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Health effects of endocrine disruptors during pregnancy and childhood

Munawar Hussain Soomro

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Spécialité

Épidémiologie

**ECOLE DOCTORALE PIERRE LOUIS DE SANTE PUBLIQUE A PARIS:
EPIDEMIOLOGIE ET SCIENCES
DE L'INFORMATION BIOMEDICALE**

Présentée par

Munawar Hussain SOOMRO

Pour obtenir le grade de

DOCTEUR de SORBONNE UNIVERSITÉ

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Effets sur la santé des perturbateurs endocriniens pendant la grossesse et l'enfance

(Health effects of endocrine disruptors during pregnancy and childhood)

Soutenue le 27 Mai 2019

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Thèse réalisée dans l'unité INSERM UMR-S 1136:
Equipe EPAR "Epidémiologie des maladies Allergiques et Respiratoires".

To my grandparents, mother, father, brother, two sisters and
my son Mubashir

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RÉSUMÉ LONG (Français)

Introduction

Tous les êtres humains sont exposés aux produits chimiques dans la vie de tous les jours, provenant des aliments, de l'environnement intérieur, des cosmétiques et d'autres produits qui nous entourent, que ce soit à la maison et au travail. De plus, l'utilisation de compléments alimentaires, de médicaments et de médicaments phytothérapeutiques peut avoir un effet chimique sur le corps, ce qui peut avoir des effets souhaités ou non. Parmi les milliers de produits chimiques auxquels nous sommes potentiellement exposés au quotidien, il a été démontré que certains agissaient en tant que perturbateurs endocriniens (PE) chez les animaux de laboratoire. Les PE sont des composés synthétiques capables de se lier aux récepteurs cellulaires et d'imiter ou de bloquer le fonctionnement des hormones naturelles, pouvant ainsi entraîner des effets néfastes sur la santé. Cependant, il existe également des composés naturels dotés de telles propriétés. Chez l'homme, il a été démontré que les PE étaient responsables de la cryptorchidie (testicules non descendus dans le scrotum) et de l'hypospadias (malformation des organes génitaux) chez les garçons, de la puberté précoce chez les filles, du cancer du sein et de la prostate, du syndrome métabolique, de l'obésité, du diabète, des maladies cardiovasculaires, d'une moindre qualité du sperme, de l'augmentation de l'incidence du cancer des testicules et de niveaux réduits de l'hormone sexuelle masculine chez l'homme. Certaines des périodes les plus sensibles de la vie humaine sont la vie fœtale et l'enfance, car le corps humain se développe de manière significative au cours de

ces périodes, ce qui nécessite un équilibre des systèmes endocriniens impliqués pendant les différents stades du développement.

Ces dernières années, des hypothèses ont été émises selon lesquelles les PE pourraient également contribuer au développement de maladies allergiques et respiratoires. En outre, il est probable que les PE pourraient déjà agir pendant la grossesse en provoquant un diabète gestationnel (DG) et une hypertension induite par la grossesse, pouvant également provoquer une hypertension et un diabète plus tard dans la vie.

Ce travail de thèse a permis de mettre en évidence que l'exposition maternelle aux phtalates et aux métaux lourds (plomb, cadmium et manganèse) pendant la grossesse pourrait être associée au diabète gestationnel, à l'hypertension induite par la grossesse et à l'eczéma/dermatite atopique chez l'enfant. Cet objectif sera atteint grâce à la mise en place d'une étude robuste, réduisant ainsi le risque d'une mauvaise classification des maladies et de l'exposition.

Matériel et méthodes

Nous avons utilisé les données de la cohorte Française de naissance mère-enfant, EDEN (Étude des déterminants pré et post-natals du développement de la santé de l'enfant) pour examiner les hypothèses de ce travail de thèse. La cohorte de naissance mère-enfant EDEN a été créée en 2003 et mise en place dans deux hôpitaux universitaires des villes de Nancy et de Poitiers, en France. La participation à cette cohorte a été proposée à toutes les femmes visitant les cliniques prénatales des hôpitaux universitaires avant la 24^{ème} semaine d'aménorrhée. Les critères d'exclusion étaient les grossesses multiples, le diabète connu avant la grossesse, l'analphabétisme ou le projet de quitter la région au cours des trois prochaines années.

Les mères ont été recrutées entre 2003 et 2006. Les mères ont été examinées à trois reprises pour des examens cliniques, une fois entre la 24^{ème} et la 28^{ème} semaines d'aménorrhée, une fois à l'accouchement et une fois 5–6 ans après l'accouchement. Les enfants ont été suivis pendant cinq ans grâce à des visites dans les centres de recherche et des questionnaires envoyés aux parents.

Les femmes ont été invitées à un examen clinique et ont fourni les urines de leur première miction matinale recueillie à la maison avant leur visite à l'hôpital entre la 24^{ème} et la 28^{ème} semaine de gestation (deuxième trimestre de la grossesse). En cas d'oubli, l'échantillon d'urine a été collecté à l'hôpital. Les analyses des échantillons ont été effectuées au laboratoire national du centre de santé environnementale, aux centres de contrôle et de prévention des maladies (CCM) à Atlanta, Géorgie (États-Unis), pour onze métabolites provenant de huit phtalates: le phtalate de diéthyle (DEP), le phtalate de di-n-butyle (DBP), le phtalate de di-isobutyle (DiBP), le phtalate de di (2-éthylhexyle) (DEHP), le phtalate de benzylbutyle (BBzP), le phtalate de di-isononyle (DiNP), le phtalate de di-n-octyle (DOP), le phtalate de di-isodécyle (DiDP).

L'eczéma a été évalué par l'administration d'une version enrichie du questionnaire standardisé et validé de l'étude internationale sur l'asthme et les allergies de l'enfance (ISAAC) complété par les parents lorsque leur enfant était âgé de 1, 2, 3, 4 et 5 ans, sur rendez-vous. Chaque année de suivi, l'eczéma était défini comme un eczéma diagnostiqué par un médecin au cours des 12 derniers mois. L'eczéma « ever » a été défini comme ayant déjà fait l'objet d'un diagnostic d'eczéma à l'âge de 1, 2, 3, 4 ou 5 ans. En outre, deux phénotypes d'eczéma ont été distingués : l'eczéma d'apparition précoce dans le cas où un eczéma a été rapporté dans un questionnaire quelconque au cours des deux premières années et l'eczéma d'apparition tardive s'il n'a été signalé qu'entre 24 et 60 mois. À l'âge de 5 ans, les IgE

sériques totales chez 293 garçons ont été évaluées. Les garçons ont été classés comme atopiques s'ils présentaient des taux d'IgE \geq 60 UI / mL.

Trois métaux, le plomb (Pb), le cadmium (Cd) et le manganèse (Mn) ont été mesurés à partir d'échantillons sanguins de grossesse pendant le deuxième trimestre. Les femmes ont été invitées à un examen clinique et à fournir un échantillon de sang veineux. Entre la 24^{ème} et la 28^{ème} semaine, les concentrations de glucose dans le sang maternel ont été mesurées une heure après une provocation de glucose à 50 g. Les femmes ayant une glycémie à 1 heure supérieure à 130 mg/dL à Nancy et à 140 mg/dL à Poitiers devaient subir un test de tolérance au glucose oral de 3 h à 100 g. Le diagnostic de diabète gestationnel a été réalisé en utilisant le test de tolérance au glucose oral selon les critères de Carpenter et Coustan.

Lorsque plus de 2 glycémies étaient supérieures aux valeurs limites suivantes: à jeun = 95 mg / dL, à 1 h = 180 mg / dL, à 2 h = 155 mg / dL et à 3 h = 140 mg / dL. Un diagnostic de tolérance altéré au glucose (IGT) a été posé si l'une des valeurs limites du test de tolérance au glucose par voie orale était atteinte ou dépassée. Les variables DG (diabète gestationnel) et IGT ont été considérées comme variables catégoriques dans l'analyse. Aux fins de l'analyse, une variable DG ou IGT a également été considérée (combinaison de DG et IGT).

La tension artérielle maternelle a été mesurée lors de visites mensuelles de routine, en position de décubitus dorsal, à l'aide d'un tensiomètre à mercure standard. Une série de mesures a été prise par la sage-femme assignée à l'étude, entre la 24^{ème} et 28^{ème} semaine en utilisant un dispositif différent (semi-automatisé), avec une moyenne calculée sur deux mesures pour déterminer les valeurs à l'issue de cette visite. Les mesures prises au cours de chaque trimestre de gestation (T1, T2 et T3) ont été moyennées pour déterminer les valeurs de pression artérielle systolique (PAS)

et de pression artérielle diastolique (PAD) assignées à chaque période. Les femmes ont été classées comme ayant une hypertension induite par la grossesse basée sur une PAS \geq 140 mmHg et / ou une PAD \geq 90 mmHg mesurée au moins deux fois après la 22ème semaine de gestation, de sorte que les mesures élevées auraient pu être observées au cours de l'une des trois périodes, à tout moment après 22 semaines. En conséquence, aucun diagnostic d'hypertension induite par la grossesse n'a été posé avant 24 semaines. Tous les cas d'hypertension induite par la grossesse ont été diagnostiqués par les médecins.

Toutes les analyses statistiques ont été réalisées à l'aide du logiciel statistique Stata 14 (Stata Corp LP, 4905 Lakeway Drive, College Station, Texas 77845, États-Unis) et la valeur $p < 0,05$ était considérée comme statistiquement significative.

Résultats

Notre premier article a montré que la prévalence de l'eczéma « ever » était respectivement de 9,7%, 15,7%, 21,0%, 26,6% et 30,4% à 1, 2, 3, 4 et 5 ans. L'incidence a diminué de 9,7% à l'âge de 1 an à 5,2% à l'âge de 5 ans. Nous avons mis en oeuvre une régression logistique multiple et avons observé que les métabolites du phtalate de di-isobutyle (DiBP) et du phtalate de di-isononyle (DiNP) étaient positivement associés à l'eczéma d'apparition précoce (de 0 à 24 mois) (15,7%) et tardive (de 24 à 60 mois) (14,7%). L'application du modèle de Cox a montré une association significative entre l'occurrence d'eczéma au cours des cinq premières années et les métabolites DiBP et DiNP. Chez les garçons sensibilisés (taux IgE supérieur au seuil), les métabolites du phtalate de di-n-butyle (DBP) et du DiBP étaient significativement associés à un eczéma (risque relatif (RR) = 1,67; intervalle de confiance à 95% (IC) 1,10-2,54; $P = 0,01$ et HR = 1,87; IC à 95% 1,01-3,48; $P = 0,04$, respectivement).

Dans notre deuxième article, nous avons montré que la prévalence du DG était de 7,06%, alors que celle de l'IGT était de 10,1%. Nous avons observé une association statistiquement significative entre l'exposition au Cd et la combinaison du DG et de l'IGT dans le modèle brut ainsi que dans le modèle ajusté (Odds-ratio (OR): 1,50, CI: 1,50-1,06 et OR: 1,61, CI: 1,05-2,48, respectivement). Des associations à la limite de la signification ont été observées dans le modèle brut et dans le modèle ajusté entre l'exposition au Cd et le DG (OR: 1,64, CI: 0,99-2,76 et 1,81, CI: 0,93-3,50 respectivement). De plus, une association significative a été observée entre l'exposition au Pb et GDM dans le modèle non ajusté (OR: 1,83, IC à 95%: 1,01-3,29), une tendance persistante après ajustement sur les facteurs de confusion (OR: 1,65, IC: 0,82 - 3,34). Tout en prenant en compte les quartiles de la distribution des métaux, une augmentation significative de l'Odds-ratio pour le DG et l'IGT combinés a été observée pour une exposition au Cd dans le 4ème quartile après ajustement sur les facteurs de confusion (OR: 2,33, IC: 1,10-4,92). Aucune association statistiquement significative n'a été observée ailleurs après ajustement sur les facteurs de confusion.

Dans le troisième article, la prévalence de l'hypertension gravidique était de 4,80%. Nous avons observé que les métabolites de phtalates de bas poids moléculaire comme le phtalate de monoéthyle (MEP) et le phtalate de mono-n-butyle (MBP) étaient positivement associés à une hypertension induite par la grossesse dans les modèles bruts (OR : 1,43, IC : 1,04-1,96, p-value = 0,02 et OR : 1,48, IC : 1,10-2,01, p-value = 0,01) et dans les modèles ajustés (OR : 1,47, IC : 1,01-2,14, p-value = 0,04 et OR : 1,66, IC : 1,11-2,47, p-value = 0,01), respectivement. Nous avons observé une association à la limite de la significativité pour l'un des métabolites du phtalate de haut poids moléculaire : le phtalate de mono (3-carboxypropyle) (MCPP) dans le modèle

brut (OR : 1,46, IC : 0,96-2,24, p-value = 0,08) ainsi qu'en modèle ajusté (OR : 1,56, IC : 0,93-2,64, p-value= 0,09). En prenant en compte les quartiles, nous n'avons pas observé d'association entre le MEP et l'hypertension gravidique. Cependant, une augmentation significative de l'Odds-ratio pour le métabolite de la MBP a été observée pour une exposition dans son 4ème quartile, dans le modèle brut (OR :3,46, IC : 1,10-10,87, p-value = 0,03) ainsi que dans le modèle ajusté sur les facteurs de confusion (OR : 5,91; IC : 0,93- 37,79, p-value = 0,06).

Conclusion

En conclusion, nos résultats apportent de nouvelles preuves potentielles sur la relation entre les perturbateurs endocriniens (phtalates et métaux lourds étudiés) et la santé de la mère et de l'enfant, selon lesquelles l'exposition prénatale à certains phtalates et métaux toxiques lourds pourrait jouer un rôle dans le développement du diabète gestationnel et de l'hypertension induite par la grossesse pendant la grossesse et, ultérieurement, un eczéma pendant la petite enfance. De futures recherches centrées sur des populations plus importantes et analysant de multiples expositions évaluées avant et après la naissance seraient nécessaires pour fournir davantage de preuves sur les contributions possibles des polluants émergents afin d'étudier le rôle des phtalates et des métaux lourds.

Mots-clés: Phthalate, exposition prénatale, la cohorte mère-enfant, marqueurs urinaires, eczéma, IgE totales, atopique, garçons, métaux-lourds, diabète sucré gestationnel, intolérance au glucose, perturbateur endocrinien, la grossesse hypertension induite par la grossesse.

ABSTRACT (ENGLISH)

All humans are exposed to chemicals in everyday life, from food, indoor environment, cosmetics and other products that surround us at home and at work. Also the use of food supplements, medicines and herbal medicines may cause a chemical effect of the body, which can have both desired and unintended effects. Among the thousands of chemicals, one might be exposed to in daily life, some have been shown to act as endocrine disrupting chemicals (EDCs) in laboratory animals. EDCs are man-made compounds that can bind to cell receptors and mimic or block the functions of natural hormones potentially resulting in adverse health effects. In humans, EDCs have been found to contribute to cause cryptorchidism (undescended testicles to scrotum) and hypospadias (malformation of the genitals) in baby boys, earlier onset of puberty in girls, breast and prostate cancer, metabolic syndrome, obesity, diabetes and cardiovascular diseases and low semen quality, increased incidence of testicular cancer and reduced levels of the male sex hormone in men. Some of the most sensitive periods in the human life are fetal life and childhood, as humans and human bodies during these periods are undergoing a significant development, which requires a balance of the endocrine systems involved in the various stages of development.

In recent years, hypotheses have been put forward that EDCs may contribute also to the development of allergic and respiratory diseases. In addition, it is likely that EDCs could act already during pregnancy by causing gestational diabetes mellitus and pregnancy induced hypertension, which may also trigger to develop hypertension and diabetes mellitus later in life.

This dissertation provides evidence that maternal exposure to phthalates and heavy metals (Lead, Cadmium and Manganese) during pregnancy may be associated with gestational diabetes mellitus, pregnancy induced hypertension and later eczema/atopic dermatitis in children. This will be reached through a robust study design thus diminishing exposure and diseases misclassification.

