	0.40	0.38	2.20	[-0.99; 5.38]	0.32	0.17
T3	0.31	[-2.75; 3.36]			-1.56	[-4.67; 1.55]			2.11	[-1.10; 5.32]		
MCOP												
T2	-3.53	[-6.55; -0.51]	0.07	0.19	-2.57	[-5.66; 0.52]	0.15	0.35	-2.92	[-6.11; 0.27]	0.17	0.22
T3	-2.06	[-5.11; 0.98]			-2.80	[-5.89; 0.30]			-0.71	[-3.91; 2.49]		
MCNP												
T2	-0.80	[-3.83; 2.23]	0.69	0.66	-0.11	[-3.21; 2.98]	1.00	0.08	-1.74	[-4.92; 0.27]	0.26	0.67
T3	0.54	[-2.46; 3.54]			-0.14	[-3.19; 2.92]			0.94	[-2.20; 4.09]		
ΣDEHP												
T2	-0.68	[-3.73; 2.37]	0.82	0.84	0.37	[-2.72; 3.47]	0.96	0.93	-0.40	[-3.59; 2.80]	0.81	0.45
T3	0.26	[-2.80; 3.33]			-0.01	[-3.12; 3.13]			0.65	[-2.58; 3.88]		

Abbreviations: CI: confidence interval, T2: tertile 2, T3: tertile 3, MnBP: mono-n-butyl phthalate, MBZP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isooctyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate and MIBP: mono-isobutyl phthalate.

^aLatent variable of methyl, ethyl, propyl and butyl parabens

^bLatent variable of di(2-ethylhexyl) phthalate metabolites: Mono(2-ethyl-5-carboxypentyl) phthalate, Mono(2-ethyl-5-hydroxyhexyl) phthalate, Mono(2-ethyl-5-oxohexyl) phthalate and Mono(2-ethylhexyl) phthalate.

3.2 Phenols, phthalates and behavior of boys in the EDEN Cohort

Title: Prenatal Exposure to Nonpersistent Endocrine Disruptors and Behavior in Boys at 3 and 5 Years

Claire Philippat¹, Dorothy Nakiwala¹, Antonia M. Calafat², Jérémie Botton,^{3,4,5} Maria De Agostini^{3,4}, Barbara Heude^{3,4}, Rémy Slama¹, and the EDEN Mother–Child Study Group

¹Institute for Advanced Biosciences, INSERM U1209, CNRS UMR 5309, University Grenoble Alpes, Grenoble, France

²Centers for Disease Control and Prevention, Atlanta, Georgia, USA

³U1153 Epidemiology and Biostatistics Sorbonne Paris Cité Research Centre (CRESS), Early Origin of the Child's Health and Development (ORCHAD) Team, Inserm, Villejuif, France

⁴Université Paris Descartes, Villejuif, France

⁵Faculty of Pharmacy, Université Paris-Sud, Université Paris-Saclay, Châtenay-Malabry, France

Published in the Environmental Health Perspectives journal

doi: <https://doi.org/10.1289/EHP1314>

3.2.1 French summary

Introduction

Des associations sexe-spécifiques ont été reportées entre une exposition aux phtalates, au bisphénol A (BPA) et le comportement de l'enfant. Il n'existe pour l'heure pas de données, issues de larges études, pour d'autres phénols susceptibles de perturber les systèmes endocriniens.

Objectifs

Notre but était d'étudier les relations entre l'exposition prénatale aux phtalates, divers phénols et le développement comportemental des jeunes garçons.

Méthodes

11 métabolites des phtalates et 9 composés issus des phénols (4 parabènes, le benzophénone-3, BPA, deux dichlorophénols et le triclosan) ont été dosés à partir d'échantillons d'urine prélevés au cours de la grossesse chez des femmes participant à la cohorte EDEN et ayant mis au monde des garçons. Les mères ont rempli le questionnaire «Strength and Difficulties Questionnaire (SDQ)» lorsque leur enfant était âgé entre 3,1 (n=529) et 5,6 ans (n=464).

Résultats

Le BPA était positivement associé aux troubles relationnels à l'âge de 3 ans [Ratio des Taux d'Incidence (IRR): 1,11; 95% Intervalle de Confiance (IC): 1,03 ; 1,20] ainsi qu'aux problèmes d'hyperactivité/inattention à 5 ans (IRR: 1,08; 95% IC: 1,01 ; 1,14). Le mono-n-butyl-phtalate (MnBP) était positivement lié aux troubles internalisés du comportement ainsi qu'aux troubles relationnels et émotionnels à 3 ans. Le mono-benzyl-phtalate (MBzP) était associé positivement aux problèmes de comportement internalisé et aux problèmes relationnels à 3 ans. Après dichotomisation des scores SDQ, le triclosan tendait à être positivement associé aux troubles émotionnels à 3 et 5 ans.

Conclusions

Les associations observées entre le BPA, le MnBP et le comportement des jeunes garçons sont cohérentes avec les résultats de précédents travaux. D'autres études d'évaluation de l'impact sur la santé, basées sur les relations dose-réponse corrigées pour les erreurs de classification de l'exposition, sont nécessaires pour évaluer les conséquences de telles associations sur la santé publique.

3.2.2 Abstract

Background

Sex-specific associations have been reported between phthalates, bisphenol A (BPA), and child behavior. No data on large study populations are available for other phenols with possible endocrine-disrupting properties.

Objectives

We aimed to study associations between prenatal exposure to phthalates and several phenols on behavior among male infants.

Methods:

We quantified 11 phthalate metabolites and nine phenols (four parabens, benzophenone-3, BPA, two dichlorophenols, triclosan) in spot urine samples collected during pregnancy among EDEN cohort mothers who delivered a boy. Mothers completed the Strength and Difficulties Questionnaire (SDQ) when their children were 3.1 ($n = 529$) and 5.6 ($n = 464$) y old.

Results

BPA was positively associated with the relationship problems subscale at 3 years [incidence rate ratio (IRR): 1.11; 95% confidence interval (CI): 1.03, 1.20] and the hyperactivity–inattention subscale scores at 5 years (IRR: 1.08; 95% CI: 1.01, 1.14). Mono-*n*-butyl phthalate (MnBP) was positively associated with internalizing behavior, relationship problem, and emotional symptom scores at 3 years. Monobenzyl phthalate (MBzP) was positively associated with internalizing behavior and relationship problems scores at 3 years. After dichotomizing SDQ scores, triclosan tended to be positively associated with emotional symptom subscales at both 3 and 5 years.

Conclusions

The observed associations between BPA, MnBP, and behavior in boys are consistent with previous findings. Further health impact assessment studies based on dose–response functions corrected for exposure misclassification are required to quantify the public health burden possibly entailed by such associations.

3.2.3 Methodology

Study population and assessmental urinary biomarker concentrations

The EDEN cohort (study population) and measurement of maternal urinary concentrations of phenols and phthalates is described section 3.2.3.

Assessment of child behavior

Behavior was assessed at 3.1 (SD: 0.1) and 5.6 (SD: 0.2) years using the Strengths and Difficulties Questionnaire (SDQ) completed by the mother²⁴⁸. This questionnaire includes 25 items scored on a 3 point-scale (0: not true; 1: somewhat true; 2: certainly true) that were combined into 4 difficulties subscore: conduct problems, hyperactivity-inattention, peer relationship problems and emotional symptoms subscores and one strength subscore: prosocial behavior. The score for each subscale ranged from 0 to 10. We then computed an externalizing (sum of the conduct problems and hyperactivity-inattention scores) and an internalizing behavior score (sum of the peer relationship problems and emotional symptoms scores). Score of the strength subscale was reversed prior to analysis so that for all SDQ subscales higher scores meant increased difficulties. Construct of each subscore is detailed in Table 3.12.

Table 3.12: Scoring of the Strengths and Difficulties Questionnaire

Clinical behaviors (score ranges:0-20)	Subscale (score ranges: 0-10)	Items used to compute each subscale (score ranges: 0-3)
Internalizing behavior	Emotional symptoms	Often complains of headaches Many worries Often unhappy, downhearted Nervous or clingy in new situations Many fears, easily scared
	Peer relationship problems	Rather solitary, tends to play alone Has at least one good friend Generally liked by other children Picked on or bullied Gets on better with adults than with other children
Externalizing behavior	Conduct problems	Often has temper tantrums or hot tempers Generally obedient Often fights with other children Often argumentative with adults Can be spiteful to others
	Hyperactivity /Inattention problems	Restless, overactive Constantly fidgeting or squirming Easily distracted, concentration wanders Can stop and think things out before acting Sees tasks through to the end
	Prosocial behavior	Considerate of other people's feelings Shares readily with other children Helpful if someone is hurt Kind to younger children Often volunteers to help others

Statistical analysis

Biomarker concentrations were standardized for creatinine and sampling conditions (e.g., hour of sampling, gestational age at sampling) prior to analysis using a two-step standardization approach developed by our team^{249,250}, as detailed in section 3.2.3.

We used adjusted negative binomial regressions to study the associations between each standardized biomarker concentration and each of SDQ subscore. Biomarker concentrations were log transformed (base 2) prior to analysis so that the Incidence Rate Ratios (IRR) corresponded to the multiplicative change in the probability of the SDQ scores increasing by one unit for a doubling in biomarker concentration.

Adjustment factors were selected based on a priori knowledge and included factors possibly related to both exposures and child behavior, or to child behavior only. These factors included child age at assessment, recruitment center (Poitiers versus Nancy), maternal age (continuous), parity (0, 1, 2 or more children), maternal body mass index (BMI, continuous), parental education (average length of the mother and father's education), breastfeeding duration (never, ≤ 3 months, > 3 months), household income (≤ 1500 , 1500 to 3000, ≥ 3000 euros per

months), smoking during pregnancy (yes/no), and maternal psychological difficulties during pregnancy (yes/no). The maternal psychological difficulties score was constructed by combining scores of the Center for Epidemiologic Studies Depression Scale Revised (CESD), a questionnaire designed to assess depression and scores of the State-Trait Anxiety Inventory (STAI) that evaluates anxiety. The CESD and the STAI were completed by the mother during pregnancy.

Sensitivity analyses

Several sensitivity analyses were conducted. To explore to which extent associations could be confounded by exposure to other phenols or phthalates, we ran analyses simultaneously adjusted for all phenols (bisphenol A, benzophenone-3, triclosan, Σ dichlorophenols, Σ parabens), and phthalate metabolites MEP, MnBP, MiBP, MBzP, MCOP, MCPP, MCNP and Σ DEHP concentrations.

Benzophenone-3 is used as a UV filter in sunscreens. Use of sunscreens is likely to vary across seasons. Because season of birth has been associated with increased risks of neurodevelopmental disorders such as autism spectrum disorders and schizophrenia²⁵¹, we performed an additional analysis in which models that looked at the associations between benzophenone-3 and SDQ scores were additionally adjusted for season of urine collection.

We also dichotomized the SDQ scores at the 85th percentile²⁵² and used adjusted logistic regression models to study the associations of these binary outcomes with biomarker concentrations.

Given the relatively limited evidence regarding possible effects of phenols and phthalates on child neurodevelopment and the breadth of data available in our cohort, we chose to adopt an exploratory approach and reported in the results section all associations (p-values < 0.05 and p-values between 0.05 and 0.1). In a sensitivity analysis we accounted for multiple comparisons by applying a false discovery rate²²⁵.

Analyses were performed using Stata/SE 14.1.

3.2.4 Results

Spearman correlation coefficients between the SDQ scores at 3 years and at 5 years were below 0.5 for the emotional, peer relationship problem and prosocial scores and ranged from 0.51 to 0.66 for the three other scores (Table 3.13).

Table 3.13: Spearman correlation coefficients between SDQ scores assessed at 3 and 5 years among boys of the EDEN mother child cohort

SDQ scores	Spearman correlation coefficients between the assessments performed at 3 and 5 years
Emotional symptoms	0.41
Conduct problems	0.59
Peer relationship problems	0.37
Hyperactivity-inattention	0.62
Prosocial behavior	0.46
Internalizing behavior	0.51
Externalising behavior	0.66

Phenols and child behavior

Maternal bisphenol A was associated with the internalizing behavior score (IRR for a doubling in concentration, 1.06, 95% CI: 1.00; 1.12) and the peer relations problem (IRR, 1.11, 95% CI, 1.03; 1.20). score at 3 years. Bisphenol A was also associated with the externalizing behavior score (IRR, 1.05, 95% CI: 1.00; 1.11) and the hyperactivity-inattention score (IRR, 1.08; 95%CI, 1.01; 1.14) at 5 years (Table 3.14 and Table 3.15).

Triclosan tended to be positively associated with several SDQ scores at 3 years with IRRs closer to one than those observed for bisphenol A: IRR of 1.01 (95%CI: 1.00; 1.03), 1.02 (95% CI: 1.00; 1.04) and 1.01 (95% CI 1.00; 1.03) for the externalizing, emotional symptoms and conduct problem scores at 3 years, respectively (Table 3.14).

No other phenol was associated with the internalizing and externalizing scores at 3 or 5 years (p-values > 0.14, Table 3.14 and Table 3.15). However, when we looked at each SDQ subscores separately, scores of the conduct problems subscale at 5 years tended to increase in association with Σ dichlorophenols (IRR, 1.03; 95% CI: 1.00; 1.07). Benzophenone-3 was associated with a decreased emotional symptoms score at 5 years (IRR, 0.96; 95% CI: 0.93; 1.00) while Σ parabens were associated with increased prosocial behavior score at 3 years (IRR, 0.97; 95% CI: 0.94; 1.00) (Table 3.14 and Table 3.15).

Phthalates and child behavior

Several phthalate metabolites were associated with SDQ scores at 3 years (Table 3.14). MnBP was associated with internalizing (IRR, 1.06, 95% CI: 1.01; 1.11) as well as with the relationship problems (IRR, 1.06, 95% CI: 1.01; 1.12) and emotional problems (IRR, 1.06, 95%CI: 1.00; 1.12) scores at 3 years. MBzP was associated with increased internalizing (IRR, 1.04, 95% CI: 1.00; 1.09) and relationship problems scores (IRR, 1.07; 95% CI: 1.01; 1.13). Two phthalates were associated with lower SDQ scores: MiBP and MCPP concentrations were indeed associated with decreased hyperactivity-inattention scores (IRR were 0.95 (95% CI: 0.91; 1.00)) and 0.96 (95%CI: 0.92; 1.00) for MiBP and MCPP, respectively). MCPP was also associated with decreased prosocial behavior scores (IRR, 0.95, 95%CI: 0.9; 1.0). None of the associations we observed between phthalate metabolites and SDQ scores at 3 years persisted at 5 years (Table 3.15)

Table 3.14: Adjusted associations between phenols, phthalate metabolites and behavior at 3 years among boys of the EDEN mother-child cohort (N = 518 to 520 mother-son pairs, depending of the subscale).

	Emotional symptoms		Conduct problems		Peer relationship problems		Hyperactivity - inattention problems		Prosocial behavior		Externalizing behavior		Internalizing behavior	
	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI
Phenols														
∑Dichlorophenols	1.02	[0.98; 1.06]	1.01	[0.99; 1.04]	0.99	[0.95; 1.03]	1.00	[0.98; 1.03]	1.00	[0.98; 1.03]	1.01	[0.99; 1.03]	1.00	[0.97; 1.03]
Bisphenol A	1.01	[0.94; 1.09]	1.03	[0.98; 1.08]	1.11	[1.03; 1.20]**	1.04	[0.99; 1.08]	1.05	[0.99; 1.10]	1.03	[0.99; 1.07]	1.06	[1.00; 1.12]*
Benzophenone-3	1.00	[0.96; 1.03]	0.99	[0.97; 1.01]	1.01	[0.98; 1.05]	1.01	[0.99; 1.03]	0.99	[0.96; 1.01]	1.00	[0.98; 1.02]	1.00	[0.98; 1.03]
Triclosan	1.02	[1.00; 1.04]*	1.01	[1.00; 1.03]*	1.00	[0.98; 1.03]	1.01	[1.00; 1.03]	1.01	[0.99; 1.03]	1.01	[1.00; 1.03]*	1.01	[0.99; 1.03]
∑Parabens	1.00	[0.97; 1.04]	0.99	[0.97; 1.02]	0.99	[0.95; 1.02]	0.98	[0.96; 1.00]	0.97	[0.94; 1.00]**	0.99	[0.97; 1.01]	1.00	[0.97; 1.03]
Phthalates														
MEP	1.03	[0.97; 1.08]	1.00	[0.97; 1.04]	0.97	[0.92; 1.03]	1.00	[0.97; 1.03]	0.99	[0.95; 1.03]	1.00	[0.97; 1.03]	1.00	[0.96; 1.04]
MnBP	1.06	[1.00; 1.12]**	1.01	[0.97; 1.04]	1.06	[1.00; 1.12]**	0.99	[0.95; 1.02]	0.99	[0.95; 1.03]	1.00	[0.97; 1.03]	1.06	[1.01; 1.11]**
MiBP	0.97	[0.90; 1.05]	1.00	[0.96; 1.05]	1.05	[0.98; 1.13]	0.95	[0.91; 1.00]**	0.99	[0.94; 1.05]	0.97	[0.94; 1.01]	1.01	[0.95; 1.07]
MCPP	1.03	[0.96; 1.11]	0.98	[0.94; 1.03]	1.04	[0.97; 1.12]	0.96	[0.92; 1.00]*	0.95	[0.90; 1.00]*	0.97	[0.93; 1.01]	1.04	[0.98; 1.10]
MBzP	1.02	[0.96; 1.09]	1.00	[0.97; 1.04]	1.07	[1.01; 1.13]**	0.98	[0.94; 1.01]	0.97	[0.93; 1.02]	0.99	[0.96; 1.02]	1.04	[1.00; 1.09]*
MCOP	1.02	[0.95; 1.10]	1.01	[0.97; 1.06]	0.99	[0.92; 1.06]	0.98	[0.94; 1.03]	1.00	[0.95; 1.05]	1.00	[0.96; 1.04]	1.01	[0.95; 1.06]
MCNP	1.01	[0.95; 1.07]	0.99	[0.95; 1.03]	1.00	[0.94; 1.06]	0.97	[0.93; 1.01]	0.98	[0.94; 1.03]	0.98	[0.95; 1.01]	1.00	[0.95; 1.05]
∑DEHP	1.04	[0.97; 1.11]	0.99	[0.95; 1.04]	1.05	[0.98; 1.12]	0.97	[0.93; 1.01]	0.99	[0.94; 1.04]	0.98	[0.94; 1.02]	1.04	[0.99; 1.10]

**p-values ≤ 0.05, *p-value ≤ 0.10,

Associations adjusted for recruitment center, maternal age, parity, parental education, breastfeeding duration, household income, smoking during pregnancy, maternal psychological difficulties during pregnancy and child age at assessment.

IRR are reported for a doubling in biomarkers concentrations.

Abbreviations: ∑DEHP: molecular sum of di(2-ethylhexyl) phthalate metabolites; CI: confidence interval, IRR: Incidence rate ratio, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isooctyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, ∑dichlorophenols: molecular sum of 2,4 and 2,5-dichlorophenols, ∑Parabens: molecular sum of methyl, ethyl, propyl and butyl parabens.

Table 3.15: Adjusted associations between phenols, phthalate metabolites and behavior at 5 years among boys of the EDEN mother-child cohort (N = 457 or 458 mother-son pairs, depending of the subscale).

	Emotional symptoms		Conduct problems		Peer relationship problems		Hyperactivity - inattention problems		Prosocial behavior		Externalizing behavior		Internalizing behavior	
	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI
Phenols														
∑Dichlorophenols	1.00	[0.97; 1.04]	1.03	[1.00; 1.07]*	0.99	[0.94; 1.04]	1.01	[0.98; 1.04]	1.00	[0.96; 1.04]	1.02	[0.99; 1.05]	1.00	[0.97; 1.03]
Bisphenol A	1.03	[0.95; 1.11]	1.02	[0.95; 1.10]	1.02	[0.93; 1.13]	1.08	[1.01; 1.14]**	1.05	[0.96; 1.14]	1.05	[1.00; 1.11]**	1.03	[0.96; 1.10]
Benzophenone-3	0.96	[0.93; 1.00]**	1.00	[0.97; 1.04]	1.01	[0.97; 1.06]	1.02	[0.99; 1.04]	1.02	[0.98; 1.06]	1.01	[0.99; 1.04]	0.98	[0.95; 1.01]
Triclosan	1.01	[0.99; 1.03]	1.00	[0.98; 1.02]	1.01	[0.98; 1.04]	1.00	[0.98; 1.01]	0.99	[0.97; 1.02]	1.00	[0.98; 1.02]	1.01	[0.99; 1.03]
∑Parabens	0.99	[0.95; 1.02]	0.99	[0.96; 1.03]	0.99	[0.94; 1.04]	0.98	[0.95; 1.00]	0.99	[0.95; 1.03]	0.98	[0.96; 1.01]	0.99	[0.96; 1.02]
Phthalates														
MEP	1.01	[0.95; 1.06]	1.03	[0.98; 1.08]	0.99	[0.92; 1.05]	0.99	[0.95; 1.03]	0.96	[0.91; 1.02]	1.01	[0.97; 1.05]	1.00	[0.95; 1.04]
MnBP	1.00	[0.94; 1.05]	1.02	[0.97; 1.07]	1.05	[0.99; 1.13]	1.01	[0.97; 1.05]	1.01	[0.95; 1.07]	1.01	[0.97; 1.06]	1.02	[0.97; 1.07]
MiBP	0.97	[0.90; 1.05]	1.01	[0.94; 1.08]	1.00	[0.91; 1.09]	0.97	[0.91; 1.02]	1.04	[0.96; 1.13]	0.98	[0.93; 1.04]	0.98	[0.92; 1.05]
M CPP	0.98	[0.91; 1.05]	1.04	[0.97; 1.11]	1.06	[0.97; 1.16]	1.01	[0.95; 1.07]	0.99	[0.91; 1.08]	1.02	[0.97; 1.08]	1.01	[0.95; 1.08]
MBzP	1.03	[0.97; 1.09]	0.98	[0.93; 1.04]	1.01	[0.94; 1.09]	1.00	[0.95; 1.04]	0.98	[0.92; 1.04]	0.99	[0.95; 1.03]	1.02	[0.97; 1.08]
MCOP	1.04	[0.97; 1.12]	1.00	[0.94; 1.06]	0.99	[0.91; 1.08]	1.01	[0.96; 1.07]	0.94	[0.87; 1.02]	1.01	[0.96; 1.06]	1.02	[0.96; 1.09]
MCNP	1.04	[0.98; 1.10]	0.99	[0.93; 1.04]	1.03	[0.96; 1.11]	0.99	[0.94; 1.04]	0.98	[0.92; 1.05]	0.99	[0.94; 1.03]	1.04	[0.98; 1.09]
∑DEHP	1.01	[0.94; 1.08]	0.99	[0.93; 1.06]	1.03	[0.94; 1.12]	1.01	[0.96; 1.07]	0.97	[0.90; 1.05]	1.00	[0.95; 1.06]	1.02	[0.95; 1.08]

** p-values ≤ 0.05, * p-value ≤ 0.10

Associations adjusted for recruitment center, maternal age, parity, parental education, breastfeeding duration, household income, smoking status, maternal psychological difficulties during pregnancy and child age at assessment.

IRR are reported for a doubling in biomarkers concentrations.

Abbreviations: ∑DEHP: molecular sum of di(2-ethylhexyl) phthalate metabolites, CI: confidence interval, IRR: Incidence rate ratio, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoocetyl phthalate, M CPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, ∑Dichlorophenols : molecular sum of 2,4 and 2,5-dichlorophenols, ∑Parabens: molecular sum of methyl, ethyl, propyl and butyl parabens.

Sensitivity analyses

Studying SDQ scores as dichotomized variables overall led to similar results as studying them as count outcomes for bisphenol A, MBzP, MnBP, and MCPP (Table 3.16 and Table 3.17).

For triclosan the positive association seen at 3 years with the emotional symptoms score persisted at 5 years when SDQ scores were dichotomized (Table 3.16 and Table 3.17).

The inverse associations we observed between benzophenone-3 and emotional symptoms at 5 years, MiBP and hyperactivity-inattention at 3 years and Σ parabens and prosocial behavior at 3 years were strongly attenuated when SDQ scores were dichotomized and did not remain significant. Odd Ratios were 0.93 (95% CI: 0.82; 1.05); 0.91 (95% CI: 0.72; 1.16) and 0.92 (95% CI: 0.80; 1.06) for benzophenone-3, MiBP and Σ parabens, respectively (Table 3.16 and Table 3.17).

Similarly, the association between Σ dichlorophenols and conduct problems at 5 years did not remain when this score was dichotomized (OR: 0.90; 95% CI: 0.80; 1.10, Table 3.17). Finally, a few associations emerged: Σ DEHP was associated with increased risk of emotional symptoms (OR, 1.27, 95% CI: 1.01; 1.60) and internalizing behavior (OR, 1.41; 95% CI: 1.13; 1.76) at 3 years while MCNP was associated with decreased risk of hyperactivity-inattention at 3 years (OR: 0.72; 95% CI: 0.55; 0.93).

Adjustment for season did not change the association observed between benzophenone-3 and the emotional subscale. Running models simultaneously adjusted for all of the biomarkers measured in our study did not strongly affect our findings and the effect estimates were close to those of the main analyses that consisted in studying each biomarker separately (Table 3.18 and Table 3.19).

When we applied a correction for multiple comparisons using a FDR method, none of the associations reported in the results section remained significant, the lowest corrected p-value being 0.42 for the association between bisphenol A and the peer relations problem score at 3 years.

Table 3.16: Adjusted associations between phenols, phthalate metabolites and dichotomized SDQ scores at 3 years (N = 518 to 520 mother-son pairs, depending of the subscale).

	Emotional symptoms		Conduct problems		Peer relationship problems		Hyperactivity - inattention problems		Prosocial behavior		Externalizing behavior		Internalizing behavior	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Phenols														
∑Dichlorophenols	1.06	[0.94; 1.20]	0.97	[0.83; 1.13]	0.99	[0.85; 1.14]	1.04	[0.92; 1.18]	1.02	[0.90; 1.17]	1.05	[0.93; 1.18]	1.04	[0.93; 1.18]
Bisphenol A	0.92	[0.70; 1.19]	1.08	[0.81; 1.43]	1.40	[1.07; 1.84]**	1.07	[0.83; 1.38]	1.07	[0.82; 1.39]	1.01	[0.79; 1.29]	1.22	[0.96; 1.55]*
Benzophenone-3	1.00	[0.89; 1.13]	1.02	[0.89; 1.16]	1.03	[0.91; 1.17]	1.01	[0.89; 1.13]	0.98	[0.86; 1.11]	0.98	[0.87; 1.10]	1.02	[0.91; 1.14]
Triclosan	1.07	[0.99; 1.17]*	1.10	[1.00; 1.21]**	1.06	[0.97; 1.16]	1.02	[0.94; 1.10]	1.07	[0.99; 1.17]*	1.05	[0.97; 1.13]	1.05	[0.97; 1.14]
Parabens	1.01	[0.89; 1.15]	1.00	[0.87; 1.16]	0.97	[0.84; 1.12]	1.01	[0.89; 1.15]	0.92	[0.80; 1.06]	0.94	[0.83; 1.07]	1.03	[0.92; 1.17]
Phthalates														
MEP	1.14	[0.95; 1.36]	0.95	[0.77; 1.17]	1.04	[0.86; 1.26]	1.07	[0.89; 1.29]	0.98	[0.81; 1.19]	1.00	[0.84; 1.20]	0.98	[0.83; 1.17]
MnBP	1.10	[0.91; 1.31]	0.99	[0.80; 1.22]	1.16	[0.95; 1.41]	0.95	[0.79; 1.16]	0.92	[0.75; 1.13]	0.93	[0.78; 1.12]	1.22	[1.03; 1.44]**
MiBP	0.87	[0.68; 1.12]	1.13	[0.86; 1.49]	1.16	[0.89; 1.53]	0.91	[0.72; 1.16]	1.10	[0.85; 1.43]	0.93	[0.74; 1.17]	1.09	[0.87; 1.38]
MCPP	1.02	[0.80; 1.31]	0.79	[0.59; 1.07]	1.13	[0.86; 1.47]	0.79	[0.61; 1.04]*	0.80	[0.61; 1.06]	0.82	[0.64; 1.05]	1.20	[0.95; 1.51]
MBzP	0.99	[0.81; 1.22]	0.99	[0.78; 1.25]	1.25	[1.00; 1.56]**	0.94	[0.76; 1.15]	0.94	[0.76; 1.17]	0.94	[0.78; 1.15]	1.19	[0.98; 1.44]
MCOP	1.02	[0.81; 1.30]	1.14	[0.89; 1.47]	1.04	[0.79; 1.35]	0.85	[0.66; 1.10]	0.98	[0.77; 1.25]	1.07	[0.86; 1.34]	1.08	[0.87; 1.35]
MCNP	0.90	[0.72; 1.14]	1.00	[0.78; 1.27]	1.09	[0.87; 1.36]	0.72	[0.55; 0.93]**	0.84	[0.65; 1.08]	0.85	[0.68; 1.07]	1.00	[0.81; 1.23]
∑DEHP	1.27	[1.01; 1.60]**	1.10	[0.85; 1.43]	1.09	[0.84; 1.41]	1.03	[0.81; 1.30]	1.18	[0.94; 1.50]	1.07	[0.85; 1.34]	1.41	[1.13; 1.76]*

** p-values ≤ 0.05, * p-value ≤ 0.10

Associations adjusted for recruitment center, maternal age, parity, parental education, breastfeeding duration, household income, smoking status, maternal psychological difficulties during pregnancy and child age at assessment.

IRR are reported for a doubling in biomarkers concentrations.

Abbreviations: ∑DEHP: molecular sum of di(2-ethylhexyl) phthalate metabolites, CI: confidence interval, OR: odds ratio, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isooctyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, ∑dichlorophenols: molecular sum of 2,4 and 2,5-dichlorophenols, ∑Parabens: molecular sum of methyl, ethyl, propyl and butyl parabens.

Table 3.17: Adjusted associations between phenols, phthalate metabolites and dichotomized SDQ scores at 5 years (N = 457 or 458 mother-son pairs, depending of the subscale).

	Emotional symptoms		Conduct problems		Peer relationship problems		Hyperactivity - inattention problems		Prosocial behavior		Externalizing behavior		Internalizing behavior	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Phenols														
∑Dichlorophenols	0.99	[0.87; 1.12]	0.94	[0.80; 1.10]	0.94	[0.79; 1.11]	1.10	[0.97; 1.24]	1.01	[0.87; 1.18]	1.05	[0.93; 1.18]	0.94	[0.83; 1.07]
Bisphenol A	1.04	[0.80; 1.36]	1.04	[0.76; 1.41]	1.10	[0.80; 1.50]	1.40	[1.08; 1.82]**	1.09	[0.80; 1.47]	1.20	[0.93; 1.54]	1.00	[0.96; 1.10]
Benzophenone-3	0.93	[0.82; 1.05]	1.00	[0.87; 1.16]	0.99	[0.86; 1.14]	1.05	[0.93; 1.19]	1.05	[0.91; 1.20]	1.06	[0.95; 1.18]	0.97	[0.95; 1.01]
Triclosan	1.08	[1.00; 1.18]*	1.06	[0.96; 1.17]	1.10	[0.99; 1.22]*	1.01	[0.93; 1.09]	1.00	[0.91; 1.09]	1.00	[0.93; 1.08]	1.07	[0.99; 1.03]
∑Parabens	0.95	[0.84; 1.08]	0.96	[0.82; 1.11]	1.01	[0.87; 1.18]	0.98	[0.86; 1.12]	0.97	[0.84; 1.13]	1.01	[0.89; 1.14]	0.96	[0.96; 1.02]
Phthalates														
MEP	1.16	[0.97; 1.39]*	1.13	[0.92; 1.38]	1.02	[0.82; 1.27]	1.05	[0.87; 1.27]	0.79	[0.63; 1.00]**	1.08	[0.91; 1.29]	0.93	[0.95; 1.04]
MnBP	1.02	[0.85; 1.23]	1.06	[0.85; 1.31]	1.24	[1.01; 1.52]**	1.03	[0.85; 1.25]	0.87	[0.68; 1.10]	1.04	[0.86; 1.25]	1.14	[0.97; 1.07]
MiBP	1.01	[0.79; 1.29]	1.02	[0.77; 1.35]	1.05	[0.79; 1.41]	0.95	[0.74; 1.23]	1.02	[0.75; 1.38]	1.00	[0.79; 1.27]	0.97	[0.92; 1.05]
MCPP	1.01	[0.79; 1.29]	1.07	[0.81; 1.43]	1.20	[0.91; 1.58]	0.98	[0.75; 1.27]	0.86	[0.63; 1.17]	1.04	[0.81; 1.33]	1.16	[0.95; 1.08]
MBzP	1.06	[0.87; 1.29]	1.02	[0.81; 1.29]	0.96	[0.75; 1.22]	1.02	[0.83; 1.25]	0.79	[0.61; 1.03]*	0.91	[0.75; 1.12]	1.04	[0.97; 1.08]
MCOP	1.15	[0.91; 1.45]	0.93	[0.70; 1.23]	1.03	[0.78; 1.36]	1.23	[0.97; 1.56]	0.88	[0.66; 1.17]	1.03	[0.82; 1.30]	1.09	[0.96; 1.09]
MCNP	1.01	[0.81; 1.25]	0.90	[0.68; 1.18]	0.99	[0.76; 1.28]	1.02	[0.82; 1.28]	1.06	[0.84; 1.35]	1.00	[0.80; 1.24]	1.04	[0.98; 1.09]
∑DEHP	1.08	[0.85; 1.36]	0.99	[0.74; 1.31]	1.18	[0.90; 1.55]	1.07	[0.84; 1.36]	0.79	[0.59; 1.07]	1.05	[0.83; 1.32]	1.15	[0.95; 1.08]

** p-values ≤ 0.05, * p-value ≤ 0.10

Associations adjusted for recruitment center, maternal age, parity, parental education, breastfeeding duration, household income, smoking status, maternal psychological difficulties during pregnancy and child age at assessment.

IRR are reported for a doubling in biomarkers concentrations.

Abbreviations: ∑DEHP: molecular sum of di(2-ethylhexyl) phthalate metabolites, CI: confidence interval, OR: odds ratio, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoocetyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, ∑dichlorophenols: molecular sum of 2,4 and 2,5-dichlorophenols, ∑Parabens: molecular sum of methyl, ethyl, propyl and butyl parabens.

Table 3.18: Adjusted associations between phenols, phthalate metabolites and behavior at 3 years among boys of the EDEN mother child cohort (N = 518 to 520 mother-son pairs, depending of the subscale). Analysis simultaneously adjusted for all phenols and phthalate metabolites assessed in our study population

	Emotional symptoms		Conduct problems		Peer relationship problems		Hyperactivity - inattention problems		Prosocial behavior		External behavior		Internal behavior	
	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI
Phenols														
∑Dichlorophenols	1.02	[0.98; 1.06]	1.02	[0.99; 1.04]	0.99	[0.95; 1.03]	1.00	[0.98; 1.03]	1.01	[0.98; 1.04]	1.01	[0.99; 1.03]	1.01	[0.98; 1.04]
Bisphenol A	1.01	[0.93; 1.09]	1.04	[0.99; 1.09]	1.11	[1.03; 1.20]	1.06	[1.01; 1.11]	1.06	[1.01; 1.13]	1.05	[1.00; 1.09]	1.05	[0.99; 1.12]
Benzophenone-3	0.99	[0.96; 1.03]	0.99	[0.97; 1.01]	1.01	[0.98; 1.05]	1.01	[0.99; 1.04]	0.99	[0.96; 1.02]	1.00	[0.98; 1.02]	1.00	[0.97; 1.03]
Triclosan	1.02	[0.99; 1.04]	1.01	[1.00; 1.03]	1.01	[0.99; 1.04]	1.01	[1.00; 1.03]	1.01	[0.99; 1.03]	1.01	[1.00; 1.03]	1.01	[1.00; 1.03]
∑Parabens	1.00	[0.96; 1.04]	0.99	[0.97; 1.02]	0.99	[0.95; 1.03]	0.98	[0.95; 1.00]	0.97	[0.94; 1.00]	0.98	[0.96; 1.01]	1.00	[0.97; 1.03]
Phthalates														
MEP	1.01	[0.95; 1.07]	1.00	[0.96; 1.03]	0.96	[0.90; 1.01]	1.00	[0.96; 1.03]	0.99	[0.95; 1.03]	0.99	[0.96; 1.03]	0.98	[0.94; 1.03]
MnBP	1.09	[1.00; 1.18]	1.04	[0.98; 1.09]	1.06	[0.97; 1.15]	1.03	[0.97; 1.08]	1.04	[0.98; 1.10]	1.03	[0.99; 1.08]	1.07	[1.01; 1.14]
MiBP	0.94	[0.86; 1.02]	1.00	[0.95; 1.05]	1.01	[0.93; 1.09]	0.96	[0.91; 1.01]	1.01	[0.95; 1.07]	0.98	[0.94; 1.02]	0.97	[0.91; 1.04]
MCPP	0.95	[0.85; 1.06]	0.94	[0.88; 1.01]	0.98	[0.88; 1.10]	0.95	[0.89; 1.02]	0.92	[0.85; 1.00]	0.95	[0.90; 1.01]	0.97	[0.89; 1.05]
MBzP	1.02	[0.95; 1.09]	1.00	[0.96; 1.05]	1.04	[0.97; 1.11]	0.99	[0.95; 1.03]	0.97	[0.92; 1.02]	1.00	[0.96; 1.03]	1.03	[0.98; 1.09]
MCOP	1.02	[0.94; 1.11]	1.03	[0.98; 1.09]	0.96	[0.88; 1.04]	1.00	[0.95; 1.05]	1.02	[0.96; 1.08]	1.02	[0.97; 1.06]	0.99	[0.93; 1.06]
MCNP	1.00	[0.93; 1.08]	0.99	[0.94; 1.03]	0.99	[0.92; 1.06]	0.98	[0.94; 1.03]	0.99	[0.94; 1.05]	0.98	[0.95; 1.02]	1.00	[0.94; 1.05]
∑DEHP	1.02	[0.94; 1.10]	0.98	[0.93; 1.03]	1.02	[0.94; 1.11]	0.98	[0.93; 1.03]	0.99	[0.93; 1.05]	0.97	[0.93; 1.02]	1.02	[0.96; 1.09]

Associations adjusted for recruitment center, maternal age, parity, parental education, breastfeeding duration, household income, smoking during pregnancy, maternal psychological difficulties during pregnancy and child age at assessment.