We used ongoing French EDEN-mother child cohort data for our three studies. Our first paper showed that the metabolites of di-isobutyl phthalate (DiBP) and di-isononyl phthalate (DiNP) were positively associated with early-onset (0-24 months of age) eczema (15.7%) and late-onset (24-60 months of age) eczema (14.7%). Applying the Cox's model showed a significant association of occurrence of eczema in the first 5 years of age with DiBP and DiNP metabolites. Among IgE sensitized boys, metabolites of di-n-butyl phthalate (DBP) and DiBP were significantly associated with ever eczema (Hazard Ratio (HR) = 1.67; 95% Confidence Interval 1.10-2.54; P = 0.01 and HR = 1.87; 95% CI 1.01-3.48; P = 0.04, respectively).

While in paper two, we observed that Lead was significantly associated with gestational diabetes mellitus (Odds-Ratio: 1.83, 95% Confidence Interval: 1.01-3.29, P-value = 0.04) and this persisted after adjusting for confounders (1.65, 0.82-3.34, P-value = 0.08). A borderline significance existed also between Cadmium and gestational diabetes mellitus. The association was statistically significant between Cadmium and the combination of gestational diabetes mellitus and impaired glucose tolerance in the adjusted model (1.61, 1.05 – 2.48, P-value = 0.03).

Third paper shows that, low molecular weight phthalates metabolites; Monoethyl phthalate (MEP) and Mono-n-butyl phthalate (MBP) were positively associated with pregnancy induced hypertension in crude (Odds Ratio: 1.43 95% Confidence Interval: 1.04-1.96, p-value = 0.02 and 1.48, 1.10-2.01, p-value = 0.01) and in adjusted (1.47, 1.01-2.14, p-value = 0.04 and 1.66, 1.11-2.47, p-value = 0.01) models respectively. We observed a borderline association with one of the high molecular weight phthalate metabolite; Mono (3-carboxypropyl) phthalate (MCPP) in crude (1.46, 0.96-2.24, p-value = 0.08) as well as in adjusted model (1.56, 0.93-2.64, p-value = 0.09).

In conclusion, our results add new suggestive evidence on the relationship between EDCs (phthalates and heavy metals we studied) and maternal and children health, that prenatal exposure to certain phthalates and heavy toxic metals may play a role in the development of gestational diabetes mellitus and pregnancy induced hypertension during pregnancy and later eczema in early childhood. Future research focusing on larger populations and addressing multiple exposures assessed prenatally and postnatally are required to provide more evidence on possible contributions of emerging pollutants to study the role of phthalates and heavy metals.

Keywords: Phthalate, prenatal exposure, mother-child cohort, urinary markers, eczema, total IgE, atopic, boys, heavy metals, mother-child cohort, gestational diabetes mellitus, impaired glucose tolerance, endocrine disruptor, pregnancy induced hypertension.

ARTICLES IN THESIS

Articles published or submitted

- **Soomro MH**, Heude B, Bornehag CG, Annesi-Maesano I. The association between maternal urinary phthalate concentrations and pregnancy induced hypertension: Results from the EDEN mother-child Cohort study. (Submitted to Env Internrtional)
- **Soomro MH**, Baiz N, Huel G, Yazbeck C, Botton J, Heude B, Bornehag CG, Annesi-Maesano I. Exposure to heavy metals during pregnancy related to gestational diabetes mellitus in diabetes-free mothers. *Sci Total Environ.* 2019; 656: 870-876.
- **Soomro MH**, Baiz N, Philippat C, Vernet C, Siroux V, Maesano CN, Sanyal S, Slama R, Bornehag CG, Annesi-Maesano I. Prenatal exposure to phthalates and the development of eczema phenotypes in male children: Results from the EDEN mother-child Cohort study. *Environ Health Persp.* 2018; 126(2): 027002.

SCIENTIFIC COMMUNICATIONS

- **2018: Soomro MH**, Bornehag CG, Heude B, Annesi-Maesano I. The association between maternal urinary phthalate concentrations and pregnancy induced hypertension. Poster presentation at Annual conference of the Doctoral School (ED393), Saint Malo-2018.
- **2017: Soomro MH**, Baiz N, Huel G, Yazbeck C, Botton J, Heude B, Bornehag CG, Annesi-Maesano I. Exposure to metals during pregnancy and the association with gestational diabetes mellitus. Poster presentation at 10Th DOHaD world Congress. Rotterdam, The Netherlands.
- **2016: Soomro MH**. Prenatal exposure to phthalates and the development of eczema phenotypes in male children. Oral presentation at HEALS Annual Meeting. Leiden, The Netherlands.
- **2016: Soomro MH**, Bornehag CG, Baiz N, Philippat C, Vernet C, Siroux V, Slama R, Annesi-Maesano I. Prenatal exposure to phthalates and eczema in male children. Poster presentation at 28th ISEE Conference, Rome, Italy.

ABBREVIATIONS

As	Arsenic
BBzP	Benzylbutyl phthalate
BMI	Body mass index
Cd	Cadmium
CI	Confidence Interval
Cr	Chromium
DBP	Di-n-butyl phthalate
DEHP	Di(2-ethylhexyl) phthalate
DiBP	Di-isobutyl phthalate
DiDP	Di-isodecyl phthalate
DiNCH	Di-iso-nonyl-cyclohexane-di-carboxylate phthalate
DiNP	Di-isononyl phthalate
EDCs	Endocrine disrupting chemicals
EDEN	Etude des Déterminants pré et post natals du développement de la santé de l'Enfant
GDM	Gestational diabetes mellitus
HMW	High molecular weight
IGT	Impaired glucose tolerance
LMW	Low molecular weight
LOD	Limit of detection
MBP	Mono-n-butyl phthalate
MBzP	Monobenzyl phthalate
MCNP	Monocarboxy-isononyl phthalate
MCOP	Monocarboxy-isoctyl phthalate

MCPP	Mono (3-carboxypropyl) phthalate
MECPP	Mono(2-ethyl-5-carboxypentyl) phthalate
MEHHP	Mono (2-ethyl-5-hydroxyhexyl) phthalate
MEHP	Mono(2-ethylhexyl) phthalate
MEOHP	Mono(2-ethyl-5-oxohexyl) phthalate
MEP	Monoethyl phthalate
MiBP	Mono-isobutyl phthalate
Mn	Manganese
OR	Odds Ratio
Pb	Lead
SD	Standard Deviation

1. INTRODUCTION

In our daily life, we widely use chemical products to facilitate ourselves. These chemical products can be found in the food wrappings, children's toys, cleaning products, medical devices, pharmaceuticals, polyvinyl chloride (PVC) flooring, building materials because of their properties of transparency, durability, and flexibility. However, the concern about its effects on health are not very clear. These chemicals included in these commonly used products include substances known as endocrine disruptors or endocrine disrupting chemicals (EDCs)¹, which have the properties to interact with the natural hormone system in humans and as well as in animals and thus impact on their health.

¹ According to United Nations Environment Programme and the World Health Organization, EDCs are defined as:

“An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations” United Nations Environment Programme, World Health Organization. 2013. State of the science of endocrine disrupting chemicals 2012 / edited by Åke Bergman, Jerrold J. Heindel, Susan Jobling, Karen A. Kidd and R. Thomas Zoeller. ISBN: 978-92-807-3274-0 (unep) and 978 92 4 150503 1 (who).

1.1 Endocrine Disrupting Chemicals and their health effects in humans

EDCs are man-made chemicals, found in various materials such as pesticides, metals, additives or contaminants in food, and personal care products (Figure 1) used as industrial solvents/lubricants and their by-products (polychlorinated biphenyls, polybrominated biphenyls, dioxins), plastics (bisphenol A), plasticizers (phthalates), heavy metals [(Lead (Pb), Cadmium (Cd), Manganese (Mn), Arsenic (As), Mercury (Hg), zinc (Zn)], pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyl-trichloroethane (DDT)], fungicides (vinclozolin) and pharmaceutical agents (diethylstilbestrol) (Iavicoli et al. 2009; Meeker 2012; United Nations Environment Programme and World Health Organization 2013; Unuvar and Buyukgebiz 2012).

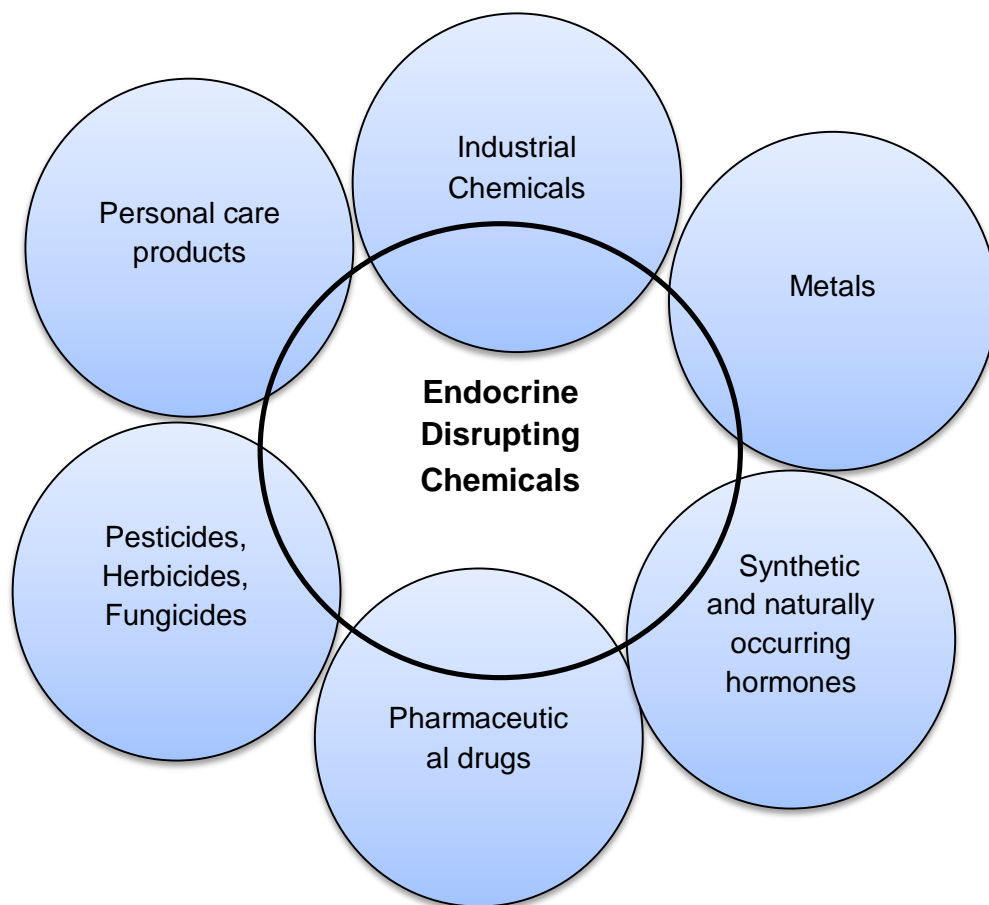


Figure 1. Endocrine disruptors are chemicals or chemical mixtures that interfere with normal hormone actions. They may be of any type of chemical substance.

1.1.1 Human exposure to phthalates

Phthalates are ubiquitous environmental toxicants, arising from contact with a wide range of consumer products. Normally, phthalates in their pure form are colorless, odorless and oily liquids with high lipophilic properties and low solubility in water. People can be exposed to phthalates through inhalation, dermal absorption (including air-to-skin transport), contact with contaminated surfaces, and use of personal care products and ingestion (including dietary ingestion and incidental ingestion) or intravenous injection (Colacino et al. 2010; Mitani et al. 2003; Mitani et al. 2004; Schettler 2006; Swan 2008).

For many phthalates main route of exposure is through food ingestion (Colacino et al. 2010), including fatty foods such as dairy products, fish and oil (Cheng et al. 2013; Kappenstein et al. 2012; Sharman et al. 1994), in drinking water (Santana et al. 2014; Wang et al. 2015), and also in wild marine organisms (Hu et al. 2016). As matrices and solvents, it has been reported that considerable amount of phthalates (up to 5%) are added to a wide range of personal care products, such as cosmetics, lotions, deodorants, perfumes, hair products and nail polish (Al-Saleh and Elkhatib 2016; Huang et al. 2018; Just et al. 2010; Koniecki et al. 2011; Silva et al. 2004). Interestingly, DEHP is present in common medical devices e.g. tubing, blood and intravenous bags, catheters, dialysis equipment, and in the manufacturing of disposable and surgical gloves (National Toxicology 2011).

Phthalates are frequently used in children toys because of their flexibility and resistant kinking properties (Andaluri et al. 2018; Ashworth et al. 2018), however

phthalates are can also found in sex toys as they are largely untested and the policies are unregulated (Zach Biesanz 2007).

Another source of exposure to phthalates are through the French wines and spirits marketed in Europe or exported has been reported, in which DBP, DEHP and BBP phthalates were detected (Chatonnet et al. 2014; Fan et al. 2014).

Based on large production volumes, widespread use, and environmental contamination, bio-monitoring data suggest that 75-100% of the population is exposed to phthalates on a daily basis (Hogberg et al. 2008; Silva et al. 2004). Once consumed by humans or animals, phthalates are rapidly metabolized in the gut, liver, and blood by esterases and lipases. Initially, the phthalate diester break down into their respective hydrolytic monoester where only one alkyl-chain remains on the phthalic acid backbone, and interestingly, it is often the monoester metabolites that induce toxicity (Braun et al. 2013; Koch and Calafat 2009). Depending on the size of the remaining monoester metabolite, the alkyl-chain can undergo further oxidative metabolism and ultimately glucuronidation depending on the species. These hydrolytic monoester and oxidative monoester metabolites, in addition to the parent phthalates, are used as biomarkers to estimate daily human exposure levels (Koch and Calafat 2009).

1.1.2 Health effects of phthalates

Human body is working on a well-functioning endocrine system to regulate hormones release. The endocrine system is made up of glands that play a central role in all vertebrates by producing and secreting hormones, chemical substances produced in the body that regulate the activity of cells or organs. These hormones regulate the body's growth, metabolism (the physical and chemical

processes of the body), and sexual development and function. In vertebrates, the centre for neural control for all endocrine systems is managed by hypothalamus (Diamanti-Kandarakis et al. 2009; Gore et al. 2015). EDCs may interfere with the body's endocrine system (Figure 2) in the production, secretion, transportation, metabolism, receptor binding, mediation of effects, and excretion of natural hormones which regulate developmental processes and support endocrine homeostasis in the organism and produce adverse developmental, reproductive, neurological, and immune effects in both humans and animals (Kavlock et al. 1996; Kuo et al. 2012; Meeker 2012).

For many years, when assessing the possible effects of EDs, according to toxicologists, it was said that “the dose makes the poison”, implying that higher doses cause greater harm and assuming that effects not seen at high doses are not expected at low doses. In contrast to this dogma in toxicology, multiple studies have revealed that neither threshold nor linear no-threshold models are applicable to the responses seen in connection with EDCs (Rhomberg and Goodman 2012; Vandenberg et al. 2012). Dose-response curves associated with EDCs often appear as Ushaped (non-monotonic) as compared with the linear dose-response curves usually displayed in toxicology (Rhomberg and Goodman 2012; Vandenberg et al. 2012). Humans are exposed to many different EDCs simultaneously, each of these chemicals may be being “safe”, but mixtures of these EDCs may have both additive and synergistic effects. Therefore, a mixture may result in more severe endocrine disruption than one chemical at a time (Fowler et al. 2012).