Abbreviations: ∑DEHP: molecular sum of di(2-ethylhexyl) phthalate metabolites, CI: confidence interval, OR: odds ratio, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoocetyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, ∑dichlorophenols: molecular sum of 2,4 and 2,5-dichlorophenols, ∑Parabens : molecular sum of methyl, ethyl, propyl and butyl parabens.

Table 3.19: Adjusted associations between phenols, phthalate metabolites and behavior at 5 years among boys of the EDEN mother child cohort (N = 457 to 458 mother-son pairs, depending of the subscale). Analysis simultaneously adjusted for all phenols and phthalate metabolites assessed in our study population

	Emotional symptoms		Conduct problems		Peer relationship problems		Hyperactivity - inattention problems		Prosocial behavior		External behavior		Internal behavior	
	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI	IRR	95% CI
Phenols														
∑Dichlorophenols	1.01	[0.97; 1.05]	1.03	[1.00; 1.07]	0.98	[0.94; 1.03]	1.01	[0.98; 1.04]	1.00	[0.96; 1.05]	1.02	[0.99; 1.05]	1.00	[0.97; 1.03]
Bisphenol A	1.03	[0.95; 1.12]	1.02	[0.95; 1.10]	1.01	[0.92; 1.12]	1.08	[1.02; 1.15]	1.07	[0.98; 1.17]	1.06	[1.00; 1.12]	1.03	[0.95; 1.10]
Benzophenone-3	0.96	[0.92; 0.99]	1.00	[0.97; 1.03]	1.02	[0.98; 1.07]	1.02	[0.99; 1.05]	1.02	[0.98; 1.06]	1.01	[0.99; 1.04]	0.98	[0.95; 1.01]
Triclosan	1.01	[0.99; 1.04]	1.00	[0.98; 1.02]	1.01	[0.98; 1.04]	1.00	[0.98; 1.02]	1.00	[0.97; 1.03]	1.00	[0.98; 1.02]	1.01	[0.99; 1.03]
∑Parabens	0.99	[0.95; 1.03]	0.99	[0.95; 1.02]	0.99	[0.94; 1.04]	0.97	[0.94; 1.01]	0.99	[0.94; 1.04]	0.98	[0.95; 1.01]	0.99	[0.96; 1.03]
Phthalates														
MEP	1.01	[0.95; 1.07]	1.04	[0.98; 1.09]	0.98	[0.91; 1.05]	0.99	[0.95; 1.04]	0.96	[0.90; 1.03]	1.01	[0.97; 1.06]	0.99	[0.94; 1.04]
MnBP	1.03	[0.95; 1.12]	1.00	[0.93; 1.08]	1.05	[0.95; 1.16]	1.02	[0.95; 1.09]	1.03	[0.94; 1.14]	1.01	[0.95; 1.08]	1.04	[0.96; 1.12]
MiBP	0.95	[0.88; 1.04]	1.02	[0.95; 1.10]	0.99	[0.89; 1.09]	0.95	[0.90; 1.02]	1.06	[0.97; 1.17]	0.98	[0.93; 1.04]	0.97	[0.90; 1.04]
MCPP	0.93	[0.83; 1.03]	1.05	[0.95; 1.16]	1.02	[0.89; 1.17]	0.99	[0.91; 1.08]	0.97	[0.86; 1.10]	1.01	[0.93; 1.09]	0.96	[0.87; 1.06]
MBzP	1.04	[0.97; 1.11]	0.98	[0.92; 1.04]	1.00	[0.92; 1.08]	1.00	[0.95; 1.05]	0.96	[0.89; 1.04]	0.99	[0.94; 1.04]	1.02	[0.96; 1.08]
MCOP	1.05	[0.97; 1.14]	1.00	[0.93; 1.08]	0.96	[0.86; 1.06]	1.02	[0.95; 1.08]	0.94	[0.85; 1.03]	1.01	[0.95; 1.07]	1.02	[0.94; 1.09]
MCNP	1.03	[0.96; 1.11]	0.97	[0.91; 1.03]	1.04	[0.95; 1.13]	0.97	[0.92; 1.03]	1.01	[0.93; 1.09]	0.97	[0.92; 1.02]	1.04	[0.97; 1.10]
∑DEHP	0.98	[0.91; 1.06]	0.97	[0.90; 1.05]	1.02	[0.92; 1.12]	1.01	[0.95; 1.07]	0.98	[0.90; 1.07]	0.99	[0.94; 1.06]	0.99	[0.92; 1.07]

Associations adjusted for recruitment center, maternal age, parity, parental education, breastfeeding duration, household income, smoking during pregnancy, maternal psychological difficulties during pregnancy and child age at assessment.

Abbreviations: ∑DEHP: molecular sum of di(2-ethylhexyl) phthalate metabolites, CI: confidence interval, OR: odds ratio, MnBP: mono-n-butyl phthalate, MBzP: monobenzyl phthalate, MCNP: monocarboxy-isononyl phthalate, MCOP: monocarboxy-isoctyl phthalate, MCPP: mono(3-carboxypropyl) phthalate, MEP: monoethyl phthalate, MiBP: mono-isobutyl phthalate, ∑dichlorophenols : molecular sum of 2,4 and 2,5-dichlorophenols, ∑Parabens : molecular sum of methyl, ethyl, propyl and butyl parabens.

3.2.5 Discussion

In our study of 546 women who delivered a boy, three phenols (bisphenol A, triclosan and Σ dichlorophenols) and two phthalates (MnBP, MBzP) biomarkers were positively associated with scores in one or more subscales of the SDQ, while inverse association was observed for other biomarkers (benzophenone-3, MiBP, MCPP).

Our study is among the first to simultaneously consider a rather large number of biomarkers from the phthalates and phenols families in relation to several behavioral scales. Because we tested many associations, some of our results might be chance finding, as suggested by the fact that none of the observed p-values remained significant after FDR correction. For this reason, in the following discussion we focused on the associations for which some consistency is observed with the previous human literature focusing on prenatal exposure, or with animal literature when no human study was available.

Phenols and child behavior

In our study population, bisphenol A was associated with increased scores on the internalizing behavior and peer relationship problem subscales at 3 years and on the externalizing behavior and hyperactivity-inattention subscales at 5 years. Associations between prenatal bisphenol A concentrations and internalizing and externalizing behavior scores in boys have been reported in most of the previous studies evaluating such behaviors^{100–103}. Regarding specific subscales, an increased score on the hyperactivity-inattention subscale in association with prenatal exposure to bisphenol A has been previously reported among boys at 4 but not at 7 years⁸⁵. Other studies have reported opposite⁹⁸ or null^{100,101} associations with this subscale.

Interpretation of the human literature on bisphenol A should be done keeping in mind that bisphenol A is one of the biomarkers with the highest within-subject variability (with intra-class coefficients of correlation of 0.1- 0.2 being typically reported during pregnancy^{253,254}, limiting the power of studies relying on one spot urine sample to assess exposure, as it is the case of most previous studies. Despite this limitation, most of the published studies (including ours) reported an association with one or several behavioral scores among boys (Table 1.6), suggesting that bisphenol A affects some aspect of behavior among boys. This is supported by experimental studies on laboratory animals that have also reported associations between perinatal exposure to bisphenol A and behavior^{255–258} and by in vitro studies that suggested that bisphenol A can interact with some pathways that are crucial for brain development. Bisphenol

A can indeed bind with estrogen and androgen receptors as well as interact with thyroid hormone pathways (reviewed by Mustieles *et al* ⁸³).

In our population, triclosan, a biocide used in some toothpastes, antibacterial soaps and detergents tended to be positively associated with the externalizing behavior score as well as with the emotional symptom and conduct problems scores at 3 years. When SDQ scores were dichotomized associations with the emotional symptom score was also observed at 5 years. To our knowledge this is the first study investigating triclosan potential effect on child neurodevelopment. Both *in vitro* studies and studies in rodents suggested that triclosan can affect pathways involved in the development of the nervous system, such as the thyroid hormone pathway ^{150,259}. In humans, a cross-sectional study reported increased total triiodothyronine concentrations in association with increased triclosan concentrations among adolescents ²⁶⁰. Other mechanisms whereby triclosan might affect child neurodevelopment include disruption of the sex hormone homeostasis because triclosan can bind, albeit with low affinity, to both the androgen and estrogen receptors (reviewed by Witorsh *et al* ²⁶¹)

Maternal urinary concentrations of Σ dichlorophenols tended to be associated with increased risk of conduct problems at 5 years. However, this association did not remain when SDQ scores were dichotomized. Σ dichlorophenols included 2,5-dichlorophenol, a metabolite of 1,4-dichlorobenzene used in mothballs, indoor deodorizers and toilet bowl disinfectants and 2,4-dichlorophenol a metabolite of 2,4-dichlorophenoxyacetic acid used as intermediate in the production of some herbicides. Our study is the first to focus on these compounds in humans for behavior-specific factors. A study in which rats were exposed during pre- and post-natal life to 2,4-dichlorophenoxyacetic acid reported deleterious effect on behaviors ²⁶² that included hyperactivity, stereotypic behavior (excessive grooming) and serotonin syndrome behaviors (forepaw tapping, sprawling of limbs, and mobility) ²⁶². Translation to epidemiological research is difficult, given the fact that behavioral outcomes and routes of exposure differ between rats and humans.

Benzophenone-3 and the Σ parabens were associated with lower SDQ scores, suggesting improved behaviors, although associations did not remain after SDQ scores were dichotomized. To our knowledge, this is the first epidemiological study that looked at the human behavioral effects of these compounds, which makes it difficult to discuss the plausibility of our associations with lower SDQ scores. However, previous studies in rats exposed to parabens pre- and post-natally (via lactation) reported increased anxiety-like behavior ²⁶³ and autism-like

symptoms⁷² as well as less social interactions and learning memory⁷² in exposed animals compared to controls, which is not in line with our findings. This difference and the fact that the association we observed with Σ parabens did not remain when SDQ scores were dichotomized, should lead to cautious interpretation of the association we observed that suggested that higher concentration of Σ parabens was associated with more prosocial behavior.

Phthalates and child behavior

We observed increased scores on the internalizing, relationship problems and emotional symptoms subscales at 3 years in association with urinary concentrations of MnBP. These associations did not remain at 5 years. In a previous study among mother-child pairs from New-York city, 3 years old boys experienced more internalizing and withdrawn behaviors as well as more emotionally reactive problems in association with maternal urinary concentrations of this phthalate metabolite⁹⁷. Other studies among older boys (4.5 to 10 years) did not report such associations^{105,106,264}. Experimental studies among rodents also suggested behavioral effects for this phthalate^{265–267}.

In our study population, MBzP was associated with increased scores on the internalizing behaviors and relationship problems subscales at 3, but not at 5 years. Associations between MBzP and behavioral scores have been reported previously among boys but with other subscales including oppositional behavior and conduct problems¹⁰⁵.

When SDQ scores were dichotomized, Σ DEHP was associated with increased risk for emotional symptoms and internalizing behavior. A few studies reported associations between DEHP biomarkers (as a sum of several metabolites or for single DEHP metabolites) and behavior in boys. These associations were seen with other subscales than those reported in our study, including increased social, delinquent, somatic and externalizing behavior subscores^{105,106}. However, these two previous studies examined behavior at older age (6 to 10 years old) and had relatively low sample size ($n < 80$ for boys). Among studies of bigger sample size, one reported no association between prenatal exposure to DEHP and behavior at 3 years⁹⁷ while the other reported better social competence and lower hyperactivity-inattention scores at 7 years in association with DEHP biomarkers²⁶⁸. Phthalates can disrupt the thyroid and sex hormone homeostasis, calcium signaling and can alter brain's lipid profile (reviewed by Miodovnok *et al.*,⁷⁸). They are also weak agonists of the aryl hydrocarbon²⁶⁹ and peroxisome proliferator-activated receptors⁷⁸ involved in numerous developmental pathways.

MiBP and MCPP were associated with lower SDQ scores, suggesting improved behaviors. When SDQ scores were dichotomized only the associations between MCPP and the prosocial behavior score at 5 years and the hyperactivity-inattention scores at 3 years remained. Previous studies that assessed this phthalate in maternal urine did not report any association with hyperactivity-inattention among boys^{106,264,268}.

Strengths and limitations

Although most studies (including ours) that examined the associations between phthalate biomarkers and child behavior in humans reported deleterious associations, the biomarkers implicated and the affected behaviors often varied across studies. This may be explained by the large heterogeneity in age, ranging from 1 to 10 years, the different instruments used to evaluate behavior, the large number of comparisons performed in each study which usually did not correct for multiple testing, and issues related to measurement error in the exposure assessment of biomarkers with short half-lives. Exposure was assessed using only one, rarely two, urine samples per participant collected at different time points during pregnancy, which is insufficient to characterize exposure for biomarkers with a high intra-individual variability, as reported for some phthalates and phenols^{254,270} and is likely to lead to attenuation bias¹⁸⁹. As in most previous studies, we assessed exposure during pregnancy and were missing exposure occurring during the first years of life, also a potentially crucial time point for brain development. Socio-economic status is likely to be a strong confounder of the associations between exposure to environmental chemicals and child neurodevelopment²⁷¹. We adjusted our analyses for many potential confounders but we cannot exclude that residual confounding remained. Among 728 Spanish women for which exposure to 81 chemicals was assessed bisphenol A was not highly correlated with other environmental biomarkers while phthalate metabolites were correlated among each other and were negatively correlated with some metals such as cadmium and copper (rho ranged between -0.3 and -0.5,²⁷²). Although correlations between exposures possibly differ across countries, this finding suggests that the associations we observed with bisphenol A and phthalate biomarkers are unlikely to be confounded by exposure to other chemicals. In line with this finding, associations varied little after adjusting our analyses for the other phenols and phthalates biomarkers measured in the study. We relied on the SDQ, a validated and widely used questionnaire in Europe, to assess child behavior at two different time points.

Strengths of our study include the sample size, larger than those of previous studies that included 122 to 460 boys and girls and among which analyses stratified for child sex have been often performed. The fact that we focused on boys limits generalizability of our findings but is not a source of bias, especially in the context of endocrine disruptors for which sex-specific effects have been reported previously^{85,102,103}. In an attempt of correcting our findings for exposure measurement error we present in the supplemental material the effect estimates corrected using an a posteriori disattenuation method¹⁸⁹. As expected, the corrected effect estimates were bigger (absolute values) than the corresponding uncorrected IRRs and ORs. We believe that these corrected effect estimates are relevant for the purpose of future meta-analyses that will combine studies relying on a different number of urine samples per participant to assess exposure.

3.2.6 Conclusion

Several of phenol and phthalate biomarkers were associated with increased scores on the SDQ subscales at 3 and/or 5 years. The associations observed for bisphenol A and MnBP were consistent with those reported previously among boys in the same age range^{97,100–102} and are further supported by the animal literature. Associations observed with MBzP and triclosan cautious interpretation since they have not been observed in previous studies (MBzP) or this study is the first to explore their potential effects on child behavior (triclosan). Harmonizing protocols across studies (including tools used to assess behavior and age at assessment) would enhance results comparability across studies. In addition, further studies that aim at looking at the potential effects of phenols and phthalates on child neurodevelopment would benefit from incorporating new approaches to improve exposure assessment, one of the main limitations of the current epidemiological literature for these biomarkers.

**CHAPTER 4 : PHENOLS AND PHTHALATES AND THYROID HORMONE
LEVELS DURING PREGNANCY AND AT BIRTH**

4 Phenols and phthalates and thyroid hormone levels during pregnancy and at birth

Title: Non-persistent environmental chemicals and thyroid hormone levels during pregnancy and at birth; an approach relying on repeated biospecimens and a priori knowledge to increase biological plausibility.

Authors: Dorothy Nakiwala¹, Amrit K. Shaki², Cathrine Thomsen², Faure Patrice³, Gauchez Anne-Marie³, Corne Christelle³, Rémy Slama¹, Claire Philippat¹ and the SEPAGES study group

¹ Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Institute for Advanced Biosciences (IAB), Inserm U1209, CNRS UMR 5309, Université Grenoble Alpes, 38000 Grenoble, France

² Norwegian Institute of Public Health, Oslo, Norway

³ Biology Department, Grenoble University Hospital 38043 Grenoble, France.

The SEPAGES study group includes E. Eyriey, A. Licinia, A. Vellement (Groupe Hospitalier Mutualiste, Grenoble), I. Pin, P. Hoffmann, E. Hullo, C. Llerena (Grenoble University Hospital, La Tronche), X. Morin (Clinique des Cèdres, Echirolles), A. Morlot (Clinique Belledonne, Saint-Martin d'Hères), J. Lepeule, S. Lyon-Caen, C. Philippat, I. Pin, J. Quentin, V. Siroux, R. Slama (Inserm, CNRS, University Grenoble Alpes IAB research center).

Manuscript not submitted yet

4.2 French summary

Introduction: Certains perturbateurs endocriniens environnementaux tels que les phénols et les phtalates ont été suggérés comme contribuant à la dérégulation de l'hormone thyroïde en période de grossesse. Les résultats des études précédentes conduites sur des femmes enceintes et des nouveaux nés sont hétérogènes ou non significatifs, ce qui pourrait être en partie dû à la multiplicité des tests d'associations ou à la qualité limitée de l'évaluation de l'exposition.

Méthodes: Cette étude incluait 418 femmes enceintes ainsi que 421 nouveaux nés issus de l'étude cohorte SEPAGES couple-enfant. Les taux de concentration dans l'urine en période de grossesse ont été évalués dans 21 échantillons de chaque femme, mis en commun, tandis que les niveaux d'hormones thyroïdiennes étaient mesurés dans le sérum de la mère et de l'enfant. Afin de limiter le nombre de tests effectués, nous nous sommes basés sur le «Adverse Outcome Pathways (AOP)» de l'activité thyroïdienne afin de sélectionner des composés précédemment liés à les «molecular initiating events» appartenant à cette AOP, dans le projet ToxCast. Nous avons utilisé une régression linéaire ajustée afin d'étudier les associations entre ces composés et les niveaux d'hormone thyroïdienne chez les femmes et les nouveau-nés.

Résultats: Quatre composés ont été identifiés comme de potentiels perturbateurs thyroïdiens : BBP, Bisphénol A et triclosan comme des inhibiteurs de thyroïde peroxydase et le DBP comme un inhibiteur du symport sodium/iodure. Le triclosan été également identifié comme inhibiteurs du déiodinade-I.

Pour les hormones maternelles, le bisphénol A était négativement associé avec $\ln(TSH)$ z-score ($\beta = -0,05$, intervalle de confiance à 95% (IC95%), $-0,15, 0,06$); MBzP, un métabolite du BBP, était positivement associé avec le TT4 z-score ($\beta = 0,12$, IC95%, $-0,01, 0,25$) et le triclosan était négativement associé avec le z-score du rapport TT3/TT4 ($\beta = -0,05$, CI95% = $-0,11, 0,00$). Lorsque les concentrations de biomarqueurs ont été catégorisées, des associations non-monotoniques entre la TSH et le triclosan (forme de U) et le MnBP, un métabolite du DBP (forme de U inversé) ont été retrouvées. Dans le sérum des nouveaux nés, seuls des effets sexe-spécifiques ont été observés. Le bisphénol A était positivement associé avec le taux de T4 chez les nouveau-nés garçons tandis que le triclosan était négativement associé au taux de T4 chez les nouveau-nés filles.

Discussion et conclusion: Les associations négatives entre triclosan et le rapport de TT3/TT4 chez les femmes enceintes et avec le TT4 chez les nouvelle-nées étaient cohérentes avec les mécanismes identifiés par ToxCast pour ce composé mais pas pour les autres. Les divergences entre nos résultats et nos hypothèses a priori peuvent résulter en partie de la complexité du système thyroïdien. Il comprend en effet des boucles de rétroaction complexes qui rendent les effets de la perturbation d'un «molecular initiating event» sur les niveaux d'hormones thyroïdiennes difficiles à prédire. De plus, nous ne pouvons pas exclure que MnBP, MBzP et le bisphénol A affectent d'autres «molecular initiating events» pertinents que ceux identifiés par ToxCast.

4.3 Abstract

Background: Certain phenols and phthalates are suspected to contribute to thyroid hormone dysregulation during pregnancy. Results from previous studies looking at the associations between these compounds and thyroid hormone levels of pregnant women and newborns are heterogenous or null, which could respectively be in part due to multiplicity in testing of associations or limited quality in exposure assessment.

Methods: We included 416 pregnant women and 421 newborns from the SEPAGES couple-child study. Pregnancy urinary concentrations of 12 phenols and 15 phthalate metabolites were assessed in pools of 21 spot samples per participant collected in the 2nd and 3rd trimesters, while thyroid hormone levels were measured in maternal and infant sera. To limit the number of tests, we relied on an existing Adverse Outcome Pathways (AOP) of thyroid activity to select phenols and phthalates previously linked to molecular initiating events belonging to this pathway in the ToxCast program. We used adjusted linear regression to study the associations of these selected compounds with levels of maternal thyroid hormones (free and total), maternal thyroid stimulating hormone (TSH), neonatal TSH and neonatal total thyroxine (TT4).

Results: Four compounds were identified from ToxCast as plausible thyroid disruptors: benzyl butyl phthalate (BBP), bisphenol A and triclosan, as inhibitors of thyroid peroxidase activity, dibutyl phthalate (DBP) as an inhibitor of the sodium / iodine symporter, and triclosan, an inhibitor of deiodinase I. Regarding the associations with maternal hormones, bisphenol A was negatively associated with the $\ln(\text{TSH})$ z-score ($\beta = -0.05$, 95% confidence interval (CI) = -0.15, 0.06); MBzP, a metabolite of BBP, was positively associated with the TT4 z-score ($\beta = 0.12$, 95% CI = -0.01, 0.25) and triclosan was negatively associated with the TT3/TT4 ratio z-score ($\beta = -0.05$, 95% CI = -0.11, 0.00). When biomarker concentrations were categorized in tertiles, we observed non-monotonic associations between TSH and triclosan (U-shape) and MnBP, a metabolite of DBP (inverse U-shape). In newborns, only sex-specific effects were observed: bisphenol A was positively associated with the TT4 z-score in male newborns while triclosan was negatively associated with the TT4 z-score in females.

Discussion and conclusion: The negative associations between triclosan and the TT3/TT4 ratio in pregnant women as well as with the TT4 concentrations in female newborns were in line with the mechanisms of action we identified through ToxCast. For the other compounds, the direction of the associations were not as hypothesized a priori. This may partly result from the complexity of the thyroid system that includes complex feedback loops that make the effects of the disruption of a molecular initiating event on the serum thyroid hormone levels difficult to predict. In addition, we cannot rule out that MnBP, MBzP and bisphenol A affect other molecular initiating events relevant for the thyroid function than those identified through ToxCast.

4.4 Methodology

Study population

Study participants included mother-child pairs of the prospective SEPAGES cohort (Suivi de l'exposition à la pollution atmosphérique durant la Grossesse et Effets sur la Santé)⁴⁶. This study originally recruited 484 pregnant women from eight obstetrical ultrasonography practices located in Grenoble area in France, between July 2014 and July 2017. Women were included basing on the following criteria: being pregnant by less than 19 gestational weeks, older than 18 years old, having a singleton pregnancy, planning to give birth in one of the four maternity clinics from Grenoble area and living in the study area. All participating women gave written consent for themselves and their children.

The SEPAGES cohort was approved by the relevant ethical committees (Commission Nationale de l'Informatique et des Libertés (CNIL), Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé(CCTIRS), Comité de Protection des Personnes Sud-Est(CPP)).

This analysis was restricted to 416 mother and 421 newborns for whom urine samples during pregnancy (for phenol, phthalate assessments) as well as either maternal or newborn blood was available (for thyroid hormone assessments) and who did not report having thyroid function problems (illustrated in Figure 4.1).

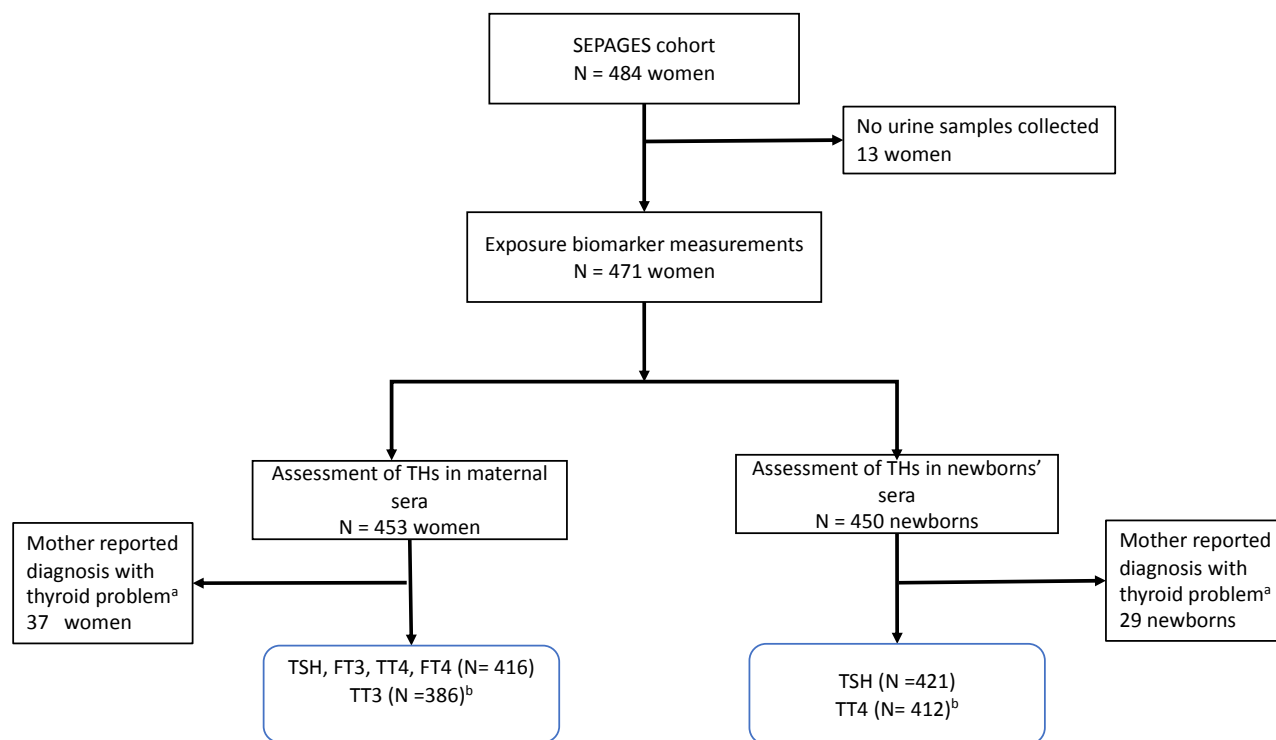


Figure 4.1 : Illustration of final sample size included in the study.

Abbreviation : THs: thyroid hormones and TSH, TSH: thyroid stimulating hormone, FT4(resp.FT3): free thyroxine (tri-iodothyronine), TT4 (resp. TT3): total thyroxine (tri-iodothyronine)

^aThyroid problems reported: hypothyroidism, hyperthyroidism and goiter.

^bNot enough serum for maternal TT3 (N= 30) and newborn TT4 (N= 9) quantification.

Sera sample collection and measurement of thyroid hormone concentrations

Maternal serum samples were collected by trained SEPAGES field workers during a study visit at the participants' homes at a median of 19 gestational weeks (min: 13.4, max: 36.3 weeks). After collection, samples were transported on ice packs to a certified biobank of the Grenoble University Hospital (bb-0033-00069); there, samples were processed and stored at -80°C. Newborn blood spots (from Guthrie cards) were collected at a median of 3 days (5th percentile: 2, 95th percentile: 4) at the maternity and stored at -20°C.

TSH, FT4, protein-bound T4, FT3 and protein-bound T3 were quantified in maternal sera by LOCI® Chemiluminescence on Dimension Vista® analyzer²⁷³. Selenium, an essential metal involved in the peripheral conversion of FT4 to FT3 and iodine, an essential element in

the synthesis of thyroid hormones, were measured in maternal sera and urine respectively, using inductively coupled plasma mass spectrometry (ICP-MS).

TSH levels of newborns were assessed as part of the national screening program on congenital hypothyroidism. Newborn T4 levels were assessed on the remaining blood using immunofluorescence (automaton GSP, Perkin Elmer). T3 was too unstable to be assessed in newborn blood spots.

Measurements were carried out at the Department of biochemistry, toxicology and pharmacology (*Département of Biochimie and Toxicologie Pharmacologie*) of Grenoble university hospital.

The ratio of T3 to T4 was calculated by dividing the concentration of TT3 by the concentration of TT4. This ratio is an indicator of T4 deiodination into the active T3 form.

Urine sample collection and phenol and phthalate concentration assessments

Urine samples were collected at two different stages of pregnancy: mid-pregnancy at a median of 17.7 weeks (minimum (min): 12.3, maximum (max): 23.4) and late pregnancy at a median of 34 weeks (min: 28, max: 37.6). At each stage, women were requested to collect 3 spot urine samples per day (in the morning, mid-day and evening) over seven consecutive days. Samples were collected in 60 ml polypropylene tubes and stored in their own freezer (-20°C) by participants. At the end of the collection week, samples were picked up by a study field-worker and brought to the biobank with ice packs, where they were stored at -20°C. Spot samples were then thawed over night at 4°C, and for each subject a pool of the same volume of all the spots collected over a measurement week were made, aliquoted and stored at -80°C¹⁹⁰.

Samples were sent on dry ice with a temperature sensor to ensure that they remain frozen during transfer to the Norwegian Institute of Public Health (NIPH), where measurements of 12 phenols and 15 phthalate metabolites concentrations were carried out using isotope dilution–high performance liquid chromatography (HPLC) – electrospray ionization-tandem mass spectrometry²⁷⁴. The free and conjugated forms of phenol biomarkers were preliminarily measured in samples from 50 women. These preliminary measurements did not suggest any external contamination⁴⁷, so that for the remaining participating women, we relied on the total form of phenol biomarkers, which was cheaper to assess.

A priori selection of phenols and phthalates tested for relations with thyroid hormones

Discrepancies in results exist across studies that assessed effects of various phenols or phthalates on thyroid hormone levels of pregnant women and neonates. These could be due to differences in study design such as timing of urine and blood collection as well as differences in exposure levels. In addition, given the multiplicity of the outcomes (several thyroid hormones, some of which are assessed at repeated time points) and exposures studied (10 phthalate metabolites and 12 phenols), chance findings cannot be ruled out. In a context where multiple exposures are studied, statistical based approaches such as Elastic net (ENET) and Deletion-Substitution-Addition algorithm (DSA) can be used to limit risk of chance findings²⁷⁵. Although these methods tend to out-perform the classical approach that relies on one model per exposure (ExWAS) in terms of controlling the false positive risk, these methods are not totally efficient²⁷⁵. In a simulation study of 237 exposures assuming that each true predictor explained 3% of the outcome variability, Agier *et al.*, reported false discovery rates of 0.86 for ExWAS, 0.37 for ENET, and 0.28 for DSA²⁷⁵.

The number of tests performed can be limited by a priori selecting the compounds for which prior information on adverse effects on the outcome of interest exists. Such an approach may rely on knowledge of the toxicological mechanisms underlying the health outcome and could be strengthened by the reliance on adverse outcome pathways (AOP) network, when they exist¹⁶³.

An AOP is defined as a conceptual framework that portrays the link between direct molecular initiating events (MIEs) (e.g., a molecular interaction between a xenobiotic and a specific biological molecule), key events (KEs, e.g. decrease in thyroid hormone levels) and adverse outcomes²⁷⁶. Toxicological data can be mapped along an AOP from early molecular events that indicate thyroid activity with high specificity to downstream biological responses and adverse outcomes²⁷⁷. In studies with large numbers of chemical exposures (exposome-studies), this concept could allow reduction in dimensionality / multiple tests by providing mechanistic plausibility²⁷⁷.

The AOP of thyroid disruption is discussed in detail by Noyes *et al*¹⁶³ (illustrated in Figure 4.2).

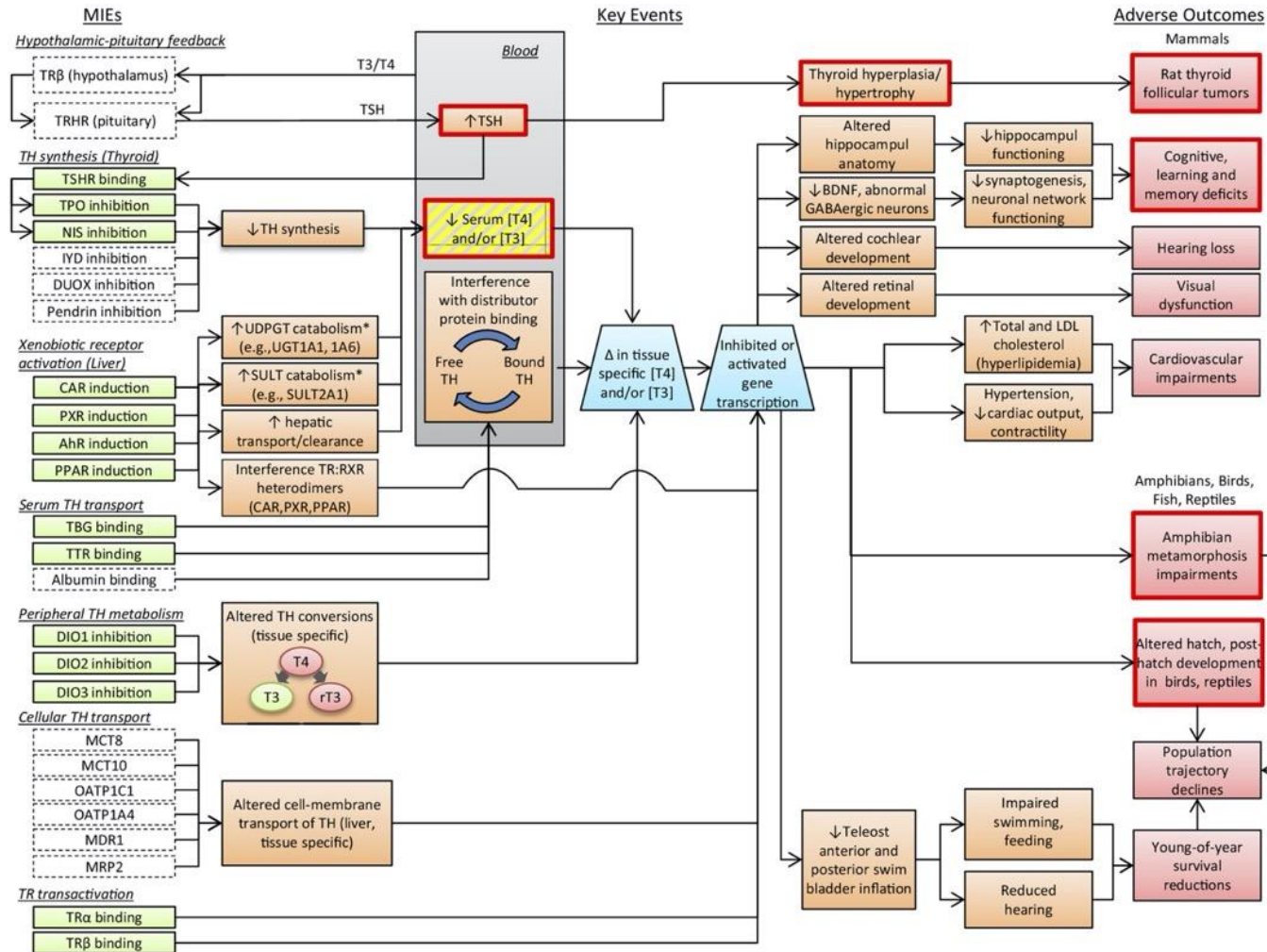


Figure 4.2: Adverse outcome pathway (AOP) network for chemically induced thyroid activity showing the integration of multiple individual AOPs under development and proposed. Adapted from Noyes et al ¹⁶³.