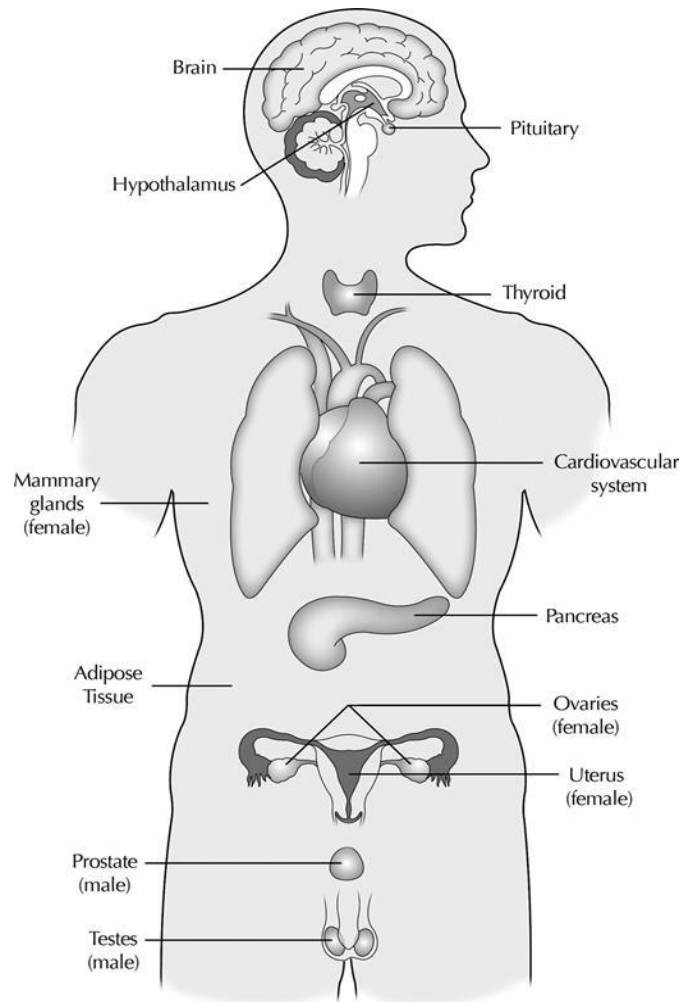


Figure 2. Model of the endocrine systems targeted by endocrine disrupting chemicals. The figure demonstrates that all hormone-sensitive physiological systems are vulnerable to EDCs, including brain and hypothalamic neuroendocrine systems; pituitary; thyroid; cardiovascular system; mammary gland; adipose tissue; pancreas; ovary and uterus in females; and testes and prostate in males.

1.1.3 Phthalates effects during pregnancy and early life

During first trimester of pregnancy, the embryo becomes a fetus and vital organs for example kidney, brain and liver are developed and start functioning. During second trimester, fetus start developing eyelids, eyebrows, eye lashes, bones becoming denser and nervous system, reproductive organs and genitalia are fully developed and start functioning. During the third trimester of the pregnancy, the lungs begin to develop and does not start working until several weeks later. If the fetus is exposed to harmful environmental chemicals during this critical developmental period, it may result in adverse health effects that may manifest later in life (Figure 3).

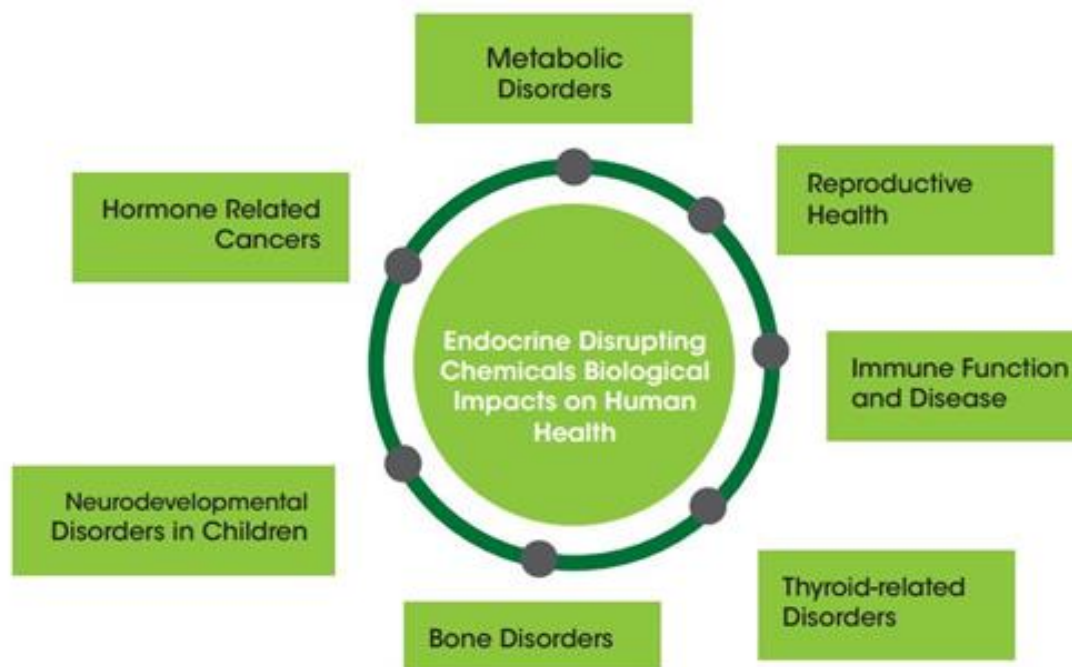


Figure 3. Impact of Endocrine Disrupting Chemicals on human health.

Prenatal and early life are critical in the development of the immune system; exposure to EDCs as a toxic pollutants during this period can result in an increased risk of adverse health outcomes later in life. Exposure to these EDCs during the pregnancy and early childhood is potentially sensitive periods for the development of adverse effects on the health of mother causing gestational diabetes mellitus (GDM) (Liu et al. 2018; Oguri et al. 2019; Romano et al. 2015; Shapiro et al. 2015), pregnancy induced hypertension (PIH) (Cantonwine et al. 2016; E Werner et al. 2015; Yazbeck et al. 2009), and reproductive system (Aung et al. 2019; Chen et al. 2018) as well for the childhood neurodevelopmental disorders and obesity (Braun 2017; Ejaredar et al. 2015; Kim and Lee 2017; Whyatt et al. 2012), and asthma and allergies (A Just et al. 2012; Stelmach et al. 2015; Whyatt et al. 2014). Hence, the exposure to chemicals with endocrine disrupting properties is a growing concern for the pregnant mother, fetus, infants, children and adults.

In recent years, growing evidences have suggested that during the different stages of pregnancy, the exposure to phthalates can alter the concentrations of naturally-produced hormones, which help to regulate and control different cells and organs in the body. The exposure during this window periods can effect adversely for the health of the mother as well as play a critical role in early fetal development.

The exposure during pregnancy may cause impaired glucose tolerance or gestational diabetes mellitus (Bellavia et al. 2017; Fisher et al. 2018; James-Todd et al. 2016; Zhang et al. 2017), pregnancy induced hypertensive disorders (clinical diagnoses of gestational hypertension, preeclampsia, eclampsia, or HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) (Cantonwine et al. 2016;

Rahmani et al. 2016; E Werner et al. 2015; Zlatnik 2016), miscarriage or pregnancy loss (Gao et al. 2017; Messerlian et al. 2016; Peng et al. 2016; Yi et al. 2016; Zhao et al. 2017), poly-cystic ovarian syndrome (Rutkowska and Diamanti-Kandarakis 2016), alteration in maternal serum thyroid hormones and sex hormones levels (Huang et al. 2016; Johns et al. 2015; Kuo et al. 2015).

In infants, toddlers, children, and adolescents, the routes and sources of phthalate exposure are related to developmental milestones and can be determined by hand-to-mouth activity, mobility, personal care or hygiene practices, diet, and health status throughout the development periods (Braun et al. 2013; Wormuth et al. 2006).

Animals as well as epidemiological studies in infants and children have found various health effects of specific phthalates, including asthma and allergies (Ait Bamai et al. 2014; Berger et al. 2018; Bornehag et al. 2004b; A Just et al. 2012; Stelmach et al. 2015; Whyatt et al. 2014), abnormal reproductive outcomes (Abdel-Maksoud et al. 2015; Aydogan and Barlas N 2015; Jurewicz and Hanke 2011; Meeker et al. 2009; Niermann et al. 2015; Watkins et al. 2017), children's neurodevelopmental, decreased cognitive development and behavioral problems (Braun et al. 2013; Ejaredar et al. 2015; Huang et al. 2015; Whyatt et al. 2012).

The prevalence of eczema in childhood, including its allergic-related phenotype, varies globally from 3% to 37% and is steadily increasing globally (Asher et al. 2006; Deckers et al. 2012; Eichenfield et al. 2014; Nutten 2015). The causes of respiratory diseases, asthma and eczema and of its increased prevalence are still unclear but are likely to be multifactorial in nature and depend on both genetic and environmental factors (Aguilar et al. 2017; Nutten 2015; Pyun 2015). During *in utero* life, the skin of

the fetus may be exposed to various products absorbed by the mothers, including phthalates given that some phthalates have been detected in amniotic fluid (Jensen et al. 2015). Later in life, children's dermal exposure can occur through the use of emollients, personal care products, and dermal contact with plastic products, soil, and dust, which can add to the total intake of certain phthalates through other routes such as inhalation and ingestion (Overgaard et al. 2017; Wormuth et al. 2006). Phthalates might influence the epidermal barrier development processes, which can take several years to occur, and this influence could be stronger in allergic individuals due to the fragility of their mucosae.

1.1.4 Replacement of phthalate and their alternatives

Over the past few decades, based on research on phthalate exposure and its effects on human and animal health, researchers and policy makers pushed companies to work on phthalate alternatives. This need for alternatives to phthalates has led manufacturers to create alternatives that share properties of phthalate plasticizers without EDCs properties. Manufacturers started replacement of DEHP with DINP and DIDP after ban on the usage of DEHP phthalate (Attina and Trasande 2015). Further, di-iso-nonyl-cyclohexane-di-carboxylate (DiNCH) is recently introduced as an alternative phthalate plasticizer. Studies have shown that DiNCH exposure to human have limited potential health effects (Machtinger et al. 2018; Van Vliet et al. 2011). However, more studies are required on large scale to see the adverse effects of these alternative phthalate on human health.

1.1.5 How to reduce phthalates exposure

Plastics commonly used to make food storage containers can inadvertently leach hormone disrupting chemicals into food and drinks. Exposure to phthalates can be reduced by considering following few steps:

- **Fresh is best:** Phthalates can migrate from the lining of cans and plastic packing into food and drinks. While it is not practical to avoid food packaging altogether, it is better to opt for fresh or frozen instead of canned food as much as possible.
- **Store it safe:** Storing food and drinks in glass or stainless steel utensils instead of plastic material.
- **Do not microwave in plastic:** Warmer temperatures increase the rate of chemical leaching into food and drinks. So use heat resistant glass or ceramic containers when microwaved or heat food on the stove.

1.2.1 Human exposure to heavy metals

Metals are one of the major category of globally distributed pollutants and are natural elements. Exposure to heavy metals remains a public health concern for longtime because of their persistence in the environment and their ability to induce toxicity even at low level. In recent decades, their contamination has been dramatically increasing particularly in the developing countries because of their continuous discharge in sewage and untreated industrial effluents (Cheng 2003; Jan et al. 2015). Naturally occurring metals like lead (Pb), cadmium (Cd) and manganese (Mn) are also

classified as EDCs (Dyer 2007; Georgescu et al. 2011; Iavicoli et al. 2009; Rahman et al. 2016).

Exposure to these heavy metals can occur through a variety of routes. Metals may be inhaled as dust or fume in tiny particulate matter, such as the lead oxide particles produced by the combustion of leaded gasoline (Hu 2002). Some metals can be vaporized for example, mercury vapor in the manufacture of fluorescent lamps and inhaled. Metals may also be ingested involuntarily through food and drink. The amount which actually absorbed from the digestive tract can vary widely. It also depends on the chemical form of the metal and the age and nutritional status of the individual (Hu 2002). Metals can escape control mechanisms such as homeostasis, transport, compartmentalization, and binding to specified cell constituents, thus they can have toxic and even lethal effects. Heavy metals can cause malfunctioning of the cellular processes via displacement of essential metals from their respective sites (Flora et al. 2008; Jan et al. 2015). Once a metal is absorbed, it distributes in tissues and organs. Excretion typically occurs primarily through the kidneys and digestive tract, however these metals tend to persist in some storage sites, like the liver, bones, and kidneys, for years or decades (Jarup 2003).

Cadmium

Cd is a non-essential, toxic heavy metal distributed widely in the environment (Barregard et al. 2013). The principal source of Cd exposure for the general non-smoking population is food (Swaddiwudhipong et al. 2012). The main source of food contamination with Cd are water wastes, industrial aerosols, fertilizers which are phosphate based and pesticides (Georgescu et al. 2011). It is hypothesized that Cd

may interfere even with low level exposure with steroid hormones in both gender's reproductive organs.

Lead

Pb is a naturally occurring toxic heavy metal found in almost all environmental domains which include soil, water and environment (Cheng et al. 2015; Georgescu et al. 2011; Marx et al. 2016; Romero-Freire et al. 2015). Contamination in human and animals can occur through ingestion, respiration or transdermal. General population is relatively exposed to low concentrations of Pb via cigarette smoking, polluted drinking water, food and air (Augustsson et al. 2015; Mohmand et al. 2015; Pinto et al. 2017). However, Pb exposure through man-made sources, such as in paint around the home, leaded pipes and fixtures that deliver water and soil contaminated with Pb from leaded gasoline. Whereas occupational exposure in welding, smelting, electroplating, painting, battery, pigments, and e-waste recycling workshops are exposed to relatively high concentrations of Pb (Julander et al. 2014; Kazi et al. 2015; Zhang et al. 2016).

Manganese

Mn is an essential micronutrient required for the activity of metabolism and antioxidant system (Livingstone 2017). However, the requirements are low and excessive intake or exposure to it can cause neurotoxicity (Hutchens et al. 2017; Pfalzer and Bowman 2017). The sources of environmental manganese are sewage sludge, wastewater discharges, mining and mineral processing, emissions from alloy, steel, and iron production, and combustion of fossil fuels (Han et al. 2017; Iavicoli et al. 2009; van Wendel de Joode et al. 2016).

1.2.2 Heavy metals effects on pregnancy, fetus, infancy and childhood

Cadmium

Over the past few decades, epidemiological studies have shown the critical role of prenatal exposure to Cd in the development of human individuals and its impact on public health. High levels of Cd during pregnancy has contributed to the risk of altering organs and tissues in the female reproductive system which leads to reproductive disorders as well as led a significant increase in premature delivery (Delemarre et al. 2008; Nishijo et al. 2002; Parodi et al. 2017).

In rodents, Cd mimic estrogenic effects, which may leads to proliferation of uterine and mammary tissues (Vaiserman 2014). Cd may also inhibit the transcription of low density lipoprotein receptor mRNA to reduce the supply of cholesterol substrate available for placental progesterone production (Ikeh-Tawari et al. 2013). Whereas, higher concentration of Cd in the body may alter the secretory patterns of pituitary hormones, including follicle stimulating hormone and luteinizing hormone (Iavicoli et al. 2009). Other adverse effects due to the exposure of Cd are GDM, PIH, low birth weight, impaired fetal growth, spontaneous abortion, still birth, neonatal death, preterm birth. Low birth weight can also leads to increased risk of developing slower cognitive development of the child (Boyce et al. 2015; Rahman et al. 2016).

Lead

Pb exposure during pregnancy can harm a pregnant woman and fetus. Pb absorbed in the body can be stored in bones for years, which can cause GDM,

miscarriage, stillbirth, Neurodevelopment problems including; lower IQ in children with learning and behavioral problems (Fatmi et al. 2017; Gulson et al. 2003; Hu and Hernandez-Avila 2002; Mendola et al. 2002). While other studies have shown that the increased levels of Pb can increase the likelihood of pre-eclampsia or pregnancy induced hypertension related diseases (Jameil 2014; Kennedy et al. 2012; Poropat et al. 2018; Zahran et al. 2014). A recent study by Wu et.al. showed significant associations between prenatal low levels of Pb exposure with newborn DNA methylation, in female infants (Wu et al. 2017).