Considering the pathways described in the AOP above, we relied on the ToxCast program ²⁷⁸ to identify compounds portraying thyroid disruption. ToxCast is a USA Environmental Protection Agency (EPA) program that builds large collections of molecular initiating events based on in vitro assay data on a diverse set of chemicals (with over 1000 chemicals in the ToxCast phase I and II lists), including 10 phenols and 4 phthalates assessed in SEPAGES.

In regards to the phenols and phthalates considered in this work, the ToxCast program only screened these compounds for disruption of 3 molecular initiating events including inhibition of thyroid peroxidase; inhibition of glandular iodide uptake by the sodium-iodide symporter and inhibition of peripheral deiodinase 1. We only included phenols and phthalates identified through the ToxCast program as disruptors of at least one of these molecular initiating events. These were BBP, bisphenol A and triclosan as inhibitors of TPO activity ¹⁴⁶ and DBP an inhibitor of the NIS ^{159,160}. Triclosan was also identified as inhibitor of deiodinase I ¹⁴⁹. The hypothesized effects on thyroid hormone levels following disruption of these MIEs are summarized in Table 4.1.

Table 4.1: Summary of the molecular initiating events that could be affected by the compounds assessed in SEPAGES and the expected resulting effect on the thyroid hormone levels

Compounds	Affected molecular initiating event^a	Expected association with TH levels
BPA, TCS, BBP	Inhibition of TPO	Decrease in T3 and T4 and increase in TSH
DBP	Inhibition of the NIS	
TCS	Inhibition of deiodinase 1	Association with the TT3/TT4 ratio

^aMolecular initiating event identified from ToxCast program.

Abbreviations: TCS: triclosan, BPA: bisphenol A, BBP: benzylbutyl phthalate, DBP: Di-n-butyl phthalate, TH: thyroid hormones, TPO: thyroid peroxidase, NIS: Sodium/Iodine symporter, T4: thyroxine, T3: triiodothyronine, TSH: thyroid stimulating hormone.

Statistical analysis

Exposure biomarker concentrations below the limit of detection (LOD) were replaced by specific instrumental reading values; when no signal was detected (i.e., instrumental reading value of 0), we replaced the value by the LOD divided by the square root of two.

Maternal and newborn TSH, phenol and phthalate concentrations were ln-transformed to limit the impact of extreme values.

To enhance results comparability across hormones, for each hormone concentrations we calculated the Z scores (Z-score for a participant I = [concentration for subject i – mean of the population]/Standard Deviation (SD) of the population).

We used adjusted linear regression to assess the association between each selected phenol and phthalate biomarker and the thyroid hormone z-scores as well as with the z-scores of the TT3/TT4 ratio.

In the models on maternal hormones, we only considered phenol and phthalate concentrations assessed in the pool of urine samples collected in early pregnancy (i.e., assessed before blood collection) while for the newborns hormones models, exposure during pregnancy was expressed as the average of the urinary concentration assessed in the pool of the urine samples collected in early pregnancy and late pregnancy.

Biomarker concentrations were considered as continuous (ln-transformed), as well as categorized into tertiles. Heterogeneity and trend tests were performed using categorical variables whose values corresponded to the tertile-specific medians of biomarker concentrations. Associations were considered non-monotonic if we observed heterogeneity (i.e., p-value for heterogeneity < 0.1) with little support for a linear trend (p-value trend tests > 0.1).

Adjustment factors were selected a priori, and included predictors of thyroid hormones concentrations^{141,174}. Maternal models were adjusted for maternal age (quadratic terms), BMI before pregnancy (continuous) and gestational age at serum collection (continuous). The maternal models of the T3/T4 ratio were further adjusted for selenium levels. Newborn models were adjusted for age of infant at blood collection in days (continuous), mode of delivery (vaginal / caesarean), sex (male / female), and vitamin use during pregnancy (yes / no). Both maternal and infant models were additionally adjusted for education level (three categories: high school (\leq high school to ≤ 2 years after high school), undergraduate (3 to 4 years after high school and postgraduate (≥ 5 years after high school)) and parity (nulliparous and parous). These two factors were not identified as predictors of thyroid hormone levels in our literature review but were included because 1) previous studies looking at the associations between phenols, phthalates and thyroid hormone levels adjusted for them¹⁷⁴ and 2) they have been associated with at least one thyroid hormone concentration (p-values < 0.2) in our study population (Table 4.5 and Table 4.6). Other predictors of newborn thyroid hormone levels such as gestational age (pre-term birth) and birthweight that were likely to lie in the causal pathway²⁷⁹ between exposure and outcome were not considered in our main analysis.

Effect estimates were reported as a SD change in T3 and T4 concentrations or a SD change in the ln-transformed TSH concentrations for a one unit increase in the ln-transformed phenol or phthalate metabolite concentrations.

Sensitivity analyses

Given that sex-specific effects in regards to phenols and phthalates were previously reported in studies of newborns ^{164,165,174}, we assessed interactions between biomarker concentrations and newborn sex, and performed stratified analysis in instances where the p-values for interaction were less than 0.1.

Smoking during pregnancy was identified as a predictor of both maternal and newborn thyroid hormone levels ^{141,174}, while alcohol consumption was identified as a predictor of newborn thyroid hormone levels ¹⁴¹. In our study sample, tobacco consumption information was missing for 39 women (9%) and alcohol consumption information for 28 (6%). To avoid lowering the sample size of our main analysis, these two factors were only considered in sensitivity analyses. In the maternal hormone models, smoking status during early pregnancy was considered while for the newborn hormone models, the status throughout pregnancy and alcohol consumption were considered.

For most women, the serum samples were collected the last day of the week of urine collection. In an instance where short-term effects are expected, including all women in our analysis may have diluted our effect estimates. For this reason, we ran sensitivity analysis restricted to the women for whom serum samples were collected immediately after collection of urine samples (less than 2 days after the collection of the last sample in the week of follow-up, N= 339).

4.5 Results

Characteristics of study participants

Mothers were on average 32 years (standard deviation (SD) = 3.9) at recruitment; the majority of them (54%) were parous and had attained graduate level education (57%). Their children were born at an average gestational age of 39.8 weeks (SD = 1.5) with an average weight of 3.3 kilograms (SD = 0.5). Majority of the infants were males (54%) (Table 4.2).

Table 4.2: Characteristics of pregnant women (N= 415) and their newborns (N = 421) included in this study; SEPAGES cohort, Grenoble.

Characteristics	N*(%)	Mean (SD)
Mothers		
Maternal age (years)		32 (3.9)
Gestational age at serum collection (weeks)		19.9 (3.4)
BMI before pregnancy (kg/m ²)		22.3 (3.6)
Education level		
≤ 2 years after high school	71 (17.1)	
3 to 4 years after high school	110 (26.4)	
≥ 5 years after high school	233 (56.0)	
Missing	2(0.5)	
Parity		
Nulliparous	189 (45.4)	
Parous	227 (54.3)	
Missing	1(0.3)	
Newborn		
Gestational age at delivery		39.8 (1.5)
Birth weight (kg)		3.3 (0.5)
Newborn age at serum sampling (days)		3 (0.7)
Child sex		
Male	226 (54)	
Female	195 (46)	
Mode of delivery		
Vaginal	345 (82)	
C-section	68 (16)	
missing	8 (2)	
Vitamin use during pregnancy		
Yes	365 (87)	
No	38 (9)	
Missing	18 (4)	
Smoking ^a		
No	354 (85)	
Yes	25 (6)	
Missing	39(9)	
Alcohol consumption during pregnancy		
No alcohol	334(80)	
Less than a glass/month	59(14)	
At least a glass/month	8(2)	
Missing	20(4)	
Alcohol consumption before knowledge of pregnancy		
No alcohol	106 (25)	
Less than a glass/month	90 (22)	
At least a glass/month	157 (37)	
Missing	68 (16)	

N*: number of women that did not report a former diagnosis of thyroid problems, had at least one hormone level assessed in maternal serum or and had exposure biomarker concentrations measured at the first visit or number of newborns for whom mothers did not report a former diagnosis of thyroid problems, had at least one hormone level assessed in cord sera or and had exposure biomarker concentrations measured at either the first or last pregnancy examinations.

^a Women that smoked at least one cigarette at any time during pregnancy.

^b Women that had at least one glass of alcohol during pregnancy

Distribution of serum thyroid hormone

On average, maternal free forms of T4 and T3 represented only 0.01% and 0.2% of total T4 and total T3 respectively. Most of the women (percentage range between 59% and 99%, depending on the hormones) were in the reference range defined by the French health authority (Table 4.3). The median urinary iodine concentration (UIC) of the women included in this study was 88µg/L, lower than the world health organization (WHO) guideline for pregnant women (median UIC range 150 to 249 µg/L)²⁸⁰.

Table 4.3: Distributions of serum thyroid hormone concentrations of the SEPAGES cohort pregnant women and their newborns

	N*	Mean (SD)	Percentiles			Within reference range N ^a (%)	>reference range N ^b (%)	< reference range N ^c (%)
			5 th	50 th	95 th			
Maternal thyroid hormones								
Total T4 (ng/mL)	416		76	95.2	122.6	354(85.1%)		62(14.9%)
Free T4 (pg/mL)	415	7.5 (1.5)	5.4	7.1	10.4	244(58.8%)	171(41.2%)	
Total T3 (ng/mL)	387		0.9	1.2	1.6	384(99.2%)		3(0.8%)
Free T3 (pg/mL)	416	2.1 (0.3)	1.7	2.1	2.6	305(73.3%)	111(26.7%)	
TSH (mUI/L)	416	1.4 (0.7)	0.6	1.3	2.5	403(96.9%)		8(1.9%)
Iodine (µg/L)	399	112(95)	30	88	272	325(81.5%)	51(12.8%)	23(5.8%)
Selenium (umole/L)	348	0.98 (0.13)	0.78	0.96	1.2			
Newborn serum levels								
Total T4 (ng/mL)	412	82.4 (21.7)	45.6	81.4	118.6			
TSH (mUI/L)	421	2.3 (1.8)	0.7	1.9	5.6			

Abbreviations: TSH: thyroid stimulating hormone, T4: thyroxine, T3: triiodothyronine

N*: number of women or newborns for whom; mothers did not report a former diagnosis of thyroid problems, had at least one hormone level assessed in maternal sera or in cord sera and had exposure biomarker concentrations measured at T1 and T3

N^a: Number of women or newborns with thyroid hormone and iodine levels within reference range

N^b: Number of women or newborns with thyroid hormone and iodine levels below reference range

N^c: Number of women or newborns with thyroid hormone and iodine levels above reference range

French reference ranges from French Haute autorité de santé: free T4 (7 – 18pg/mL), total T4 (45 – 110ng/mL), free T3 (2 - 4.3pg/mL), total T3(0.6 - 1.9ng/mL), TSH (0.36 – 2.5 and 0.36 – 3.74 mUI/L for mothers and newborns respectively), WHO guidelines for pregnant population (median urinary concentration: 150 – 249 µg/L) ²⁸⁰

Within-sample correlation coefficients were low for all hormones, with the highest correlation observed between TT4 and TT3 ($\rho = 0.33$) and between TT3 and FT3 ($\rho = 0.33$ Table 4.4). TSH was weakly negatively correlated with TT4, FT4 and FT3 in maternal samples (ρ between -0.06 and -0.01) as well as with TT4 in the newborn samples. Newborn TSH was also negatively correlated to newborn TT4 ($\rho = -0.07$, Supplemental material Table 4.4). Correlation between maternal and newborn thyroid hormone concentrations were overall low, with the highest correlation coefficients observed between maternal TT3 and newborn TT4 ($\rho = 0.14$).

Table 4.4: Spearman correlation coefficients between maternal and newborn thyroid hormones

		Maternal ^a					Newborn ^b	
		TT4	FT4	TT3	FT3	TSH	TT4	TSH
Maternal	TT4	1.00						
	FT4	0.18	1.00					
	TT3	0.33	-0.16	1.00				
	FT3	0.20	0.31	0.33	1.00			
	TSH	-0.06	-0.07	0.03	-0.01	1.00		
Newborn	TT4	-0.07	0.02	0.14	0.06	-0.06	1.00	
	TSH	0.02	0.06	0.02	0.03	0.08	0.09	1.00

Abbreviations: TT4 :total thyroxine, FT4 :free thyroxine, TT3 :total triiodothyronine, FT3 : Free triiodothyronine, TSH: thyroid stimulating hormone

^aThyroid hormone levels assessed at mean of 19.9 (standard deviation = 3.4) gestational weeks.

^bThyroid hormone levels assessed at 3 (standard deviation = 0.7) days.

Distribution of maternal urinary phenol and phthalate urinary concentrations

MnBP, MBzP, bisphenol A and triclosan were detected in more than 96% of the pooled urine samples assessed, with highest median values observed for MnBP followed by MBzP, bisphenol A and triclosan (Figure 4.3). Correlations between biomarker concentrations were low ($\rho < 0.23$), except between MnBP and MBzP, which were positively correlated ($\rho = 0.61$).

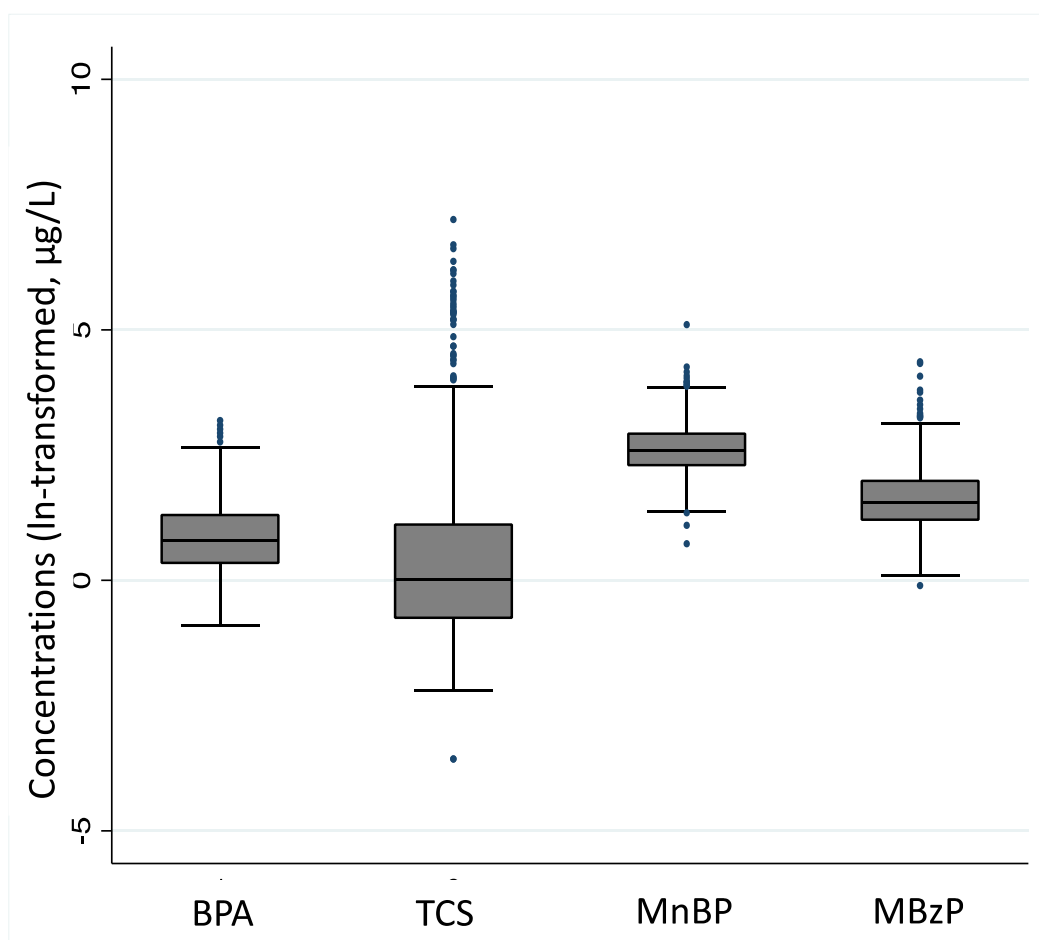


Figure 4.3: Distributions of the average (mid- and late pregnancy) phenol and phthalate urinary concentrations in weekly pools (average of 2 within-subject pool of 21 spot samples) of pregnant women in the SEPAGES cohort (N =418).

Abbreviations; BPA: bisphenol A, TCS: Triclosan, MnBP: mono-n-butyl phthalate, MBzP: mono benzyl phthalate.

Limits of detection : 0.04 for bisphenol A and Triclosan and 0.2 for MnBP and MBzP.

Predictors of maternal and newborn thyroid hormone levels in our study population

Maternal age was inversely associated with TT4. For this same hormone, mothers that had one or more children had lower serum concentrations compared to those in their first pregnancy. Maternal pre-pregnancy body mass index (BMI) and gestational age at serum sampling were positively associated TT4. Mothers with lower education levels (high school and undergraduate) had higher levels of TT4 (p-value for heterogeneity = 0.03). Only one factor was significantly associated with maternal TSH: maternal age at conception was positively associated with this hormone.

In relation to newborn TT4 levels, most predictors tended to be positively associated with this hormone, including gestational age at birth, being born of a mother that took vitamins

during pregnancy or that had a high school education level. Newborns of mothers that smoked however had lower TT4 levels (p-value = 0.01). Newborn TSH levels were positively associated with gestational duration and levels of this hormone were also higher among infants delivered by caesarean section. Newborns of older mothers had lower TSH levels (Table 4.6).

Table 4.5: Multivariate analysis: potential predictors of thyroid hormone levels during pregnancy in our study population (N =416)

Predictors	TT4			TT3			Ln (TSH)		
	β	95% CI	Phet	β	95% CI	Phet	β	95% CI	Phet
Maternal age at conception (years)	-0.03	[-0.05 ; 0.00]		-0.01	[-0.04 ; 0.02]		0.02	[-0.01 ; 0.04]	
Gestational age at sampling (weeks)	0.08	[0.05 ; 0.11]		0.01	[-0.03 ; 0.04]		0.00	[-0.03 ; 0.03]	
Pre-pregnancy BMI (continuous, kg/m ²)	0.02	[0.00 ; 0.05]		0.06	[0.03 ; 0.09]		0.01	[-0.02 ; 0.03]	
Maternal education			0.03			0.52			0.91
\geq 5 years after high school	(ref)			(ref)			(ref)		
3 to 4 years after high school	0.24	[-0.03 ; 0.51]		0.12	[-0.17 ; 0.40]		0.05	[-0.20 ; 0.29]	
\leq 2 years after high school	0.17	[-0.04 ; 0.37]		0.01	[-0.20 ; 0.23]		-0.19	[-0.38 ; -0.01]	
Parity									
Nulliparous	(ref)			(ref)			(ref)		
Parous	-0.16	[-0.40 ; 0.07]		-0.06	[-0.31 ; 0.19]		-0.01	[-0.22 ; 0.20]	
Maternal smoking in early pregnancy									
No	(ref)			(ref)			(ref)		
Yes	-0.26	[-0.70 ; 0.18]		-0.22	[-0.70 ; 0.25]		0.13	[-0.27 ; 0.53]	

Abbreviations; CI: confidence interval, ref: reference category, Phet: P-value of heterogeneity

Table 4.6: Multivariate analysis: potential predictors of thyroid hormone levels in SEPAGES newborns (N = 421).

Predictors	TT4 (N = 345)			LnTSH (N =352)		
	β	95% CI	Phet	β	95% CI	Phet
Age of child at blood sampling (days)	-0.01	[-0.14 ; 0.13]		0.02	[-0.13 ; 0.16]	
Gestational age at birth (weeks)	0.07	[-0.02 ; 0.15]		0.13	[0.04 ; 0.22]	
Birth weight (grams)	0.00	[0.00 ; 0.00]		0.00	[0.00 ; 0.00]	
Maternal age (years)	0.00	[-0.03 ; 0.03]		-0.04	[-0.07 ; -0.01]	
Maternal pre-pregnancy BMI (kg/m ²)	0.00	[-0.03 ; 0.03]		0.01	[-0.02 ; 0.04]	
Child sex						
Male	(ref)			(ref)		
Female	0.21	[-0.15 ; 0.57]		0.19	[-0.19 ; 0.58]	
Vitamins use						
No	(ref)			(ref)		
Yes	0.13	[-0.07 ; 0.34]		-0.01	[-0.22 ; 0.21]	
Education			0.14			0.99
≥ 5 years after high school	(ref)			(ref)		
3 to 4 years after high school	0.11	[-0.13 ; 0.36]		0.00	[-0.25 ; 0.26]	
≤ 2 years after high school	0.29	[0.00 ; 0.58]		-0.02	[-0.32 ; 0.28]	
Parity						
Nulliparous	(ref)			(ref)		
Multiparous	0.13	[-0.09 ; 0.36]		-0.06	[-0.30 ; 0.17]	
Mode of delivery						
Vaginal	(ref)			(ref)		
Caesarean section	-0.03	[-0.32 ; 0.26]		0.45	[0.15 ; 0.75]	
Alcohol consumption during pregnancy						
No	(ref)			(ref)		
Yes	0.07	[-0.16 ; 0.29]		0.10	[-0.13 ; 0.34]	
Active smoking at T1 or during pregnancy						
No	(ref)			(ref)		
Yes	-0.51	[-0.91 ; -0.11]		0.07	[-0.35 ; 0.49]	

Abbreviations; CI: confidence interval, ref: reference, Phet: P-value of heterogeneity

Associations of urinary phenol and phthalate metabolite concentrations with maternal thyroid hormone levels during early pregnancy

We observed a negative association ($p\text{-het} = 0.09$, $p\text{-trend} = 0.03$) between bisphenol A and maternal $\ln\text{-TSH}$, which decreased by 0.11 SD (95% CI = -0.33, 0.10) and 0.24 (95% CI = -0.46, -0.02) in the second and third tertiles of bisphenol A concentration, respectively, compared to the first tertile. Our results were also suggestive of non-monotonic associations ($p\text{-het} < 0.05$, $p\text{-trend} \geq 0.13$) between triclosan (U-shape), MnBP (inverse U-shape) and TSH (Table 4.7 and Figure 4.4).

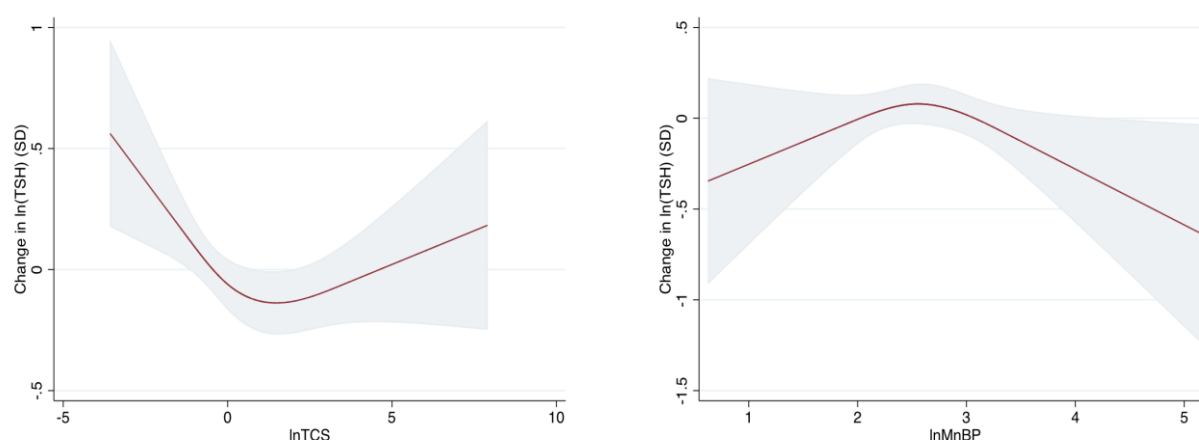


Figure 4.4: TSH concentrations as a function of urinary triclosan and MnBP (ln-transformed) concentrations assessed in a pool of urine samples collected in early pregnancy, coded as restricted cubic splines (3 knots). The predicted curves were adjusted for maternal age, body mass index, gestational age at serum sampling, maternal education level and parity. Shading indicate 95% confidence intervals.

Abbreviations: MnBP: mono-n-butyl phthalate, TSH: thyroid stimulating hormone

No clear association was observed with FT4, TT3 or FT3 ($p\text{-het} \geq 0.21$) in our main analysis (Table 4.7).

Regarding TT4, our main analysis was suggestive of a positive association between MBzP (coded in continuous) and TT4 maternal concentration ($\beta = 0.12$ SD for an increase by 1 in ln-transformed MBzP urine concentrations, 95% confidence interval (CI) = -0.01, 0.25, Table 4.7). This positive association was stronger when analysis was restricted to the 339 women for whom blood sample for hormone assessment was collected at the end of the urine collection week used to assess exposure ($\beta = 0.15$, 95% CI = 0.02, 0.28, Supplemental material Table 4.10).

While not significantly associated with TT3 and TT4 , triclosan (coded in continuous) was inversely associated with the TT3/TT4 ratio, which decreased by 0.05 (95% CI = -0.11, 0.00) for each increase by one in the ln-transformed triclosan concentration (Supplemental material Table 4.9).

Additional adjustment for maternal smoking status did not affect our results (Supplemental material Table 4.9, Table 4.11 and Table 4.12).

Table 4.7 : Adjusted associations of maternal thyroid hormone levels with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior to blood collection (N= 416, SEPAGES cohort).

	Total T4 (TT4)				Free T4				Total T3 (TT3)				Free T3				TSH			
	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b
Bisphenol A			0.76	0.99			0.52	0.62			0.34	0.16			0.50	0.71			0.09	0.03
Tertile 1	(ref)																			
Tertile 2	0.08	[-0.14 ; 0.31]			0.10	[-0.13 ; 0.32]			0.10	[-0.12 ; 0.36]			0.12	[-0.12 ; 0.36]			-0.11	[-0.33 ; 0.10]		
Tertile 3	0.02	[-0.20 ; 0.25]			-0.02	[-0.25 ; 0.20]			0.18	[-0.25 ; 0.23]			-0.01	[-0.25 ; 0.23]			-0.24	[-0.46 ; -0.02]		
<i>Ln (BPA)</i>	0.05	[-0.06 ; 0.16]			-0.04	[-0.16 ; 0.07]			0.09	[-0.14 ; 0.10]			-0.02	[-0.14 ; 0.10]			-0.05	[-0.15 ; 0.06]		
Triclosan			0.44	0.81			0.59	0.36			0.37	0.23			0.24	0.73			0.01	0.13
Tertile 1	(ref)																			
Tertile 2	0.14	[-0.08 ; 0.36]			0.04	[-0.18 ; 0.26]			0.08	[-0.04 ; 0.43]			0.19	[-0.04 ; 0.43]			-0.28	[-0.49 ; -0.07]		
Tertile 3	0.09	[-0.14 ; 0.31]			-0.07	[-0.30 ; 0.15]			-0.10	[-0.20 ; 0.29]			0.05	[-0.20 ; 0.29]			-0.27	[-0.48 ; -0.05]		
<i>Ln (TCS)</i>	0.00	[-0.04 ; 0.05]			-0.03	[-0.08 ; 0.02]			-0.04	[-0.07 ; 0.04]			-0.01	[-0.07 ; 0.04]			-0.03	[-0.07 ; 0.02]		
MnBP			0.46	0.95			0.21	0.09			0.92	0.94			0.43	0.33			0.02	0.15
Tertile 1	(ref)																			
Tertile 2	0.14	[-0.09 ; 0.36]			-0.01	[-0.23 ; 0.22]			0.05	[-0.31 ; 0.18]			-0.06	[-0.31 ; 0.18]			0.20	[-0.02 ; 0.41]		
Tertile 3	0.04	[-0.20 ; 0.28]			-0.19	[-0.43 ; 0.05]			0.02	[-0.16 ; 0.36]			0.10	[-0.16 ; 0.36]			-0.11	[-0.34 ; 0.12]		
<i>Ln (MnBP)</i>	0.13	[-0.04 ; 0.30]			-0.09	[-0.26 ; 0.08]			0.05	[-0.09 ; 0.28]			0.10	[-0.09 ; 0.28]			-0.12	[-0.28 ; 0.04]		
MBzP			0.41	0.25			0.77	0.47			0.88	0.70			0.93	0.81			0.56	0.41
Tertile 1	(ref)																			
Tertile 2	0.12	[-0.10 ; 0.34]			-0.03	[-0.25 ; 0.20]			0.02	[-0.20 ; 0.28]			0.04	[-0.20 ; 0.28]			0.04	[-0.17 ; 0.25]		
Tertile 3	0.15	[-0.09 ; 0.38]			-0.08	[-0.32 ; 0.15]			-0.04	[-0.21 ; 0.29]			0.04	[-0.21 ; 0.29]			-0.08	[-0.30 ; 0.14]		
<i>Ln (MBzP)</i>	0.12	[-0.01 ; 0.25]			-0.07	[-0.20 ; 0.06]			0.01	[-0.22 ; 0.07]			-0.07	[-0.22 ; 0.07]			-0.06	[-0.19 ; 0.06]		

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), TT4: total thyroxine, FT3: free thyroxine, TT3: total triiodothyronine, TSH : thyrotropin, CI: confidence interval, ref: reference category

Adjustment factors: maternal age, body mass index, gestational age at serum sampling, maternal education level, parity and urine specific gravity.

^aP: P-value of heterogeneity test.

^bP: P-value of trend test.

Associations of urinary phenol and phthalate metabolite concentrations with newborn thyroid hormone levels

We observed a monotonic association between MnBP and TSH (p-het = 0.09, p-trend = 0.07). Newborn ln-TSH z-score increased by 0.2 (95% CI = 0.01, 0.40,) for each increase by one in the ln-transformed maternal MnBP concentrations (Table 4.8).

No other exposure biomarker was associated with TSH or TT4 in newborns in our main analysis when boys and girls were considered together (p-value for heterogeneity were ≥ 0.19 , Table 4.8)

However, for TT4, we observed sex-specific associations with bisphenol A and triclosan (p-value of interaction = 0.03 and 0.11 respectively, Table 4.8). After stratification for child sex, TT4 concentration was positively associated with bisphenol A concentration among boys (beta = 0.24, 95% CI = 0.02, 0.45), while TT4 decreased with an increase in triclosan concentration among girls only (beta = - 0.07, 95% CI = -0.14; - 0.00, Figure 4.5).

Additional adjustment for smoking and alcohol consumption during pregnancy did not change these results (Supplemental material, Table 4.13).

Table 4.8: Adjusted associations between maternal urinary phenol and phthalate metabolite concentrations and newborn thyroid hormone levels, SEPAGES cohort.

	Total T4					TSH				
	β	95% CI	P-het ^a	P-trend	P for interaction ^c	β	95% CI	P ^a	P ^b	P ^c
Bisphenol A			0.20	0.16				0.41	0.73	
Tertile 1	(ref)					(ref)				
Tertile 2	0.18	[-0.07 ; 0.42]				-0.14	[-0.39 ; 0.10]			
Tertile 3	0.21	[-0.04 ; 0.45]				0.00	[-0.25 ; 0.25]			
Ln(BPA)	0.07	[-0.07 ; 0.22]			0.03	-0.03	[-0.18 ; 0.12]			0.61
Triclosan			0.26	0.27				0.43	0.19	
Tertile 1	(ref)					(ref)				
Tertile 2	-0.16	[-0.40 ; 0.08]				0.00	[-0.24 ; 0.25]			
Tertile 3	-0.19	[-0.44 ; 0.05]				-0.14	[-0.39 ; 0.10]			
Ln(TCS)	-0.03	[-0.08 ; 0.02]			0.11	-0.03	[-0.08 ; 0.02]			0.33
MnBP			0.27	0.93				0.09	0.07	
Tertile 1	(ref)					(ref)				
Tertile 2	0.19	[-0.05 ; 0.43]				-0.04	[-0.29 ; 0.20]			
Tertile 3	0.04	[-0.22 ; 0.31]				0.22	[-0.04 ; 0.49]			
Ln(MnBP)	0.07	[-0.12 ; 0.26]			0.66	0.20	[0.01 ; 0.40]			0.89
MBzP			0.93	0.71				0.94	0.79	
Tertile 1	(ref)					(ref)				
Tertile 2	0.02	[-0.23 ; 0.27]				-0.02	[-0.27 ; 0.23]			
Tertile 3	0.05	[-0.20 ; 0.30]				0.03	[-0.23 ; 0.28]			
Ln(MBzP)	0.04	[-0.11 ; 0.18]			0.79	0.02	[-0.13 ; 0.18]			0.87

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), TT4: total thyroxine, TSH: thyrotropin.

Adjustment factors: Age of child, type of delivery, child sex, parity, maternal age, maternal education level, vitamin use, age of mother and urine specific gravity.

^aP: P-value of heterogeneity test.

^bP: P-value of trend test.

^cP: p-value for interaction term for child sex and urinary biomarker concentrations.

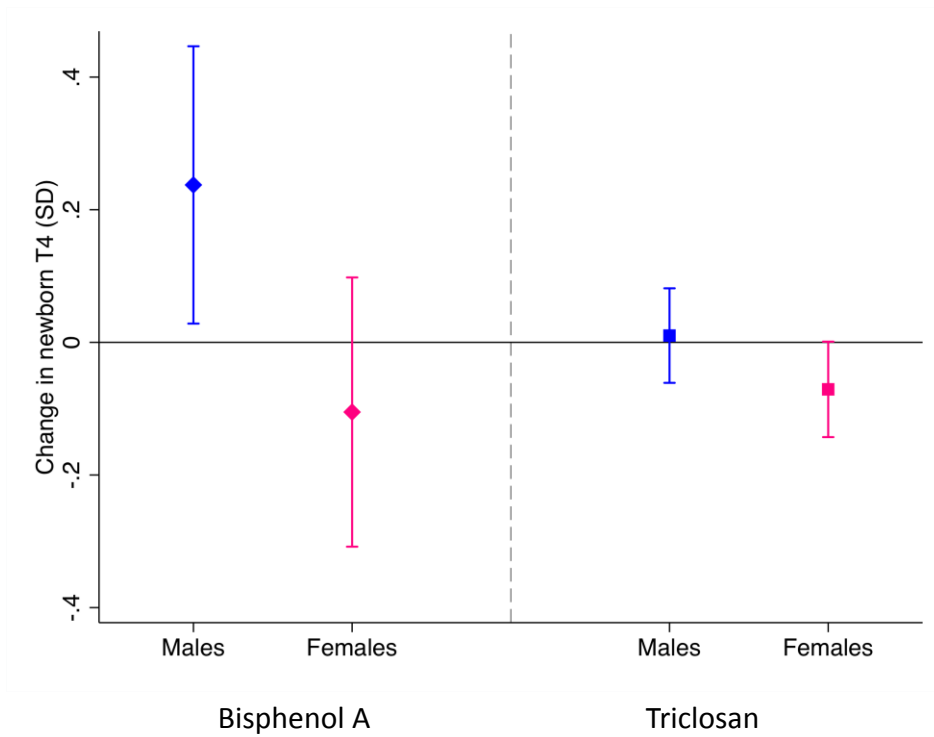


Figure 4.5: Adjusted associations between newborn thyroid hormone levels and maternal urinary bisphenol A and triclosan concentrations, stratified by sex.

Abbreviations: T4: total thyroxine

Adjustment factors: Age of child, type of delivery, child sex, parity, maternal age, maternal education level, vitamin use, age of mother and urine specific gravity.