Manganese

Mn crosses the placenta, which may be adversely affect fetus and infants particularly during high concentrations of Mn, because of homeostatic mechanisms in young children, who absorb and retain a larger fraction of ingested Mn than adults (Aschner and Aschner 2005; Yoon et al. 2009; Yoon et al. 2011). Based on animal studies, exposure to Mn may affect the normal endocrine system functions, particularly the secretion and production of sexual hormones (Hiney et al. 2016; Lee et al. 2006; Pine et al. 2005). Further in rats an increase in serum luteinizing hormones, follicular stimulating hormones and testosterone, sperm production and efficiency of spermatogenesis has been observed (Lee et al. 2006).

1.3 Rationale and hypotheses of thesis

Humans are exposed to various chemicals in everyday life, from food, indoor environment, cosmetics and other products that surround us at work and at home. Further the use of food supplements and medicines which can have both desired and unintended effects on human body. Studies in humans and laboratory animals have shown that the phthalates and heavy metals can cause a variety of reproductive and developmental problems in offspring, in part by disrupting the normal hormonal functioning systems. The epidemiologic evidence are not strong, however some studies have shown associations with neurodevelopment and reproductive as well as for asthma and allergic conditions. Exposure to phthalates and heavy metals is a global public health concern because several of them have been identified as reproductive and developmental toxicants and EDCs. Even low doses of these chemicals in the environment pose a significant risk to health. Further, the mother's health problems during pregnancy may have repercussions on child's health in early life and later. Because the development of the immune and organs largely takes place in utero and during early childhood. Exposure to environmental factors during these time windows may be particularly critical for the occurrence of diseases in both mother and children. More specifically, in recent years, hypotheses have been put forward that EDCs may also contribute to the development of allergic and respiratory diseases. In addition, it is likely that EDCs could act already during pregnancy by causing gestational diabetes mellitus (GDM) and pregnancy induced hypertension, which may also trigger to develop hypertension and diabetes mellitus later in life.

However, as a whole, a limited number of studies have been conducted on the impact of EDCs on the health of the childbearing mother and the child in the early years

of life. The mother's health is of importance because it can influence child's health later in life, so we conducted three studies and tested our hypotheses by using the French EDEN mother-child prospective cohort study.

The overall aim of this thesis is to improve the knowledge basis for risk assessment and address how exposure to phthalates and heavy metals during pregnancy contributes to the critical issues of maternal and child health. Based on the evidences that EDCs like phthalates and heavy metals may disrupt immunological, and hormonal normal functions to development of the epidermal barrier defects processes. It improves on previous studies of phthalates and heavy metals which we studied adds substantially to the literature by investigating a poorly understood mechanism that has not been previously tested in humans.

1.4 Objectives of the thesis

The objectives of this thesis work are as follows:

General:

To determine the role of EDCs in the childbearing mothers and the children in their first five years of life using birth cohort data.

Specific:

1. To test the hypothesis that exposure to phthalates during *in utero* increase the risk of eczema in young children through the production of immune markers at various stages of life (Paper I).
2. To test the hypothesis that exposure to heavy metals during pregnancy and its association with gestational diabetes mellitus and impaired glucose tolerance (Paper II).
3. To test the hypothesis that exposure to phthalates during pregnancy and its association with pregnancy induced hypertension (Paper III).

To this extent, we used longitudinal population based data of the pregnant women and children living in two cities of France.

2. MATERIALS AND METHODS

2.1 Study population

The population data set used in this thesis is from the French EDEN (Etude des Déterminants pré et post natals du développement de la santé de l'Enfant) mother-child prospective birth cohort (<https://eden.vjf.inserm.fr>) to examine the hypotheses of the thesis.

2.2 Introduction of the study protocols

EDEN mother-child prospective birth cohort was set up in 2003 in two university hospitals of the cities, Nancy and Poitiers, in France (see Figure 4), by the local university hospital teams in collaboration with research teams from the National Institute of Health and Medical Research (INSERM).

Participation in this cohort was proposed to all women visiting the prenatal clinics at the university hospitals before their 24th week of amenorrhea. Exclusion criteria were multiple pregnancies, known diabetes before pregnancy, French illiteracy or planning to move out of the region within the next 3 years. Among the 3758 women invited to participate, 2002 (53%) were enrolled in the cohort (1034 women from Nancy and 968 from Poitiers). Mothers were recruited during the period of 2003 to 2006. On average, women were included at 15 weeks of amenorrhea (range: 8–26). Mean age was 29 years (range: 18–44). Whereas, 30% of the women were pregnant for the first time.

Children were followed for up to 5 years, by visits to research centres and questionnaires mailed to parents. Clinical examinations from pregnancy to 5–6 years of age were performed by specifically trained midwives.



Figure 4. Map shows the study centers Nancy and Poitiers.

2.3 Assessments schedule

Mothers were examined three times for clinical examinations, one between 24 and 28 weeks of amenorrhea, one at delivery and one 5–6 years after delivery (Figure 5). The children were clinically examined for four times: at birth, at 1 year and 3 years and during the 6th year (at 5 years and 8 months, on average), with specific cognitive assessments by psychologists at 3 and 5–6 years. Concomitantly and in between clinical visits, mothers and fathers answered questionnaires, self-administered or

administered by midwives, about their offspring, themselves and their household. Questionnaires were filled up twice during the first year (at 4 and 8 months), at 1 year and every year thereafter until 5–6 years. At 4, 8 and 12 months, the parents provided a 3-day food record for their infants. Biological samples were collected from the mother during pregnancy and at birth, and from the child (or cord) at birth and at 5–6 years.

Blood samples were also collected from fathers at the child’s birth. The mothers’ recruitment and follow-up procedures, and methodological assumptions have been published in earlier studies.

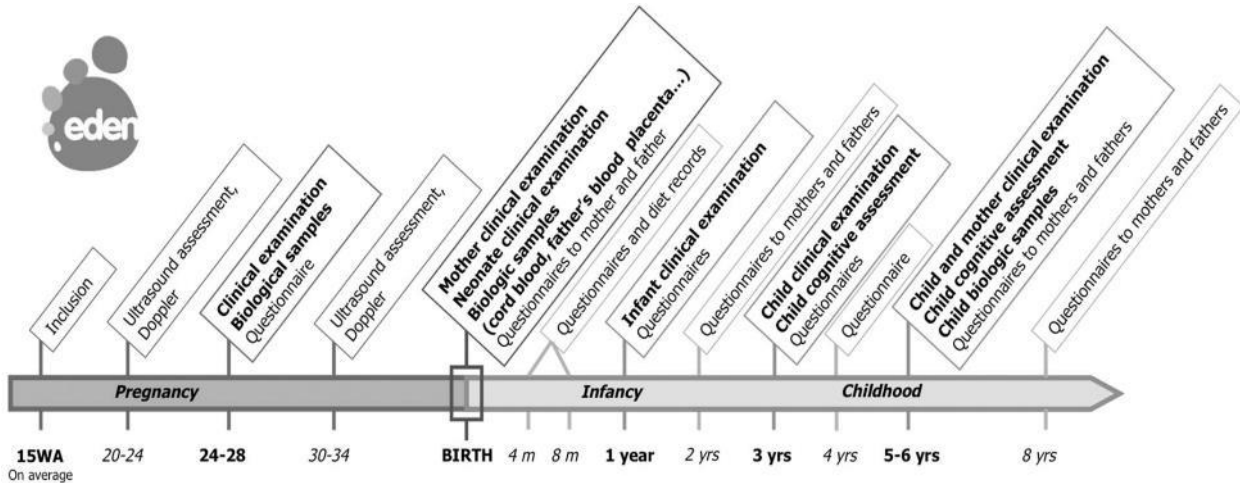


Figure 5. Assessments and data collection -from mothers included during pregnancy to children 8 years after birth.

2.4 Ethical Approval

The study was approved by the relevant ethical committees (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale, Le Kremlin Bicêtre University Hospital, and Commission Nationale de l’Informatique et des Libertés), and

all participating women gave informed written consent for their own participation and both parents for newborn child after delivery.

Table 1. Overview of the study aims, subjects and methods.

Study	Aim	Study design	Study population	Analysis
I	To test the hypothesis that exposure to phthalates during in utero increase the risk of eczema in young children through the production of immune markers at various stages of life.	Prospective Cohort	604	Logistic regression, survival analysis
II	To test the hypothesis that exposure to heavy metals during pregnancy and its association with gestational diabetes mellitus and impaired glucose tolerance.	Nested Cross-sectional Cohort	623	Logistic regression
III	To test the hypothesis that exposure to phthalates during pregnancy and its association with pregnancy induced hypertension.	Nested Cross-sectional Cohort	604	Logistic regression

2.5 Data collection

The population in our studies is a subgroup of the French EDEN mother-child prospective birth cohort. The mothers' recruitment and follow-up procedures, and methodological assumptions have been published in earlier studies (Heude et al. 2016; Philippat et al. 2012).

Women were given an appointment with a study midwife between the 24th and 28th gestational weeks, during which an interview on life style factors was conducted and biological samples were collected. The information on the mothers and their newborns, including parity, mode of delivery, birth weight, gestational age, season of birth, behavioral and environmental data were collected by the use of questionnaires, interviews and from obstetric and pediatric records. Children were followed from birth to the age of 5 years. However, in our first paper only includes all male offspring for whom maternal urine samples had been analyzed for metabolites of phthalates as a part of previous studies (Botton et al. 2016; Chevrier et al. 2012; Mortamais et al. 2012), which explains why we had only boys in the study. Restricting to one sex could be an advantage in the context where sex-specific effects of exposures are expected (Harley et al. 2013; Wolff et al. 2008); a study restricted to one sex likely has a higher statistical power than a study including both.

2.6 EDCs investigated in this thesis

In this thesis we studied the effects of eleven metabolites of eight phthalates: Diethyl phthalate (DEP), Di-n-butyl phthalate (DBP), Di-isobutyl phthalate (DiBP), Di(2-ethylhexyl) phthalate (DEHP), Benzylbutyl phthalate (BBzP), Di-isononyl phthalate

(DiNP), Di-n-octyl phthalate (DOP), and Di-isodecyl phthalate (DiDP) and three heavy metals: Lead (Pb), Cadmium (Cd) and Manganese (Mn) are presented (Table 2).

Phthalates

Phthalates or phthalate esters, are esters of phthalic acid are one of the EDCs, which are mainly used as plasticizers. Phthalates are ubiquitous man-made chemical substances, widely used for a variety of purposes in our daily life i.e. medical devices, pharmaceuticals, PVCs, food wrappings, cosmetic products, children's toys and cleaning products. They are added to plastics in order to increase their flexibility, transparency, durability, and longevity (Bornehag et al. 2005; Meeker et al. 2009; Meeker 2012; Shelby 2006).

Phthalates are classified into two major categories based on their number of carbon atoms in the main carbon chains, where different phthalates with different carbon atom numbers have different physical, chemical and even toxicological properties:

- Low Molecular Weight (LMW) phthalates: LMW phthalates includes; DEP, DBP, BBzP, and DiBP and their metabolites, which contain three to six carbon atoms in the backbone.
- High Molecular Weight (HMW) phthalates: HMW phthalates includes; DiNP, DOP, DiDP, DEHP and their metabolites. HMW phthalates have more than six carbon atoms in their backbone.

Table 2. EDCs investigated in the thesis.

Parent compound	Abbrev.	Phthalate metabolites	Abbrev.
Diethyl phthalate	(DEP)	Monoethyl phthalate	MEP
Di-n-butyl phthalate	(DBP)	Mono-n-butyl phthalate	MBP
Di-isobutyl phthalate	(DiBP)	Mono-isobutyl phthalate	MiBP
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethyl-5-carboxypentyl) phthalate	MECPP
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethylhexyl) phthalate	MEHP
Benzylbutyl phthalate	(BBzP)	Monobenzyl phthalate	MBzP
Di-isononyl phthalate	(DiNP)	Monocarboxy-isoctyl phthalate	MCOP
Di-n-octyl phthalate	(DOP)	Mono(3-carboxypropyl) phthalate	MCPP
Di-isodecyl phthalate	(DiDP)	Monocarboxy-isononyl phthalate	MCNP
Lead	Pb		
Cadmium	Cd		
Manganese	Mn		

Women were invited for a clinical examination and to provide a first morning urine at home before the hospital study visit between the 24th and 28th gestational weeks (second trimester of the pregnancy). If forgotten, the urine sample was collected at the hospital. A polypropylene container (FP40VPS) manufactured by Centre Européen de Biotechnologie SA (CEB) in Angers, France was used. The urine samples were aliquoted and then frozen at -80° C. The analyses of the samples were conducted at the National Center for Environment Health Laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA) for eleven metabolites of eight phthalates: Diethyl phthalate (DEP), Di-n-butyl phthalate (DBP), Di-isobutyl phthalate (DiBP), Di(2-ethylhexyl) phthalate (DEHP), Benzylbutyl phthalate (BBzP), Di-isononyl phthalate (DiNP), Di-n-octyl phthalate (DOP), and Di-isodecyl phthalate (DiDP). The targeted phthalate metabolites were monoethyl phthalate (MEP), mono-n-butyl phthalate (MBP), 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-isoctyl phthalate (MCOP), mono(3-carboxypropyl) phthalate (MCP) and monocarboxy-isononyl phthalate (MCNP).

Quantification of the phthalate metabolites concentrations was performed using online solid phase extraction high performance liquid chromatography electrospray ionization isotope dilution tandem mass spectrometry (Silva et al. 2007). Creatinine concentrations were also quantified following identical analytical procedure as described previously (Mortamais et al. 2012). The concentrations of metabolites below the limit of detection (LOD) were replaced by instrumental reading values or by the compound specific lowest instrumental reading value divided by the square root of two

($LOD/\sqrt{2}$) when the instrumental value found missing (Hornung and Reed 1990). The vast majority of the samples/metabolites (>97%) were above LOD. Two-step standardization was performed to reduce variability in biomarkers urinary concentrations owed to sampling conditions as described previously (Mortamais et al. 2012). There was no significant difference in the concentrations of phthalates in the urines collected at homes compared to those of the urines collected in the hospital.

Heavy metals

Three metals Pb, Cd and Mn were measured from second trimester blood samples of pregnancy. Women were invited for a clinical examination and to provide a venous blood sample. EDTA tubes were used to collect the blood for assay. The blood tubes were prepared within an hour of sample collection. After aliquoting, all biological material was frozen at -80°C . After centrifugation at 3000 rpm for 5 min, maternal blood was aliquoted and frozen at -80°C for later analysis. The blood Pb, Cd and Mn concentrations were measured by electrothermal atomic-absorption spectrometry (model 4100 ZL; Perkin-Elmer, Courtaboeuf, France) with Zeeman background correction as previously described (Bonithon-Kopp et al. 1986; Huel et al. 1986; Mergler et al. 1996). The limit of detection was 100%.

2.7 Eczema phenotypes

Eczema was assessed by administration of an enriched version of the International Study of Asthma and Allergies in Childhood (ISAAC) standardized and validated questionnaire (ISAAC Steering Committee 1998; Williams et al. 1999) to the

parents at 1, 2, 3, 4 and 5 years of age by giving appointment. At each year of follow-up, eczema was defined as eczema diagnosed by a doctor in the last 12 months. Ever eczema was then defined as having ever reported a diagnosis of eczema at 1, 2, 3, 4 or 5 years of age. Additionally, two eczema phenotypes were distinguished: early-onset eczema if eczema was reported on any questionnaire in the first 2 years of age and late-onset eczema if it was reported only between age 24 months and 60 months. Further, the prevalence and the incidence of eczema were computed.

2.8 Immunoglobulin E (IgE) and atopic status

At the age of 5 years, total serum IgE in 293 boys was assessed by using the CAP assay according to the manufacturers' instructions (Pharmacia CAP System™, Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden). Boys were classified as atopic if they had IgE levels of ≥ 60 IU/mL (Baiz et al. 2017).

2.9 Gestational diabetes mellitus and Impaired Glucose Tolerance

At 24–28 weeks, maternal blood glucose concentrations were measured 1 hour after a 50-g glucose challenge. Women with 1-h glucose concentrations over 130 mg/dL in Nancy and 140 mg/dL in Poitiers were scheduled for a 3-h 100-g oral-glucose tolerance test. The GDM was diagnosed by using the oral-glucose-tolerance test according to the Carpenter and Coustan criteria (Steer et al. 2004).

When there were >2 blood glucose concentrations greater than the following cut points: fasting = 95 mg/dL, at 1 h = 180 mg/dL, at 2 h = 155 mg/dL, and at 3 h = 140 mg/dL. While a diagnosis of IGT was assigned if one of the oral-glucose-tolerance test cut-off values was met or exceeded.

2.10 Maternal Blood Pressure Measurements

Maternal blood pressure was measured during routine monthly visits, with the subject in supine position, using a standard mercury sphygmomanometer. One set of measures was taken by the study midwife between 24 and 28 weeks using a different (semi-automated) device, with two measurements averaged to determine the values for that visit.

Measurements taken within each period of gestational trimester (T1, T2, and T3) were averaged to determine the values for systolic blood pressure (SBP) and diastolic blood pressure (DBP) assigned to each period.

Women were classified as having pregnancy induced hypertension based on SBP \geq 140 mmHg and/or DBP \geq 90 mmHg measured during at least two visits after the 22nd week of gestation, such that the elevated measures could have occurred during or across any of the three periods, at any point after 22 weeks. Consequently, no diagnosis of pregnancy induced hypertension was made before 24 weeks. All cases of pregnancy induced hypertension were diagnosed by the physicians.

2.11 Other Variables

Information on potential confounders was collected which includes; birth weight, gestational age (two-class variable: < 37 gestational week, \geq 37 gestational week), season of birth, parity, number of siblings (0, 1, 2 \geq 3), exclusive breast-feeding for 4 or more months (yes/no), maternal age at delivery (< 25 years, 25-29 years, 30 – 34 years, \geq 35 years), pre-pregnancy maternal body mass index (BMI, < 18.5, 18.5-24.9, 25.0-30.0, > 30 kg/m²), maternal and paternal history of allergies, maternal and

paternal educational level (< 2 years after high school, High school +2 years, ≥ High school +3 years), household income, city of residence (Nancy / Poitiers), mode of delivery (vaginal, caesarean section), smoking during pregnancy (yes/no), maternal alcohol use during pregnancy (yes/no), maternal and paternal physician-diagnosis of asthma, rhinitis, eczema, and food allergies (yes/no), maternal physician-diagnosis of GDM (yes/no) and pregnancy induced hypertension (yes/no).

2.12 Statistical analysis

Urinary concentrations of phthalate metabolites and metals were log natural (ln) transformed because of non-normality of the distribution. For the purpose of analysis, MECPP, MEHHP, MEOHP and MEHP were grouped as sum of Σ DEHP (DEHP being their parent compound). We calculated the molar sum of these DEHP metabolites (nanomoles per milliliter) by dividing each metabolite concentration by its molecular weight and then summing the individual concentrations.

The prevalence of eczema, GDM and pregnancy induced hypertension was computed. Additionally incidence was measured for eczema in boys. We applied multiple logistic regression analysis to examine the association between each phthalate metabolite and Σ DEHP and eczema/pregnancy induced hypertension outcomes adjusted for potential confounders. The potential confounders were determined a priori from literature review. Further in our first paper we used Directed Acyclic Graph (<http://www.dagitty.net/dags.html>) to show the hypothesis relations between biomarkers concentration, confounders and eczema outcomes. The confounders included in our final model were parental asthma/rhinitis/eczema, maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, and number of siblings. Models were additionally adjusted for recruitment centre.

We also applied the Cox proportional hazard discrete time survival model to estimate the association between in utero exposure to phthalates and eczema occurrence in the first 5 years of life after adjustment for the confounding factors. Results were further stratified in order to see whether phthalate biomarkers were associated with atopic (presence of allergic sensitization according to IgE) or non-atopic (absence of allergic sensitization to IgE) eczema. We also took IgE as an additional variable to see its association with phthalate biomarkers by applying multiple logistic regression analysis. As our statistical analysis involved multiple comparisons so that it became increasingly likely that at least one comparison would have been statistically different due to random sampling error alone, the multiple testing hypothesis was considered through Bonferroni test.

We also applied multiple logistic regression analysis to examine the association between each In metal concentration and GDM or IGT outcomes adjusted for potential confounders. The confounders included in our final model were maternal smoking, maternal age, maternal BMI, maternal education level, pregnancy induced hypertension, number of siblings and recruitment centre. In addition, quartiles of the concentrations of each metal were estimated to describe mothers' exposure to metals according to 4 levels, namely none, light, moderate and high. Other categorical variables were expressed as the number and percentages (%) and continuous variables were expressed as the mean \pm standard deviation (SD). The study population was also compared according to the recruitment centre by applying a chi-squared test (for categorical variables) or a Mann–Whitney U test (for continuous variables).

All the statistical analysis was conducted using Stata statistical software 14 (Stata Corp LP, 4905 Lakeway Drive, College Station, Texas 77845, USA) where p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Paper 1: Prenatal exposure to phthalates and the development of eczema phenotypes in male children: Results from the EDEN Mother-Child Cohort Study.

In this part, we show the results on relationships between prenatal exposure to phthalates and development of eczema phenotypes in male children. We published our paper in *Environmental Health Perspectives*. We present the details of this paper in the thesis.

Prenatal Exposure to Phthalates and the Development of Eczema Phenotypes in Male Children: Results from the EDEN Mother–Child Cohort Study

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BACKGROUND: Contradictory results exist regarding the importance of early-life exposure to phthalates for development of childhood eczema.

OBJECTIVES: We evaluated the association between maternal urinary concentrations of phthalate metabolites between the 24th and 28th week of gestation and occurrence of eczema in their sons up to 5 y of age, according to allergic sensitization as assessed by total immunoglobulin E (IgE) in a subsample of individuals.

METHODS: Data on health outcomes and background factors were collected using five standardized annual questionnaires completed by parents at the children's ages of 1–5 y, and their associations with phthalate metabolite urinary concentrations were assessed in 604 mother–son pairs with adjusted multiple logistic regression and Cox's survival model. Several eczema phenotypes were considered. Atopic status was assessed at 5 y of age in 293 boys through total IgE assessment.

RESULTS: At 5 y of age, the prevalence of ever eczema was 30.4%. Metabolites of di-isobutyl phthalate (DiBP) and di-isononyl phthalate (DiNP) were positively associated with early-onset (0–24 mo of age) eczema (15.7%) and late-onset (24–60 mo of age) eczema (14.7%). Applying the Cox's model showed a significant association of occurrence of eczema in the first 5 y of life with DiBP and DiNP metabolites. Among IgE-sensitized boys, metabolites of di-*n*-butyl phthalate (DBP) and DiBP were significantly associated with ever eczema [hazard ratio (HR) = 1.67 [95% confidence interval (CI): 1.10, 2.54], $p = 0.01$ and HR = 1.87 (95% CI: 1.01, 3.48), $p = 0.04$, respectively].

CONCLUSIONS: Occurrence of eczema in early childhood may be influenced by prenatal exposure to certain phthalates in boys. Further investigations are needed to confirm this observation. <https://doi.org/10.1289/EHP1829>

Introduction

Phthalates are man-made chemicals widely used in our daily life [e.g., food wrappings, cosmetic products, children's toys, cleaning products, medical devices, pharmaceuticals, polyvinyl chloride (PVC) flooring, building materials] because of their properties of transparency, durability, and flexibility (Meeker et al. 2009; Bornehag et al. 2005; Arbuckle et al. 2014). People can be exposed to phthalates through inhalation, dermal absorption (including air-to-skin transport), contact with contaminated surfaces, and use of personal care products and ingestion (including dietary ingestion and incidental ingestion) (Swan 2008). Urinary concentrations of phthalate metabolite are the most reliable measures for exposure assessment (Swan 2008). The ubiquitous presence of phthalates in the environment and the potential consequences of human exposure to phthalates have raised concerns, particularly

in vulnerable populations such as pregnant women and infants. Prenatal and early life are critical in the development of the immune system; exposure to toxic pollutants during this period can result in an increased risk of adverse health outcomes later in life.

Animal as well as epidemiological studies in infants and children have found various health effects of specific phthalates, including abnormal reproductive outcomes (Meeker et al. 2009; Abdel-Maksoud et al. 2015; Niemann et al. 2015; Aydoğan Ahab and Barlas 2015; Jurewicz and Hanke 2011), children's neurodevelopmental and behavioral problems (Whyatt et al. 2012; Ejaredar et al. 2015; Braun et al. 2013), and asthma and allergies (Bornehag et al. 2004b; Whyatt et al. 2014; Stelmach et al. 2015; Just et al. 2012; Hsu et al. 2012), although not consistently.

The prevalence of eczema in childhood, including its allergic-related phenotype, varies globally from 3% to 37% and is steadily increasing worldwide (Asher et al. 2006; Nutten 2015; Eichenfield et al. 2014; Deckers et al. 2012). The causes of eczema and of its increased prevalence are still unclear but are likely to be multifactorial in nature and depend on both genetic and environmental factors (Nutten 2015; Pyun 2015). During *in utero* life, the skin of the fetus may be exposed to various products absorbed by the mothers, including phthalates given that some phthalates have been detected in amniotic fluid (Jensen et al. 2015). Later in life, children's dermal exposure can occur through the use of emollients, personal care products, and dermal contact with plastic products, soil, and dust, which can add to the total intake of certain phthalates through other routes such as inhalation and ingestion (Wormuth et al. 2006; Overgaard et al. 2017).

Eczema pathogenesis involves immunologic dysfunction and skin barrier defects (Pyun 2015). Phthalates might influence the epidermal barrier development processes, which can take several

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years to occur, and this influence could be stronger in allergic individuals due to the fragility of their mucosae. Although dermal absorption of phthalates and its impact on child health is established, contradictory results exist regarding eczema development after phthalate exposure *in utero* (Just et al. 2012; Smit et al. 2015; Weschler et al. 2015; Gong et al. 2015; Pan et al. 2014).

The objective of the present study was to evaluate the potential associations between maternal exposure to phthalates during the second trimester (between the 24th and 28th weeks) of pregnancy and occurrence of eczema phenotypes in male children in their first 5 y of life using data collected in the French EDEN mother-child prospective cohort study. The association was explored according to atopic status as defined by total immunoglobulin E (IgE) in a subsample of boys for which this assessment was available.

Methods

Study Population and Data Collection

The population in this study is a subgroup of the French EDEN (Etude des Déterminants pré et post natals du développement de la santé de l'Enfant) mother-child prospective birth cohort restricted to boys (<https://eden.vjf.inserm.fr>). The mothers' recruitment and follow-up procedures and methodological assumptions have been published in earlier studies (Heude et al. 2015; Philippat et al. 2012). Briefly, women were given an appointment with a study midwife between the 24th and 28th gestational weeks, during which an interview on lifestyle factors was conducted and biological samples were collected. The information on the mothers and their newborns, including parity, mode of delivery, birth weight, gestational age, season of birth, behavioral, and environmental data were collected by the use of questionnaires, interviews, and obstetric and pediatric records. Boys were followed from birth to 5 y of age. The present study includes all male offspring for whom maternal urine samples had been analyzed for metabolites of phthalates as a part of previous studies (Chevrier et al. 2012; Mortamais et al. 2012; Botton et al. 2016), which explains why we had only boys in the study. Restricting to one sex could be an advantage in the context where sex-specific effects of exposures are expected (Harley et al. 2013; Wolff et al. 2008); a study restricted to one sex likely has a higher statistical power than a study including both.

Urine Collection and Analysis

Women were invited for a clinical examination and to provide a first morning urine at home before the hospital study visit between the 24th and 28th gestational weeks (second trimester of the pregnancy). If forgotten, the urine sample was collected at the hospital. A polypropylene container [FP40VPS; Centre Européen de Biotechnologie SA (CEB), Angers, France] was used. The urine samples were aliquoted and then frozen at -80°C . The analyses of the samples were conducted at the National Center for Environment Health Laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA) for 11 metabolites of 8 phthalates: diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), di-isobutyl phthalate (diBP), di(2-ethylhexyl) phthalate (DEHP), benzylbutyl phthalate (BBzP), di-isononyl phthalate (diNP), di-*n*-octyl phthalate (DOP), and di-isodecyl phthalate (DiDP).

The targeted phthalate metabolites were monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), 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 (MCPP), and monocarboxy-isononyl phthalate (MCNP).

Quantification of the phthalate metabolites concentrations was performed using on-line solid phase extraction-high-performance liquid chromatography-electrospray ionization isotope dilution-tandem mass spectrometry (Silva et al. 2007). Creatinine concentrations were also quantified following the same analytical procedure described previously (Mortamais et al. 2012). The concentrations of metabolites below the limit of detection (LOD) were replaced by instrumental reading values or by the compound-specific lowest instrumental reading value divided by the square root of 2 when the instrumental value was missing (Hornung and Reed 1990). The vast majority of the samples/metabolites (>97%) were above LOD. Two-step standardization was performed to reduce variability in the urinary concentrations of biomarkers due to sampling conditions, as described previously (Mortamais et al. 2012). There was no significant difference in the concentrations of phthalates in the urine samples collected at homes compared with those collected in the hospital.

Eczema Phenotypes

Eczema was assessed by administration of an enriched version of the International Study of Asthma and Allergies in Childhood (ISAAC) standardized and validated questionnaire (ISAAC Steering Committee 1998; Williams et al. 1999) to the parents at the children's ages of 1, 2, 3, 4, and 5 y by appointment. At each year of follow-up, eczema was defined as eczema diagnosed by a doctor in the last 12 mo. Ever eczema was then defined as having ever reported a diagnosis of eczema at 1, 2, 3, 4, or 5 y of age. Additionally, two eczema phenotypes were distinguished: early-onset eczema if eczema was reported on any questionnaire in the first 2 y of life, and late-onset eczema if it was reported only between 24 and 60 mo of age.

IgE and Atopic Status

Total serum IgE was assessed in 293 boys at 5 y of age by using the CAP assay according to the manufacturers' instructions (Pharmacia CAP System™, Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden). Boys were classified as atopic if they had IgE levels of ≥ 60 IU/mL (Baiz et al. 2017).

Other Variables

Information on potential confounders related to the children's eczema included birth weight, gestational age (two-class variable: <37 gestational week, ≥ 37 gestational week), season of birth, parity, number of siblings (0, 1, 2, ≥ 3), exclusive breast-feeding for ≥ 4 mo (yes/no), maternal age at delivery (<25 y, 25–29 y, 30–34 y, ≥ 35 y), pre-pregnancy maternal body mass index (BMI, <18.5 , 18.5–24.9, 25.0–30.0, >30 kg/m²), maternal and paternal history of allergies, maternal and paternal educational level (<2 y after high school, high school + 2 y, \geq high school + 3 y), household income, city of residence (Nancy/Poitiers), mode of delivery (vaginal, cesarean section), smoking during pregnancy (yes/no), maternal alcohol use during pregnancy (yes/no), maternal and paternal physician-diagnosed cases of asthma, rhinitis, eczema, and food allergies (yes/no).

Statistical Analysis

Urinary concentrations of phthalate metabolites were log-transformed because of nonnormality of the distribution. For the

Table 1. Characteristics of the study population and comparison with the entire EDEN cohort of boys.