4.6 Discussion

In this study, maternal urinary bisphenol A, triclosan and MnBP pregnancy urinary levels were associated with maternal TSH. The negative association with bisphenol A was monotonic, while associations with triclosan (U-shape) and MnBP (inverse U-shape) were non-monotonic. Urinary MBzP concentrations was positively associated with maternal TT4 while triclosan was negatively associated with the TT3/TT4 ratio. No significant associations were observed for maternal FT4, TT3 and FT3.

Regarding the newborn TSH and TT4 levels, maternal urinary MnBP concentrations were positively associated with newborn TSH. When stratified for sex, bisphenol A was positively associated with TT4 in boys while triclosan was negatively associated with the TT4 in girls.

Strengths and limitations

Discrepancies exist in results of previous studies assessing exposure to bisphenol A, triclosan, MnBP and MBzP in relation to thyroid hormone levels during pregnancy and at birth. This could partly be a consequence of differences in study designs such as timing of urine and blood sample collection, differences in exposure levels and as well as due to the multiplicity of associations tested that could have led to chance findings. In this study, we relied on an AOP concept^{163,281} to a priori select four compounds among the 27 assayed in maternal urine, that have been previously identified as disruptors of molecular initiating events involved in thyroid hormone regulation¹⁶³. This selection based on prior knowledge from in-vitro studies allowed us to decrease the number of tests carried out, compared to previous studies on this topic. One limitation of this approach was that we only considered molecular initiating events tested in the ToxCast program^{146,149,160,170}, namely inhibition of TPO, NIS and deiodinase I. There is a chance that some of the compounds assessed in SEPAGES act on thyroid hormones and TSH through other mechanisms not considered in ToxCast, such as induction of receptors like the pregnane X, constitutive androstane, aryl hydrocarbon and peroxisome proliferator-activated and binding to the thyroid hormone receptor-beta¹⁶³ (Figure 4.2). In addition, not all the compounds assessed in SEPAGES were previously screened for thyroid disruption in the ToxCast program.

We relied on robust methods to assess exposure to phenols and phthalates during pregnancy¹⁹⁰. Concentrations were indeed assessed in two pools of 21 spot urine per participant collected over a week at two time points of pregnancy. This protocol was previously validated by our research team using a simulation approach¹⁸⁹ and real data¹⁹⁰, showing its efficiency

to limit classical measurement error and consequently reduce bias in the effect estimates and improve power, compared to approaches relying on 1 to 3 spot samples to estimate exposure throughout pregnancy.

We also assessed thyroid hormone levels and TSH at two time points relevant for brain development (pregnancy and birth). This is key in identifying pathways through which phenols and phthalates could disrupt brain development. Various phenols and phthalates, including those considered in this study, have previously been linked to adverse neurodevelopmental effects^{82,83,86,87,104}. Additionally, for the majority of women (81.5%, N =339), urine samples were collected in the week preceding blood collection, allowing us to assess short-term effect of bisphenol A, triclosan, DBP and BBP on thyroid function of pregnant women.

SEPAGES cohort mainly consisted of highly educated women, compared to the French population of pregnant women (all had a high school diploma). Although this limits generalizability of results to the French population of pregnant women, it is not a limitation in etiologic studies^{282,283}. Focusing on homogeneous samples also lowers risk of confounding bias from between-subject socio-economic differences.

Associations of thyroid hormones and TSH levels with maternal phenol and phthalate urinary concentrations

Although triclosan tested positive for TPO inhibition in the ToxCast EPA program, no inverse associations were observed in our study between this compound and either T4 or T3 levels in pregnant women. We found an inverse association between triclosan and the TT3/TT4 ratio in pregnant women, which was in line with results of the ToxCast program reporting inhibition of deiodinase I¹⁴⁹ by this compound. Triclosan was also associated with lower TT4 concentrations among newborn girls. This negative association might be a consequence of triclosan's ability to inhibit TPO¹⁴⁶, as well as other mechanisms reported in toxicological studies. Triclosan has been proven to be able to influence activity of various enzymes involved in metabolism of thyroid hormones, leading to increased elimination of T4¹⁵⁰. In our study population, triclosan was also inversely associated with maternal TSH levels. We did not find any relevant information on the mechanisms that could underlie such an association.

Among the five epidemiological studies assessing the associations between triclosan and thyroid hormones and TSH concentrations during pregnancy, only one reported an inverse association with TSH¹⁷⁰, while other associations reported with this compound were an inverse association with T3¹⁷⁰ and T4^{170,171}, which we did not observe. Only one study assessed cord

blood thyroid hormone levels in relation to triclosan, and reported a decrease in free T3 with increased exposure to triclosan ¹⁷⁰. No study has considered any effects of gestational exposure to triclosan on T4 levels at birth.

Bisphenol A was identified as a TPO inhibitor in ToxCast program ¹⁴⁶ and so, if acting solely through this mechanism, increased bisphenol A exposure is expected to decrease T4 and/or T3 levels. Our results did not evidence such associations with T3 or T4. We observed a decrease in maternal TSH and an increase in TT4 concentrations of male newborns only in relation to bisphenol A exposure. These observations were suggestive that any effect of this compound on the thyroid hormone pathway would mainly be mediated by other mechanisms than TPO inhibition.

In line with our results in male newborns, a toxicological study in rats exposed to bisphenol A during gestation reported increase in serum T4, but not TSH, of pups ¹⁴⁴. This was explained by the fact that bisphenol A is a thyroid receptor (TR-beta) receptor antagonist ^{144,145,284}, found among others, in the pituitary and hypothalamus, which mediate the negative feedback effect of thyroid hormones in the pituitary gland.

In regards to epidemiological studies looking at effects of gestational exposure to bisphenol A on thyroid function in newborns, only two studies assessed levels of TSH ^{164,165} or thyroid hormones ¹⁶⁵ of newborns or in cord blood. One study (N= 249) did not report any association with TT4 cord blood level but reported increased TT3 levels in girls ¹⁶⁵, while the other (N = 364) only assessed TSH and reported inverse associations in boys ¹⁶⁴.

Regarding thyroid hormone and TSH levels during pregnancy, among the six studies exploring this issue, similar to our results, only one reported an inverse association between bisphenol A and TSH ¹⁶⁷. We did not observe any other association with levels of TT4 or FT4, although a few studies have reported inverse ¹⁶⁸ or positive associations ^{166,167,169}

While we did not observe any association between MnBP and the thyroid hormone levels, this phthalate was however associated with both maternal (non-monotonic, U-shape) and newborn (positively) TSH. DBP, the parent compound of this metabolite was reported to disrupt NIS activity ¹⁵⁹ and so may affect TSH levels through the hypothalamic-pituitary feedback. Studies of pregnant women only reported inverse associations with FT4 and TT4 in relation to this metabolite ^{178-180,285} but not with TSH.

No past study found similar results as those observed in our study in regards to newborn TSH levels.

Contrarily to our initial hypothesis that relied on the observation that BBP, the parent compounds of MBzP inhibits TPO, MBzP was positively associated with maternal TT4 in our study population. No previous epidemiological study reported similar findings with TT4, however, after stratifying for gestational age at serum collection, Johns *et al*, reported positive association for this compound with FT4¹⁷⁹ during later pregnancy. Other findings in epidemiology studies were either negative in relation to TT4 and FT4¹⁷⁶ or null^{173–178}.

Conclusion

The negative associations between triclosan and the TT3/TT4 ratio in pregnant women as well as with the TT4 concentrations in female newborns were in line with the mechanisms of action we identified through ToxCast for this compound (Inhibition of deiodinase I and TPO and respectively). This was not the case for the negative associations of bisphenol A with maternal TSH, nor for the positive associations of bisphenol A with the male newborns' TT4, MnBP with maternal TSH and MBzP with maternal TT4. Discrepancies between our results and our a priori hypotheses may partly result from the complexity of the thyroid system. It indeed includes complex feedback loops that make the effects of the disruption of a molecular initiating event on the thyroid hormone levels difficult to predict. In addition, we cannot rule out that MnBP, MBzP and bisphenol A affect other relevant molecular initiating events than those identified through ToxCast.

4.7 Supplemental Materials

List of tables

- Table 4.9: Adjusted associations of the maternal TT3/TT4 ratio with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior blood collection
- Table 4.10: Sensitivity analysis: Adjusted associations of maternal thyroid hormone levels with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior blood collection. Analysis restricted to women for whom blood samples were collected at the end of the urine collection week (within 2 days) urine collection, N =339
- Table 4.11: Complete case analysis: Association between maternal thyroid hormone levels and urinary phenol and phthalate metabolite concentrations additionally adjusted for smoking
- Table 4.12: Complete case analysis: Association between maternal thyroid hormone levels and urinary phenol and phthalate metabolite concentrations additionally not adjusted for smoking
- Table 4.13: Complete case analysis: Association between newborn thyroid hormone levels and maternal urinary phenol and phthalate metabolite concentrations additionally adjusted for or not smoking alcohol consumption during pregnancy (N=350)

Table 4.9: Adjusted associations of the maternal TT3/TT4 ratio with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior blood collection.

	T3/T4 (N= 416, main analysis)				T3/T4 (complete cases ^c analysis, not adjusted for smoking, N= 335)				T3/T4 (complete cases analysis ^c , adjusted for smoking, N= 335)			
	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b
Bisphenol A			0.43	0.19			0.37	0.18			0.41	0.20
Tertile 1	(ref)											
Tertile 2	0.04	[-0.20 ; 0.29]			0.10	[-0.17 ; 0.36]			0.09	[-0.17 ; 0.36]		
Tertile 3	0.16	[-0.09 ; 0.40]			0.19	[-0.07 ; 0.45]			0.18	[-0.08 ; 0.44]		
Ln (BPA)	0.10	[-0.03 ; 0.23]			0.06	[-0.07 ; 0.18]			0.05	[-0.07 ; 0.18]		
Triclosan			0.42	0.19			0.33	0.14			0.33	0.14
Tertile 1	(ref)											
Tertile 2	-0.01	[-0.25 ; 0.24]			0.00	[-0.26 ; 0.25]			0.00	[-0.26 ; 0.25]		
Tertile 3	-0.15	[-0.39 ; 0.10]			-0.18	[-0.44 ; 0.09]			-0.18	[-0.44 ; 0.09]		
Ln (TCS)	-0.05	[-0.11 ; 0.00]			-0.05	[-0.10 ; 0.01]			-0.05	[-0.10 ; 0.01]		
MnBP			0.85	0.71			0.81	0.82			0.79	0.86
Tertile 1	(ref)											
Tertile 2	-0.07	[-0.31 ; 0.18]			-0.09	[-0.35 ; 0.18]			-0.09	[-0.35 ; 0.17]		
Tertile 3	-0.06	[-0.33 ; 0.20]			-0.05	[-0.33 ; 0.24]			-0.04	[-0.33 ; 0.24]		
Ln (MnBP)	-0.06	[-0.25 ; 0.14]			-0.08	[-0.28 ; 0.12]			-0.08	[-0.28 ; 0.13]		
MBzP			0.31	0.13			0.41	0.18			0.41	0.18
Tertile 1	(ref)											
Tertile 2	-0.09	[-0.33 ; 0.16]			-0.06	[-0.31 ; 0.20]			-0.06	[-0.31 ; 0.20]		
Tertile 3	-0.20	[-0.45 ; 0.06]			-0.18	[-0.45 ; 0.09]			-0.18	[-0.45 ; 0.09]		
Ln (MBzP)	-0.13	[-0.28 ; 0.03]			-0.09	[-0.25 ; 0.07]			-0.09	[-0.25 ; 0.06]		

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), TT4: total thyroxine, FT3: free thyroxine, TT3: total triiodothyronine, TSH : thyrotropin, CI: confidence interval, ref: reference category

Adjustment factors: maternal age, body mass index, gestational age at serum sampling, maternal education level and parity

^aP: P-value of heterogeneity test.

^bP: P-value of trend test.

^cComplete case analysis: women with information on smoking

Table 4.10: Sensitivity analysis: Adjusted associations of maternal thyroid hormone levels with urinary phenol and phthalate metabolite concentrations (ln-transformed) assessed in a pool of repeated urine samples collected prior blood collection. Analysis restricted to women for whom blood samples were collected at the end (within 2 days) of the urine collection week, N =339; SEPAGES cohort.

	TT4				FT4				TT3				FT3				TSH			
	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b
Bisphenol A			0.91	0.67			0.75	0.70			0.17	0.06			0.35	0.61			0.11	0.06
Tertile 1	(ref)																			
Tertile 2	0.02	[-0.20 ; 0.25]			0.10	[-0.16 ; 0.35]			0.10	[-0.07 ; 0.47]			0.20	[-0.07 ; 0.47]			-0.16	[-0.40 ; 0.07]		
Tertile 3	0.05	[-0.17 ; 0.27]			0.07	[-0.19 ; 0.33]			0.25	[-0.16 ; 0.38]			0.11	[-0.16 ; 0.38]			-0.25	[-0.48 ; -0.01]		
Ln (BPA)	0.03	[-0.07 ; 0.14]			-0.01	[-0.14 ; 0.12]			0.11	[-0.08 ; 0.18]			0.05	[-0.08 ; 0.18]			-0.03	[-0.15 ; 0.08]		
Triclosan			0.49	0.43			0.65	0.40			0.15	0.17			0.50	0.70			0.05	0.13
Tertile 1	(ref)																			
Tertile 2	0.11	[-0.11 ; 0.32]			0.04	[-0.21 ; 0.29]			0.16	[-0.12 ; 0.40]			0.14	[-0.12 ; 0.40]			-0.25	[-0.47 ; -0.02]		
Tertile 3	0.12	[-0.10 ; 0.34]			-0.08	[-0.34 ; 0.18]			-0.10	[-0.26 ; 0.28]			0.01	[-0.26 ; 0.28]			-0.26	[-0.49 ; -0.03]		
Ln (TCS)	0.01	[-0.04 ; 0.06]			-0.03	[-0.08 ; 0.03]			-0.05	[-0.07 ; 0.04]			-0.02	[-0.07 ; 0.04]			-0.03	[-0.08 ; 0.01]		
MnBP			0.54	0.92			0.46	0.24			0.94	0.86			0.31	0.25			0.03	0.36
Tertile 1	(ref)																			
Tertile 2	0.12	[-0.10 ; 0.34]			0.00	[-0.25 ; 0.26]			0.03	[-0.35 ; 0.19]			-0.08	[-0.35 ; 0.19]			0.25	[0.02 ; 0.48]		
Tertile 3	0.04	[-0.20 ; 0.28]			-0.15	[-0.43 ; 0.13]			-0.02	[-0.16 ; 0.42]			0.13	[-0.16 ; 0.42]			-0.04	[-0.30 ; 0.21]		
Ln (MnBP)	0.14	[-0.03 ; 0.30]			-0.08	[-0.27 ; 0.11]			0.03	[-0.09 ; 0.31]			0.11	[-0.09 ; 0.31]			-0.08	[-0.25 ; 0.10]		
MBzP			0.27	0.26			0.66	0.37			0.76	0.46			0.63	0.39			0.76	0.64
Tertile 1	(ref)																			
Tertile 2	0.16	[-0.05 ; 0.38]			-0.03	[-0.28 ; 0.23]			-0.03	[-0.17 ; 0.36]			0.09	[-0.17 ; 0.36]			0.04	[-0.19 ; 0.27]		
Tertile 3	0.15	[-0.08 ; 0.38]			-0.12	[-0.38 ; 0.15]			-0.10	[-0.15 ; 0.40]			0.13	[-0.15 ; 0.40]			-0.05	[-0.29 ; 0.20]		
Ln (MBzP)	0.15	[0.02 ; 0.28]			-0.07	[-0.22 ; 0.08]			-0.01	[-0.20 ; 0.11]			-0.04	[-0.20 ; 0.11]			-0.05	[-0.19 ; 0.08]		

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), TT4: total thyroxine, FT3: free thyroxine, TT3: total triiodothyronine, TSH: thyroid stimulating hormone, CI: confidence interval, ref: reference category

Adjustment factors: maternal age, body mass index, gestational age at serum sampling, maternal education level and parity

^aP: P-value of heterogeneity test.

^bP: P-value of trend test.

Table 4.11: Complete cases analysis*: Association between maternal thyroid hormone levels and urinary phenol and phthalate metabolite concentrations additionally adjusted for smoking (N=335, SEPAGES cohort).

	TT4				FT4				TT3				FT3				TSH			
	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b
Bisphenol A																				
Tertile 1	(ref)																			
Tertile 2	0.01	[-0.23 ; 0.26]	0.99	0.97	0.03	[-0.20 ; 0.27]	0.94	0.90	0.09	[-0.13 ; 0.38]	0.32	0.13	0.12	[-0.13 ; 0.38]	0.63	0.76	-0.01	[-0.23 ; 0.21]	0.08	0.03
Tertile 3	0.00	[-0.24 ; 0.24]			0.00	[-0.24 ; 0.23]			0.20	[-0.19 ; 0.32]			0.07	[-0.19 ; 0.32]			-0.22	[-0.44 ; 0.00]		
<i>Ln (BPA)</i>	<i>0.05</i>	<i>[-0.07 ; 0.17]</i>			<i>-0.04</i>	<i>[-0.15 ; 0.08]</i>			<i>0.09</i>	<i>[-0.12 ; 0.13]</i>			<i>0.01</i>	<i>[-0.12 ; 0.13]</i>			<i>-0.03</i>	<i>[-0.14 ; 0.07]</i>		
Triclosan																				
Tertile 1	(ref)																			
Tertile 2	0.10	[-0.13 ; 0.34]	0.68	0.99	0.08	[-0.15 ; 0.31]	0.63	0.52	0.05	[-0.04 ; 0.45]	0.24	0.11	0.21	[-0.04 ; 0.45]	0.19	0.60	-0.28	[-0.49 ; -0.07]	0.03	0.35
Tertile 3	0.05	[-0.20 ; 0.29]			-0.04	[-0.28 ; 0.20]			-0.16	[-0.23 ; 0.28]			0.03	[-0.23 ; 0.28]			-0.21	[-0.43 ; 0.01]		
<i>Ln (TCS)</i>	<i>-0.01</i>	<i>[-0.06 ; 0.04]</i>			<i>-0.02</i>	<i>[-0.07 ; 0.03]</i>			<i>-0.05</i>	<i>[-0.07 ; 0.04]</i>			<i>-0.02</i>	<i>[-0.07 ; 0.04]</i>			<i>-0.01</i>	<i>[-0.06 ; 0.03]</i>		
MnBP																				
Tertile 1	(ref)																			
Tertile 2	0.11	[-0.13 ; 0.35]	0.61	0.86	0.03	[-0.20 ; 0.26]	0.11	0.06	0.00	[-0.28 ; 0.22]	1.00	0.97	-0.03	[-0.28 ; 0.22]	0.83	0.64	0.22	[0.01 ; 0.44]	0.02	0.28
Tertile 3	0.00	[-0.26 ; 0.26]			-0.22	[-0.48 ; 0.03]			0.00	[-0.22 ; 0.33]			0.05	[-0.22 ; 0.33]			-0.07	[-0.31 ; 0.17]		
<i>Ln (MnBP)</i>	<i>0.11</i>	<i>[-0.07 ; 0.30]</i>			<i>-0.12</i>	<i>[-0.30 ; 0.06]</i>			<i>0.04</i>	<i>[-0.11 ; 0.28]</i>			<i>0.09</i>	<i>[-0.11 ; 0.28]</i>			<i>-0.08</i>	<i>[-0.25 ; 0.09]</i>		
MBzP																				
Tertile 1	(ref)																			
Tertile 2	0.12	[-0.12 ; 0.36]	0.50	0.35	-0.07	[-0.30 ; 0.16]	0.77	0.56	0.05	[-0.26 ; 0.23]	0.76	0.65	-0.02	[-0.26 ; 0.23]	0.98	0.92	0.05	[-0.17 ; 0.26]	0.52	0.41
Tertile 3	0.13	[-0.12 ; 0.38]			-0.08	[-0.32 ; 0.16]			-0.05	[-0.25 ; 0.27]			0.01	[-0.25 ; 0.27]			-0.08	[-0.31 ; 0.14]		
<i>Ln (MBzP)</i>	<i>0.10</i>	<i>[-0.04 ; 0.25]</i>			<i>-0.05</i>	<i>[-0.19 ; 0.09]</i>			<i>0.02</i>	<i>[-0.21 ; 0.09]</i>			<i>-0.06</i>	<i>[-0.21 ; 0.09]</i>			<i>-0.05</i>	<i>[-0.18 ; 0.08]</i>		

*Women with complete data on smoking status, thyroid hormone levels, urinary biomarker concentrations and other confounders.

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), TT4: total thyroxine, FT3: free thyroxine, TT3: total triiodothyronine, TSH : thyroid stimulating hormone, CI: confidence interval, ref: reference category

Adjustment factors: maternal age, body mass index, gestational age at serum sampling, maternal education level, parity and smoking

^aP: P-value of heterogeneity test.

^bP: P-value of trend test.

Table 4.12: Complete cases analysis*: Association between maternal thyroid hormone levels and urinary phenol and phthalate metabolite concentrations not adjusted for smoking but adjusted for other potential confounders (N=335).

	TT4				FT4				TT3				FT3				TSH			
	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b	β	95% CI	P ^a	P ^b
Bisphenol A																				
Tertile 1	(ref)																			
Tertile 2	0.02	[-0.23 ; 0.26]	0.99	0.95	0.03	[-0.20 ; 0.27]	0.94	0.89	0.09	[-0.13 ; 0.37]	0.27	0.11	0.12	[-0.13 ; 0.37]	0.65	0.80	-0.01	[-0.23 ; 0.21]	0.07	0.02
Tertile 3	0.01	[-0.23 ; 0.25]			-0.01	[-0.24 ; 0.23]			0.21	[-0.19 ; 0.31]			0.06	[-0.19 ; 0.31]			-0.23	[-0.44 ; -0.01]		
Ln (BPA)	0.05	[-0.06 ; 0.17]			-0.04	[-0.15 ; 0.08]			0.09	[-0.12 ; 0.13]			0.01	[-0.12 ; 0.13]			-0.03	[-0.14 ; 0.07]		
Triclosan																				
Tertile 1	(ref)																			
Tertile 2	0.10	[-0.13 ; 0.34]	0.68	0.98	0.08	[-0.15 ; 0.31]	0.63	0.52	0.05	[-0.03 ; 0.45]	0.24	0.11	0.21	[-0.03 ; 0.45]	0.19	0.59	-0.28	[-0.49 ; -0.07]	0.03	0.35
Tertile 3	0.05	[-0.20 ; 0.29]			-0.04	[-0.27 ; 0.20]			-0.17	[-0.23 ; 0.28]			0.03	[-0.23 ; 0.28]			-0.21	[-0.43 ; 0.01]		
Ln (TCS)	-0.01	[-0.06 ; 0.05]			-0.02	[-0.07 ; 0.03]			-0.05	[-0.07 ; 0.04]			-0.02	[-0.07 ; 0.04]			-0.01	[-0.06 ; 0.03]		
MnBP																				
Tertile 1	(ref)																			
Tertile 2	0.12	[-0.12 ; 0.36]	0.51	0.78	0.03	[-0.21 ; 0.26]	0.12	0.06	0.01	[-0.28 ; 0.22]	0.99	0.95	-0.03	[-0.28 ; 0.22]	0.79	0.60	0.21	[0.00 ; 0.43]	0.03	0.31
Tertile 3	-0.01	[-0.27 ; 0.25]			-0.22	[-0.47 ; 0.04]			-0.01	[-0.21 ; 0.33]			0.06	[-0.21 ; 0.33]			-0.06	[-0.30 ; 0.17]		
Ln (MnBP)	0.10	[-0.08 ; 0.29]			-0.12	[-0.30 ; 0.06]			0.03	[-0.10 ; 0.29]			0.09	[-0.10 ; 0.29]			-0.08	[-0.24 ; 0.09]		
MBzP																				
Tertile 1	(ref)																			
Tertile 2	0.11	[-0.12 ; 0.35]	0.50	0.32	-0.07	[-0.30 ; 0.16]	0.77	0.56	0.04	[-0.26 ; 0.23]	0.79	0.67	-0.01	[-0.26 ; 0.23]	0.99	0.94	0.05	[-0.16 ; 0.26]	0.50	0.39
Tertile 3	0.13	[-0.11 ; 0.38]			-0.08	[-0.32 ; 0.16]			-0.05	[-0.25 ; 0.27]			0.01	[-0.25 ; 0.27]			-0.08	[-0.31 ; 0.14]		
Ln (MBzP)	0.11	[-0.04 ; 0.25]			-0.05	[-0.19 ; 0.09]			0.02	[-0.22 ; 0.08]			-0.07	[-0.22 ; 0.08]			-0.05	[-0.18 ; 0.08]		

*Women with complete data on smoking status, thyroid hormone levels, urinary biomarker concentrations and other confounders.

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), TT4: total thyroxine, FT3: free thyroxine, TT3: total triiodothyronine, TSH : thyrotropin, CI: confidence interval, ref: reference category

Adjustment factors: maternal age, body mass index, gestational age at serum sampling, maternal education level, parity.

^aP: P-value of heterogeneity test.

^bP: P-value of trend test.

Table 4.13: Complete case analysis*: Association between newborn thyroid hormone levels and maternal urinary phenol and phthalate metabolite concentrations additionally adjusted or not for smoking and alcohol consumption during pregnancy (N=350, SEPAGES cohort).

	Adjusted for smoking and alcohol				Not adjusted for smoking and alcohol			
	TT4		TSH		TT4		TSH	
	β	95%CI	β	95%CI	β	95%CI	β	95%CI
BPA	0.06	[-0.09 ; 0.21]	-0.05	[-0.20 ; 0.11]	0.06	[-0.08 ; 0.21]	-0.05	[-0.20 ; 0.10]
TCS	-0.03	[-0.09 ; 0.02]	-0.03	[-0.08 ; 0.02]	-0.03	[-0.09 ; 0.02]	-0.03	[-0.08 ; 0.02]
MnBP	0.02	[-0.18 ; 0.22]	0.25	[0.04 ; 0.45]	-0.02	[-0.22 ; 0.18]	0.24	[0.04 ; 0.45]
MBzP	0.06	[-0.10 ; 0.21]	0.07	[-0.09 ; 0.23]	0.05	[-0.11 ; 0.20]	0.07	[-0.09 ; 0.23]

*Newborns with complete data on maternal smoking status and alcohol consumption, thyroid hormone levels, maternal urinary biomarker concentrations and other adjustment factors.

Abbreviations: BPA: bisphenol A, TCS: triclosan, MnBP: mono-n-butyl phthalate (metabolite of Di-n-butyl phthalate), MBzP: mono benzyl phthalate (metabolite of benzybutyl phthalate), T4: total thyroxine, TSH: thyroid stimulating hormone.

Adjustment factors: Age of child, type of delivery, child sex, parity, maternal age, maternal education level, vitamin use, age of mother and simple gravity.

CHAPTER 5: GENERAL DISCUSSION

5 General discussion

The goal of this chapter is to put the topics of this PhD thesis into general context. We give a brief summary of our main findings and their relevance to public health, and then discuss some methodological issues and how they were overcome, as well as present recommendations for further research.

5.1 Summary of findings, consistency with the literature and relevance to public health

5.1.1 Use of personal care products and phenol urinary concentrations during pregnancy

In chapter 2, we assessed how use of personal care products (PCP) influenced urinary concentrations of four parabens, bisphenol A and S, two dichlorophenols, triclosan, and benzophenone-3. We characterized use of PCPs as the total number of PCP applications as well as the use of specific PCPs (yes / no) in shorter time windows than in former studies (0 to 6, 6 to 12 and 12 to 24 hours before each urine sample collection). This was essential, given the short half-life of the studied compounds (< 24 hours)³³⁻³⁹. Studies that considered longer time windows only were more likely to miss the rise in urinary concentrations following PCP use^{35,67}, leading to dilution of the associations.

In line with previous studies, we observed that the use of many^{59,62} or specific PCPs was associated with a short-term increase in urinary concentration of parabens (methylparaben, ethylparaben, propylparaben and butylparaben)^{61,62,64-66}. This further emphasized that the use of PCPs is indeed a source of exposure to these compounds. Colored cosmetics and creams or lotions were consistent across studies as positively associated with the parabens' urinary concentrations in studies of pregnant women in north America^{61,62,64,66} and Europe⁶⁵.

Due to increasing public awareness and stricter regulations on use of endocrine disruptors in personal care products, some companies now advertise products as “low chemical” or paraben -free”.

Our analysis revealed that between 2012 and 2013, PCPs were still contributors to individual exposure to parabens, a family of broad-spectrum preservatives. Cosmetic products are regulated at European level through Cosmetic Regulation (EC) No 1223/2009 “to ensure consumer safety and the integrity of the internal market”. When mixed with other esters, parabens are by regulation limited to 0.4% of the weight of the product, while in singular use,

the limit is 0.8%. Although considered safe by the Scientific Committee on Consumer Safety, parabens are reported to demonstrate estrogenic²⁸⁶, antiandrogenic²⁸⁷ and thyroid disruption¹⁵¹ effects. In terms of adverse health outcomes in humans, a recent study reported that methylparaben was associated with a lower mental developmental index among girls⁸⁹ while another study suggested prenatal exposure to ethylparaben was associated with an increased asthma rate among boys²⁸⁸. Meeker *et al.*, also reported that exposure to butylparaben was associated with sperm DNA damage²⁸⁹.

Although our study focused on exposure from personal care products or cosmetics, it is also important to note that parabens are also allowed in pre-packed foods and drugs with a group acceptable daily intake of 10 mg/kg body weight per day³², if one seeks to reduce exposure to these chemicals.

This study further revealed that personal care products are likely sources of bisphenol S, while we did not observe any evidence of PCP-induced exposure to bisphenol A. Bisphenols are usually not directly used in PCPs formulation²⁹⁰ but are likely to migrate from PCP packaging. Recent PCP survey studies in China and America found that, second to bisphenol F, bisphenol S was one of the most commonly detected bisphenol substitute in PCPs^{54,71}. This is the first epidemiological study to consider bisphenol S in relation to PCP use.

To date, bisphenol A substitutes are not very well identified or regulated. For example, bisphenols F and S are not included in Annex VI of the Classification, Labelling and Packaging Regulation, a regulation that mandates manufacturers, importers or downstream users of substances or mixtures to classify, label and package their hazardous chemicals appropriately before placing them on the market (regulation described in Annex 7.2), and therefore do not have harmonized labeling at EU level. In Europe, bisphenol S is allowed in plastics and articles intended to come into contact with food, with a migration rate of up to 0.05 mg / kg of food (Commission Regulation 10/2011).

Bisphenol S is not a safer alternative to bisphenol A. A review of toxicological literature revealed that this compound has somewhat similar endocrine disrupting effects and systemic endpoints to its predecessor bisphenol A (illustrated in Figure 5.1)²¹¹.

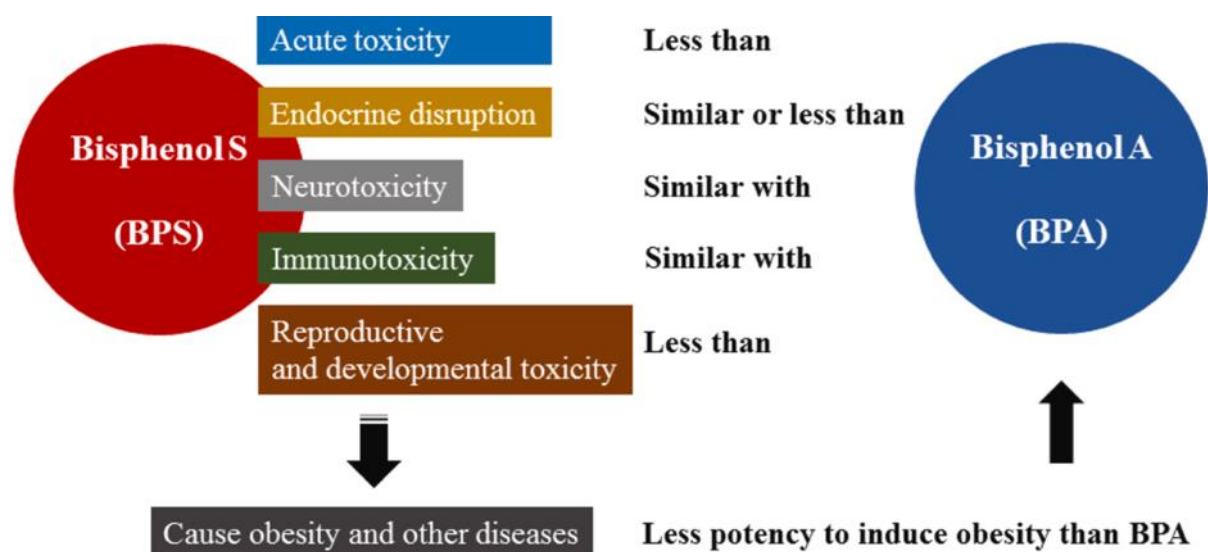


Figure 5.1: Potential toxicities of bisphenol S compared with bisphenol A (Adapted from Qiu *et al.*,²¹¹)

5.1.2 In-utero exposure to phenols, phthalates and child neurodevelopment and disruption of the thyroid axis as a mechanism of neurotoxicity

The growing burden of neurodevelopmental disorders among children globally is a matter of public health importance. It calls for a clear understanding of the modifiable underlying environmental risk factors (chapter 3 of the thesis) and their potential mechanisms of action (chapter 4).

Experimental animal models demonstrated that the growing fetal brain is sensitive to gestational exposure to varying concentrations of phenols and phthalates, even at human exposure levels^{75,81}.

Assessment of neurodevelopmental effects of gestational exposure to phenols and phthalates in humans is a relatively new but rapidly growing field, with the first birth cohort studies results published in 2010^{82,83,291,292}. Previous epidemiological studies mainly focused on phthalates and bisphenol A^{82,83}; as of 2018, when we published the manuscripts presented in chapter 3, no study had considered other phenols such as benzophenone-3, triclosan, parabens and dichlorophenols, even though they are potential EDs^{79,286,293,294} with some neurodevelopmental effects reported in animal studies^{72,262}. Additionally, although studies of phthalates overall covered both the cognitive and behavioral domains, studies of bisphenol A mostly focused on the behavioral domain of neurodevelopment. Stratification for sex in various studies revealed that adverse effects observed may differ between male and female births, with possibly clearer associations among boys in association with bisphenol A exposure.

In the scope of this PhD, we covered two main neurodevelopmental domains, cognition and behaviors, related to intellectual and adaptive behavior disabilities, respectively. We assessed associations between maternal urinary concentrations of 9 phenols and 11 phthalate metabolites with IQ and behavior of boys at 3 and 5 years, in a relatively large sample (N= 452 to 546 boys). None of the compounds assessed showed a significant negative association with the boys' verbal or performance IQ. Regarding behavior, bisphenol A, triclosan and two phthalate metabolites (MnBP, MBzP) were adversely associated with behaviors on the internalizing or externalizing spectrums among boys at 3 and (or) 5 years.

In relation to prenatal exposure to bisphenol A, results from this study and from past mother-child studies suggested no significant adverse effects on the IQ of boys between 3 and 6 years^{44,84,88}. However, when Tanner *et al.*, assessed effects of gestational exposure to a mixture of 26 endocrine disrupting compounds on child IQ, including bisphenol A, they found a decrease in full scale IQ of children at 7 years²⁹⁵. This study did not assess the effect of bisphenol A independently as in our study. When considering postnatal exposure (not assessed in our work because no urine sample was collected in early childhood in the EDEN cohort), two studies reported that childhood bisphenol A exposure was associated with a decrease of IQ in boys between age of 5 and 8 years^{84,88,68,72,68,72}, while another study did not find any associations²⁹⁶. Exposure data in early postnatal life is available in the SEPAGES cohort and this may allow to further consider this question in the future.

Our findings in regards to effects of bisphenol A on child behavior were consistent with most of the previous studies that also reported adverse effects mainly among boys¹⁰⁰⁻¹⁰³ rather than girls (Table 1.5).

We observed (in chapter 4) that bisphenol A was positively associated with neonatal TT4 and negatively associated with maternal TSH. The increase in maternal TSH was to our knowledge only previously reported by Aung *et al*¹⁶⁷, among six studies that assessed maternal TSH levels in relation to bisphenol A exposure. TSH is regulated via a negative feedback, with low TSH levels possibly resulting from an increase in thyroid hormones levels. High levels of thyroid hormones during pregnancy have been linked to an increased risk for behavioral disorders^{130,131} and lower IQ¹²⁵. This suggests that, if bisphenol A indeed increases thyroid hormone levels and consequently decreases TSH levels, this could be a plausible pathway through which this compound could disrupt child behavior.