Characteristics	All male EDEN cohort (n = 998)		Present study (n = 604)		p-Value
	n	%	n	%	
Maternal age (y)					
<25	200	20.0	79	13.0	
25–29	378	37.9	218	36.1	
30–34	285	28.6	205	34.0	
≥35	135	13.5	102	16.9	0.0006
Parity					
0	436	43.7	269	44.5	
1	374	37.5	222	36.8	
≥2	187	18.7	112	18.6	
Missing	1	0.1	1	0.1	0.9
BMI (kg/m ²)					
<18.5	92	9.2	57	9.4	
18.5–24.9	634	63.6	379	62.9	
25–30	171	17.1	110	18.3	
>30	80	8.0	49	8.1	
Missing	21	2.1	9	1.5	0.8
Maternal education					
<2 y after high school	459	46.0	263	43.5	
High school + 2 y	219	21.9	132	21.9	
≥High school + 3 y	297	29.8	199	32.9	
Missing	23	2.3	10	1.7	0.4
Active smoking (cigarettes/day)					
0	827	82.9	518	86.0	
1–5	82	8.2	45	7.5	
>5	86	8.6	37	6.2	
Missing	3	0.3	2	0.3	0.2
Passive smoking					
Yes	278	27.9	192	31.8	
No	711	71.2	405	67.1	
Missing	9	0.9	7	1.1	0.2
Alcohol during pregnancy					
Yes	278	27.9	151	25.0	
No	714	71.5	450	74.5	
Missing	6	0.6	3	0.5	0.4
Center					
Poitiers	533	53.4	347	57.4	
Nancy	465	46.6	257	42.6	0.1
Gestational age					
<37 gestational week	63	6.3	24	4.0	
≥37 gestational week	935	93.7	580	96.0	0.04
Birth weight					
<2,500 g	47	4.7	15	2.5	
≥2,500 g	951	95.3	589	97.5	0.02
Year of birth					
2003–2004	560	56.1	330	54.6	
2005–2006	438	43.9	274	45.4	0.5
Breast-feeding					
Yes	720	72.2	432	71.5	
No	270	27.0	172	28.5	
Missing	8	0.8	0	0	0.07
Employment					
Yes	727	72.9	458	75.9	
No	262	26.2	142	23.5	
Missing	9	0.90	4	0.6	0.3
Cesarean section					
Yes	175	17.5	95	15.7	
No	822	82.4	509	84.3	
Missing	1	0.1	0	0	0.4
Marital status					
Married	534	53.6	329	54.5	
Unmarried/single	435	43.5	257	42.6	
Divorced	19	1.9	12	1.9	
Separated	2	0.2	2	0.3	
Widowed	2	0.2	1	0.2	
Missing	6	0.6	3	0.5	0.9

Table 1. (Continued.)

Characteristics	All male EDEN cohort (n = 998)		Present study (n = 604)		p-Value
	n	%	n	%	
Season of urine sample collection					
Winter	262	26.2	189	31.3	
Spring	301	30.2	146	24.2	
Summer	249	24.9	129	21.3	
Fall	186	18.7	140	23.2	0.002
Time of sampling					
2400–0759 hours	500	50.1	310	51.3	
0800–1159 hours	272	27.3	149	24.7	
1200–1900 hours	7	0.7	5	0.8	
Missing	219	21.9	140	23.2	0.7
Maternal history of asthma					
Yes	110	11.0	66	10.9	
No	884	88.6	536	88.7	
Missing	4	0.4	2	0.3	0.9
Maternal history of eczema					
Yes	142	14.2	87	14.4	
No	852	85.4	515	85.3	
Missing	4	0.4	2	0.3	0.9
Maternal history of rhinitis					
Yes	167	16.7	101	16.7	
No	827	82.9	501	83.0	
Missing	4	0.4	2	0.3	0.9
Paternal history of asthma					
Yes	99	9.9	58	9.6	
No	895	89.7	544	90.1	
Missing	4	0.4	2	0.3	0.9
Paternal history of eczema					
Yes	48	4.8	24	4.0	
No	946	94.8	578	95.7	
Missing	4	0.4	2	0.3	0.7
Paternal history of rhinitis					
Yes	100	10.0	60	9.9	
No	894	89.6	542	89.8	
Missing	4	0.4	2	0.3	0.3

Note: BMI; body mass index.

purpose of present analysis, MECPP, MEHHP, MEOHP, and MEHP were grouped as the sum of ΣDEHP (DEHP being their parent compound). We calculated the molar sum of these DEHP metabolites (nanomoles per milliliter) by dividing each metabolite concentration by its molecular weight and then summing the individual concentrations.

The prevalence and the incidence of eczema were computed. We applied multiple logistic regression analysis to examine the association between each phthalate metabolite and ΣDEHP and eczema outcomes adjusted for potential confounders. The potential confounders were determined *a priori* from literature review. Then we used Directed Acyclic Graph (<http://www.dagitty.net/dags.html>) to show the hypothesis relations between biomarkers concentration, confounders, and eczema outcomes (see Figure S1). The confounders included in our final model were parental asthma/rhinitis/eczema, maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, and number of siblings. Models were additionally adjusted for recruitment center.

We also applied the Cox proportional hazard discrete time survival model to estimate the association between *in utero* exposure to phthalates and eczema occurrence in the first 5 y of life after adjustment for the confounding factors. Results were further

stratified in order to see whether phthalate biomarkers were associated with atopic (presence of allergic sensitization according to IgE) or nonatopic (absence of allergic sensitization to IgE) eczema. We also took IgE as an additional variable to see its association with phthalate biomarkers by applying multiple logistic regression analysis. Because our statistical analysis involved multiple comparisons so that it became increasingly likely that at least one comparison would have been statistically different due to random sampling error alone, the multiple testing hypothesis was considered using the Bonferroni test. All the statistical analysis was conducted using Stata statistical software (version 14; Stata Corporation), where $p < 0.05$ was considered statistically significant.

Ethics Approval

The study was approved by the relevant ethical committees (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale, Le Kremlin Bicêtre University Hospital, and Commission Nationale de l'Informatique et des Libertés), and all participating women gave informed written consent for their own participation, and both parents for a newborn child after delivery.

Results

Characteristics of the Population

There were 998 male offspring in the cohort, among whom 604 had phthalates assessment. The participants included in this study analysis did not differ in major demographic characteristics from the other EDEN boys (Table 1) except for maternal age. However, results did not change when adjusting for maternal age in the final model. Average maternal age was 29.9 y, 26.3% women were overweight or obese ($BMI > 25 \text{ kg/m}^2$), and 85.8% did not smoke during pregnancy. There were 24 (4.0%) boys born before the 37th week of gestation, and 15 (3.0%) had a birth weight $< 2,500 \text{ g}$. Ninety-five (15.7%) mothers delivered through cesarean section. Urine was reported as being collected before 0800 hours 51.3% of the time, and between 0800 and 1200 hours 24.7% of the time. We also observed that during pregnancy 14.4% of the mothers had eczema, 10.9% had asthma, and 16.7% had rhinitis.

Eczema Prevalence and Incidence

The prevalence of ever eczema was 9.7%, 15.7%, 21.0%, 26.6%, and 30.4% at 1, 2, 3, 4, and 5 y of age, respectively. The incidence decreased from 9.7% at 1 y of age to 5.2% at 5 y of age (Figure 1). The prevalence of early-onset and late-onset eczema were 15.7% and 14.7%, respectively.

Prenatal Exposure to Phthalates

Phthalate metabolites were detected in the urine of more than 97% of the mothers. MEP had the highest geometric mean ($110.7 \mu\text{g/L}$), whereas MCNP had the lowest ($1.5 \mu\text{g/L}$). The LOD ranged from 0.2 to $0.6 \mu\text{g/L}$. A summary of detection frequency as well as geometric means and select percentiles of urinary concentrations of phthalate metabolites are outlined in Table 2.

Spearman correlation coefficients between ln-transformed phthalate metabolites urinary concentrations are shown in Table S1. The majority of biomarkers were positively correlated except for the correlation between MEP and MiBP, MECPP, MEHHP, MCOP, MCP, or MCNP. All the other biomarkers were significantly correlated with each other (with correlation coefficients ranging from $r = 0.08$, $p < 0.05$ to $r = 0.97$, $p < 0.05$). As expected, DEHP metabolites were highly correlated with each other ($r \geq 0.77$, $p < 0.05$).

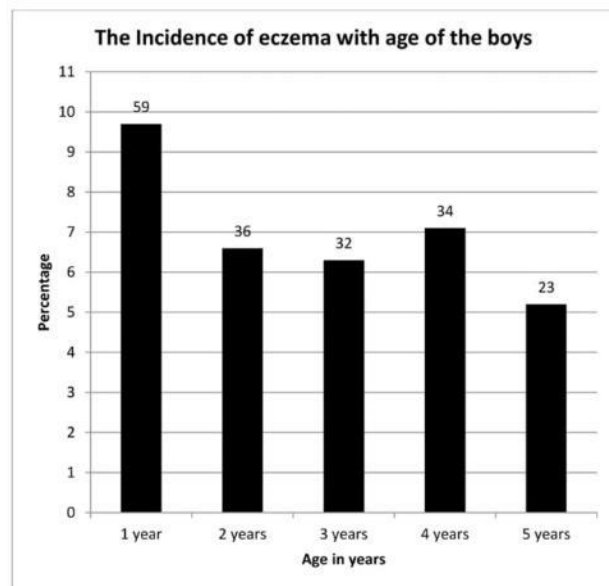


Figure 1: The incidence of eczema with age of the boys. The y-axis shows the percentage, and the numbers above each bar indicate the number of boys diagnosed at each year of age.

Associations between Phthalates Metabolites and Eczema Phenotypes

Table 3 presents the relationship of prenatal phthalate exposure (based on phthalate metabolite urinary concentrations) to the occurrence of ever eczema outcomes in boys between 1 and 5 y of age. Based on a multiple logistic regression model that was adjusted for confounding factors, we observed an increased risk of eczema at 1 y of age in association with increased MEP concentrations [odds ratio (OR) = 1.69; 95% confidence interval (CI): 1.05, 2.73; $p < 0.05$] and at 1, 3, 4, and 5 y of age for MiBP.

MBP and MCP did not show significant associations at any age. MEHHP and MEOHP showed borderline significance at 4 and 5 y of age, respectively; whereas MECPP was of borderline significance at 4 y of age and significant at 5 y of age. MEHP was only significant at 5 y of age [OR = 1.38 (95% CI: 1.03, 1.85), $p < 0.05$]. Σ DEHP was also of borderline significance at 4 y of age and significant at 5 y of age. Interestingly, MBzP was only found to be marginally significant at 5 y [OR = 1.30 (95% CI: 0.96, 1.76), $p < 0.10$]. We also observed that MCOP was marginally significant at 4 y of age and significant at 5 y of age, whereas MCNP showed significant results at 3 and 5 y of age.

In both phenotypes of eczema, MiBP and MCOP were positively associated with early-onset eczema [OR = 1.27 (95% CI: 1.00, 1.72), $p < 0.05$ and OR = 1.29 (95% CI: 1.04, 1.60), $p < 0.05$, respectively] as well as late-onset eczema [OR = 1.55 (95% CI: 1.10, 2.18), $p < 0.05$ and OR = 1.63 (95% CI: 1.20, 2.21), $p < 0.05$, respectively] after adjusting for potential confounders (Table 3). Other phthalate metabolites were not significantly associated with early-onset eczema. However in the case of late-onset eczema, MECPP and MCNP were positively associated [OR = 1.34 (95% CI: 1.00, 1.81), $p < 0.05$ and OR = 1.29 (95% CI: 1.02, 1.64), $p < 0.05$, respectively], whereas the association with MEHHP, MEOHP, and Σ DEHP were of borderline significance ($p < 0.10$).

We also applied survival analysis to compute the hazard ratio (HR) of the occurrence of eczema in the first 5 y of life with respect to exposure to phthalates (Table 4). Both MiBP [adjusted HR = 1.16 (95% CI: 1.01, 1.34), $p = 0.03$] and MCOP [HR = 1.09

Table 2. Distribution of phthalate metabolites concentration ($\mu\text{g/L}$) in maternal prenatal urine ($n = 604$).

Parent compound	Abbrev	Phthalate metabolites ($\mu\text{g/L}$)	Abbrev	LOD ($\mu\text{g/L}$)	% > LOD	GM	Minimum	Percentile					Maximum
								5th	25th	50th	75th	95th	
Diethyl phthalate	DEP	Monoethyl phthalate	MEP	0.6	100	110.7	7.9	21	51	96	195	714	4093.4
Di- <i>n</i> -butyl phthalate	DBP	Mono- <i>n</i> -butyl phthalate	MBP	0.2	100	51.6	2.8	12	28	43	73	438	2418.8
Di-isobutyl phthalate	DiBP	Mono-isobutyl phthalate	MiBP	0.2	100	42.7	1.5	12	25	39	69	166	689.9
Di(2-ethylhexyl) phthalate	DEHP	Mono(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.2	100	40.5	2.6	12	25	38	62	160	2640.5
Di(2-ethylhexyl) phthalate	DEHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	0.2	100	26.9	1.7	6.8	16	27	44	103	2103.8
Di(2-ethylhexyl) phthalate	DEHP	Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	0.2	99.7	22.1	0.6	5.6	14	23	36	84	1441.4
Di(2-ethylhexyl) phthalate	DEHP	Mono(2-ethylhexyl) phthalate	MEHP	0.5	98	7.2	0.1	1.6	4.4	7.7	15	35	292.3
Benzylbutyl phthalate	BBzP	Monobenzyl phthalate	MBzP	0.3	100	19.7	1.6	4.5	11	18	33	104	970.4
Di-isononyl phthalate	DiNP	Monocarboxy-isononyl phthalate	MCOP	0.2	99	4.1	0.1	1.2	2.4	3.9	6.5	19	375.3
Di- <i>n</i> -octyl phthalate	DOP	Mono(3-carboxypropyl) phthalate	MCPP	0.2	100	2.1	0.1	0.7	1.3	1.9	3.4	9.1	78.7
Di-isodecyl phthalate	DiDP	Monocarboxy-isononyl phthalate	MCNP	0.2	97.5	1.5	0.1	0.5	0.8	1.2	2.2	9.5	686.8

Note: Abbrev, abbreviation; GM, geometric mean; LOD, limit of detection.

(95% CI: 0.95, 1.25), $p = 0.05$] were related to ever eczema after adjustment for potential confounders. No other phthalate was associated with the occurrence of eczema in this analysis.

Associations between Phthalate Metabolites and Ever Eczema According to Atopic Status

We found significant associations between total IgE and phthalate metabolites, including Σ DEHP ($p = 0.01$) and its metabolites MECPP ($p = 0.03$), MEHHP ($p = 0.005$), MEOHP ($p = 0.008$), and MEHP ($p = 0.04$). We further stratified eczema outcome by atopic status. Of the 293 boys tested for IgE, we observed that 60 (20.48%) were sensitized and 233 (79.52%) were not. Table S2 shows the associations between maternal urinary concentrations of phthalate metabolite and eczema outcomes in boys according to their atopic status. Among IgE-sensitized boys, MBP and MiBP were significantly associated with ever eczema [HR = 1.67 (95% CI: 1.10, 2.54), $p = 0.01$ and HR = 1.87 (95% CI: 1.01, 3.48), $p = 0.04$, respectively]. Σ DEHP and MECPP, MEHHP, MEOHP, and MEHP individually were also positively associated with ever eczema in sensitized boys. MBzP was partially associated [HR = 1.47 (95% CI: 0.98, 2.20), $p = 0.06$]. However, the association for MEP, MCOP, MCPP, and MCNP was not significant. We did not find any association between prenatal phthalate exposure and eczema in boys without IgE sensitization. Using the multiple hypothesis testing did not modify the results except for MBP, MiBP, and MBzP, for which only a trend was observed.

Discussion

Major Findings

In this study, we report an association between maternal prenatal urinary concentration of metabolites of DiBP and DiNP and eczema development in offspring preschool boys followed-up between birth and 5 y of age. Metabolites of DiBP and DiNP were significantly associated with both phenotypes of eczema (early and late onset) (Table 4). DiBP is used in nitrocellulose plastic, nail polish, lacquer manufacturing and used with methyl methacrylate applications, whereas DiNP is used in a diverse range of industrial products (such as electrical wire and cables, flexible PVC sheeting, coated fabrics, automotive parts), building and construction, and several categories of toys (plastic books, balls, dolls, and cartoon characters) (Johns et al. 2015).

MECPP, a DEHP metabolite, and MCNP, a DiDP metabolite, were positively associated with late-onset eczema, whereas MEHHP and MEOHP, two metabolites of DEHP, were marginally associated. Our study also suggests a significant association between prenatal exposure to various phthalates and allergic eczema in boys at 5 y of age based on IgE levels. The metabolites of DBP, DiBP, and DEHP were significantly associated with ever eczema in IgE-sensitized boys, and borderline significance was associated with MBzP, the major BBzP metabolite [HR = 1.47 (95% CI: 0.98, 2.20), $p = 0.06$].

Comparison of Findings with Other Published Work

Several studies have reported a potential relationship between phthalates exposure and asthma (Bornehag et al. 2004b; Hsu et al. 2012; Smit et al. 2015; Ait Bamai et al. 2014) and eczema (Bornehag et al. 2004b; Stelmach et al. 2015; Just et al. 2012; Hsu et al. 2012; Smit et al. 2015; Ait Bamai et al. 2014) related symptoms in children. However, most of these studies used a case-control (Bornehag et al. 2004a, 2004b, 2005, 2006) or cross-sectional (Hsu et al. 2012; Ait Bamai et al. 2014; Hoppin et al. 2013) design or relied on phthalate levels in dust as a marker of phthalate exposure (Bornehag et al. 2005; Ait Bamai et al. 2014; Langer et al. 2014; Kolarik et al. 2008). This limits the conclusions that can be drawn because dust is not the only source of exposure to these chemicals.

In a birth cohort study in New York City where maternal urine was collected during the third trimester of pregnancy, MBzP, the major BBzP metabolite, was associated with the development of eczema by 5 y of age in 407 children (Just et al. 2012). In Sweden, an association between BBzP levels in dust and atopic dermatitis was observed in cross-sectional data (Bornehag et al. 2004b). In a cross-sectional study among 101 Taiwanese children between 3 and 9 y of age, BBzP levels in house dust were associated with eczema and asthma (Hsu et al. 2012). However, in the present study we observed only partial association between the main metabolite of BBzP and eczema at 5 y of age, even in boys with allergic eczema. In our study the metabolites of DiBP and DiNP were significantly associated with early-onset and late-onset eczema as well as ever eczema in boys. In contrast, in a birth cohort with 1,024 mother-child pairs from Greenland and Ukraine, oxidative metabolites of DiNP in maternal serum were negatively associated with current eczema as well as with ever eczema in boys and girls from Greenland (Smit et al. 2015). One limitation of that study is that it was conducted in

Table 3. Associations [adjusted odds ratio and 95% confidence interval [OR (95% CI)]] between maternal urinary concentrations of phthalate metabolites and occurrence of eczema phenotypes in boys (*n* = 604).

Year, eczema	MEP	MBP	MiBP	MECPP	MEHHP	MEOHP	MEHP	MBzP	MCOP	MCPP	MCNP	ΣDEHP
1	1.69 (1.05, 2.73) [*]	1.17 (0.74, 1.84)	1.78 (0.95, 3.33) [†]	1.04 (0.55, 1.98)	1.06 (0.61, 1.85)	1.07 (0.60, 1.92)	1.06 (0.61, 1.83)	1.10 (0.60, 2.00)	1.25 (0.70, 2.20)	1.15 (0.66, 2.00)	0.88 (0.59, 1.32)	1.01 (0.87, 1.18)
2	1.08 (0.74, 1.57)	1.12 (0.76, 1.63)	1.15 (0.64, 2.09)	0.94 (0.58, 1.52)	0.94 (0.60, 1.46)	0.97 (0.61, 1.54)	0.88 (0.57, 1.36)	1.14 (0.70, 1.87)	0.85 (0.52, 1.37)	1.18 (0.75, 1.87)	0.84 (0.55, 1.29)	0.98 (0.87, 1.10)
3	1.46 (0.89, 2.40)	1.12 (0.74, 1.69)	1.81 (0.94, 3.49) [†]	0.72 (0.38, 1.34)	0.88 (0.50, 1.54)	0.90 (0.50, 1.60)	0.94 (0.61, 1.47)	1.14 (0.70, 1.86)	1.28 (0.79, 2.09)	1.09 (0.64, 1.85)	1.61 (1.00, 2.59) [*]	0.96 (0.84, 1.11)
4	1.03 (0.79, 1.33)	1.15 (0.87, 1.85)	1.68 (1.16, 2.45) [*]	1.32 (0.96, 1.83) [†]	1.29 (0.95, 1.74) [†]	1.30 (0.95, 1.77) [†]	1.21 (0.91, 1.61)	1.24 (0.91, 1.69)	1.37 (0.99, 1.89) [†]	1.11 (0.79, 1.56)	1.22 (0.95, 1.57)	1.07 (0.99, 1.15) [†]
5	1.10 (0.86, 1.42)	1.10 (0.83, 1.47)	1.63 (1.12, 2.36) [*]	1.46 (1.04, 2.06) [*]	1.32 (0.96, 1.81) [†]	1.33 (0.96, 1.85) [†]	1.38 (1.03, 1.85) [*]	1.30 (0.96, 1.70) [†]	1.60 (1.16, 2.23) [*]	1.19 (0.84, 1.69)	1.37 (1.04, 1.80) [*]	1.08 (1.00, 1.18) [*]
Early-onset eczema	1.06 (0.89, 1.25)	1.10 (0.92, 1.32)	1.27 (1.00, 1.72) [*]	1.03 (0.77, 1.36)	1.02 (0.79, 1.32)	1.04 (0.80, 1.36)	1.06 (0.85, 1.32)	1.13 (0.88, 1.47)	1.29 (1.04, 1.60) [*]	1.18 (0.93, 1.49)	1.08 (0.90, 1.30)	0.98 (0.92, 1.04)
Late-onset eczema	1.13 (0.90, 1.42)	1.17 (0.96, 1.42)	1.55 (1.10, 2.18) [*]	1.34 (1.00, 1.81) [*]	1.26 (0.96, 1.67) [†]	1.28 (0.96, 1.70) [†]	1.18 (0.92, 1.52)	1.19 (0.90, 1.57)	1.63 (1.20, 2.21) [*]	1.14 (0.83, 1.57)	1.29 (1.02, 1.64) [*]	1.06 (0.99, 1.13) [†]

Note: Models were adjusted for parental asthma/rhinitis/eczema, maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, number of siblings, and recruitment center. Note: BMI, body mass index; CI, confidence interval; DEHP, di(2-ethylhexyl) phthalate; MBP, mono-*n*-butyl phthalate; MiBP, monobenzyl phthalate; MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MBzP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MCOP, monocarboxy-isooctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MCNP, monocarboxy-isooctyl phthalate; MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MBP, mono-isobutyl phthalate; OR, odds ratio. ^{*}*p* < 0.05, [†]*p* < 0.10.

Table 4. Association (hazard ratio and 95% confidence interval) between maternal urinary concentrations of phthalate metabolites and occurrence of ever eczema in boys (*n* = 184/604).

Phthalate metabolite	Unadjusted			Adjusted ^a		
	HR ^b	95% CI	<i>p</i> -Value	HR	95% CI	<i>p</i> -Value
MEP	1.04	0.93, 1.15	0.47	1.04	0.94, 1.16	0.42
MBP	1.00	0.89, 1.12	0.96	1.03	0.92, 1.15	0.60
MiBP	1.16	1.01, 1.33 [*]	0.03	1.16	1.01, 1.34 [*]	0.03
MECPP	0.97	0.84, 1.12	0.73	0.98	0.84, 1.13	0.77
MEHHP	0.99	0.86, 1.13	0.90	0.99	0.88, 1.14	0.93
MEOHP	1.01	0.88, 1.15	0.91	1.01	0.88, 1.16	0.86
MEHP	0.96	0.86, 1.07	0.50	0.96	0.85, 1.08	0.47
MBzP	0.99	0.88, 1.12	0.99	1.02	0.90, 1.15	0.69
MCOP	1.04	0.91, 1.19	0.48	1.09	0.95, 1.25 [†]	0.05
MCPP	1.02	0.88, 1.17	0.81	1.05	0.91, 1.22	0.50
MCNP	1.02	0.90, 1.14	0.74	1.03	0.92, 1.17	0.56
ΣDEHP	0.99	0.96, 1.03	0.77	0.98	0.94, 1.03	0.57

Note: CI, confidence interval; DEHP, di(2-ethylhexyl) phthalate; HR, hazard ratio; MBP, mono-*n*-butyl phthalate; MiBP, monobenzyl phthalate; MCNP, monocarboxy-isooctyl phthalate; MCPP, mono(3-carboxypropyl) phthalate; MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MBzP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MCOP, monocarboxy-isooctyl phthalate; MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MBP, mono-isobutyl phthalate. ^{*}*p* < 0.05, [†]*p* < 0.10.

^aHR adjusted for parental asthma/rhinitis/eczema, maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, number of siblings, and recruitment center.

^bHR according to the proportional Cox's model.

children between 5 and 9 y of age, because approximately 40–70% of childhood eczema cases resolve by the time the children reach 6–7 y of age (Pyun 2015).

Although phthalates are not highly sensitizing, they may promote the development of allergy or eczema (Wang and Karmaus 2015). Studies on mouse models have shown that phthalates, including DEHP and DiNP at low doses, can aggravate atopic dermatitis/eczema-like skin lesions by direct or indirect activation of the immune cells, which can be responsible, at least in part, for the increase in the prevalence of atopic dermatitis/eczema (Koike et al. 2010; Sadakane et al. 2014; Yanagisawa et al. 2008). To date, a large amount of work has been done on DEHP and allergy; DEHP is banned in many industrialized countries in PVC toys that may be placed in the mouth by children <3 y of age (Sadakane et al. 2014). DiNP is a substitute for DEHP, and this might be a reason for its increasing levels with time and the adverse effects associated with DiNP.

A case-control study from Taipei, Taiwan, on the effects of phthalate exposure and filaggrin gene variants on atopic dermatitis showed that children with specific filaggrin genotypes had higher urinary concentrations of phthalates, suggesting that such genotypes may increase skin permeability and so may lead to the higher absorption of phthalates and result in increased susceptibility to atopic dermatitis in such children (Wang and Karmaus 2015). In terms of mechanisms, phthalates might impair the epidermal barrier development processes. In addition, skin barrier defects caused by filaggrin gene mutations may allow allergens or irritants to penetrate the epidermis and to interact with antigen-presenting cells, phenomena which lead to the development of atopic dermatitis (Osawa et al. 2011; van den Oord and Sheikh 2009). A Danish study in children between 3 and 5 y of age found a relationship between atopic dermatitis and MEP in urine, but only in the fourth quartile, and no association with MiBP, a DiBP metabolite, while other metabolites of DiNP were not investigated (Callesen et al. 2014). Our study shows associations between prenatal phthalate exposure and atopy as assessed by total IgE at 5 y of age based on total IgE levels; however, findings in a study of another birth cohort did not observe an association based on specific IgE levels (Gascon et al. 2015). In our

study, the information on atopy was available for only 48.5% of the study population; hence the results should be interpreted with caution. Studies have suggested that two-thirds of the cases with eczema do not have measurable levels of circulating IgE antibodies (Flohr et al. 2004). In our study, about one-third (32%) of the boys had allergic eczema and two-thirds (68%) had nonallergic eczema.

Strengths and Limitations

Our study has several strengths. The population-based prospective cohort design with nutritional, environmental, biological, and social factors starting during prenatal life is a strength of our study that allowed us to control for potential confounders and to assess exposure before the health outcomes. Furthermore, to reduce subject variability, values of the exposure biomarkers were standardized for urine sampling conditions (Mortamais et al. 2012). However, we were limited to one urine sample per mother during the second trimester of pregnancy; thus we were unable to assess the variability in concentrations of each metabolite across the pregnancy. But it is also not clearly known at what point(s) in gestation the infants are at higher risk of exposure. Other studies have also used second-trimester maternal urinary concentration for biomarkers (Whyatt et al. 2014; Gascon et al. 2015; Bertelsen et al. 2013). Consistency in urinary concentrations during pregnancy has been observed for some environmental exposure biomarkers (Muckle et al. 2001; Longnecker et al. 1999), and other studies have suggested that phthalate biomarkers are relatively stable for a period of weeks to months (Hoppin et al. 2002; Hauser et al. 2004; Teitelbaum et al. 2008). Increasing the number of urine samples collected would have provided a more accurate estimate of the average exposure during the gestation period. We also adjusted for many potential confounders; however, residual confounding (if any) cannot be ruled out.

Regarding exposure assessment, it might be important to explore other techniques for assessment of the exposure, particularly those that may offer a more exhaustive and coordinated picture of the individual environment, for instance, by assessing the use of particular items in conjunction with indoor air levels and additional biomarkers of exposure.

Previous studies have reveal sex/gender-specific relationships between phthalates and health (Bertelsen et al. 2013; Vaidya and Kulkarni 2012). In our study, we considered only male offspring because the initial purpose was to see the effects of phthalates on malformations of male genitalia (Philippat et al. 2012). Although considering only males limits the generalization of our results, it is not a source of bias. From a statistical point of view, focusing on one sex is another way to increase the study accuracy when the sample size is limited.

The questionnaire data were self-reported and thus could have been subject to misclassification; in addition, recall bias was a possibility that might in part account for the high prevalence of eczema in our study population. The prevalence of early- and late-onset eczema in the present study was 15.7% and 14.7%, respectively, both of which are higher than that found (12.2%) in a cohort study from Poland in children at 2 y of age (Stelmach et al. 2015). However, a study by Augustin et al. (2015) showed a prevalence of 17.13% and 12.87% in children between 0–2 and 3–6 y of age, respectively.

Creatinine correction is most often used for urinary biomarkers of phthalates, phenols, and pesticides. There are limitations to the usage to creatinine to normalize for urine dilution; different investigators have used specific gravity as an alternative to creatinine to adjust phthalate urinary biomarkers for urine dilution (Just et al. 2012; Whyatt et al. 2014), but we did not have specific gravity measurements. Nevertheless, specific gravity is

highly correlated with creatinine (Barr et al. 2005) and, consequently, we likely overcorrected for urine dilution.

The exposures we studied are relatively prevalent, and some biomarker urinary concentrations approach those with significant effects in experimental models. In a healthy cohort such as ours, effects of hormonally active environmental exposures on skin may be small, yet a continuous exposure can have adverse health effects.

A further dimension to consider in future research is multiple exposures of hormonally active agents such as these phthalates. In terms of prevention, exposure to these chemicals can be avoided if the product contents are already known or are listed on the product label; unfortunately, they are often not listed on the label because of their identification as inactive ingredients.

Conclusions

Our data provide new suggestive evidence that prenatal exposure to certain phthalates, including DiBP and DiNP, may play a role in the development of eczema in early childhood. Future research focusing on larger populations and addressing multiple exposures assessed prenatally and postnatally are required to provide more evidence on possible contributions of emerging pollutants to study the role of phthalates on eczema in children.

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Supplemental Material

Prenatal Exposure to Phthalates and the Development of Eczema Phenotypes in Male Children: Results from the EDEN Mother–Child Cohort Study

Munawar Hussain Soomro, Nour Baiz, Claire Philippat, Celine Vernet, Valerie Siroux, Cara Nichole Maesano, Shreosi Sanyal, Remy Slama, Carl-Gustaf Bornehag, and Isabella Annesi-Maesano

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Figure S1. Directed acyclic graph (DAG) for evaluation of covariate selection in the analysis of phthalates and eczema. Green nodes: Variables associated to phthalates levels, Blue nodes: Variables associated to eczema occurrence, Red nodes: Variables associated to phthalates and eczema outcomes.

Table S1. Spearman correlation between ln-transformed standardized urinary concentrations of phthalate metabolites.