We were not able to perform mediation analysis in the framework of this PhD because chapter 2 (endocrine disruptors and behavior) and chapter 3 (endocrine disruptors and thyroid

hormones) were not based on the same study population (EDEN versus SEPAGES cohorts), but such an analysis may be undertaken in the future in SEPAGES cohort as behavioral assessment results in childhood become available.

In the EU, bisphenol A is banned from food contact materials for children aged until three years. In 2015, this compound was also banned from food contact materials (Table 1.2). The European Chemicals Agency recognized bisphenol A as a substance of very high concern, but has yet to take action to add this compound to the REACH Annex XIV list, a list of substances that cannot be placed on the market or used unless an authorization is granted for their specific use ³².

Effects of triclosan on child behavior reported in this study have only been replicated in one recently published study ⁸⁷. Triclosan was recently found to contribute significantly (weight contribution = 9% of the weighted quantile sum index) to a decrease in IQ among seven year old children exposed to a cocktail of 26 endocrine disruptors ²⁹⁵. We did not observe such associations with IQ in our work as we considered this compound individually.

Regarding the association with thyroid hormones and TSH, in the SEPAGES cohort, triclosan was non-monotonously associated (U-shape) associated with TSH and the TT3 to TT4 ratio during pregnancy, as well as with decreased TT4 levels at birth among girls. As a basis of newborn screening programs, low TT4 (or high TSH levels (congenital hypothyroidism)) has been associated with downstream neurodevelopmental effects ranging from decreased functioning on epidemiological scales to clinical disorders ²⁹⁷. Triclosan exposure is ubiquitous among pregnant women in Europe ²⁹⁸ and is still allowed, with some restrictions, in various cosmetics (Table 1.2).

In our study, MnBP and MBzP, metabolites DBP and BBP respectively, were associated with adverse behaviors but not IQ. Consistent with our study, Whyatt *et al.*, reported higher emotional problems among boys in relation to DBP exposure, while 5 other studies that assessed this compound did not suggest any associations among males ^{105–108,299}. MBzP was associated with increased scores on the internalizing behaviors and relationship problems subscales of boys. None of the six studies previous studies found similar findings among boys ^{97,105–107,268,299}, except one that found associations with another scale (conduct problems) among boys ¹⁰⁵.

When studying associations of these two phthalate metabolites with thyroid hormone levels during pregnancy and at birth, we found that MnBP portrayed a non-monotonic (inverse U-shape) association with maternal TSH and a positive association with newborn TSH. MBzP

was only associated with an increase in maternal TT4. Previous studies of pregnant women found that higher levels of maternal TSH¹²⁸ or TT4^{130,131} during pregnancy predicted adverse neurobehavioral outcomes later in childhood, independent of diagnosis of a maternal thyroid dysfunction, suggesting that this could be a mechanism through which these compounds affect behavioral programming.

DBP is a low molecular weight phthalate, likely to be used as a solvent in cosmetics (Table 1.1), while BBP is a high molecular weight compound, likely to be used as a plasticizer (Table 1.1). DBP and BBP are listed in the REACH annex XVII and require authorization before use in some products³². Other restrictions include use in food contact materials pediatric / neonatal / maternity ward medical tubes and 2), and electrical and electronic equipment (regulations detailed in Annex 1). The European Union has prohibited, via European regulation 1223/2009, the placing on the market of cosmetics containing DBP or BBP (Table 1.2). However, in spite of these regulations, exposure to these compounds is still ubiquitous, especially among vulnerable populations (with 100% detection levels in pregnant women of SEPAGES, a more recent biomonitoring study in France)⁴⁷.

5.2 Methodological considerations

5.2.1 Multiple testing and type I error

In this thesis, various tests were performed in chapters 2 and 3. We made several efforts to limit chance findings. This was done by relying on both statistical methods (e.g., SEM and correction for false discovery rate, chapter 3) and prior knowledge (chapter 4)³⁰⁰.

In chapter 2 (PCP use), we approximately carried out 408 tests (8 phenols x 17 PCPs x 3 time windows excluding the dichlorophenols that were included into the analysis as a control). We opted not to correct for multiple testing because we had strong a priori knowledge, these compounds being previously detected in PCPs, except the 2 dichlorophenols studied as negative controls.

In chapter 3, we used Structural Equation Models³⁰¹ to limit the number of tests in the study of the associations of gestational exposure to phenols and phthalates with IQ of boys at 5 years. SEM permitted grouping of compounds from the same group (e.g., parabens) or metabolites from similar parent compounds (e.g., DEHP metabolites) as well as IQ subscores (six subscales of IQ were grouped into verbal and performance IQ) through latent variables. Use of latent variables lowered the number of associations tested, compared to the classical approaches that considered one regression model per outcome and exposure (from 46 to 26 tests). Furthermore, because Structural Equation Models set an error rate per parameter (ERPP) tested³⁰², we used the Benjamini and Hochberg false discovery rate method to control for multiple testing³⁰³. This method defines a new significance level taking into account the number of tests performed³⁰³ while providing a compromise between power and reduction of type I error.

In chapter 4, assessing the effects of phenols and phthalates on thyroid function, we used an a priori based approach to limit the number of associations tested. Using prior information from the thyroid disruption screening carried out by the ToxCast program^{146,149,159,160,304} and a published adverse outcome pathway for the thyroid function³⁰⁴, we a priori selected four compounds bisphenol A, triclosan, DBP and BBP among the 27 assessed in SEPAGES, to be included in our main analysis.

5.2.2 Selection bias and generalizability

In chapter 2, our study included only eight women of the SEPAGES-feasibility study that collected the majority of their urine samples (95%) over the three weeks of follow up¹⁸⁶. Given the intensive protocol, it is likely that highly educated women with interest in science or with a health issue were more likely to participate. Such women may have specific behaviors in terms of PCP use, and might for example be more likely to use PCPs labeled as organic or not containing specific chemicals suspected to be harmful, as varying patterns of behaviors pertaining to use of personal care products have been reported among women of different social economic status³⁰⁵. For this matter, results from this study cannot be generalized to the population of pregnant women in France nor the Grenoble area.

Additionally, both SEPAGES and EDEN recruited more highly educated women than the French population of pregnant women^{46,191}. Over representation of highly-educated participants is common in environmental health cohorts. This might be a problem if the original goal was to assess exposure across social-economic classes, which was not the case here. Lack of representativeness is generally not a limitation in etiologic studies^{282,283}, as no effect is expected on the dose-response relationships. Focusing on homogeneous samples instead lowers risk of confounding bias from between-subject socio-economic differences.

The analysis based on the EDEN cohort only included boys; for that matter, results from this study cannot be generalized to females, but this not a source of bias.^{97,217,219,220}. One perk of this choice was that for a given budget, we had a larger sex-specific sample size, and in turn a higher statistical power compared to two sex-stratified analyses, each with have half the sample size of our analysis.

5.2.3 Confounding bias

A confounding factor can be defined as being associated with both the exposure and the studied outcome without being affected by them³⁰⁶. In this thesis, in order to reduce confounding bias, we relied on regressions adjusted for confounders that we identified a priori.

To more accurately account for confounding, we carefully considered their coding so as to limit residual confounding arising from coding of covariates³⁰⁷. In the case of quantitative potential confounders, we explored associations using different coding approaches to identify potential non-linear relations, as illustrated in Figure 5.2 below for the relation between maternal age and gestational TSH levels.

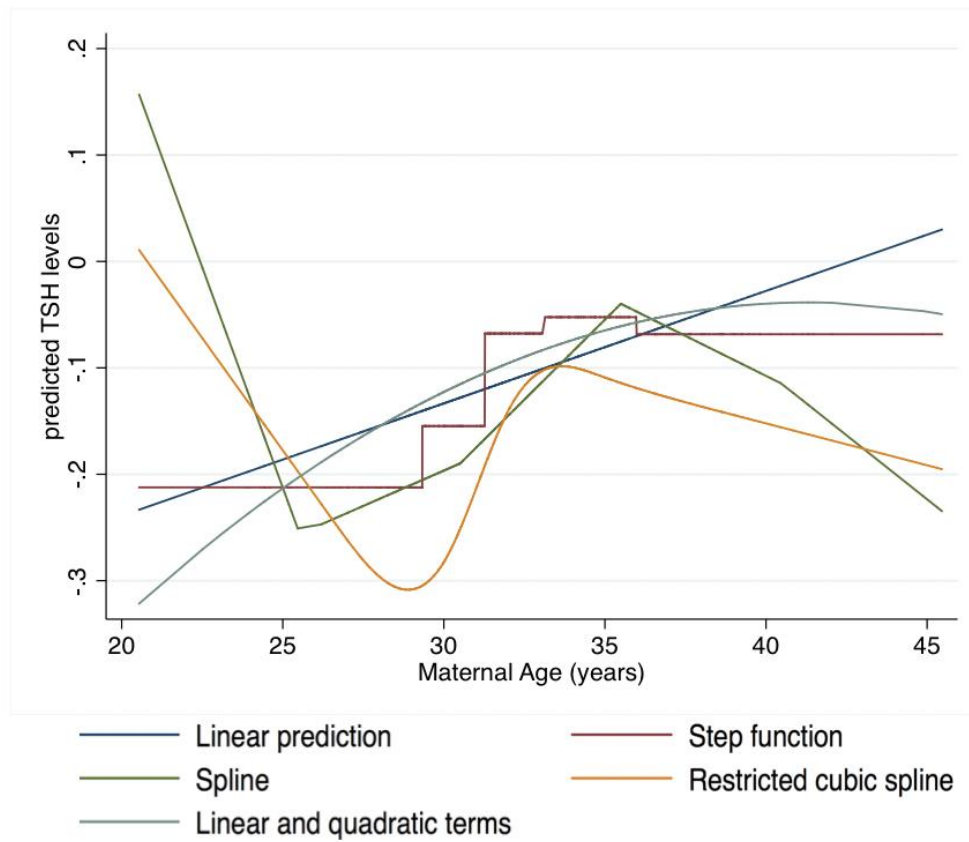


Figure 5.2 Multivariate models exploring various coding of maternal age in association with TSH pregnancy levels (N=416 women from SEPAGES cohort). All models were adjusted for maternal age, gestational age at serum sample collection, pre-pregnancy BMI and education level.

Despite the measures mentioned above, residual confounding from unmeasured variables cannot be ruled out. In this study we focused on phenols and phthalates, while other chemicals might affect child neurodevelopment²⁶ or thyroid function¹⁸¹. A previous study that assessed exposure to 87 environmental factors among women from 6 European countries did not highlight strong correlations across environmental exposure groups (illustrated in Figure 5.3 below)³⁰⁸.

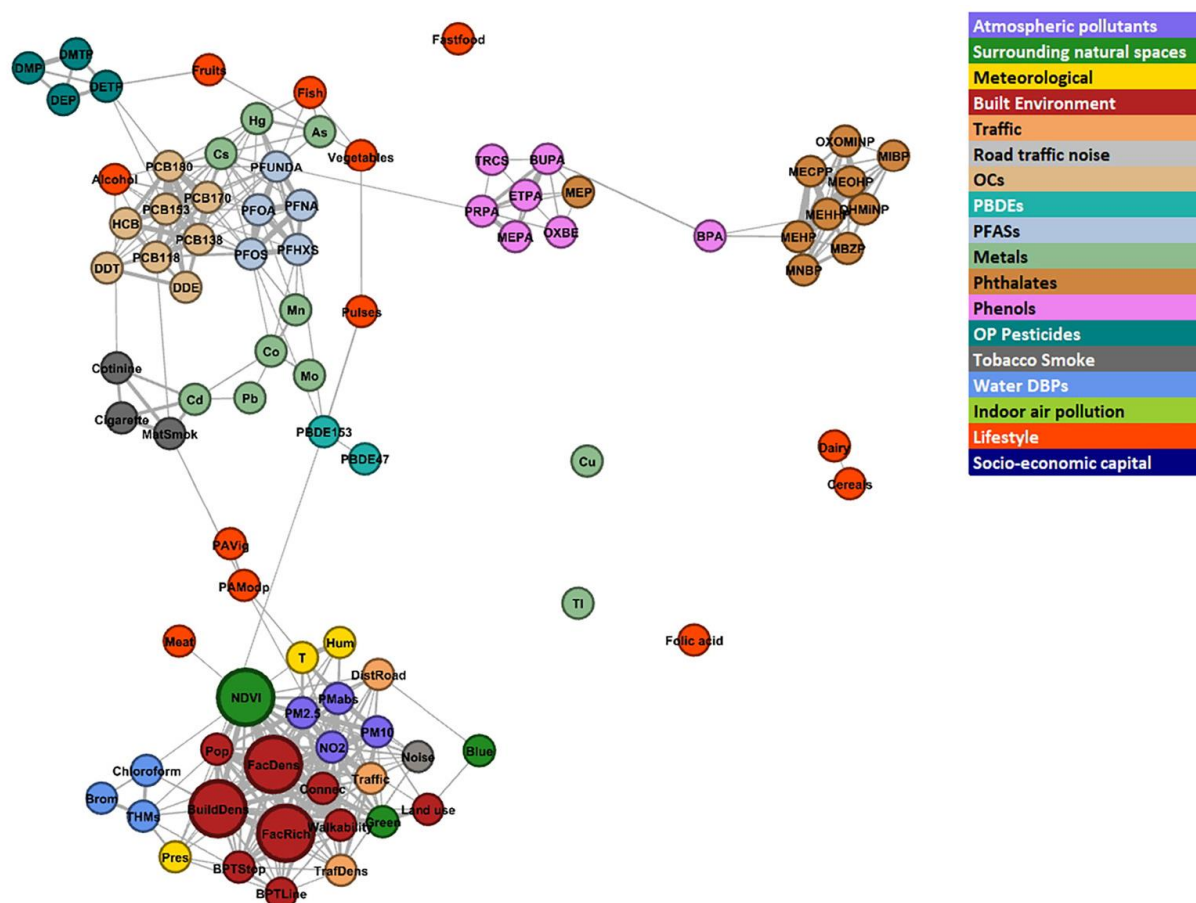


Figure 5.3: Correlations across environmental exposures in the Human Early-Life Exposome (HELIX) project, in which 87 environmental exposures during pregnancy (grouped in 19 exposure groups) were assessed in 1301 pregnant mothers in six European birth cohorts.

The size of the nodes is proportional to the number of correlations were > 0.5 outside the exposure group and the length of the edges is proportional to the inverse of the correlation (the higher the correlation, the shorter the edge length) between exposures³⁰⁸.

In chapter 2, the SEPAGES-feasibility study relied on within-subject contrasts other than between-subject contrasts. Such studies are less prone to confounding bias (for example due to differences between subjects using and those not using specific PCPs). However since the concentrations of phenols vary widely intra-individually, we adjusted for factors that could affect this temporal variability.

5.2.4 Measurement error

In chapter 3, we relied on the strengths and difficulties questionnaire (SDQ) completed by the mothers to assess behavioral outcomes of boys. This assessment tool is subject to recall or reporter bias (with questions such as “*often complains of headaches*”, “*Nervous or clingy in new situations*”), depending on the mothers’ or caretakers’ ability to recall past events³⁰⁶. In developmental psychopathology, use of multiple informants is usually recommended as each observant contributes unique information about internalizing and externalizing behavior problems³⁰⁹. This tool can be completed by either parents or teachers. In EDEN, it was only administered to parents²⁴⁸.

In the same chapter, our assessment of exposure was based on a single spot urine sample collected during pregnancy. Relying on the same study population as in chapter 2, Vernet *et al.*, demonstrated that in the context of non-persistent compounds, a spot sample was inadequate at estimating exposure over long time windows¹⁸⁶ such as the pregnancy, as well as generally for shorter time windows. The expected structure of measurement error is of classical type and is expected to lead to attenuation bias in the effect estimate and to power loss⁴², which may partly explain our null findings¹⁸⁹. In chapter 4, we attempted to overcome this limitation by relying on pools of 21 samples per participant collected in two weeks of the pregnancy. Another solution to this limitation could be assessment of biomarkers in other matrices such as hair that would aggregate exposure over longer time period. Beranger *et al.*, assessed exposure to non-persistent pesticides and observed lower intra-individual variability across segments of individual bundles of hair strands³¹⁰ compared to assessment in urine.

5.3 Conclusion and perspectives

The growing burden of neurodevelopmental disorders among children reported in several areas of the world^{5,7,10,11} is a matter of public health importance, so that identifying modifiable environmental risk factors influencing neurodevelopment is a relevant aim for public health.

This PhD relied on complementary cohorts to assess if prenatal exposure to select phenols and phthalates could be associated with neurodevelopmental dysfunction during childhood and underlying biological mechanisms i.e. disruption of the thyroid hormone axis. We further characterized the role of use of personal care products in exposure to these phenols during pregnancy.

Findings in this study concur with previous literature indicating that PCPs are sources of exposure to parabens; they also suggest that PCPs could contribute to bisphenol S exposure. This is the first study to report such an association between PCP use and bisphenol S urinary concentrations. Larger epidemiological studies are needed to confirm this finding. One has however to keep in mind the cost of such studies, which may have to rely on thousands of chemical assays (our study relied on 178 assays of 10 phenolic compounds for 8 women) to allow finer information on phenol concentrations in urine. In addition or alternatively, the constitution of studies or databases documenting the composition and contamination of PCPs in France and in the EU would help to answer this question.

In line with previous studies, we reported that bisphenol A and various phthalates, including DBP and BBP exposures during pregnancy, could be associated with behavioral changes among boys. At the start of this thesis, triclosan neurodevelopmental effects had not been assessed in human studies. Our study suggested that this compound could possibly affect neurobehavior of boys, a finding recently replicated by Jackson-Browne *et al.*, among boys⁸⁷. Our results did not suggest any adverse association between phenols or phthalates and the IQ of boys in the EDEN cohort, which is consistent with the literature, nor effects of parabens on neurodevelopment. The null associations observed with cognition could be due to inadequacy in assessment of exposure or assessment in a non-toxicologically-relevant window, as negative associations have been reported in relation to childhood exposures to some of the compounds we considered⁸⁸ and at birth³¹¹. Furthermore, adverse neurodevelopmental effects among girls were reported in other studies (Table 1.5 and Table 1.6), but we only considered boys.

Future studies on this topic should consider use of multiple urine samples per individual to assess exposure during pre and post-natal life among boys and girls. All the above shortcomings are set to be corrected in the SEPAGES study described in chapter 4. The SEPAGES participants provided multiple samples which were pooled for exposure quantification. Assessment of childhood exposures was also carried out and neurodevelopment will be assessed during this window including assessment of effects in girls.

All four compounds (bisphenol A, triclosan, DBP and BBP metabolites) associated with behavior of boys in EDEN were associated with one or more thyroid hormone levels of mothers and newborns in the SEPAGES cohort, suggesting possible disruption of thyroid homeostasis during pregnancy. Following results from this PhD, we hypothesize that the effects of bisphenol A, triclosan, DBP and BBP metabolites on neurodevelopment could be mediated by disruption of thyroid function during pregnancy. Future studies with information on gestational exposure to these four compounds, thyroid hormone levels during pregnancy, and neurodevelopmental outcomes in the same study sample, could focus on assessing the extent to which effects of these compounds on neurodevelopment could be mediated by their effects on thyroid hormone homeostasis. In the SEPAGES cohort, the information necessary for this mediation analysis is available (exposure data and thyroid hormone levels) or scheduled to be collected (neurobehavioural data), therefore this analysis will be carried out in the future.

REFERENCES

6 References

- (1) Rice, D.; Barone, S. Critical Periods of Vulnerability for the Developing Nervous System: Evidence from Humans and Animal Models. *Environmental health perspectives* **2000**, *108 Suppl*, 511–33.
- (2) Rodier, P. M. Vulnerable Periods and Processes during Central Nervous System Development. *Environ. Health Perspect.* **1994**, *102 Suppl 2*, 121–124. <https://doi.org/10.1289/ehp.94102121>.
- (3) Knuesel, I.; Chicha, L.; Britschgi, M.; Schobel, S. A.; Bodmer, M.; Hellings, J. A.; Toovey, S.; Prinssen, E. P. Maternal Immune Activation and Abnormal Brain Development across CNS Disorders. *Nature Reviews Neurology* **2014**, *10*, 643–660. <https://doi.org/10.1038/nrneurol.2014.187>.
- (4) Neurodevelopmental Disorders. In *Diagnostic and Statistical Manual of Mental Disorders; DSM Library; American Psychiatric Association*, 2013. <https://doi.org/10.1176/appi.books.9780890425596.dsm01>.
- (5) Bloom, B.; Cohen, R.; Freeman, G. *Summary Health Statistics for U.S. Children: National Health Interview Survey, 2009*; 2009.
- (6) van Bakel, M. M. E.; Delobel-Ayoub, M.; Cans, C.; Assouline, B.; Jouk, P.-S.; Raynaud, J.-P.; Arnaud, C. Low but Increasing Prevalence of Autism Spectrum Disorders in a French Area from Register-Based Data. *Journal of autism and developmental disorders* **2015**, *45* (10), 3255–61. <https://doi.org/10.1007/s10803-015-2486-6>.
- (7) Zablotsky, B.; Black, L. I.; Maenner, M. J.; Schieve, L. A.; Danielson, M. L.; Bitsko, R. H.; Blumberg, S. J.; Kogan, M. D.; Boyle, C. A. Prevalence and Trends of Developmental Disabilities among Children in the United States: 2009–2017. *Pediatrics* **2019**, *144* (4), e20190811. <https://doi.org/10.1542/peds.2019-0811>.
- (8) Matson, J. L.; Kozlowski, A. M. The Increasing Prevalence of Autism Spectrum Disorders. *Research in Autism Spectrum Disorders* **2011**, *5* (1), 418–425. <https://doi.org/10.1016/j.rasd.2010.06.004>.
- (9) Xu, G.; Strathearn, L.; Liu, B.; Yang, B.; Bao, W. Twenty-Year Trends in Diagnosed Attention-Deficit/Hyperactivity Disorder Among US Children and Adolescents, 1997-2016. *JAMA Netw Open* **2018**, *1* (4), e181471. <https://doi.org/10.1001/jamanetworkopen.2018.1471>.
- (10) Elsabbagh, M.; Divan, G.; Koh, Y.-J.; Kim, Y. S.; Kauchali, S.; Marcín, C.; Montiel-Nava, C.; Patel, V.; Paula, C. S.; Wang, C.; et al. Global Prevalence of Autism and Other Pervasive Developmental Disorders. *Autism Res* **2012**, *5* (3), 160–179. <https://doi.org/10.1002/aur.239>.
- (11) Thomas, R.; Sanders, S.; Doust, J.; Beller, E.; Glasziou, P. Prevalence of Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-Analysis. *Pediatrics* **2015**, *135* (4), e994–e1001. <https://doi.org/10.1542/peds.2014-3482>.
- (12) King, M.; Bearman, P. Diagnostic Change and the Increased Prevalence of Autism. *Int J Epidemiol* **2009**, *38* (5), 1224–1234. <https://doi.org/10.1093/ije/dyp261>.
- (13) Hertz-Picciotto, I.; Delwiche, L. The Rise in Autism and the Role of Age at Diagnosis. *Epidemiology* **2009**, *20* (1), 84–90. <https://doi.org/10.1097/EDE.0b013e3181902d15>.
- (14) Hansen, S. N.; Schendel, D. E.; Parner, E. T. Explaining the Increase in the Prevalence of Autism Spectrum Disorders: The Proportion Attributable to Changes in Reporting Practices. *JAMA Pediatr* **2015**, *169* (1), 56–62. <https://doi.org/10.1001/jamapediatrics.2014.1893>.
- (15) Nassar, N.; Dixon, G.; Bourke, J.; Bower, C.; Glasson, E.; De Klerk, N.; Leonard, H. Autism Spectrum Disorders in Young Children: Effect of Changes in Diagnostic Practices. *International journal of epidemiology* **2009**, *38* (5), 1245–1254.
- (16) Gustavsson, A.; Svensson, M.; Jacobi, F.; Allgulander, C.; Alonso, J.; Beghi, E.; Dodel, R.; Ekman, M.; Faravelli, C.; Fratiglioni, L.; et al. Cost of Disorders of the Brain in Europe 2010. *Eur Neuropsychopharmacol* **2011**, *21* (10), 718–779. <https://doi.org/10.1016/j.euroneuro.2011.08.008>.
- (17) Braun, J. M. Early Life Exposure to Endocrine Disrupting Chemicals and Childhood Obesity and Neurodevelopment. *Nat Rev Endocrinol* **2017**, *13* (3), 161–173. <https://doi.org/10.1038/nrendo.2016.186>.

- (18) Forns, J.; Aranbarri, A.; Grellier, J.; Julvez, J.; Vrijheid, M.; Sunyer, J. A Conceptual Framework in the Study of Neuropsychological Development in Epidemiological Studies. *Neuroepidemiology* **2012**, *38* (4), 203–208. <https://doi.org/10.1159/000337169>.
- (19) van Loo, K. M. J.; Martens, G. J. M. Genetic and Environmental Factors in Complex Neurodevelopmental Disorders. *Current genomics* **2007**, *8* (7), 429–444. <https://doi.org/10.2174/138920207783591717>.
- (20) Sandin, S.; Lichtenstein, P.; Kuja-Halkola, R.; Larsson, H.; Hultman, C. M.; Reichenberg, A. The Familial Risk of Autism. *JAMA* **2014**, *311* (17), 1770–1777. <https://doi.org/10.1001/jama.2014.4144>.
- (21) Penagarikano, O.; Mulle, J. G.; Warren, S. T. The Pathophysiology of Fragile x Syndrome. *Annu Rev Genomics Hum Genet* **2007**, *8*, 109–129. <https://doi.org/10.1146/annurev.genom.8.080706.092249>.
- (22) Ghosh, S.; Feingold, E.; Dey, S. K. Etiology of Down Syndrome: Evidence for Consistent Association among Altered Meiotic Recombination, Nondisjunction, and Maternal Age across Populations. *Am. J. Med. Genet. A* **2009**, *149A* (7), 1415–1420. <https://doi.org/10.1002/ajmg.a.32932>.
- (23) Schroeder, S. R. Mental Retardation and Developmental Disabilities Influenced by Environmental Neurotoxic Insults. *Environmental Health Perspectives* **2000**, *108* (Suppl 3), 395. <https://doi.org/10.1289/ehp.00108s3395>.
- (24) Grandjean, P.; Landrigan, P. J. Developmental Neurotoxicity of Industrial Chemicals. *The Lancet* **2006**, *368* (9553), 2167–2178. [https://doi.org/10.1016/S0140-6736\(06\)69665-7](https://doi.org/10.1016/S0140-6736(06)69665-7).
- (25) Rauh, V. A.; Margolis, A. Research Review: Environmental Exposures, Neurodevelopment and Child Mental Health – New Paradigms for the Study of Brain and Behavioral Effects. *J Child Psychol Psychiatry* **2016**, *57* (7), 775–793. <https://doi.org/10.1111/jcpp.12537>.
- (26) Grandjean, P.; Landrigan, P. J. Neurobehavioural Effects of Developmental Toxicity. *The Lancet. Neurology* **2014**, *13* (3), 330–338. [https://doi.org/10.1016/S1474-4422\(13\)70278-3](https://doi.org/10.1016/S1474-4422(13)70278-3).
- (27) Haug, L. S.; Sakhi, A. K.; Cequier, E.; Casas, M.; Maitre, L.; Basagana, X.; Andrusaityte, S.; Chalkiadaki, G.; Chatzi, L.; Coen, M.; et al. In-Utero and Childhood Chemical Exposome in Six European Mother-Child Cohorts. *Environ Int* **2018**, *121* (Pt 1), 751–763. <https://doi.org/10.1016/j.envint.2018.09.056>.
- (28) Hauser, R.; Calafat, A. M. Phthalates and Human Health. *Occup Environ Med* **2005**, *62* (11), 806–818. <https://doi.org/10.1136/oem.2004.017590>.
- (29) Lintelmann, J.; Katayama, A.; Kurihara, N.; Shore, L.; Wenzel, A. Endocrine Disruptors in the Environment (IUPAC Technical Report). *Pure and Applied Chemistry* **2003**, *75* (5), 631–681. <https://doi.org/10.1351/pac200375050631>.
- (30) Rudel, R. A.; Perovich, L. J. Endocrine Disrupting Chemicals in Indoor and Outdoor Air. *Atmospheric environment (Oxford, England: 1994)* **2009**, *43* (1), 170–181. <https://doi.org/10.1016/j.atmosenv.2008.09.025>.
- (31) EC Regulation, C. Amending Annexes II and V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on Cosmetic Products. In 358/2014. *Official Journal of the European Union* **2014**.
- (32) Demeneix, B.; Slama, R. *Endocrine Disruptors: From Scientific Evidence to Human Health Protection*; Study PE 608.866; European Parliament, 2019; p 132.
- (33) Janjua, N. R.; Frederiksen, H.; Skakkebaek, N. E.; Wulf, H. C.; Andersson, A.-M. Urinary Excretion of Phthalates and Paraben after Repeated Whole-Body Topical Application in Humans. *Int. J. Androl.* **2008**, *31* (2), 118–130. <https://doi.org/10.1111/j.1365-2605.2007.00841.x>.
- (34) Janjua, N. R.; Mortensen, G. K.; Andersson, A.-M.; Kongshoj, B.; Skakkebaek, N. E.; Wulf, H. C. Systemic Uptake of Diethyl Phthalate, Dibutyl Phthalate, and Butyl Paraben Following Whole-Body Topical Application and Reproductive and Thyroid Hormone Levels in Humans. *Environ. Sci. Technol.* **2007**, *41* (15), 5564–5570.
- (35) Koch, H. M.; Lorber, M.; Christensen, K. L. Y.; Pålmeke, C.; Koslitz, S.; Brüning, T. Identifying Sources of Phthalate Exposure with Human Biomonitoring: Results of a 48h Fasting Study with Urine Collection and Personal Activity Patterns. *International Journal of Hygiene and Environmental Health* **2013**, *216* (6), 672–681. <https://doi.org/10.1016/j.ijheh.2012.12.002>.

- (36) Ye, X.; Kuklennyik, Z.; Needham, L. L.; Calafat, A. M. Quantification of Urinary Conjugates of Bisphenol A, 2,5-Dichlorophenol, and 2-Hydroxy-4-Methoxybenzophenone in Humans by Online Solid Phase Extraction-High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Analytical and bioanalytical chemistry* **2005**, 383 (4), 638–44. <https://doi.org/10.1007/s00216-005-0019-4>.
- (37) Queckenberg, C.; Meins, J.; Wachall, B.; Doroshenko, O.; Tomalik-Scharte, D.; Bastian, B.; Abdel-Tawab, M.; Fuhr, U. Absorption, Pharmacokinetics, and Safety of Triclosan after Dermal Administration. *Antimicrobial Agents and Chemotherapy* **2010**, 54 (1), 570–572. <https://doi.org/10.1128/AAC.00615-09>.
- (38) Völkel, W.; Colnot, T.; Csanády, G. A.; Filser, J. G.; Dekant, W. Metabolism and Kinetics of Bisphenol A in Humans at Low Doses Following Oral Administration. *Chem. Res. Toxicol.* **2002**, 15 (10), 1281–1287. <https://doi.org/10.1021/tx025548t>.
- (39) Janjua, N. R.; Mogensen, B.; Andersson, A.-M.; Petersen, J. H.; Henriksen, M.; Skakkebaek, N. E.; Wulf, H. C. Systemic Absorption of the Sunscreens Benzophenone-3, Octyl-Methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor after Whole-Body Topical Application and Reproductive Hormone Levels in Humans. *J. Invest. Dermatol.* **2004**, 123 (1), 57–61. <https://doi.org/10.1111/j.0022-202X.2004.22725.x>.
- (40) Casas, M.; Chevrier, C.; Hond, E. Den; Fernandez, M. F.; Pierik, F.; Philippat, C.; Slama, R.; Toft, G.; Vandentorren, S.; Wilhelm, M.; et al. Exposure to Brominated Flame Retardants, Perfluorinated Compounds, Phthalates and Phenols in European Birth Cohorts: ENRIECO Evaluation, First Human Biomonitoring Results, and Recommendations. *International Journal of Hygiene and Environmental Health* **2013**, 216 (3), 230–242. <https://doi.org/10.1016/j.ijheh.2012.05.009>.
- (41) Philippat, C.; Wolff, M. S.; Calafat, A. M.; Ye, X.; Bausell, R.; Meadows, M.; Stone, J.; Slama, R.; Engel, S. M. Prenatal Exposure to Environmental Phenols: Concentrations in Amniotic Fluid and Variability in Urinary Concentrations during Pregnancy. *Environmental Health Perspectives* **2013**, 121 (10). <https://doi.org/10.1289/ehp.1206335>.
- (42) Jensen, M. S.; Nørgaard-Pedersen, B.; Toft, G.; Hougaard, D. M.; Bonde, J. P.; Cohen, A.; Thulstrup, A. M.; Ivell, R.; Anand-Ivell, R.; Lindh, C. H.; et al. Phthalates and Perfluorooctanesulfonic Acid in Human Amniotic Fluid: Temporal Trends and Timing of Amniocentesis in Pregnancy. *Environmental health perspectives* **2012**, 120 (6), 897–903. <https://doi.org/10.1289/ehp.1104522>.
- (43) Chou, W.-C.; Chen, J.-L.; Lin, C.-F.; Chen, Y.-C.; Shih, F.-C.; Chuang, C.-Y. Biomonitoring of Bisphenol A Concentrations in Maternal and Umbilical Cord Blood in Regard to Birth Outcomes and Adipokine Expression: A Birth Cohort Study in Taiwan. *Environmental health : a global access science source* **2011**, 10, 94. <https://doi.org/10.1186/1476-069X-10-94>.
- (44) Nakiwala, D.; Peyre, H.; Heude, B.; Bernard, J. Y.; Béranger, R.; Slama, R.; Philippat, C. In-Utero Exposure to Phenols and Phthalates and the Intelligence Quotient of Boys at 5 Years. *Environ Health* **2018**, 17. <https://doi.org/10.1186/s12940-018-0359-0>.
- (45) Dereumeaux, C.; Fillol, C.; Charles, M.-A.; Denys, S. The French Human Biomonitoring Program: First Lessons from the Perinatal Component and Future Needs. *International Journal of Hygiene and Environmental Health* **2017**, 220 (2, Part A), 64–70. <https://doi.org/10.1016/j.ijheh.2016.11.005>.
- (46) Lyon-Caen, S.; Siroux, V.; Lepeule, J.; Lorimier, P.; Hainaut, P.; Mossuz, P.; Quentin, J.; Supernant, K.; Meary, D.; Chaperot, L.; et al. Deciphering the Impact of Early-Life Exposures to Highly Variable Environmental Factors on Foetal and Child Health: Design of SEPAGES Couple-Child Cohort. *Int J Environ Res Public Health* **2019**, 16 (20). <https://doi.org/10.3390/ijerph16203888>.
- (47) Rolland, M.; Lyon-Caen, S.; Sakhi, A. K.; Sabaredzovic, A.; Thomsen, C.; Slama, R.; Philippat, C. Exposure to Phenols during Pregnancy and the First Year of Life in a New Type of Mother-Child Cohort Relying on Repeated Urine Biospecimen Collection. **In preparation.**
- (48) Fillol, C.; Balicco, A.; Bidondo, M.-L.; Gane, J.; Oleko, A.; Saoudi, A.; Zeghnoun, A. *Imprégnation de La Population Française Par Les Parabènes; Programme National de Biosurveillance, Esteban 2014-2016*; ISSN: 2609-2174; Santé publique France: France, 2019; p 41 p.

- (49) Campioli, E.; Lee, S.; Lau, M.; Marques, L.; Papadopoulos, V. Effect of Prenatal DINCH Plasticizer Exposure on Rat Offspring Testicular Function and Metabolism. *Sci Rep* **2017**, *7*. <https://doi.org/10.1038/s41598-017-11325-7>.
- (50) Moon, M. K. Concern about the Safety of Bisphenol A Substitutes. *Diabetes Metab J* **2019**, *43* (1), 46–48. <https://doi.org/10.4093/dmj.2019.0027>.
- (51) Dodson, R. E.; Nishioka, M.; Standley, L. J.; Perovich, L. J.; Brody, J. G.; Rudel, R. A. Endocrine Disruptors and Asthma-Associated Chemicals in Consumer Products. *Environmental health perspectives* **2012**, *120* (7), 935–943. <https://doi.org/10.1289/ehp.1104052>.
- (52) Guo, Y.; Wang, L.; Kannan, K. Phthalates and Parabens in Personal Care Products From China: Concentrations and Human Exposure. *Archives of Environmental Contamination and Toxicology* **2014**, *66* (1), 113–119. <https://doi.org/10.1007/s00244-013-9937-x>.
- (53) Liao, C.; Kannan, K. Widespread Occurrence of Benzophenone-Type UV Light Filters in Personal Care Products from China and the United States: An Assessment of Human Exposure. *Environ. Sci. Technol.* **2014**, *48* (7), 4103–4109. <https://doi.org/10.1021/es405450n>.
- (54) Lu, S.; Yu, Y.; Ren, L.; Zhang, X.; Liu, G.; Yu, Y. Estimation of Intake and Uptake of Bisphenols and Triclosan from Personal Care Products by Dermal Contact. *Sci. Total Environ.* **2018**, *621*, 1389–1396. <https://doi.org/10.1016/j.scitotenv.2017.10.088>.
- (55) Shen, H.-Y.; Jiang, H.-L.; Mao, H.-L.; Pan, G.; Zhou, L.; Cao, Y.-F. Simultaneous Determination of Seven Phthalates and Four Parabens in Cosmetic Products Using HPLC-DAD and GC-MS Methods. *J Sep Sci* **2007**, *30* (1), 48–54.
- (56) Ferguson, K. K.; Colacino, J. A.; Lewis, R. C.; Meeker, J. D. Personal Care Product Use among Adults in NHANES: Associations between Urinary Phthalate Metabolites and Phenols and Use of Mouthwash and Sunscreen. *Journal of Exposure Science and Environmental Epidemiology* **2017**, *27* (3), 326–332. <https://doi.org/10.1038/jes.2016.27>.
- (57) Husøy, T.; Andreassen, M.; Hjertholm, H.; Carlsen, M. H.; Norberg, N.; Sprong, C.; Papadopoulou, E.; Sakhi, A. K.; Sabaredzovic, A.; Dirven, H. a. a. M. The Norwegian Biomonitoring Study from the EU Project EuroMix: Levels of Phenols and Phthalates in 24-Hour Urine Samples and Exposure Sources from Food and Personal Care Products. *Environ Int* **2019**, *132*, 105103. <https://doi.org/10.1016/j.envint.2019.105103>.
- (58) Nassan, F. L.; Coull, B. A.; Gaskins, A. J.; Williams, M. A.; Skakkebaek, N. E.; Ford, J. B.; Ye, X.; Calafat, A. M.; Braun, J. M.; Hauser, R. Personal Care Product Use in Men and Urinary Concentrations of Select Phthalate Metabolites and Parabens: Results from the Environment And Reproductive Health (EARTH) Study. *Environ Health Perspect* **2017**, *125* (8). <https://doi.org/10.1289/EHP1374>.
- (59) Philippat, C.; Bennett, D.; Calafat, A. M.; Picciotto, I. H. Exposure to Select Phthalates and Phenols through Use of Personal Care Products among Californian Adults and Their Children. *Environmental Research* **2015**, *140*, 369–376. <https://doi.org/10.1016/j.envres.2015.04.009>.
- (60) Sakhi, A. K.; Sabaredzovic, A.; Cequier, E.; Thomsen, C. Phthalate Metabolites in Norwegian Mothers and Children: Levels, Diurnal Variation and Use of Personal Care Products. *Science of The Total Environment* **2017**, *599–600*, 1984–1992. <https://doi.org/10.1016/j.scitotenv.2017.05.109>.
- (61) Ashrap, P.; Watkins, D. J.; Calafat, A. M.; Ye, X.; Rosario, Z.; Brown, P.; Vélez-Vega, C. M.; Alshwabkeh, A.; Cordero, J. F.; Meeker, J. D. Elevated Concentrations of Urinary Triclocarban, Phenol and Paraben among Pregnant Women in Northern Puerto Rico: Predictors and Trends. *Environment International* **2018**, *121*, 990–1002. <https://doi.org/10.1016/j.envint.2018.08.020>.
- (62) Braun, J. M.; Just, A. C.; Williams, P. L.; Smith, K. W.; Calafat, A. M.; Hauser, R. Personal Care Product Use and Urinary Phthalate Metabolite and Paraben Concentrations during Pregnancy among Women from a Fertility Clinic. *Journal of Exposure Science and Environmental Epidemiology* **2014**, *24* (5), 459–466. <https://doi.org/10.1038/jes.2013.69>.
- (63) Buckley, J. P.; Palmieri, R. T.; Matuszewski, J. M.; Herring, A. H.; Baird, D. D.; Hartmann, K. E.; Hoppin, J. A. Consumer Product Exposures Associated with Urinary Phthalate Levels in Pregnant Women. *J Expo Sci Environ Epidemiol* **2012**, *22* (5), 468–475. <https://doi.org/10.1038/jes.2012.33>.

- (64) Fisher, M.; MacPherson, S.; Braun, J. M.; Hauser, R.; Walker, M.; Feeley, M.; Mallick, R.; Bérubé, R.; Arbuckle, T. E. Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product Use. *Environmental Science & Technology* **2017**, *51* (7), 4009–4017. <https://doi.org/10.1021/acs.est.6b04302>.
- (65) Larsson, K.; Ljung Björklund, K.; Palm, B.; Wennberg, M.; Kaj, L.; Lindh, C. H.; Jönsson, B. A. G.; Berglund, M. Exposure Determinants of Phthalates, Parabens, Bisphenol A and Triclosan in Swedish Mothers and Their Children. *Environment International* **2014**, *73*, 323–333. <https://doi.org/10.1016/j.envint.2014.08.014>.
- (66) Meeker, J. D.; Cantonwine, D. E.; Rivera-González, L. O.; Ferguson, K. K.; Mukherjee, B.; Calafat, A. M.; Ye, X.; Anzalota Del Toro, L. V.; Crespo-Hernández, N.; Jiménez-Vélez, B.; et al. Distribution, Variability, and Predictors of Urinary Concentrations of Phenols and Parabens among Pregnant Women in Puerto Rico. *Environmental science & technology* **2013**, *47* (7), 3439–47. <https://doi.org/10.1021/es400510g>.
- (67) Koch, H. M.; Aylward, L. L.; Hays, S. M.; Smolders, R.; Moos, R. K.; Cocker, J.; Jones, K.; Warren, N.; Levy, L.; Bevan, R. Inter- and Intra-Individual Variation in Urinary Biomarker Concentrations over a 6-Day Sampling Period. Part 2: Personal Care Product Ingredients. *Toxicology Letters* **2014**, *231* (2), 261–269. <https://doi.org/10.1016/j.toxlet.2014.06.023>.
- (68) Morrison, G. C.; Bekö, G.; Weschler, C. J.; Schripp, T.; Salthammer, T.; Hill, J.; Andersson, A.-M.; Toftum, J.; Clausen, G.; Frederiksen, H. Dermal Uptake of Benzophenone-3 from Clothing. *Environ. Sci. Technol.* **2017**, *51* (19), 11371–11379. <https://doi.org/10.1021/acs.est.7b02623>.
- (69) Shin, M.-Y.; Shin, C.; Choi, J. W.; Lee, J.; Lee, S.; Kim, S. Pharmacokinetic Profile of Propyl Paraben in Humans after Oral Administration. *Environ Int* **2019**, *130*, 104917. <https://doi.org/10.1016/j.envint.2019.104917>.
- (70) Dodson, R. E.; Nishioka, M.; Standley, L. J.; Perovich, L. J.; Brody, J. G.; Rudel, R. A. Endocrine Disruptors and Asthma-Associated Chemicals in Consumer Products. *Environmental health perspectives* **2012**, *120* (7), 935–43. <https://doi.org/10.1289/ehp.1104052>.
- (71) Liao, C.; Kannan, K. A Survey of Alkylphenols, Bisphenols, and Triclosan in Personal Care Products from China and the United States. *Arch. Environ. Contam. Toxicol.* **2014**, *67* (1), 50–59. <https://doi.org/10.1007/s00244-014-0016-8>.
- (72) Ali, E. H. A.; Elgoly, A. H. M. Combined Prenatal and Postnatal Butyl Paraben Exposure Produces Autism-like Symptoms in Offspring: Comparison with Valproic Acid Autistic Model. *Pharmacology, biochemistry, and behavior* **2013**, *111*, 102–10. <https://doi.org/10.1016/j.pbb.2013.08.016>.
- (73) Barakat, R.; Lin, P.-C.; Park, C. J.; Best-Popescu, C.; Bakry, H. H.; Abosalem, M. E.; Abdelaleem, N. M.; Flaws, J. A.; Ko, C. Prenatal Exposure to DEHP Induces Neuronal Degeneration and Neurobehavioral Abnormalities in Adult Male Mice. *Toxicol Sci* **2018**, *164* (2), 439–452. <https://doi.org/10.1093/toxsci/kfy103>.
- (74) Bortolozzi, A. A.; Duffard, R. O.; Evangelista De Duffard, A. M. Behavioral Alterations Induced in Rats by a Pre- and Postnatal Exposure to 2,4-Dichlorophenoxyacetic Acid. *Neurotoxicology and Teratology* **1999**, *21* (4), 451–465. [https://doi.org/10.1016/S0892-0362\(98\)00059-2](https://doi.org/10.1016/S0892-0362(98)00059-2).
- (75) Inadera, H. Neurological Effects of Bisphenol A and Its Analogues. *Int J Med Sci* **2015**, *12* (12), 926–936. <https://doi.org/10.7150/ijms.13267>.
- (76) Rochester, J. R.; Bolden, A. L.; Kwiatkowski, C. F. Prenatal Exposure to Bisphenol A and Hyperactivity in Children: A Systematic Review and Meta-Analysis. *Environment International* **2018**, *114*, 343–356. <https://doi.org/10.1016/j.envint.2017.12.028>.
- (77) Wnuk, A.; Rzemieniec, J.; Staroń, J.; Litwa, E.; Lasoń, W.; Bojarski, A.; Kajta, M. Prenatal Exposure to Benzophenone-3 Impairs Autophagy, Disrupts RXRs/PPAR γ Signaling, and Alters Epigenetic and Post-Translational Statuses in Brain Neurons. *Mol. Neurobiol.* **2019**, *56* (7), 4820–4837. <https://doi.org/10.1007/s12035-018-1401-5>.
- (78) Miodovnik, A.; Edwards, A.; Bellinger, D. C.; Hauser, R. *Developmental Neurotoxicity of Ortho-Phthalate Diesters: Review of Human and Experimental Evidence*; 2014; Vol. 41. <https://doi.org/10.1016/j.neuro.2014.01.007>.

- (79) Ruszkiewicz, J. A.; Li, S.; Rodriguez, M. B.; Aschner, M. Is Triclosan a Neurotoxic Agent? *J Toxicol Environ Health B Crit Rev* **2017**, *20* (2), 104–117. <https://doi.org/10.1080/10937404.2017.1281181>.
- (80) Ruszkiewicz, J. A.; Pinkas, A.; Ferrer, B.; Peres, T. V.; Tsatsakis, A.; Aschner, M. Neurotoxic Effect of Active Ingredients in Sunscreen Products, a Contemporary Review. *Toxicol Rep* **2017**, *4*, 245–259. <https://doi.org/10.1016/j.toxrep.2017.05.006>.
- (81) Talsness, C. E.; Andrade, A. J. M.; Kuriyama, S. N.; Taylor, J. A.; vom Saal, F. S. Components of Plastic: Experimental Studies in Animals and Relevance for Human Health. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **2009**, *364* (1526), 2079–2096. <https://doi.org/10.1098/rstb.2008.0281>.
- (82) Zhang, Q.; Chen, X.-Z.; Huang, X.; Wang, M.; Wu, J. The Association between Prenatal Exposure to Phthalates and Cognition and Neurobehavior of Children—Evidence from Birth Cohorts. *Neurotoxicology* **2019**, *73*, 199–212. <https://doi.org/10.1016/j.neuro.2019.04.007>.
- (83) Mustieles, V.; Pérez-Lobato, R.; Olea, N.; Fernández, M. F. Bisphenol A: Human Exposure and Neurobehavior. *Neurotoxicology* **2015**, *49*, 174–84. <https://doi.org/10.1016/j.neuro.2015.06.002>.
- (84) Braun, J. M.; Muckle, G.; Arbuckle, T.; Bouchard, M. F.; Fraser, W. D.; Ouellet, E.; Séguin, J. R.; Oulhote, Y.; Webster, G. M.; Lanphear, B. P. Associations of Prenatal Urinary Bisphenol A Concentrations with Child Behaviors and Cognitive Abilities. *Environmental Health Perspectives* **2017**, *125* (06), 067008. <https://doi.org/10.1289/EHP984>.
- (85) Casas, M.; Forns, J.; Martínez, D.; Avella-García, C.; Valvi, D.; Ballesteros-Gómez, A.; Luque, N.; Rubio, S.; Julvez, J.; Sunyer, J.; et al. Exposure to Bisphenol A during Pregnancy and Child Neuropsychological Development in the INMA-Sabadell Cohort. *Environmental research* **2015**, *142*, 671–9. <https://doi.org/10.1016/j.envres.2015.07.024>.
- (86) Etzel, T.; Muckle, G.; Arbuckle, T. E.; Fraser, W. D.; Ouellet, E.; Séguin, J. R.; Lanphear, B.; Braun, J. M. Prenatal Urinary Triclosan Concentrations and Child Neurobehavior. *Environ Int* **2018**, *114*, 152–159. <https://doi.org/10.1016/j.envint.2018.02.032>.
- (87) Jackson-Browne, M. S.; Papandonatos, G. D.; Chen, A.; Yolton, K.; Lanphear, B. P.; Braun, J. M. Early-Life Triclosan Exposure and Parent-Reported Behavior Problems in 8-Year-Old Children. *Environment International* **2019**. <https://doi.org/10.1016/j.envint.2019.01.021>.
- (88) Stacy, S. L.; Papandonatos, G. D.; Calafat, A. M.; Chen, A.; Yolton, K.; Lanphear, B. P.; Braun, J. M. Early Life Bisphenol A Exposure and Neurobehavior at 8 Years of Age: Identifying Windows of Heightened Vulnerability. *Environment International* **2017**, *107*, 258–265. <https://doi.org/10.1016/j.envint.2017.07.021>.
- (89) Jiang, Y.; Zhao, H.; Xia, W.; Li, Y.; Liu, H.; Hao, K.; Chen, J.; Sun, X.; Liu, W.; Li, J.; et al. Prenatal Exposure to Benzophenones, Parabens and Triclosan and Neurocognitive Development at 2 years. *Environment International* **2019**, *126*, 413–421. <https://doi.org/10.1016/j.envint.2019.01.023>.
- (90) Factor-Litvak, P.; Insel, B.; Calafat, A. M.; Liu, X.; Perera, F.; Rauh, V. A.; Whyatt, R. M. Persistent Associations between Maternal Prenatal Exposure to Phthalates on Child IQ at Age 7 Years. *PLoS ONE* **2014**, *9* (12), e114003. <https://doi.org/10.1371/journal.pone.0114003>.
- (91) Huang, H.-B.; Chen, H.-Y.; Su, P.-H.; Huang, P.-C.; Sun, C.-W.; Wang, C.-J.; Chen, H.-Y.; Hsiung, C. A.; Wang, S.-L.; Koch, H.; et al. Fetal and Childhood Exposure to Phthalate Diesters and Cognitive Function in Children Up to 12 Years of Age: Taiwanese Maternal and Infant Cohort Study. *PLOS ONE* **2015**, *10* (6), e0131910. <https://doi.org/10.1371/journal.pone.0131910>.
- (92) Kim, J. I.; Hong, Y.-C.; Shin, C. H.; Lee, Y. A.; Lim, Y.-H.; Kim, B.-N. The Effects of Maternal and Children Phthalate Exposure on the Neurocognitive Function of 6-Year-Old Children. *Environmental Research* **2017**, *156*, 519–525. <https://doi.org/10.1016/j.envres.2017.04.003>.
- (93) Kim, Y.; Ha, E.-H.; Kim, E.-J.; Park, H.; Ha, M.; Kim, J.-H.; Hong, Y.-C.; Chang, N.; Kim, B.-N. Prenatal Exposure to Phthalates and Infant Development at 6 Months: Prospective Mothers and Children’s Environmental Health (MOCEH) Study. *Environ. Health Perspect.* **2011**, *119* (10), 1495–1500. <https://doi.org/10.1289/ehp.1003178>.
- (94) Doherty, B. T.; Engel, S. M.; Buckley, J. P.; Silva, M. J.; Calafat, A. M.; Wolff, M. S. Prenatal Phthalate Biomarker Concentrations and Performance on the Bayley Scales of Infant

- Development-II in a Population of Young Urban Children. *Environmental Research* **2017**, *152*, 51–58. <https://doi.org/10.1016/j.envres.2016.09.021>.
- (95) Polanska, K.; Ligocka, D.; Sobala, W.; Hanke, W. Phthalate Exposure and Child Development: The Polish Mother and Child Cohort Study. *Early Human Development* **2014**, *90* (9), 477–485. <https://doi.org/10.1016/j.earlhumdev.2014.06.006>.
- (96) Téllez-Rojo, M. M.; Cantoral, A.; Cantonwine, D. E.; Schnaas, L.; Peterson, K.; Hu, H.; Meeker, J. D. Prenatal Urinary Phthalate Metabolites Levels and Neurodevelopment in Children at Two and Three Years of Age. *Science of The Total Environment* **2013**, *461–462*, 386–390. <https://doi.org/10.1016/j.scitotenv.2013.05.021>.
- (97) Whyatt, R. M.; Liu, X.; Rauh, V. A.; Calafat, A. M.; Just, A. C.; Hoepner, L.; Diaz, D.; Quinn, J.; Adibi, J.; Perera, F. P.; et al. Maternal Prenatal Urinary Phthalate Metabolite Concentrations and Child Mental, Psychomotor, and Behavioral Development at 3 Years of Age. *Environmental Health Perspectives* **2011**, *120* (2), 290–295. <https://doi.org/10.1289/ehp.1103705>.
- (98) Braun, J. M.; Kalkbrenner, A. E.; Calafat, A. M.; Yolton, K.; Ye, X.; Dietrich, K. N.; Lanphear, B. P. Impact of Early-Life Bisphenol A Exposure on Behavior and Executive Function in Children. *PEDIATRICS* **2011**, *128* (5), 873–882. <https://doi.org/10.1542/peds.2011-1335>.
- (99) Braun, J. M.; Yolton, K.; Dietrich, K. N.; Hornung, R.; Ye, X.; Calafat, A. M.; Lanphear, B. P. Prenatal Bisphenol A Exposure and Early Childhood Behavior. *Environmental health perspectives* **2009**, *117* (12), 1945–52. <https://doi.org/10.1289/ehp.0900979>.
- (100) Evans, S. F.; Kobrosly, R. W.; Barrett, E. S.; Thurston, S. W.; Calafat, A. M.; Weiss, B.; Stahlhut, R.; Yolton, K.; Swan, S. H. Prenatal Bisphenol A Exposure and Maternally Reported Behavior in Boys and Girls. *Neurotoxicology* **2014**, *45*, 91–9. <https://doi.org/10.1016/j.neuro.2014.10.003>.
- (101) Harley, K. G.; Gunier, R. B.; Kogut, K.; Johnson, C.; Bradman, A.; Calafat, A. M.; Eskenazi, B. Prenatal and Early Childhood Bisphenol A Concentrations and Behavior in School-Aged Children. *Environmental research* **2013**, *126*, 43–50. <https://doi.org/10.1016/j.envres.2013.06.004>.
- (102) Perera, F.; Vishnevetsky, J.; Herbstman, J. B.; Calafat, A. M.; Xiong, W.; Rauh, V.; Wang, S. Prenatal Bisphenol a Exposure and Child Behavior in an Inner-City Cohort. *Environmental health perspectives* **2012**, *120* (8), 1190–4. <https://doi.org/10.1289/ehp.1104492>.
- (103) Roen, E. L.; Wang, Y.; Calafat, A. M.; Wang, S.; Margolis, A.; Herbstman, J.; Hoepner, L. A.; Rauh, V.; Perera, F. P. Bisphenol A Exposure and Behavioral Problems among Inner City Children at 7-9 Years of Age. *Environmental research* **2015**, *142*, 739–45. <https://doi.org/10.1016/j.envres.2015.01.014>.
- (104) Philippat, C.; Nakiwala, D.; Calafat, A. M.; Botton, J.; De Agostini, M.; Heude, B.; Slama, R. Prenatal Exposure to Nonpersistent Endocrine Disruptors and Behavior in Boys at 3 and 5 Years. *Environ Health Perspect* **2017**, *125* (9). <https://doi.org/10.1289/EHP1314>.
- (105) Kobrosly, R. W.; Evans, S.; Miodovnik, A.; Barrett, E. S.; Thurston, S. W.; Calafat, A. M.; Swan, S. H. Prenatal Phthalate Exposures and Neurobehavioral Development Scores in Boys and Girls at 6-10 Years of Age. *Environmental Health Perspectives* **2014**, *122* (5). <https://doi.org/10.1289/ehp.1307063>.
- (106) Lien, Y.-J.; Ku, H.-Y.; Su, P.-H.; Chen, S.-J.; Chen, H.-Y.; Liao, P.-C.; Chen, W.-J.; Wang, S.-L. Prenatal Exposure to Phthalate Esters and Behavioral Syndromes in Children at 8 Years of Age: Taiwan Maternal and Infant Cohort Study. *Environ. Health Perspect.* **2015**, *123* (1), 95–100. <https://doi.org/10.1289/ehp.1307154>.
- (107) Engel, S. M.; Miodovnik, A.; Canfield, R. L.; Zhu, C.; Silva, M. J.; Calafat, A. M.; Wolff, M. S. Prenatal Phthalate Exposure Is Associated with Childhood Behavior and Executive Functioning. *Environ. Health Perspect.* **2010**, *118* (4), 565–571. <https://doi.org/10.1289/ehp.0901470>.
- (108) Kim, S.; Eom, S.; Kim, H.-J.; Lee, J. J.; Choi, G.; Choi, S.; Kim, S.; Kim, S. Y.; Cho, G.; Kim, Y. D.; et al. Association between Maternal Exposure to Major Phthalates, Heavy Metals, and Persistent Organic Pollutants, and the Neurodevelopmental Performances of Their Children at 1 to 2years of Age- CHECK Cohort Study. *Science of The Total Environment* **2018**, *624*, 377–384. <https://doi.org/10.1016/j.scitotenv.2017.12.058>.

- (109) Golden, R.; Gandy, J.; Vollmer, G. A Review of the Endocrine Activity of Parabens and Implications for Potential Risks to Human Health. *Critical reviews in toxicology* **2005**, *35* (5), 435–458.
- (110) Meeker, J. D. Exposure to Environmental Endocrine Disruptors and Child Development. *Archives of pediatrics & adolescent medicine* **2012**, *166* (6), E1-7. <https://doi.org/10.1001/archpediatrics.2012.241>.
- (111) Miodovnik, A.; Edwards, A.; Bellinger, D. C.; Hauser, R. Developmental Neurotoxicity of Ortho-Phthalate Diesters: Review of Human and Experimental Evidence. *NeuroToxicology*. 2014. <https://doi.org/10.1016/j.neuro.2014.01.007>.
- (112) Mustieles, V.; Pérez-Lobato, R.; Olea, N.; Fernández, M. F. Bisphenol A: Human Exposure and Neurobehavior. *Neurotoxicology* **2015**, *49*, 174–184. <https://doi.org/10.1016/j.neuro.2015.06.002>.
- (113) Schug, T. T.; Blawas, A. M.; Gray, K.; Heindel, J. J.; Lawler, C. P. *Elucidating the Links between Endocrine Disruptors and Neurodevelopment*; 2015; Vol. 156. <https://doi.org/10.1210/en.2014-1734>.
- (114) Boberg, J.; Taxvig, C.; Christiansen, S.; Hass, U. Possible Endocrine Disrupting Effects of Parabens and Their Metabolites. *Reproductive toxicology (Elmsford, N.Y.)* **2010**, *30* (2), 301–312. <https://doi.org/10.1016/j.reprotox.2010.03.011>.
- (115) Krause, M.; Klit, A.; Blomberg Jensen, M.; Sjøborg, T.; Frederiksen, H.; Schlumpf, M.; Lichtensteiger, W.; Skakkebaek, N. E.; Drzewiecki, K. T. Sunscreens: Are They Beneficial for Health? An Overview of Endocrine Disrupting Properties of UV-Filters. *International journal of andrology* **2012**, *35* (3), 424–436. <https://doi.org/10.1111/j.1365-2605.2012.01280.x>.
- (116) Bernal, J. *Thyroid Hormones in Brain Development and Function*; MDText.com, Inc., 2015.
- (117) Lombardo, M. V.; Ashwin, E.; Auyeung, B.; Chakrabarti, B.; Taylor, K.; Hackett, G.; Bullmore, E. T.; Baron-Cohen, S. Fetal Testosterone Influences Sexually Dimorphic Gray Matter in the Human Brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **2012**, *32* (2), 674–680. <https://doi.org/10.1523/JNEUROSCI.4389-11.2012>.
- (118) Kundakovic, M.; Champagne, F. A. Epigenetic Perspective on the Developmental Effects of Bisphenol A. *Brain, behavior, and immunity* **2011**, *25* (6), 1084–1093. <https://doi.org/10.1016/j.bbi.2011.02.005>.
- (119) Bernal, J. Thyroid Hormones and Brain Development. *Vitam. Horm.* **2005**, *71*, 95–122. [https://doi.org/10.1016/S0083-6729\(05\)71004-9](https://doi.org/10.1016/S0083-6729(05)71004-9).
- (120) Chen, Z.-P.; Hetzel, B. S. Cretinism Revisited. *Best Practice & Research Clinical Endocrinology & Metabolism* **2010**, *24* (1), 39–50. <https://doi.org/10.1016/j.beem.2009.08.014>.
- (121) Ghassabian, A.; Henrichs, J.; Tiemeier, H. Impact of Mild Thyroid Hormone Deficiency in Pregnancy on Cognitive Function in Children: Lessons from the Generation R Study. *Best Pract. Res. Clin. Endocrinol. Metab.* **2014**, *28* (2), 221–232. <https://doi.org/10.1016/j.beem.2013.04.008>.
- (122) Haddow, J. E.; Palomaki, G. E.; Allan, W. C.; Williams, J. R.; Knight, G. J.; Gagnon, J.; O’Heir, C. E.; Mitchell, M. L.; Hermos, R. J.; Waisbren, S. E.; et al. Maternal Thyroid Deficiency during Pregnancy and Subsequent Neuropsychological Development of the Child. *New England Journal of Medicine* **1999**, *341* (8), 549–555. <https://doi.org/10.1056/NEJM199908193410801>.
- (123) Henrichs, J.; Bongers-Schokking, J. J.; Schenk, J. J.; Ghassabian, A.; Schmidt, H. G.; Visser, T. J.; Hooijkaas, H.; de Muinck Keizer-Schrama, S. M. P. F.; Hofman, A.; Jaddoe, V. V. W.; et al. Maternal Thyroid Function during Early Pregnancy and Cognitive Functioning in Early Childhood: The Generation R Study. *J. Clin. Endocrinol. Metab.* **2010**, *95* (9), 4227–4234. <https://doi.org/10.1210/jc.2010-0415>.
- (124) Julvez, J.; Alvarez-Pedrerol, M.; Rebagliato, M.; Murcia, M.; Forns, J.; Garcia-Esteban, R.; Lertxundi, N.; Espada, M.; Tardón, A.; Riaño Galán, I.; et al. Thyroxine Levels during Pregnancy in Healthy Women and Early Child Neurodevelopment. *Epidemiology* **2013**, *24* (1), 150–157. <https://doi.org/10.1097/EDE.0b013e318276ccd3>.
- (125) Korevaar, T. I. M.; Muetzel, R.; Medici, M.; Chaker, L.; Jaddoe, V. W. V.; Rijke, Y. B. de; Steegers, E. A. P.; Visser, T. J.; White, T.; Tiemeier, H.; et al. Association of Maternal Thyroid Function during Early Pregnancy with Offspring IQ and Brain Morphology in Childhood: A

- Population-Based Prospective Cohort Study. *The Lancet Diabetes & Endocrinology* **2016**, *4* (1), 35–43. [https://doi.org/10.1016/S2213-8587\(15\)00327-7](https://doi.org/10.1016/S2213-8587(15)00327-7).
- (126) Pop, V. J.; Brouwers, E. P.; Vader, H. L.; Vulmsa, T.; van Baar, A. L.; de Vijlder, J. J. Maternal Hypothyroxinaemia during Early Pregnancy and Subsequent Child Development: A 3-Year Follow-up Study. *Clin. Endocrinol. (Oxf)* **2003**, *59* (3), 282–288. <https://doi.org/10.1046/j.1365-2265.2003.01822.x>.
- (127) Román, G. C.; Ghassabian, A.; Bongers-Schokking, J. J.; Jaddoe, V. W. V.; Hofman, A.; de Rijke, Y. B.; Verhulst, F. C.; Tiemeier, H. Association of Gestational Maternal Hypothyroxinemia and Increased Autism Risk. *Ann. Neurol.* **2013**, *74* (5), 733–742. <https://doi.org/10.1002/ana.23976>.
- (128) Ghassabian, A.; Bongers-Schokking, J. J.; Henrichs, J.; Jaddoe, V. W. V.; Visser, T. J.; Visser, W.; de Muinck Keizer-Schrama, S. M. P. F.; Hooijkaas, H.; Steegers, E. A. P.; Hofman, A.; et al. Maternal Thyroid Function during Pregnancy and Behavioral Problems in the Offspring: The Generation R Study. *Pediatr. Res.* **2011**, *69* (5 Pt 1), 454–459. <https://doi.org/10.1203/PDR.0b013e3182125b0c>.
- (129) Pop, V. J.; Kuijpers, J. L.; van Baar, A. L.; Verkerk, G.; van Son, M. M.; de Vijlder, J. J.; Vulmsa, T.; Wiersinga, W. M.; Drexhage, H. A.; Vader, H. L. Low Maternal Free Thyroxine Concentrations during Early Pregnancy Are Associated with Impaired Psychomotor Development in Infancy. *Clin. Endocrinol. (Oxf)* **1999**, *50* (2), 149–155. <https://doi.org/10.1046/j.1365-2265.1999.00639.x>.
- (130) Andersen, S. L.; Laurberg, P.; Wu, C. S.; Olsen, J. Attention Deficit Hyperactivity Disorder and Autism Spectrum Disorder in Children Born to Mothers with Thyroid Dysfunction: A Danish Nationwide Cohort Study. *BJOG* **2014**, *121* (11), 1365–1374. <https://doi.org/10.1111/1471-0528.12681>.
- (131) Andersen, S. L.; Carlé, A.; Karmisholt, J.; Pedersen, I. B.; Andersen, S. MECHANISMS IN ENDOCRINOLOGY: Neurodevelopmental Disorders in Children Born to Mothers with Thyroid Dysfunction: Evidence of Fetal Programming? *Eur. J. Endocrinol.* **2017**, *177* (1), R27–R36. <https://doi.org/10.1530/EJE-16-0947>.
- (132) Howdeshell, K. L. A Model of the Development of the Brain as a Construct of the Thyroid System. *Environ Health Perspect* **2002**, *110* (Suppl 3), 337–348.
- (133) Segni, M. Disorders of the Thyroid Gland in Infancy, Childhood and Adolescence. In *Endotext*; Feingold, K. R., Anawalt, B., Boyce, A., Chrousos, G., Dungan, K., Grossman, A., Hershman, J. M., Kaltsas, G., Koch, C., Kopp, P., et al., Eds.; MDText.com, Inc.: South Dartmouth (MA), 2000.
- (134) Calsolaro, V.; Pasqualetti, G.; Niccolai, F.; Caraccio, N.; Monzani, F. Thyroid Disrupting Chemicals. *Int J Mol Sci* **2017**, *18* (12). <https://doi.org/10.3390/ijms18122583>.
- (135) Ferguson, A. V.; Latchford, K. J.; Samson, W. K. The Paraventricular Nucleus of the Hypothalamus A Potential Target for Integrative Treatment of Autonomic Dysfunction. *Expert Opin Ther Targets* **2008**, *12* (6), 717–727. <https://doi.org/10.1517/14728222.12.6.717>.
- (136) Gereben, B.; Zavacki, A. M.; Ribich, S.; Kim, B. W.; Huang, S. A.; Simonides, W. S.; Zeöld, A.; Bianco, A. C. Cellular and Molecular Basis of Deiodinase-Regulated Thyroid Hormone Signaling. *Endocr Rev* **2008**, *29* (7), 898–938. <https://doi.org/10.1210/er.2008-0019>.
- (137) Yan, Y.-Q.; Dong, Z.-L.; Dong, L.; Wang, F.-R.; Yang, X.-M.; Jin, X.-Y.; Lin, L.-X.; Sun, Y.-N.; Chen, Z.-P. Trimester- and Method-Specific Reference Intervals for Thyroid Tests in Pregnant Chinese Women: Methodology, Euthyroid Definition and Iodine Status Can Influence the Setting of Reference Intervals. *Clin. Endocrinol. (Oxf)* **2011**, *74* (2), 262–269. <https://doi.org/10.1111/j.1365-2265.2010.03910.x>.
- (138) Reinehr, T. Obesity and Thyroid Function. *Molecular and Cellular Endocrinology* **2010**, *316* (2), 165–171. <https://doi.org/10.1016/j.mce.2009.06.005>.
- (139) Wiersinga, W. M. Smoking and Thyroid. *Clin. Endocrinol. (Oxf)* **2013**, *79* (2), 145–151. <https://doi.org/10.1111/cen.12222>.
- (140) Ahad, F.; Ganie, S. A. Iodine, Iodine Metabolism and Iodine Deficiency Disorders Revisited. *Indian J Endocrinol Metab* **2010**, *14* (1), 13–17.

- (141) Herbstman, J.; Apelberg, B. J.; Witter, F. R.; Panny, S.; Goldman, L. R. Maternal, Infant, and Delivery Factors Associated with Neonatal Thyroid Hormone Status. *Thyroid* **2008**, *18* (1), 67–76. <https://doi.org/10.1089/thy.2007.0180>.
- (142) Miyamoto, N.; Tsuji, M.; Imataki, T.; Nagamachi, N.; Hirose, S.; Hamada, Y. Influence of Mode of Delivery on Fetal Pituitary-Thyroid Axis. *Acta Paediatr Jpn* **1991**, *33* (3), 363–368. <https://doi.org/10.1111/j.1442-200x.1991.tb01567.x>.
- (143) Turan, S.; Bereket, A.; Angaji, M.; Koroglu, O. A.; Bilgen, H.; Onver, T.; Akman, I.; Ozek, E. The Effect of the Mode of Delivery on Neonatal Thyroid Function. *J. Matern. Fetal. Neonatal. Med.* **2007**, *20* (6), 473–476. <https://doi.org/10.1080/14767050701398314>.
- (144) Zoeller, R. T.; Bansal, R.; Parris, C. Bisphenol-A, an Environmental Contaminant That Acts as a Thyroid Hormone Receptor Antagonist in Vitro, Increases Serum Thyroxine, and Alters RC3/Neurogranin Expression in the Developing Rat Brain. *Endocrinology* **2005**, *146* (2), 607–612. <https://doi.org/10.1210/en.2004-1018>.
- (145) Moriyama, K.; Tagami, T.; Akamizu, T.; Usui, T.; Saijo, M.; Kanamoto, N.; Hataya, Y.; Shimatsu, A.; Kuzuya, H.; Nakao, K. Thyroid Hormone Action Is Disrupted by Bisphenol A as an Antagonist. *The Journal of Clinical Endocrinology & Metabolism* **2002**, *87* (11), 5185–5190. <https://doi.org/10.1210/jc.2002-020209>.
- (146) Paul Friedman, K.; Watt, E. D.; Hornung, M. W.; Hedge, J. M.; Judson, R. S.; Crofton, K. M.; Houck, K. A.; Simmons, S. O. Tiered High-Throughput Screening Approach to Identify Thyroperoxidase Inhibitors Within the ToxCast Phase I and II Chemical Libraries. *Toxicol. Sci.* **2016**, *151* (1), 160–180. <https://doi.org/10.1093/toxsci/kfw034>.
- (147) Silva, M. M. D.; Xavier, L. L. F.; Gonçalves, C. F. L.; Santos-Silva, A. P.; Paiva-Melo, F. D.; Freitas, M. L. D.; Fortunato, R. S.; Alves, L. M.; Ferreira, A. C. F. Bisphenol A Increases Hydrogen Peroxide Generation by Thyrocytes Both in Vivo and in Vitro. *Endocr Connect* **2018**. <https://doi.org/10.1530/EC-18-0348>.
- (148) Butt, C. M.; Stapleton, H. M. Inhibition of Thyroid Hormone Sulfotransferase Activity by Brominated Flame Retardants and Halogenated Phenolics. *Chem Res Toxicol* **2013**, *26* (11). <https://doi.org/10.1021/tx400342k>.
- (149) Hornung, M. W.; Korte, J. J.; Olker, J. H.; Denny, J. S.; Knutsen, C.; Hartig, P. C.; Cardon, M. C.; Degitz, S. J. Screening the ToxCast Phase 1 Chemical Library for Inhibition of Deiodinase Type 1 Activity. *Toxicol. Sci.* **2018**, *162* (2), 570–581. <https://doi.org/10.1093/toxsci/kfx279>.
- (150) Paul, K. B.; Hedge, J. M.; DeVito, M. J.; Crofton, K. M. Short-Term Exposure to Triclosan Decreases Thyroxine In Vivo via Upregulation of Hepatic Catabolism in Young Long-Evans Rats. *Toxicol Sci* **2010**, *113* (2), 367–379. <https://doi.org/10.1093/toxsci/kfp271>.
- (151) Taxvig, C.; Vinggaard, A. M.; Hass, U.; Axelstad, M.; Boberg, J.; Hansen, P. R.; Frederiksen, H.; Nellemann, C. Do Parabens Have the Ability to Interfere with Steroidogenesis? *Toxicol. Sci.* **2008**, *106* (1), 206–213. <https://doi.org/10.1093/toxsci/kfn148>.
- (152) Schmutzler, C.; Gotthardt, I.; Hofmann, P. J.; Radovic, B.; Kovacs, G.; Stemmler, L.; Nobis, I.; Bacinski, A.; Mentrup, B.; Ambrugger, P.; et al. Endocrine Disruptors and the Thyroid Gland—A Combined in Vitro and in Vivo Analysis of Potential New Biomarkers. *Environ Health Perspect* **2007**, *115* (Suppl 1), 77–83. <https://doi.org/10.1289/ehp.9369>.
- (153) van den Berg, K. J. Interaction of Chlorinated Phenols with Thyroxine Binding Sites of Human Transthyretin, Albumin and Thyroid Binding Globulin. *Chem. Biol. Interact.* **1990**, *76* (1), 63–75.
- (154) Shen, O.; Wu, W.; Du, G.; Liu, R.; Yu, L.; Sun, H.; Han, X.; Jiang, Y.; Shi, W.; Hu, W.; et al. Thyroid Disruption by Di-n-Butyl Phthalate (DBP) and Mono-n-Butyl Phthalate (MBP) in *Xenopus Laevis*. *PLoS One* **2011**, *6* (4). <https://doi.org/10.1371/journal.pone.0019159>.
- (155) Shen, O.; Du, G.; Sun, H.; Wu, W.; Jiang, Y.; Song, L.; Wang, X. Comparison of in Vitro Hormone Activities of Selected Phthalates Using Reporter Gene Assays. *Toxicol. Lett.* **2009**, *191* (1), 9–14. <https://doi.org/10.1016/j.toxlet.2009.07.019>.
- (156) Sugiyama, S.-I.; Shimada, N.; Miyoshi, H.; Yamauchi, K. Detection of Thyroid System-Disrupting Chemicals Using in Vitro and in Vivo Screening Assays in *Xenopus Laevis*. *Toxicol. Sci.* **2005**, *88* (2), 367–374. <https://doi.org/10.1093/toxsci/kfi330>.

- (157) Wenzel, A.; Franz, C.; Breous, E.; Loos, U. Modulation of Iodide Uptake by Dialkyl Phthalate Plasticisers in FRTL-5 Rat Thyroid Follicular Cells. *Mol. Cell. Endocrinol.* **2005**, *244* (1–2), 63–71. <https://doi.org/10.1016/j.mce.2005.02.008>.
- (158) Breous, E.; Wenzel, A.; Loos, U. The Promoter of the Human Sodium/Iodide Symporter Responds to Certain Phthalate Plasticisers. *Mol. Cell. Endocrinol.* **2005**, *244* (1–2), 75–78. <https://doi.org/10.1016/j.mce.2005.06.009>.
- (159) Wang, J.; Hallinger, D. R.; Murr, A. S.; Buckalew, A. R.; Lougee, R. R.; Richard, A. M.; Laws, S. C.; Stoker, T. E. High-Throughput Screening and Chemotype-Enrichment Analysis of ToxCast Phase II Chemicals Evaluated for Human Sodium-Iodide Symporter (NIS) Inhibition. *Environment International* **2019**, *126*, 377–386. <https://doi.org/10.1016/j.envint.2019.02.024>.
- (160) Hallinger, D. R.; Murr, A. S.; Buckalew, A. R.; Simmons, S. O.; Stoker, T. E.; Laws, S. C. Development of a Screening Approach to Detect Thyroid Disrupting Chemicals That Inhibit the Human Sodium Iodide Symporter (NIS). *Toxicology in Vitro* **2017**, *40*, 66–78. <https://doi.org/10.1016/j.tiv.2016.12.006>.
- (161) Ye, H.; Ha, M.; Yang, M.; Yue, P.; Xie, Z.; Liu, C. Di2-Ethylhexyl Phthalate Disrupts Thyroid Hormone Homeostasis through Activating the Ras/Akt/TRHr Pathway and Inducing Hepatic Enzymes. *Sci Rep* **2017**, *7*. <https://doi.org/10.1038/srep40153>.
- (162) Liu, C.; Zhao, L.; Wei, L.; Li, L. DEHP Reduces Thyroid Hormones via Interacting with Hormone Synthesis-Related Proteins, Deiodinases, Transthyretin, Receptors, and Hepatic Enzymes in Rats. *Environ Sci Pollut Res Int* **2015**, *22* (16), 12711–12719. <https://doi.org/10.1007/s11356-015-4567-7>.
- (163) Noyes Pamela D.; Friedman Katie Paul; Browne Patience; Haselman Jonathan T.; Gilbert Mary E.; Hornung Michael W.; Barone Stan; Crofton Kevin M.; Laws Susan C.; Stoker Tammy E.; et al. Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. *Environmental Health Perspectives* *127* (9), 095001. <https://doi.org/10.1289/EHP5297>.
- (164) Chevrier, J.; Gunier, R. B.; Bradman, A.; Holland, N. T.; Calafat, A. M.; Eskenazi, B.; Harley, K. G. Maternal Urinary Bisphenol a during Pregnancy and Maternal and Neonatal Thyroid Function in the CHAMACOS Study. *Environmental health perspectives* **2013**, *121* (1), 138–44. <https://doi.org/10.1289/ehp.1205092>.
- (165) Romano, M. E.; Webster, G. M.; Vuong, A. M.; Thomas Zoeller, R.; Chen, A.; Hoofnagle, A. N.; Calafat, A. M.; Karagas, M. R.; Yolton, K.; Lanphear, B. P.; et al. Gestational Urinary Bisphenol A and Maternal and Newborn Thyroid Hormone Concentrations: The HOME Study. *Environmental Research* **2015**, *138*. <https://doi.org/10.1016/j.envres.2015.03.003>.
- (166) Aker, A. M.; Watkins, D. J.; Johns, L. E.; Ferguson, K. K.; Soldin, O. P.; Anzalota Del Toro, L. V.; Alshwabkeh, A. N.; Cordero, J. F.; Meeker, J. D. Phenols and Parabens in Relation to Reproductive and Thyroid Hormones in Pregnant Women. *Environmental Research* **2016**, *151*, 30–37. <https://doi.org/10.1016/j.envres.2016.07.002>.
- (167) Aung, M. T.; Johns, L. E.; Ferguson, K. K.; Mukherjee, B.; McElrath, T. F.; Meeker, J. D. Thyroid Hormone Parameters during Pregnancy in Relation to Urinary Bisphenol A Concentrations: A Repeated Measures Study. *Environ Int* **2017**, *104*, 33–40. <https://doi.org/10.1016/j.envint.2017.04.001>.
- (168) Derakhshan, A.; Shu, H.; Peeters, R. P.; Kortenkamp, A.; Lindh, C. H.; Demeneix, B.; Bornehag, C.-G.; Korevaar, T. I. M. Association of Urinary Bisphenols and Triclosan with Thyroid Function during Early Pregnancy. *Environment International* **2019**, *133*, 105123. <https://doi.org/10.1016/j.envint.2019.105123>.
- (169) Aker, A. M.; Ferguson, K. K.; Rosario, Z. Y.; Mukherjee, B.; Alshwabkeh, A. N.; Calafat, A. M.; Cordero, J. F.; Meeker, J. D. A Repeated Measures Study of Phenol, Paraben and Triclocarban Urinary Biomarkers and Circulating Maternal Hormones during Gestation in the Puerto Rico PROTECT Cohort. *Environmental Health* **2019**, *18* (1), 28. <https://doi.org/10.1186/s12940-019-0459-5>.
- (170) Wang, X.; Ouyang, F.; Feng, L.; Wang, X.; Liu, Z.; Zhang, J. Maternal Urinary Triclosan Concentration in Relation to Maternal and Neonatal Thyroid Hormone Levels: A Prospective Study. *Environ. Health Perspect.* **2017**, *125* (6), 067017. <https://doi.org/10.1289/EHP500>.

- (171) Berger, K.; Gunier, R. B.; Chevrier, J.; Calafat, A. M.; Ye, X.; Eskenazi, B.; Harley, K. G. Associations of Maternal Exposure to Triclosan, Parabens, and Other Phenols with Prenatal Maternal and Neonatal Thyroid Hormone Levels. *Environ. Res.* **2018**, *165*, 379–386. <https://doi.org/10.1016/j.envres.2018.05.005>.
- (172) Krause, M.; Frederiksen, H.; Sundberg, K.; Jørgensen, F. S.; Jensen, L. N.; Nørgaard, P.; Jørgensen, C.; Ertberg, P.; Petersen, J. H.; Feldt-Rasmussen, U.; et al. Maternal Exposure to UV Filters: Associations with Maternal Thyroid Hormones, IGF-I/IGFBP3 and Birth Outcomes. *Endocr Connect* **2018**, *7* (2), 334–346. <https://doi.org/10.1530/EC-17-0375>.
- (173) Kuo, F.-C.; Su, S.-W.; Wu, C.-F.; Huang, M.-C.; Shiea, J.; Chen, B.-H.; Chen, Y.-L.; Wu, M.-T. Relationship of Urinary Phthalate Metabolites with Serum Thyroid Hormones in Pregnant Women and Their Newborns: A Prospective Birth Cohort in Taiwan. *PLoS One* **2015**, *10* (6). <https://doi.org/10.1371/journal.pone.0123884>.
- (174) Romano, M. E.; Eliot, M. N.; Zoeller, R. T.; Hoofnagle, A. N.; Calafat, A. M.; Karagas, M. R.; Yolton, K.; Chen, A.; Lanphear, B. P.; Braun, J. M. Maternal Urinary Phthalate Metabolites during Pregnancy and Thyroid Hormone Concentrations in Maternal and Cord Sera: The HOME Study. *Int J Hyg Environ Health* **2018**, *221* (4), 623–631. <https://doi.org/10.1016/j.ijheh.2018.03.010>.
- (175) Huang, H.-B.; Kuo, P.-L.; Chang, J.-W.; Jaakkola, J. J. K.; Liao, K.-W.; Huang, P.-C. Longitudinal Assessment of Prenatal Phthalate Exposure on Serum and Cord Thyroid Hormones Homeostasis during Pregnancy - Tainan Birth Cohort Study (TBCS). *Science of The Total Environment* **2018**, *619–620*, 1058–1065. <https://doi.org/10.1016/j.scitotenv.2017.11.048>.
- (176) Yao, H.-Y.; Han, Y.; Gao, H.; Huang, K.; Ge, X.; Xu, Y.-Y.; Xu, Y.-Q.; Jin, Z.-X.; Sheng, J.; Yan, S.-Q.; et al. Maternal Phthalate Exposure during the First Trimester and Serum Thyroid Hormones in Pregnant Women and Their Newborns. *Chemosphere* **2016**, *157*, 42–48. <https://doi.org/10.1016/j.chemosphere.2016.05.023>.
- (177) Johns, L. E.; Ferguson, K. K.; Soldin, O. P.; Cantonwine, D. E.; Rivera-González, L. O.; Del Toro, L. V. A.; Calafat, A. M.; Ye, X.; Alshwabkeh, A. N.; Cordero, J. F.; et al. Urinary Phthalate Metabolites in Relation to Maternal Serum Thyroid and Sex Hormone Levels during Pregnancy: A Longitudinal Analysis. *Reprod Biol Endocrinol* **2015**, *13*. <https://doi.org/10.1186/1477-7827-13-4>.
- (178) Huang, P.-C.; Kuo, P.-L.; Guo, Y.-L.; Liao, P.-C.; Lee, C.-C. Associations between Urinary Phthalate Monoesters and Thyroid Hormones in Pregnant Women. *Human Reproduction* **2007**, *22* (10), 2715–2722. <https://doi.org/10.1093/humrep/dem205>.
- (179) Johns, L. E.; Ferguson, K. K.; McElrath, T. F.; Mukherjee, B.; Meeker, J. D. Associations between Repeated Measures of Maternal Urinary Phthalate Metabolites and Thyroid Hormone Parameters during Pregnancy. *Environ. Health Perspect.* **2016**, *124* (11), 1808–1815. <https://doi.org/10.1289/EHP170>.
- (180) Huang, P.-C.; Tsai, C.-H.; Liang, W.-Y.; Li, S.-S.; Huang, H.-B.; Kuo, P.-L. Early Phthalates Exposure in Pregnant Women Is Associated with Alteration of Thyroid Hormones. *PLOS ONE* **2016**, *11* (7), e0159398. <https://doi.org/10.1371/journal.pone.0159398>.
- (181) Ghassabian, A.; Trasande, L. Disruption in Thyroid Signaling Pathway: A Mechanism for the Effect of Endocrine-Disrupting Chemicals on Child Neurodevelopment. *Front Endocrinol (Lausanne)* **2018**, *9*. <https://doi.org/10.3389/fendo.2018.00204>.
- (182) Harris, C. A.; Henttu, P.; Parker, M. G.; Sumpster, J. P. The Estrogenic Activity of Phthalate Esters in Vitro. *Environ Health Perspect* **1997**, *105* (8), 802–811.
- (183) Le, H. H.; Carlson, E. M.; Chua, J. P.; Belcher, S. M. Bisphenol A Is Released from Polycarbonate Drinking Bottles and Mimics the Neurotoxic Actions of Estrogen in Developing Cerebellar Neurons. *Toxicol Lett* **2008**, *176* (2), 149–156. <https://doi.org/10.1016/j.toxlet.2007.11.001>.
- (184) Ain, K. B.; Refetoff, S.; Sarne, D. H.; Murata, Y. Effect of Estrogen on the Synthesis and Secretion of Thyroxine-Binding Globulin by a Human Hepatoma Cell Line, Hep G2. *Mol. Endocrinol.* **1988**, *2* (4), 313–323. <https://doi.org/10.1210/mend-2-4-313>.
- (185) Vernet Céline; Philippat Claire; Calafat Antonia M.; Ye Xiaoyun; Lyon-Caen Sarah; Siroux Valérie; Schisterman Enrique F.; Slama Rémy. Within-Day, Between-Day, and Between-Week

- Variability of Urinary Concentrations of Phenol Biomarkers in Pregnant Women. *Environmental Health Perspectives* **2018**, *126* (3), 037005. <https://doi.org/10.1289/EHP1994>.
- (186) Vernet, C.; Philippat, C.; Calafat, A. M.; Ye, X.; Lyon-Caen, S.; Siroux, V.; Schisterman, E. F.; 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), 037005. <https://doi.org/10.1289/EHP1994>.
- (187) Shin, H.-M.; Bennett, D. H.; Barkoski, J.; Ye, X.; Calafat, A. M.; Tancredi, D.; Hertz-Picciotto, I. Variability of Urinary Concentrations of Phthalate Metabolites during Pregnancy in First Morning Voids and Pooled Samples. *Environment International* **2019**, *122*, 222–230. <https://doi.org/10.1016/j.envint.2018.11.012>.
- (188) Rappaport, S. M.; Symanski, E.; Yager, J. W.; Kupper, L. L. The Relationship between Environmental Monitoring and Biological Markers in Exposure Assessment. *Environmental health perspectives* **1995**, 49–53.
- (189) Perrier, F.; Giorgis-Allemand, L.; Slama, R.; Philippat, C. Within-Subject Pooling of Biological Samples to Reduce Exposure Misclassification in Biomarker-Based Studies. *Epidemiology (Cambridge, Mass.)* **2016**, *27* (3), 378–88. <https://doi.org/10.1097/EDE.0000000000000460>.
- (190) Vernet, C.; Philippat, C.; Agier, L.; Calafat, A. M.; Ye, X.; Lyon-Caen, S.; Hainaut, P.; Siroux, V.; Schisterman, E. F.; Slama, R. An Empirical Validation of the Within-Subject Biospecimens Pooling Approach to Minimize Exposure Misclassification in Biomarker-Based Studies. *Epidemiology* **2019**, *30* (5), 756–767. <https://doi.org/10.1097/EDE.0000000000001056>.
- (191) Heude, B.; Forhan, A.; Slama, R.; Douhaud, L.; Bedel, S.; Saurel-Cubizolles, M.-J.; Hankard, R.; Thiebaugeorges, O.; De Agostini, M.; Annesi-Maesano, I.; et al. Cohort Profile: The EDEN Mother-Child Cohort on the Prenatal and Early Postnatal Determinants of Child Health and Development. *International journal of epidemiology* **2016**, *45* (2), 353–63. <https://doi.org/10.1093/ije/dyv151>.
- (192) Ouidir, M.; Giorgis-Allemand, L.; Lyon-Caen, S.; Morelli, X.; Cracowski, C.; Pontet, S.; Pin, I.; Lepeule, J.; Siroux, V.; Slama, R. Estimation of Exposure to Atmospheric Pollutants during Pregnancy Integrating Space-Time Activity and Indoor Air Levels: Does It Make a Difference? *Environment international* **2015**, *84*, 161–73. <https://doi.org/10.1016/j.envint.2015.07.021>.
- (193) Zhou, X.; Kramer, J. P.; Calafat, A. M.; Ye, X. Automated On-Line Column-Switching High Performance Liquid Chromatography Isotope Dilution Tandem Mass Spectrometry Method for the Quantification of Bisphenol A, Bisphenol F, Bisphenol S, and 11 Other Phenols in Urine. *J Chromatogr B Analyt Technol Biomed Life Sci* **2014**, *944*, 152–156. <https://doi.org/10.1016/j.jchromb.2013.11.009>.
- (194) Helsel, D. R. *Statistics for Censored Environmental Data Using Minitab and R*; John Wiley & Sons, 2011; Vol. 77.
- (195) Lubin, J. H.; Colt, J. S.; Camann, D.; Davis, S.; Cerhan, J. R.; Severson, R. K.; Bernstein, L.; Hartge, P. Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. *Environ Health Perspect* **2004**, *112* (17), 1691–1696. <https://doi.org/10.1289/ehp.7199>.
- (196) Rudel Ruthann A.; Gray Janet M.; Engel Connie L.; Rawsthorne Teresa W.; Dodson Robin E.; Ackerman Janet M.; Rizzo Jeanne; Nudelman Janet L.; Brody Julia Green. Food Packaging and Bisphenol A and Bis(2-Ethyhexyl) Phthalate Exposure: Findings from a Dietary Intervention. *Environmental Health Perspectives* **2011**, *119* (7), 914–920. <https://doi.org/10.1289/ehp.1003170>.
- (197) Liu, J.; Wattar, N.; Field, C. J.; Dinu, I.; Dewey, D.; Martin, J. W.; APrON study team. Exposure and Dietary Sources of Bisphenol A (BPA) and BPA-Alternatives among Mothers in the APrON Cohort Study. *Environ Int* **2018**, *119*, 319–326. <https://doi.org/10.1016/j.envint.2018.07.001>.
- (198) Liao, C.; Liu, F.; Kannan, K. Occurrence of and Dietary Exposure to Parabens in Foodstuffs from the United States. *Environ. Sci. Technol.* **2013**, *47* (8), 3918–3925. <https://doi.org/10.1021/es400724s>.
- (199) Berger, K. P.; Kogut, K. R.; Bradman, A.; She, J.; Gavin, Q.; Zahedi, R.; Parra, K. L.; Harley, K. G. Personal Care Product Use as a Predictor of Urinary Concentrations of Certain Phthalates, Parabens, and Phenols in the HERMOSA Study. *J Expo Sci Environ Epidemiol* **2018**. <https://doi.org/10.1038/s41370-017-0003-z>.

- (200) Braun, J. M.; Just, A. C.; Williams, P. L.; Smith, K. W.; Calafat, A. M.; Hauser, R. Personal Care Product Use and Urinary Phthalate Metabolite and Paraben Concentrations during Pregnancy among Women from a Fertility Clinic. *J Expo Sci Environ Epidemiol* **2014**, *24* (5), 459–466. <https://doi.org/10.1038/jes.2013.69>.
- (201) Kim, S.; Lee, S.; Shin, C.; Lee, J.; Kim, S.; Lee, A.; Park, J.; Kho, Y.; Moos, R. K.; Koch, H. M.; et al. Urinary Parabens and Triclosan Concentrations and Associated Exposure Characteristics in a Korean Population—A Comparison between Night-Time and First-Morning Urine. *International Journal of Hygiene and Environmental Health* **2018**, *221* (4), 632–641. <https://doi.org/10.1016/j.ijheh.2018.03.009>.
- (202) Larsson, K.; Lindh, C. H.; Jönsson, B. A.; Giovanoulis, G.; Bibi, M.; Bottai, M.; Bergström, A.; Berglund, M. Phthalates, Non-Phthalate Plasticizers and Bisphenols in Swedish Preschool Dust in Relation to Children’s Exposure. *Environment International* **2017**, *102*, 114–124. <https://doi.org/10.1016/j.envint.2017.02.006>.
- (203) Rothman, K. J. No Adjustments Are Needed for Multiple Comparisons. *Epidemiology* **1990**, *1* (1), 43–46.
- (204) Stacy, S. L.; Eliot, M.; Etzel, T.; Papandonatos, G.; Calafat, A. M.; Chen, A.; Hauser, R.; Lanphear, B. P.; Sathyanarayana, S.; Ye, X.; et al. Patterns, Variability, and Predictors of Urinary Triclosan Concentrations during Pregnancy and Childhood. *Environmental Science & Technology* **2017**, *51* (11), 6404–6413. <https://doi.org/10.1021/acs.est.7b00325>.
- (205) Moos, R. K.; Angerer, J.; Dierkes, G.; Brüning, T.; Koch, H. M. Metabolism and Elimination of Methyl, Iso- and n-Butyl Paraben in Human Urine after Single Oral Dosage. *Arch. Toxicol.* **2016**, *90* (11), 2699–2709. <https://doi.org/10.1007/s00204-015-1636-0>.
- (206) Sandborgh-Englund, G.; Adolfsson-Erici, M.; Odham, G.; Ekstrand, J. Pharmacokinetics of Triclosan Following Oral Ingestion in Humans. *J. Toxicol. Environ. Health Part A* **2006**, *69* (20), 1861–1873. <https://doi.org/10.1080/15287390600631706>.
- (207) Dodge, L. E.; Kelley, K. E.; Williams, P. L.; Williams, M. A.; Hernández-Díaz, S.; Missmer, S. A.; Hauser, R. Medications as a Source of Paraben Exposure. *Reprod Toxicol* **2015**, *52*, 93–100. <https://doi.org/10.1016/j.reprotox.2015.02.002>.
- (208) Soni, M. G.; Carabin, I. G.; Burdock, G. A. Safety Assessment of Esters of P-Hydroxybenzoic Acid (Parabens). *Food Chem. Toxicol.* **2005**, *43* (7), 985–1015. <https://doi.org/10.1016/j.fct.2005.01.020>.
- (209) Liao, C.; Kannan, K. Widespread Occurrence of Bisphenol A in Paper and Paper Products: Implications for Human Exposure. *Environ. Sci. Technol.* **2011**, *45* (21), 9372–9379. <https://doi.org/10.1021/es202507f>.
- (210) ECHA. 4,4'-isopropylidenediphenol - Candidate List of substances of very high concern for Authorisation - ECHA <https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e180e22414> (accessed Oct 29, 2019).
- (211) Qiu, W.; Zhan, H.; Hu, J.; Zhang, T.; Xu, H.; Wong, M.; Xu, B.; Zheng, C. The Occurrence, Potential Toxicity, and Toxicity Mechanism of Bisphenol S, a Substitute of Bisphenol A: A Critical Review of Recent Progress. *Ecotoxicology and Environmental Safety* **2019**, *173*, 192–202. <https://doi.org/10.1016/j.ecoenv.2019.01.114>.
- (212) Heude, B.; Forhan, A.; Slama, R.; Douhaud, L.; Bedel, S.; Saurel-Cubizolles, M.-J.; Hankard, R.; Thiebaugeorges, O.; De Agostini, M.; Annesi-Maesano, I.; et al. Cohort Profile: The EDEN Mother-Child Cohort on the Prenatal and Early Postnatal Determinants of Child Health and Development. *International journal of epidemiology* **2016**, *45* (2), 353–363. <https://doi.org/10.1093/ije/dyv151>.
- (213) Chevrier, C.; Petit, C.; Philippat, C.; Mortamais, M.; Slama, R.; Rouget, F.; Calafat, A. M.; Ye, X.; Silva, M. J.; Charles, M.-A.; et al. Maternal Urinary Phthalates and Phenols and Male Genital Anomalies. *Epidemiology (Cambridge, Mass.)* **2012**, *23* (2), 353–356. <https://doi.org/10.1097/EDE.0b013e318246073e>.
- (214) Botton, J.; Philippat, C.; Calafat, A. M.; Carles, S.; Charles, M.-A.; Slama, R.; The Eden Mother-Child Cohort Study Group. Phthalate Pregnancy Exposure and Male Offspring Growth from the Intra-Uterine Period to Five Years of Age. *Environmental research* **2016**, *151*, 601–609. <https://doi.org/10.1016/j.envres.2016.08.033>.

- (215) Philippat, C.; Botton, J.; Calafat, A. M.; Ye, X.; Charles, M.-A.; Slama, R. Prenatal Exposure to Phenols and Growth in Boys. *Epidemiology (Cambridge, Mass.)* **2014**, *25* (5), 625–635. <https://doi.org/10.1097/EDE.0000000000000132>.
- (216) Philippat, C.; Mortamais, M.; Chevrier, C.; Petit, C.; Calafat, A. M.; Ye, X.; Silva, M. J.; Brambilla, C.; Pin, I.; Charles, M.-A.; et al. Exposure to Phthalates and Phenols during Pregnancy and Offspring Size at Birth. In *Environmental health perspectives*; 2012; Vol. 120, pp 464–470. <https://doi.org/10.1289/ehp.1103634>.
- (217) Kim, Y.; Ha, E.-H.; Kim, E.-J.; Park, H.; Ha, M.; Kim, J.-H.; Hong, Y.-C.; Chang, N.; Kim, B.-N. Prenatal Exposure to Phthalates and Infant Development at 6 Months: Prospective Mothers and Children’s Environmental Health (MOCEH) Study. *Environmental health perspectives* **2011**, *119* (10), 1495–1500. <https://doi.org/10.1289/ehp.1003178>.
- (218) Whyatt, R. M.; Liu, X.; Rauh, V. A.; Calafat, A. M.; Just, A. C.; Hoepner, L.; Diaz, D.; Quinn, J.; Adibi, J.; Perera, F. P.; et al. Maternal Prenatal Urinary Phthalate Metabolite Concentrations and Child Mental, Psychomotor, and Behavioral Development at 3 Years of Age. *Environmental Health Perspectives* **2011**, *120* (2), 290–295. <https://doi.org/10.1289/ehp.1103705>.
- (219) Doherty, B. T.; Engel, S. M.; Buckley, J. P.; Silva, M. J.; Calafat, A. M.; Wolff, M. S. Prenatal Phthalate Biomarker Concentrations and Performance on the Bayley Scales of Infant Development-II in a Population of Young Urban Children. *Environmental Research* **2017**, *152*, 51–58. <https://doi.org/10.1016/j.envres.2016.09.021>.
- (220) Braun, J. M.; Kalkbrenner, A. E.; Calafat, A. M.; Yolton, K.; Ye, X.; Dietrich, K. N.; Lanphear, B. P. Impact of Early-Life Bisphenol A Exposure on Behavior and Executive Function in Children. *PEDIATRICS* **2011**, *128* (5), 873–882. <https://doi.org/10.1542/peds.2011-1335>.
- (221) Wechsler, D. *Wechsler Intelligence Scale for Children: Administration and Scoring Manual.*, 3rd ed.; The Psychological Corporation.: San Antonio, TX, 2002.
- (222) Wechsler, D. *WPPSI-III : Échelle d’intelligence de Wechsler Pour La Période Préscolaire et Primaire. 3ème Édition*; Paris, France, 2004.
- (223) Peyre, H.; Bernard, J. Y.; Hoertel, N.; Forhan, A.; Charles, M.-A.; De Agostini, M.; Heude, B.; Ramus, F. Differential Effects of Factors Influencing Cognitive Development at the Age of 5-to-6 Years. *Cognitive Development* **2016**, *40*, 152–162. <https://doi.org/10.1016/j.cogdev.2016.10.001>.
- (224) Ye, X.; Kuklennyik, Z.; Needham, L. L.; Calafat, A. M. Measuring Environmental Phenols and Chlorinated Organic Chemicals in Breast Milk Using Automated On-Line Column-Switching-High Performance Liquid Chromatography-Isotope Dilution Tandem Mass Spectrometry. *Journal of chromatography. B, Analytical technologies in the biomedical and life sciences* **2006**, *831* (1–2), 110–115. <https://doi.org/10.1016/j.jchromb.2005.11.050>.
- (225) SILVA, M.; SAMANDAR, E.; PREAUJR, J.; REIDY, J.; NEEDHAM, L.; CALAFAT, A. Quantification of 22 Phthalate Metabolites in Human Urine☆. *Journal of Chromatography B* **2007**, *860* (1), 106–112. <https://doi.org/10.1016/j.jchromb.2007.10.023>.
- (226) Mortamais, M.; Chevrier, C.; Philippat, C.; Petit, C.; Calafat, A. M.; Ye, X.; Silva, M. J.; Brambilla, C.; Eijkemans, M. J. C.; Charles, M.-A.; et al. Correcting for the Influence of Sampling Conditions on Biomarkers of Exposure to Phenols and Phthalates: A 2-Step Standardization Method Based on Regression Residuals. *Environmental health: a global access science source* **2012**, *11*, 29. <https://doi.org/10.1186/1476-069X-11-29>.
- (227) White, I. R.; Royston, P.; Wood, A. M. Multiple Imputation Using Chained Equations: Issues and Guidance for Practice. *Statistics in Medicine* **2011**, *30* (4), 377–399. <https://doi.org/10.1002/sim.4067>.
- (228) O’Grady, K. E. A Confirmatory Maximum Likelihood Factor Analysis of the WPPSI. *Personality and Individual Differences* **1990**, *11* (2), 135–140. [https://doi.org/10.1016/0191-8869\(90\)90005-C](https://doi.org/10.1016/0191-8869(90)90005-C).
- (229) Stone, B. J.; Gridley, B. E.; Gyurke, J. S. Confirmatory Factor Analysis of the WPPSI-R at the Extreme End of the Age Range. *Journal of Psychoeducational Assessment* **1991**, *9* (3), 263–270. <https://doi.org/10.1177/073428299100900309>.

- (230) Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing on JSTOR. *Journal of the Royal Statistical Society* **1995**, *57* (1), 289–300.
- (231) Sánchez, B. N.; Budtz-Jørgensen, E.; Ryan, L. M.; Hu, H. Structural Equation Models. *Journal of the American Statistical Association* **2005**, *100* (472), 1443–1455. <https://doi.org/10.1198/016214505000001005>.
- (232) Budtz-Jørgensen, E.; Keiding, N.; Grandjean, P.; Weihe, P. Estimation of Health Effects of Prenatal Methylmercury Exposure Using Structural Equation Models. *Environmental Health* **2002**, *1* (1), 2. <https://doi.org/10.1186/1476-069X-1-2>.
- (233) Kline, Rex. B. *Principles and Practice of Structural Equation Modeling*, 3rd ed.; The Guilford press, Ed.; 2003.
- (234) Calafat, A. M.; Silva, M. J.; Reidy, J. A.; Earl Gray, L.; Samandar, E.; Preau, J. L.; Herbert, A. R.; Needham, L. L. Mono-(3-Carboxypropyl) Phthalate, A Metabolite of Di-*n*-Octyl Phthalate. *Journal of Toxicology and Environmental Health, Part A* **2006**, *69* (3), 215–227. <https://doi.org/10.1080/15287390500227381>.
- (235) Polanska, K.; Ligocka, D.; Sobala, W.; Hanke, W. Phthalate Exposure and Child Development: The Polish Mother and Child Cohort Study. *Early Human Development* **2014**, *90* (9), 477–485. <https://doi.org/10.1016/j.earlhumdev.2014.06.006>.
- (236) Téllez-Rojo, M. M.; Cantoral, A.; Cantonwine, D. E.; Schnaas, L.; Peterson, K.; Hu, H.; Meeker, J. D. Prenatal Urinary Phthalate Metabolites Levels and Neurodevelopment in Children at Two and Three Years of Age. *Science of The Total Environment* **2013**, *461–462*, 386–390. <https://doi.org/10.1016/j.scitotenv.2013.05.021>.
- (237) Gascon, M.; Valvi, D.; Forns, J.; Casas, M.; Martínez, D.; Julvez, J.; Monfort, N.; Ventura, R.; Sunyer, J.; Vrijheid, M. Prenatal Exposure to Phthalates and Neuropsychological Development during Childhood. *International Journal of Hygiene and Environmental Health* **2015**, *218* (6). <https://doi.org/10.1016/j.ijheh.2015.05.006>.
- (238) Stacy, S. L.; Papandonatos, G. D.; Calafat, A. M.; Chen, A.; Yolton, K.; Lanphear, B. P.; Braun, J. M. Early Life Bisphenol A Exposure and Neurobehavior at 8 Years of Age: Identifying Windows of Heightened Vulnerability. *Environment International* **2017**, *107*, 258–265. <https://doi.org/10.1016/j.envint.2017.07.021>.
- (239) Braun, J. M.; Muckle, G.; Arbuckle, T.; Bouchard, M. F.; Fraser, W. D.; Ouellet, E.; Séguin, J. R.; Oulhote, Y.; Webster, G. M.; Lanphear, B. P. Associations of Prenatal Urinary Bisphenol A Concentrations with Child Behaviors and Cognitive Abilities. *Environmental Health Perspectives* **2017**, *125* (06), 067008. <https://doi.org/10.1289/EHP984>.
- (240) Lin, C.-C.; Chien, C.-J.; Tsai, M.-S.; Hsieh, C.-J.; Hsieh, W.-S.; Chen, P.-C. Prenatal Phenolic Compounds Exposure and Neurobehavioral Development at 2 and 7 Years of Age. *Science of The Total Environment* **2017**, *605–606*, 801–810. <https://doi.org/10.1016/j.scitotenv.2017.06.160>.
- (241) Casas, M.; Forns, J.; Martínez, D.; Avella-García, C.; Valvi, D.; Ballesteros-Gómez, A.; Luque, N.; Rubio, S.; Julvez, J.; Sunyer, J.; et al. Exposure to Bisphenol A during Pregnancy and Child Neuropsychological Development in the INMA-Sabadell Cohort. *Environmental research* **2015**, *142*, 671–679. <https://doi.org/10.1016/j.envres.2015.07.024>.
- (242) Ali, E. H. A.; Elgoly, A. H. M. Combined Prenatal and Postnatal Butyl Paraben Exposure Produces Autism-like Symptoms in Offspring: Comparison with Valproic Acid Autistic Model. *Pharmacology, biochemistry, and behavior* **2013**, *111*, 102–110. <https://doi.org/10.1016/j.pbb.2013.08.016>.
- (243) Kim, Y.; Ha, E.; Kim, E.; Park, H.; Ha, M.; Kim, J.; Hong, Y.; Chang, N.; Kim, B. Prenatal Exposure to Phthalates and Infant Development at 6 Months: Prospective Mothers and Children’s Environmental Health (MOCEH) Study. *Environmental Health Perspectives* **2011**, *119* (10), 1495–1500. <https://doi.org/10.1289/ehp.1003178>.
- (244) Polanska, K.; Ligocka, D.; Sobala, W.; Hanke, W. Phthalate Exposure and Child Development: The Polish Mother and Child Cohort Study. *Early human development* **2014**, *90* (9), 477–485. <https://doi.org/10.1016/j.earlhumdev.2014.06.006>.

- (245) Perrier, F.; Giorgis-Allemand, L.; Slama, R.; Philippat, C. Within-Subject Pooling of Biological Samples to Reduce Exposure Misclassification in Biomarker-Based Studies. *Epidemiology (Cambridge, Mass.)* **2016**, *27* (3), 378–388. <https://doi.org/10.1097/EDE.0000000000000460>.
- (246) Rappaport, S. M.; Symanski, E.; Yager, J. W.; Kupper, L. L. The Relationship between Environmental Monitoring and Biological Markers in Exposure Assessment. *Environmental health perspectives* **1995**, 49–53.
- (247) Robinson, O.; Basagaña, X.; Agier, L.; de Castro, M.; Hernandez-Ferrer, C.; Gonzalez, J. R.; Grimalt, J. O.; Nieuwenhuijsen, M.; Sunyer, J.; Slama, R.; et al. The Pregnancy Exposome: Multiple Environmental Exposures in the INMA-Sabadell Birth Cohort. *Environmental Science & Technology* **2015**, *49* (17), 10632–10641. <https://doi.org/10.1021/acs.est.5b01782>.
- (248) Goodman, A. The Strengths and Difficulties Questionnaire: A Research Note. *Journal of Child Psychology and Psychiatry* **1997**, *38*, 581–586.
- (249) Mortamais, M.; Chevrier, C.; Philippat, C.; Petit, C.; Calafat, A. M.; Ye, X.; Silva, M. J.; Brambilla, C.; Eijkemans, M. J. C.; Charles, M.-A.; et al. Correcting for the Influence of Sampling Conditions on Biomarkers of Exposure to Phenols and Phthalates: A 2-Step Standardization Method Based on Regression Residuals. *Environmental health: a global access science source* **2012**, *11*, 29. <https://doi.org/10.1186/1476-069X-11-29>.
- (250) Philippat, C.; Botton, J.; Calafat, A. M.; Ye, X.; Charles, M.-A.; Slama, R. Prenatal Exposure to Phenols and Growth in Boys. *Epidemiology (Cambridge, Mass.)* **2014**, *25* (5), 625–35. <https://doi.org/10.1097/EDE.0000000000000132>.
- (251) Torrey, E. F.; Miller, J.; Rawlings, R.; Yolken, R. H. Seasonality of Births in Schizophrenia and Bipolar Disorder: A Review of the Literature. *Schizophr. Res.* **1997**, *28* (1), 1–38. [https://doi.org/10.1016/s0920-9964\(97\)00092-3](https://doi.org/10.1016/s0920-9964(97)00092-3).
- (252) Melchior, M.; Hersi, R.; van der Waerden, J.; Larroque, B.; Saurel-Cubizolles, M.-J.; Chollet, A.; Galéra, C. Maternal Tobacco Smoking in Pregnancy and Children’s Socio-Emotional Development at Age 5: The EDEN Mother-Child Birth Cohort Study. *European psychiatry: the journal of the Association of European Psychiatrists* **2015**, *30* (5), 562–8. <https://doi.org/10.1016/j.eurpsy.2015.03.005>.
- (253) Braun, J. M.; Smith, K. W.; Williams, P. L.; Calafat, A. M.; Berry, K.; Ehrlich, S.; Hauser, R. Variability of Urinary Phthalate Metabolite and Bisphenol A Concentrations before and during Pregnancy. *Environ. Health Perspect.* **2012**, *120* (5), 739–745. <https://doi.org/10.1289/ehp.1104139>.
- (254) Philippat, C.; Wolff, M. S.; Calafat, A. M.; Ye, X.; Bausell, R.; Meadows, M.; Stone, J.; Slama, R.; Engel, S. M. Prenatal Exposure to Environmental Phenols: Concentrations in Amniotic Fluid and Variability in Urinary Concentrations during Pregnancy. *Environmental Health Perspectives* **2013**, *121* (10), 1225–31. <https://doi.org/10.1289/ehp.1206335>.
- (255) Anderson, O. S.; Peterson, K. E.; Sanchez, B. N.; Zhang, Z.; Mancuso, P.; Dolinoy, D. C. Perinatal Bisphenol A Exposure Promotes Hyperactivity, Lean Body Composition, and Hormonal Responses across the Murine Life Course. *FASEB J.* **2013**, *27* (4), 1784–1792. <https://doi.org/10.1096/fj.12-223545>.
- (256) Ishido, M.; Masuo, Y.; Terasaki, M.; Morita, M. Rat Hyperactivity by Bisphenol A, but Not by Its Derivatives, 3-Hydroxybisphenol A or Bisphenol A 3,4-Quinone. *Toxicol. Lett.* **2011**, *206* (3), 300–305. <https://doi.org/10.1016/j.toxlet.2011.08.011>.
- (257) Komada, M.; Itoh, S.; Kawachi, K.; Kagawa, N.; Ikeda, Y.; Nagao, T. Newborn Mice Exposed Prenatally to Bisphenol A Show Hyperactivity and Defective Neocortical Development. *Toxicology* **2014**, *323*, 51–60. <https://doi.org/10.1016/j.tox.2014.06.009>.
- (258) Nakagami, A.; Negishi, T.; Kawasaki, K.; Imai, N.; Nishida, Y.; Ihara, T.; Kuroda, Y.; Yoshikawa, Y.; Koyama, T. Alterations in Male Infant Behaviors towards Its Mother by Prenatal Exposure to Bisphenol A in Cynomolgus Monkeys (*Macaca Fascicularis*) during Early Suckling Period. *Psychoneuroendocrinology* **2009**, *34* (8), 1189–1197. <https://doi.org/10.1016/j.psyneuen.2009.03.005>.
- (259) Paul, K. B.; Hedge, J. M.; Bansal, R.; Zoeller, R. T.; Peter, R.; DeVito, M. J.; Crofton, K. M. Developmental Triclosan Exposure Decreases Maternal, Fetal, and Early Neonatal Thyroxine: A Dynamic and Kinetic Evaluation of a Putative Mode-of-Action. *Toxicology* **2012**, *300* (1–2), 31–45. <https://doi.org/10.1016/j.tox.2012.05.023>.

- (260) Koeppe, E. S.; Ferguson, K. K.; Colacino, J. A.; Meeker, J. D. Relationship between Urinary Triclosan and Paraben Concentrations and Serum Thyroid Measures in NHANES 2007-2008. *The Science of the total environment* **2013**, *445–446*, 299–305. <https://doi.org/10.1016/j.scitotenv.2012.12.052>.
- (261) Witorsch, R. J. Critical Analysis of Endocrine Disruptive Activity of Triclosan and Its Relevance to Human Exposure through the Use of Personal Care Products. *Crit. Rev. Toxicol.* **2014**, *44* (6), 535–555. <https://doi.org/10.3109/10408444.2014.910754>.
- (262) Bortolozzi, A. A.; Duffard, R. O.; Evangelista De Duffard, A. M. Behavioral Alterations Induced in Rats by a Pre- and Postnatal Exposure to 2,4-Dichlorophenoxyacetic Acid. *Neurotoxicology and Teratology* **1999**, *21* (4), 451–465. [https://doi.org/10.1016/S0892-0362\(98\)00059-2](https://doi.org/10.1016/S0892-0362(98)00059-2).
- (263) Kawaguchi, M.; Irie, K.; Morohoshi, K.; Watanabe, G.; Taya, K.; Morita, M.; Kondo, Y.; Imai, H.; Himi, T. Maternal Isobutyl-Paraben Exposure Alters Anxiety and Passive Avoidance Test Performance in Adult Male Rats. *Neurosci. Res.* **2009**, *65* (2), 136–140. <https://doi.org/10.1016/j.neures.2009.06.011>.
- (264) Engel, S. M.; Levy, B.; Liu, Z.; Kaplan, D.; Wolff, M. S. Xenobiotic Phenols in Early Pregnancy Amniotic Fluid. *Reproductive toxicology (Elmsford, N.Y.)* **2006**, *21* (1), 110–2. <https://doi.org/10.1016/j.reprotox.2005.07.007>.
- (265) Farzanehfar, V.; Naderi, N.; Kobarfard, F.; Faizi, M. Determination of Dibutyl Phthalate Neurobehavioral Toxicity in Mice. *Food Chem. Toxicol.* **2016**, *94*, 221–226. <https://doi.org/10.1016/j.fct.2016.05.006>.
- (266) Hoshi, H.; Ohtsuka, T. Adult Rats Exposed to Low-Doses of Di-n-Butyl Phthalate during Gestation Exhibit Decreased Grooming Behavior. *Bull Environ Contam Toxicol* **2009**, *83* (1), 62–66. <https://doi.org/10.1007/s00128-009-9729-1>.
- (267) Yan, B.; Guo, J.; Liu, X.; Li, J.; Yang, X.; Ma, P.; Wu, Y. Oxidative Stress Mediates Dibutyl Phthalate-induced Anxiety-like Behavior in Kunming Mice. *Environ. Toxicol. Pharmacol.* **2016**, *45*, 45–51. <https://doi.org/10.1016/j.etap.2016.05.013>.
- (268) Gascon, M.; Valvi, D.; Forns, J.; Casas, M.; Martnez, D.; J?lvez, J.; Monfort, N.; Ventura, R.; Sunyer, J.; Vrijheid, M. Prenatal Exposure to Phthalates and Neuropsychological Development during Childhood. *International Journal of Hygiene and Environmental Health* **2015**, *218* (6). <https://doi.org/10.1016/j.ijheh.2015.05.006>.
- (269) Krüger, T.; Long, M.; Bonefeld-Jørgensen, E. C. Plastic Components Affect the Activation of the Aryl Hydrocarbon and the Androgen Receptor. *Toxicology* **2008**, *246* (2–3), 112–123. <https://doi.org/10.1016/j.tox.2007.12.028>.
- (270) Adibi Jennifer J.; Whyatt Robin M.; Williams Paige L.; Calafat Antonia M.; Camann David; Herrick Robert; Nelson Heather; Bhat Hari K.; Perera Frederica P.; Silva Manori J.; et al. Characterization of Phthalate Exposure among Pregnant Women Assessed by Repeat Air and Urine Samples. *Environmental Health Perspectives* **2008**, *116* (4), 467–473. <https://doi.org/10.1289/ehp.10749>.
- (271) Bellinger, D. C. Assessing Environmental Neurotoxicant Exposures and Child Neurobehavior: Confounded by Confounding? *Epidemiology* **2004**, *15* (4), 383–384. <https://doi.org/10.1097/01.ede.0000129525.15064.a4>.
- (272) Robinson, O.; Basagaña, X.; Agier, L.; de Castro, M.; Hernandez-Ferrer, C.; Gonzalez, J. R.; Grimalt, J. O.; Nieuwenhuijsen, M.; Sunyer, J.; Slama, R.; et al. The Pregnancy Exposome: Multiple Environmental Exposures in the INMA-Sabadell Birth Cohort. *Environmental Science & Technology* **2015**, *49* (17), 10632–10641. <https://doi.org/10.1021/acs.est.5b01782>.
- (273) Monneret, D.; Guergour, D.; Vergnaud, S.; Laporte, F.; Faure, P.; Gauchez, A. S. Evaluation of LOCI Technology-Based Thyroid Blood Tests on the Dimension Vista Analyzer. *Clin. Biochem.* **2013**, *46* (13–14), 1290–1297. <https://doi.org/10.1016/j.clinbiochem.2012.11.011>.
- (274) Sabaredzovic, A.; Sakhi, A. K.; Brantsæter, A. L.; Thomsen, C. Determination of 12 Urinary Phthalate Metabolites in Norwegian Pregnant Women by Core-Shell High Performance Liquid Chromatography with on-Line Solid-Phase Extraction, Column Switching and Tandem Mass Spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2015**, *1002*, 343–352. <https://doi.org/10.1016/j.jchromb.2015.08.040>.

- (275) Agier, L.; Portengen, L.; Chadeau-Hyam, M.; Basagaña, X.; Giorgis-Allemand, L.; Siroux, V.; Robinson, O.; Vlaanderen, J.; González, J. R.; Nieuwenhuijsen, M. J.; et al. A Systematic Comparison of Linear Regression–Based Statistical Methods to Assess Exposome–Health Associations. *Environmental Health Perspectives* **2016**, *124* (12). <https://doi.org/10.1289/EHP172>.
- (276) Ankley, G. T.; Bennett, R. S.; Erickson, R. J.; Hoff, D. J.; Hornung, M. W.; Johnson, R. D.; Mount, D. R.; Nichols, J. W.; Russom, C. L.; Schmieder, P. K.; et al. Adverse Outcome Pathways: A Conceptual Framework to Support Ecotoxicology Research and Risk Assessment. *Environ. Toxicol. Chem.* **2010**, *29* (3), 730–741. <https://doi.org/10.1002/etc.34>.
- (277) Escher, B. I.; Hackermüller, J.; Polte, T.; Scholz, S.; Aigner, A.; Altenburger, R.; Böhme, A.; Bopp, S. K.; Brack, W.; Busch, W.; et al. From the Exposome to Mechanistic Understanding of Chemical-Induced Adverse Effects. *Environment International* **2017**, *99*, 97–106. <https://doi.org/10.1016/j.envint.2016.11.029>.
- (278) Dix, D. J.; Houck, K. A.; Martin, M. T.; Richard, A. M.; Setzer, R. W.; Kavlock, R. J. The ToxCast Program for Prioritizing Toxicity Testing of Environmental Chemicals. *Toxicol. Sci.* **2007**, *95* (1), 5–12. <https://doi.org/10.1093/toxsci/kfl103>.
- (279) Consortium on Thyroid and Pregnancy—Study Group on Preterm Birth; Korevaar, T. I. M.; Derakhshan, A.; Taylor, P. N.; Meima, M.; Chen, L.; Bliddal, S.; Carty, D. M.; Meems, M.; Vaidya, B.; et al. Association of Thyroid Function Test Abnormalities and Thyroid Autoimmunity With Preterm Birth: A Systematic Review and Meta-Analysis. *JAMA* **2019**, *322* (7), 632–641. <https://doi.org/10.1001/jama.2019.10931>.
- (280) World Health Organization; others. *Urinary Iodine Concentrations for Determining Iodine Status in Populations*; World Health Organizations, 2013.
- (281) Aguayo-Orozco, A.; Audouze, K.; Siggaard, T.; Barouki, R.; Brunak, S.; Taboureau, O. SAOP: Linking Chemical Stressors to Adverse Outcomes Pathway Networks. *Bioinformatics* **2019**. <https://doi.org/10.1093/bioinformatics/btz570>.
- (282) Rothman, K. J.; Gallacher, J. E. J.; Hatch, E. E. Why Representativeness Should Be Avoided. *Int J Epidemiol* **2013**, *42* (4), 1012–1014. <https://doi.org/10.1093/ije/dys223>.
- (283) Richiardi, L.; Pizzi, C.; Pearce, N. Representativeness. *Int J Epidemiol* **2014**, *43* (2), 632–633. <https://doi.org/10.1093/ije/dyt271>.
- (284) Lee, S.; Kim, C.; Youn, H.; Choi, K. Thyroid Hormone Disrupting Potentials of Bisphenol A and Its Analogues - in Vitro Comparison Study Employing Rat Pituitary (GH3) and Thyroid Follicular (FRTL-5) Cells. *Toxicol In Vitro* **2017**, *40*, 297–304. <https://doi.org/10.1016/j.tiv.2017.02.004>.
- (285) Gao, H.; Wu, W.; Xu, Y.; Jin, Z.; Bao, H.; Zhu, P.; Su, P.; Sheng, J.; Hao, J.; Tao, F. Effects of Prenatal Phthalate Exposure on Thyroid Hormone Concentrations Beginning at The Embryonic Stage. *Sci Rep* **2017**, *7*. <https://doi.org/10.1038/s41598-017-13672-x>.
- (286) Boberg, J.; Taxvig, C.; Christiansen, S.; Hass, U. Possible Endocrine Disrupting Effects of Parabens and Their Metabolites. *Reproductive toxicology (Elmsford, N.Y.)* **2010**, *30* (2), 301–12. <https://doi.org/10.1016/j.reprotox.2010.03.011>.
- (287) Kortenkamp, A.; Faust, M. Combined Exposures to Anti-Androgenic Chemicals: Steps towards Cumulative Risk Assessment. *International Journal of Andrology* **2010**, *33* (2), 463–474. <https://doi.org/10.1111/j.1365-2605.2009.01047.x>.
- (288) Vernet, C.; Pin, I.; Giorgis-Allemand, L.; Philippat, C.; Benmerad, M.; Quentin, J.; Calafat, A. M.; Ye, X.; Annesi-Maesano, I.; Siroux, V.; et al. 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). <https://doi.org/10.1289/EHP1015>.
- (289) Meeker, J. D.; Yang, T.; Ye, X.; Calafat, A. M.; Hauser, R. Urinary Concentrations of Parabens and Serum Hormone Levels, Semen Quality Parameters, and Sperm DNA Damage. *Environ. Health Perspect.* **2011**, *119* (2), 252–257. <https://doi.org/10.1289/ehp.1002238>.
- (290) Goodson, A.; Robin, H.; Summerfield, W.; Cooper, I. Migration of Bisphenol A from Can Coatings--Effects of Damage, Storage Conditions and Heating. *Food Addit Contam* **2004**, *21* (10), 1015–1026. <https://doi.org/10.1080/02652030400011387>.

- (291) Ejaredar, M.; Lee, Y.; Roberts, D. J.; Sauve, R.; Dewey, D. Bisphenol A Exposure and Children's Behavior: A Systematic Review. *J Expo Sci Environ Epidemiol* **2017**, *27* (2), 175–183. <https://doi.org/10.1038/jes.2016.8>.
- (292) Ejaredar, M.; Nyanza, E. C.; Ten Eycke, K.; Dewey, D. Phthalate Exposure and Childrens Neurodevelopment: A Systematic Review. *Environmental Research* **2015**, *142*, 51–60. <https://doi.org/10.1016/j.envres.2015.06.014>.
- (293) Krause, M.; Klit, A.; Blomberg Jensen, M.; Sjøborg, T.; Frederiksen, H.; Schlumpf, M.; Lichtensteiger, W.; Skakkebaek, N. E.; Drzewiecki, K. T. Sunscreens: Are They Beneficial for Health? An Overview of Endocrine Disrupting Properties of UV-Filters. *International journal of andrology* **2012**, *35* (3), 424–36. <https://doi.org/10.1111/j.1365-2605.2012.01280.x>.
- (294) Li, J.; Ma, M.; Wang, Z. In Vitro Profiling of Endocrine Disrupting Effects of Phenols. *Toxicol In Vitro* **2010**, *24* (1), 201–207. <https://doi.org/10.1016/j.tiv.2009.09.008>.
- (295) Tanner, E. M.; Hallerbäck, M. U.; Wikström, S.; Lindh, C.; Kiviranta, H.; Gennings, C.; Bornehag, C.-G. Early Prenatal Exposure to Suspected Endocrine Disruptor Mixtures Is Associated with Lower IQ at Age Seven. *Environment International* **2019**, 105185. <https://doi.org/10.1016/j.envint.2019.105185>.
- (296) Rodríguez-Carrillo, A.; Mustieles, V.; Pérez-Lobato, R.; Molina-Molina, J. M.; Reina-Pérez, I.; Vela-Soria, F.; Rubio, S.; Olea, N.; Fernández, M. F. Bisphenol A and Cognitive Function in School-Age Boys: Is BPA Predominantly Related to Behavior? *NeuroToxicology* **2019**, *74*, 162–171. <https://doi.org/10.1016/j.neuro.2019.06.006>.
- (297) Thompson, W.; Russell, G.; Baragwanath, G.; Matthews, J.; Vaidya, B.; Thompson-Coon, J. Maternal Thyroid Hormone Insufficiency during Pregnancy and Risk of Neurodevelopmental Disorders in Offspring: A Systematic Review and Meta-analysis. *Clin Endocrinol (Oxf)* **2018**, *88* (4), 575–584. <https://doi.org/10.1111/cen.13550>.
- (298) Casas, M.; Basagaña, X.; Sakhi, A. K.; Haug, L. S.; Philippat, C.; Granum, B.; Manzano-Salgado, C. B.; Brochot, C.; Zeman, F.; de Bont, J.; et al. Variability of Urinary Concentrations of Non-Persistent Chemicals in Pregnant Women and School-Aged Children. *Environment International* **2018**, *121*, 561–573. <https://doi.org/10.1016/j.envint.2018.09.046>.
- (299) Huang, H.-B.; Kuo, P.-H.; Su, P.-H.; Sun, C.-W.; Chen, W. J.; Wang, S.-L. Prenatal and Childhood Exposure to Phthalate Diesters and Neurobehavioral Development in a 15-Year Follow-up Birth Cohort Study. *Environmental research* **2019**.
- (300) Rothman, K. J. Six Persistent Research Misconceptions. *J Gen Intern Med* **2014**, *29* (7), 1060–1064. <https://doi.org/10.1007/s11606-013-2755-z>.
- (301) Cribbie, R. A. Multiplicity Control in Structural Equation Modeling. *Structural Equation Modeling: A Multidisciplinary Journal* **2007**, *14* (1), 98–112. <https://doi.org/10.1080/10705510709336738>.
- (302) Cribbie, R. A. Evaluating the Importance of Individual Parameters in Structural Equation Modeling: The Need for Type I Error Control. *Personality and Individual Differences* **2000**, *29* (3), 567–577. [https://doi.org/10.1016/S0191-8869\(99\)00219-6](https://doi.org/10.1016/S0191-8869(99)00219-6).
- (303) Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing on JSTOR. *ournal of the Royal Statistical Society* **1995**, *57* (1), 289–300.
- (304) Noyes, P. D.; Friedman, K. P.; Browne, P.; Haselman, J. T.; Gilbert, M. E.; Hornung, M. W.; Barone, S.; Crofton, K. M.; Laws, S. C.; Stoker, T. E.; et al. Evaluating Chemicals for Thyroid Disruption: Opportunities and Challenges with in Vitro Testing and Adverse Outcome Pathway Approaches. *Environ. Health Perspect.* **2019**, *127* (9), 95001. <https://doi.org/10.1289/EHP5297>.
- (305) Lang, C.; Fisher, M.; Neisa, A.; MacKinnon, L.; Kuchta, S.; MacPherson, S.; Probert, A.; Arbuckle, T. E. Personal Care Product Use in Pregnancy and the Postpartum Period: Implications for Exposure Assessment. *Int J Environ Res Public Health* **2016**, *13* (1). <https://doi.org/10.3390/ijerph13010105>.
- (306) Rothman, K.; Greenland, S.; Lash, T. *Modern Epidemiology*, 3rd Editio.; Lippincott Williams & Wilkins: Philadelphia, PA, 2008.
- (307) Slama, R.; Werwatz, A. Controlling for Continuous Confounding Factors: Non- and Semiparametric Approaches. *Rev Epidemiol Sante Publique* **2005**, *53 Spec No 2*, 2S65-80.

- (308) Tamayo-Uria, I.; Maitre, L.; Thomsen, C.; Nieuwenhuijsen, M. J.; Chatzi, L.; Siroux, V.; Aasvang, G. M.; Agier, L.; Andrusaityte, S.; Casas, M.; et al. The Early-Life Exposome: Description and Patterns in Six European Countries. *Environment International* **2019**, *123*, 189–200. <https://doi.org/10.1016/j.envint.2018.11.067>.
- (309) Möricke, E.; Buitelaar, J. K.; Rommelse, N. N. J. Do We Need Multiple Informants When Assessing Autistic Traits? The Degree of Report Bias on Offspring, Self, and Spouse Ratings. *J Autism Dev Disord* **2016**, *46*, 164–175. <https://doi.org/10.1007/s10803-015-2562-y>.
- (310) Béranger, R.; Hardy, E. M.; Dexet, C.; Guldner, L.; Zaros, C.; Nougadère, A.; Metten, M.-A.; Chevrier, C.; Appenzeller, B. M. R. Multiple Pesticide Analysis in Hair Samples of Pregnant French Women: Results from the ELFE National Birth Cohort. *Environment International* **2018**, *120*, 43–53. <https://doi.org/10.1016/j.envint.2018.07.023>.
- (311) Jackson-Browne, M. S.; Papandonatos, G. D.; Chen, A.; Calafat, A. M.; Yolton, K.; Lanphear, B. P.; Braun, J. M. Identifying Vulnerable Periods of Neurotoxicity to Triclosan Exposure in Children. *Environ Health Perspect* **2018**, *126* (5). <https://doi.org/10.1289/EHP2777>.
- (312) Understanding REACH - ECHA <https://echa.europa.eu/regulations/reach/understanding-reach> (accessed Nov 12, 2019).
- (313) Understanding CLP - ECHA <https://echa.europa.eu/regulations/clp/understanding-clp> (accessed Nov 12, 2019).

7 Annex

7.2 Regulations of use of phenols and phthalates in France and in the European Union

Other regulations key regulations

a. REACH regulation (1907/2006)

The EC regulation REACH, which stands for registration, evaluation, authorization and restriction of chemicals in the EU, aims to improve the protection of human health and the environment from the risks that can be posed by chemicals.

REACH applies to most chemical substances, not only those used in industrial processes but also in our day-to-day lives, for example in cleaning products, paints as well as in articles such as clothes, furniture and electrical appliances (but not to active substances used in pesticides).

Under this regulation, the first step of restriction is listing of a substance a substance of very high concern (SVHC). According to article 57 of the REACH regulation, a chemical substance may be considered a SVHC if it is carcinogenic, mutagenic, toxic for reproduction, persistent, bioaccumulative, or if there is scientific evidence of adverse effects on human health of similar concern, such as neurotoxicity and endocrine disruption.

Following the EC committees' decision, substances have a possibility to be added to restriction list (Annex XIV) and, for substances on this list, authorization before placement on market is needed ³¹².

Specific phthalate compounds, including BBP, DEHP, DIBP and DBP, are listed in the annex XIV, while bisphenol A is recognized as a SVHC.

b. CLP directive (1272/2008)

The Classification, Labelling and Packaging (CLP) Regulation mandates manufacturers, importers or downstream users of substances or mixtures to classify, label and package their hazardous chemicals appropriately before placing them on the market.

Substances that meet the CLP criteria assigned a certain hazard class and category that cover physical, health, environmental and additional hazards and are labelled as such on product packaging ³¹³. This directive defines specific health hazards, such as carcinogens or reprotoxicants, but not endocrine disruptors. Although Bisphenol A is classified as a reprotoxic substance, category 1B in the Annex VI of the CLP regulation, no labelling requirements apply to this compound yet.

7.3 Publications and oral communication

Publications

Nakiwala D, Shaki A.K, Thomsen C, Patrice F, Gauchez A.M, Corne C, Slama R, Philippat C and the SEPAGES study group et al. Non-persistent environmental chemicals and thyroid hormone levels during pregnancy and at birth; an approach relying on repeated biospecimens and a priori knowledge to reduce the size of the exposome, *in preparation*.

Nakiwala D, Vernet C, Lyon-Caen S, Lavorel A, Rolland M, Cracowski C, Pin I, Calafat AM, Slama R, Philippat C and the SEPAGES study group. Use of personal care products during pregnancy in relation to urinary concentrations of select phenols: a longitudinal analysis from the SEPAGES feasibility study. *Submitted*.

Nakiwala D, Peyre H, Heude B, Bernard JY, Béranger R, Slama R, et al. In-utero exposure to phenols and phthalates and the intelligence quotient of boys at 5 years. *Environ Health*. 2018.

Philippat C, **Nakiwala D**, Calafat AM, Botton J, De Agostini M, Heude B, et al. Prenatal Exposure to Nonpersistent Endocrine Disruptors and Behavior in Boys at 3 and 5 Years. *Environ Health Perspect*. 2017 Sep 15;125.

Conferences and seminars

- 8–9 November, 2018 SF-Dohad, **Poster presentation**, Nakiwala D, Vernet C, Slama R, Philippat C et al. Phenol urinary concentrations in relation to use of personal care products in pregnant women: a longitudinal analysis from SEPAGES feasibility study, Grenoble, France.
- 26–30 August, 2018 ISES-ISEE, **Poster presentation**, Nakiwala D, Vernet C, Slama R (presenter), Philippat C et al. Phenol urinary concentrations in relation to use of personal care products in pregnant women: a longitudinal analysis from SEPAGES feasibility study, Ottawa, Canada.
- 19–21 March, 2018 ISEE Europe Young and Early Career Conference, **Oral presentation**, Nakiwala D, Slama R, Philippat C et al. In-utero exposure to phenols and phthalates and the intelligence quotient of boys at 5 years, Munich, German.
- 5 October 2017 Immuno-psychiatry conference, **Blitz data session**, Dorothy Nakiwala, Rémy Slama, Claire Philippat et al. In-utero exposure to phenols and phthalates and the intelligence quotient of boys at 5 years, Paris, France.

PhD traing and Teaching

Courses taken (141 hours)

Scientific (54)

- i. Advanced Regression Modeling, Université Grenoble Alpes (30 hours)
- ii. Statistical strategies for multi-pollutant modeling, ISEE young careers, Munich, Germany (12 hours)
 - o Principal component analysis (PCA)
 - o Univariate / single-pollutant modelling and corrections for multiple testing
 - o Partial least squares regression (PLSR)
 - o Sparse PLS (SPLS)
 - o Penalized regression models (variable selection using Lasso regression, Ridge regression, and elastic net regression)
- iii. EDISCE Scientific day, Université Grenoble Alpes (12 hours)

Professional integration (47 hours)

- i. Project management, Université Grenoble Alpes (19 hours)
- ii. Think and build your career, Université Grenoble Alpes (7 hours)
- iii. Job Search Techniques, Université Grenoble Alpes (21 hours)

Languages, Université Grenoble Alpes (40 hours)

- i. French courses for non-francophone scientists - winter session (20 hours)
- ii. French courses for non-francophone scientists - spring session (20 hours)

Teaching

Master of Health Engineering, Université Grenoble Alpes (November 2017)

Classes given: Methods of research in environmental health epidemiology (regression modelling and data management).

THESIS SUMMARY

Prenatal exposure to phenols and phthalates, child neurodevelopment and the role of the thyroid hormones

The increase in the prevalence of neurodevelopmental disorders warrants identification of the possible underlying modifiable risk factors. Pregnant women are constantly exposed to several phenols and phthalates that are endocrine disrupting chemicals and have been linked to various adverse neurodevelopmental outcomes in toxicological studies. One of the suggested mechanisms of neurotoxicity of these compounds is by disruption of the thyroid hormone pathway.

The objectives of this thesis were; 1) To document if the use of personal care products (PCP) could influence phenols biomarkers concentrations during pregnancy; 2) to assess associations between prenatal exposure to phenols and phthalates and child neurodevelopment; and 3) to assess associations between prenatal exposure to phenols and phthalates on the thyroid hormone levels of pregnant women and their newborns.

We relied on three complementary cohorts; the SEPAGES-feasibility study with multiple urine samples and detailed, time-resolved information on PCP use, the EDEN cohort with maternal urinary concentrations of phenols, phthalates and information on child neurodevelopment and the SEPAGES cohort with information on gestational exposure to phenols and phthalates (assessed in 2 pools of 21 urine sample per participant) and levels of thyroid hormones levels during pregnancy and at birth.

The total number of PCP applications (without distinction) was positively associated with the parabens' and bisphenol S urinary concentrations during pregnancy but not with other phenols. No phenol or phthalate metabolite pregnancy concentration was negatively associated with IQ. Bisphenol A was with the increased relationship problems subscale at 3 years and the hyperactivity-inattention problems at 5 years. MnBP was associated with increased internalizing behavior, relationship problems, and emotional problems at 3 years. MBzP was associated with internalizing behavior and relationship problems scores at 3 years. After dichotomizing behavioral scores, triclosan tended to be associated with increased emotional problems at both 3 and 5 years. Regarding the associations with maternal hormones, bisphenol A was negatively associated TSH; MBzP was positively associated with the TT4 and triclosan was negatively associated with the TT3/TT4 ratio. When biomarker concentrations were categorized in tertiles, we observed non-monotonic associations between TSH and triclosan (U-shape) and MnBP (inverse U-shape). In newborns, only sex-specific effects were observed: bisphenol A was positively associated with the TT4 z-score in male newborns while triclosan was negatively associated with the TT4 z-score in females.

Findings in this study concur with previous literature that PCPs use may contribute to exposure to parabens. Our study was the first to report associations between PCP use and bisphenol S, a substitute of bisphenol A. In line with previous studies, bisphenol A and various phthalates, including DBP and BBP exposure during pregnancy, were associated adverse behavioral symptoms among boys. Our study was the first to report adverse neurobehavioral effects in relation to triclosan exposure. The four compounds associated with adverse behavioral effects in EDEN were also associated with one or more thyroid hormone levels of mothers or newborns in the SEPAGES cohort, suggesting possible disruption of thyroid hormones homeostasis.

RESUME DE LA THESE

Exposition prénatale aux phénols et aux phtalates, développement neurologique de l'enfant et rôle des hormones thyroïdiennes

L'augmentation rapide de la prévalence de certains troubles du neurodéveloppement nécessite une meilleure compréhension des facteurs de risque modifiables de ces troubles. Les phénols et les phtalates sont des perturbateurs endocriniens dont l'exposition est prévalente en population générale et pour lesquels des effets neurotoxiques sont suspectés. L'un des mécanismes sous-jacents à ces effets pourrait être une action de ces composés sur l'axe thyroïdien

Les objectifs étaient de documenter si l'utilisation de produits de soin personnel pourrait influencer les concentrations urinaires de biomarqueurs d'exposition aux phénols chez les femmes enceintes; 2) d'évaluer les associations entre l'exposition prénatale aux phénols et aux phtalates et le neurodéveloppement des enfants; et d'évaluer les associations ces expositions prénatales et les concentrations d'hormones thyroïdiennes chez la femme enceinte et le nouveau-né.

Ce travail s'appuie sur trois cohortes françaises complémentaires; l'étude SEPAGES-faisabilité pour laquelle nous disposons de multiples échantillons d'urine et d'informations détaillées sur l'utilisation de produits de soin, la cohorte EDEN pour laquelle nous disposons des concentrations urinaires maternelles de phénols et phtalates et d'information sur le neurodéveloppement et la cohorte SEPAGES au sein de laquelle nous avons étudié les effets de l'exposition aux phénols et phtalates sur les niveaux d'hormones thyroïdiennes pendant la grossesse et à la naissance. Dans cette étude, un recueil intensif d'échantillons d'urine (42 échantillons d'urine par grossesse) a permis d'évaluer finement les expositions.

Le nombre total d'utilisation de produits de soin était associé à une augmentation des concentrations urinaires de bisphénol S et des parabènes mais pas des autres phénols. Aucun des composés étudiés n'était associé négativement aux scores de quotient intellectuel. Bisphénol A étaient associées à plus de symptômes de type troubles relationnels à 3 ans et de comportements de type hyperactif à 5 ans. Le MnBP était associé à une augmentation des scores liés aux troubles émotionnels et relationnels à 3 ans, tandis que le MBzP était lui associé à une augmentation des scores liés aux troubles internalisés et les troubles relationnels. Enfin lorsque les scores de comportement étaient étudiés en tant que variables binaires, une association entre le triclosan et les scores liés aux troubles émotionnels était observée à 3 et 5 ans. Dans la cohorte SEPAGES, Les concentrations urinaires de bisphénol A étaient négativement associées aux concentrations de thyrostimuline (TSH) maternelles tandis que le MBzP, était lui positivement associé aux Z-scores de thyroxine totale et le triclosan négativement associé au ratio TT3/TT4. Lorsque les expositions étaient étudiées en tertiles, des relations non linéaires ont aussi été observées entre le triclosan et la TSH (relation en U) et le MnBP et la TSH (U inversé). Pour les nouveau-nés, après stratification sur le sexe, les concentrations urinaires maternelles de bisphénol A étaient positivement associées au niveau de TT4 chez les garçons tandis que le triclosan était négativement associé à cette hormone chez les filles.

Les associations positives entre l'utilisation de produits de soin et les concentrations urinaires de parabènes étaient en accord avec les études précédentes sur le sujet. Notre étude était la première à mettre en évidence de telles associations avec le bisphénol S. Les associations observées entre les expositions prénatales au bisphénol A et au DBP et le comportement avaient déjà été mis en évidence dans des études précédentes chez de jeunes garçons. Pour ce qui est du triclosan notre étude était la première à s'intéresser aux associations avec le comportement. Les composés associés au comportement dans la cohorte EDEN ont tous été associés à au moins une hormone thyroïdienne dans la cohorte SEPAGES, suggérant une possible perturbation de l'homéostasie des hormones thyroïdiennes.