Table S2. Association between maternal urinary concentrations of phthalate metabolites and occurrence of ever eczema in boys according to atopic status as assessed by Total IgE.

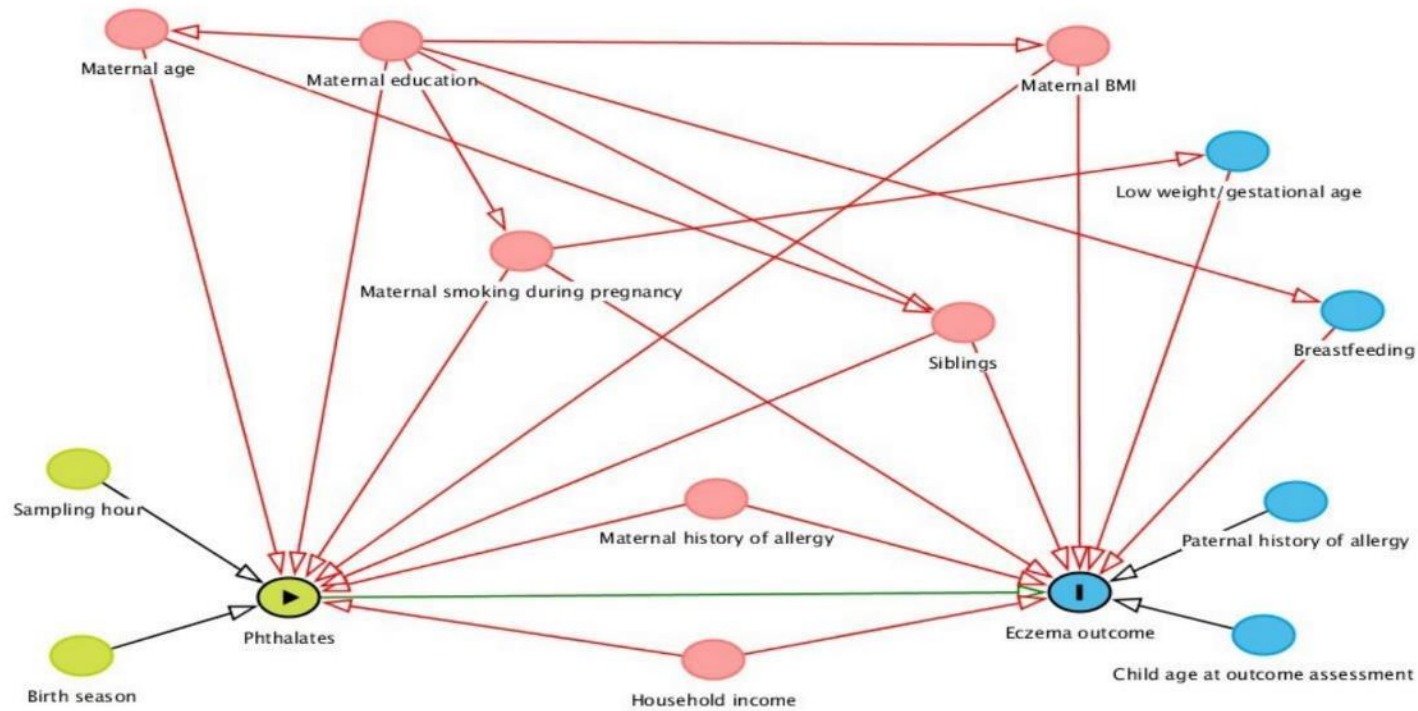


Figure-S1: Directed acyclic graph (DAG) for evaluation of covariate selection in the analysis of phthalates and eczema. Green nodes: Variables associated to phthalates levels, Blue nodes: Variables associated to eczema occurrence, Red nodes: Variables associated to phthalates and eczema outcomes

Table S1. Spearman correlation between ln-transformed standardized[‡] urinary concentrations of phthalate metabolites.

	MBP	MiBP	MECPP	MEHHP	MEOHP	MEHP	MBzP	MCOP	M CPP	MCNP
MEP	0.13*	0.03	0.04	0.06	0.08*	0.08*	0.10*	0.07	0.02	0.01
MBP		0.37*	0.26*	0.31*	0.32*	0.24*	0.40*	0.13*	0.62*	0.13*
MiBP			0.26*	0.29*	0.30*	0.27*	0.38*	0.20*	0.24*	0.11*
MECPP				0.90*	0.92*	0.77*	0.31*	0.36*	0.31*	0.27*
MEHHP					0.97*	0.83*	0.36*	0.33*	0.35*	0.25*
MEOHP						0.84*	0.38*	0.32*	0.36*	0.24*
MEHP							0.31*	0.25*	0.27*	0.19*
MBzP								0.19*	0.28*	0.17*
MCOP									0.28*	0.42*
M CPP										0.33*

MEP = Monoethyl phthalate; MBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; 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; MBzP = Monobenzyl phthalate; MCOP = Monocarboxy-isooctyl phthalate; M CPP = Mono(3-carboxypropyl) phthalate; MCNP = Monocarboxy-isononyl phthalate

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

* P<0.05

Table S2. Association between maternal urinary concentrations of phthalate metabolites and occurrence of ever eczema in boys according to atopic status as assessed by Total IgE.

Phthalate metabolite	Non-Sensitized Boys n=233/293			Sensitized Boys n=60/293		
	HR ^a	95% CI	P-Value	HR	95% CI	P-Value
MEP	1.13	0.82-01.56	0.44	1.17	0.71-1.93	0.53
MBP	0.90	0.63-1.28	0.55	1.67	1.10-2.54*	0.01
MIBP	1.32	0.86-2.01	0.19	1.87	1.01-3.48*	0.04
MECPP	1.05	0.68-1.62	0.81	2.15	1.32-3.50*	0.002
MEHHP	1.07	0.72-1.57	0.73	2.02	1.27-3.19*	0.003
MEOHP	1.08	0.73-1.62	0.68	2.02	1.26-3.25*	0.004
MEHP	1.14	0.82-1.59	0.42	2.35	1.35-4.08*	0.002
MBzP	1.13	0.80-1.60	0.48	1.47	0.98-2.20[†]	0.06
MCOP	1.20	0.76-1.77	0.49	1.51	0.85-2.67	0.16
M CPP	1.15	0.70-1.89	0.57	1.51	0.81-2.81	0.18
MCNP	1.14	0.82-1.56	0.41	1.41	0.89-2.21	0.14
ΣDEHP	1.02	0.93-1.13	0.66	1.22	1.07-1.38*	0.002

^aHR: Hazard Ratio according to the proportional cox's model.

HRadjusted for maternal smoking, maternal age, maternal BMI, education level, gestational age, number of siblings, and recruitment Centre

MEP = Monoethyl phthalate; MBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; 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; MBzP = Monobenzyl phthalate; MCOP = Monocarboxy-isoctyl phthalate; M CPP = Mono (3-carboxypropyl) phthalate; MCNP = Monocarboxy-isononyl phthalate; DEHP = di(2-ethylhexyl) phthalate.

*P<0.05

[†]P<0.10

3.2 Paper 2: Exposure to heavy metals during pregnancy related to gestational diabetes mellitus in diabetes-free mothers.

In our second paper, we show the results on association between maternal exposure to heavy metals during pregnancy and gestational diabetes mellitus. We published our paper in the journal Science of Total Environment. We present the details of this paper in the thesis.



Exposure to heavy metals during pregnancy related to gestational diabetes mellitus in diabetes-free mothers



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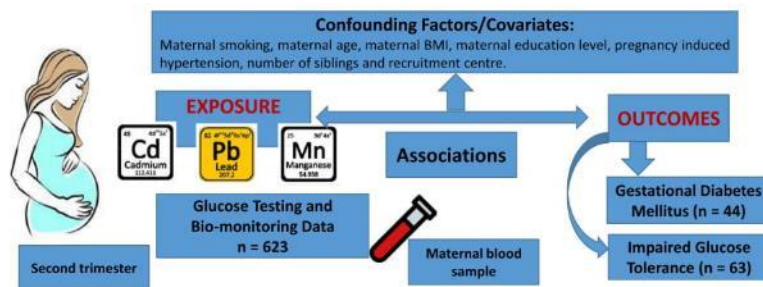
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HIGHLIGHTS

- Does maternal exposure to heavy metals namely, Pb, Cd and Mn play a role in the development of GDM?
- In a sample of 623 diabetes-free mothers, exposure during pregnancy to Pb or Cd was associated with GDM development.
- Findings add to the growing evidence supporting the role of maternal exposure during pregnancy to heavy toxic metals.
- Heavy toxic metals that persist longtime in the environment as a risk factor for GDM, which opens actions for prevention.

GRAPHICAL ABSTRACT



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ABSTRACT

Evidence is cumulating on the adverse health effects of environmental exposures on health of the fetus and the childbearing mothers. Among mother's conditions, gestational diabetes mellitus has been considered rarely in spite of its importance for both mother and child. We determined the role of maternal exposure to lead (Pb), cadmium (Cd) and manganese (Mn) to gestational diabetes mellitus (GDM) on diagnosed GDM and impaired glucose tolerance (IGT) in diabetes-free mothers from the French EDEN mother-child cohort. 623 pregnant women without pre-existing diabetes were included in the study. GDM and IGT were diagnosed by a gynecologist during consultations after blood analysis. Pb, Cd and Mn were measured in second-trimester blood samples. Associations between ln-transformed concentrations of metals and GDM and IGT respectively were examined

Abbreviations: BMI, body mass index; Cd, cadmium; CI, confidence interval; GDM, gestational diabetes mellitus; IGT, impaired glucose tolerance; LOD, limit of detection; Mn, manganese; OR, odds ratio; Pb, lead; SD, standard deviation.

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using multiple logistic regression analysis adjusted for potential confounders. The prevalences of GDM and IGT were 7.1% and 10.1% respectively. After adjustment for confounders, Cd was statistically related to having had a diagnosis of GDM or IGT (Adjusted Odds-Ratio (AOR): 1.61, 1.05–2.48), and Pb to GDM at borderline significance (AOR: 1.65, 0.82–3.34). Our findings add to the growing evidence supporting the role of maternal exposure to heavy toxic metals that persist longtime in the environment as a risk factor for GDM.

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1. Introduction

Gestational diabetes mellitus (GDM), which is one of the most common complications of the pregnancy, is defined as a glucose impaired tolerance resulting in hyperglycemia of variable severity with onset or first recognition during pregnancy (Baz et al., 2016). Studies have also shown that the intrauterine environment of maternal diabetes can have significant influence on health in later life in both mother and child (Kubo et al., 2014; Nehring et al., 2013). GDM prevalence has increased worldwide in the past decades and there is evidence that such increase cannot be fully explained by lifestyle factors such as sedentary behavior and caloric intake alone (Ferrara, 2007; Moses et al., 2017; Yeung et al., 2017). Recently, epidemiological studies have shown involvement of maternal exposure during pregnancy to environmental chemicals (Arrebola et al., 2015; Ehrlich et al., 2016; Shapiro et al., 2015).

Exposure to heavy metals remains a public health concern for long-time because of their persistence in the environment and their ability to induce toxicity even at low level. Naturally occurring metals like lead (Pb), cadmium (Cd) and manganese (Mn) are also classified as endocrine disruptors (EDCs) (Iavicoli et al., 2009). The EDCs, alter the functions of endocrine system and consequently cause adverse health effects. The mechanisms by which the risk of GDM may increase includes oxidative stress, up-regulation of inflammatory markers (tumor necrosis factor alpha and interleukin 6), inhibition of peroxisome proliferator-activated receptor gamma (PPAR- γ) and alteration of methylation patterns of diabetes-related genes. In particular, metals may influence glucose homeostasis via other toxic mechanisms, such as inducing oxidative stress. Women are challenged during the pregnancy with multiple EDCs. The main sources of occupational exposure to heavy metals are from foundry, mining, and/or manufacturing industries, while exposure in general population occurs through the ingestion of contaminated food or contact with the consumer products containing these metals (Iavicoli et al., 2009). The epidemiological studies have shown positive associations between metals (Arsenic, Cd, Pb) and GDM (Peng et al., 2015; Romano et al., 2015; Romano et al., 2015). On the other hand, current knowledge on EDCs like metals and GDM is still limited with contradictory results, which can depend on differences in populations and methods and potential biases.

The objective of this study was to evaluate the associations between maternal exposure to heavy metals during pregnancy and GDM or impaired glucose tolerance (IGT), as intermediate phenotype, using data collected in the French EDEN mother-child prospective cohort study conducted in the general population.

2. Methods

2.1. Study population and data collection

The EDEN study (Etude des Déterminants pré et post natus du développement de la santé de l'Enfant) is a mother-child prospective birth cohort study (Heude et al., 2016). This study enrolled 2002 pregnant women from February 2003 to January 2006, before their 24th week of gestation in two French university hospitals in the cities of Poitiers and Nancy. The mothers' recruitment, follow-up procedures, and methodological assumptions have been published elsewhere (Heude

et al., 2016). Briefly, women were given an appointment with a study midwife between the 24th and 28th gestational weeks, during which an interview on life style factors was conducted and biological samples were collected. The information on the mothers and their newborns, including parity, mode of delivery, birth weight, gestational age, season of birth, behavioral and environmental data were collected by the use of questionnaires, interviews and from obstetric and pediatric records. The children were followed from birth to the age of 5 years. The current study includes all diabetes-free mothers ($n = 623$) for whom maternal blood samples had been analyzed for the metals (Fig. 1). The absence of diabetes was one of the inclusion criteria of the study (Heude et al., 2016).

2.2. Chemical bio-monitoring data

Three metals Pb, Cd and Mn were measured from second trimester (between the 24th and 28th gestational weeks) blood samples of pregnancy. Women were invited for a clinical examination and to provide a venous blood sample. EDTA tubes were used to collect the blood for assay. The blood tubes were prepared within an hour of sample collection. After aliquoting, all biological material was frozen at $-80\text{ }^{\circ}\text{C}$. After centrifugation at 3000 rpm for 5min, maternal blood was aliquoted and frozen at $-80\text{ }^{\circ}\text{C}$ for later analysis. The blood Pb, Cd and Mn concentrations were measured by electrothermal atomic-absorption spectrometry (model 4100 ZL; Perkin-Elmer, Courtaboeuf, France) with Zeeman background correction as previously described (Bonithon-Kopp et al., 1986; Huel et al., 1986; Mergler et al., 1996). The limit of detection was 100%.

2.3. Gestational diabetes mellitus and impaired glucose tolerance

At 24–28 weeks, maternal blood glucose concentrations were measured 1 h after a 50-g glucose challenge. Women with 1-h glucose concentrations over 130 mg/dL in Nancy and 140 mg/dL in Poitiers were scheduled for a 3-h 100-g oral-glucose tolerance test. The GDM was diagnosed by using the oral-glucose-tolerance test according to the Carpenter and Coustan criteria (Steer et al., 2004) when there were >2 blood glucose concentrations greater than the following cut points: fasting = 95 mg/dL, at 1 h = 180 mg/dL, at 2 h = 155 mg/dL, and at 3 h = 140 mg/dL. While a diagnosis of IGT was assigned if one of the oral-glucose-tolerance test cut-off values was met or exceeded. The GDM and IGT were taken as categorical variables in the analysis. For the purpose of the analysis, an additional variable resulting from the combination of GDM and IGT in the case of pregnant women diagnosed with either GDM or IGT was also considered (named "Combination of GDM and IGT").

2.4. Other variables

Information on potential confounders included: parity, number of siblings (0, 1, 2 \geq 3), maternal age at delivery (<25 years, 25–29 years, 30–34 years, ≥ 35 years), pre-pregnancy maternal body mass index (BMI, <18.5 , 18.5–24.9, 25.0–30.0, $>30\text{ kg/m}^2$), maternal and paternal history of allergies, maternal and paternal educational level (<2 years after high school, High school + 2 years, \geq High school + 3 years), city of residence (Nancy/Poitiers), mode of delivery (vaginal, caesarean

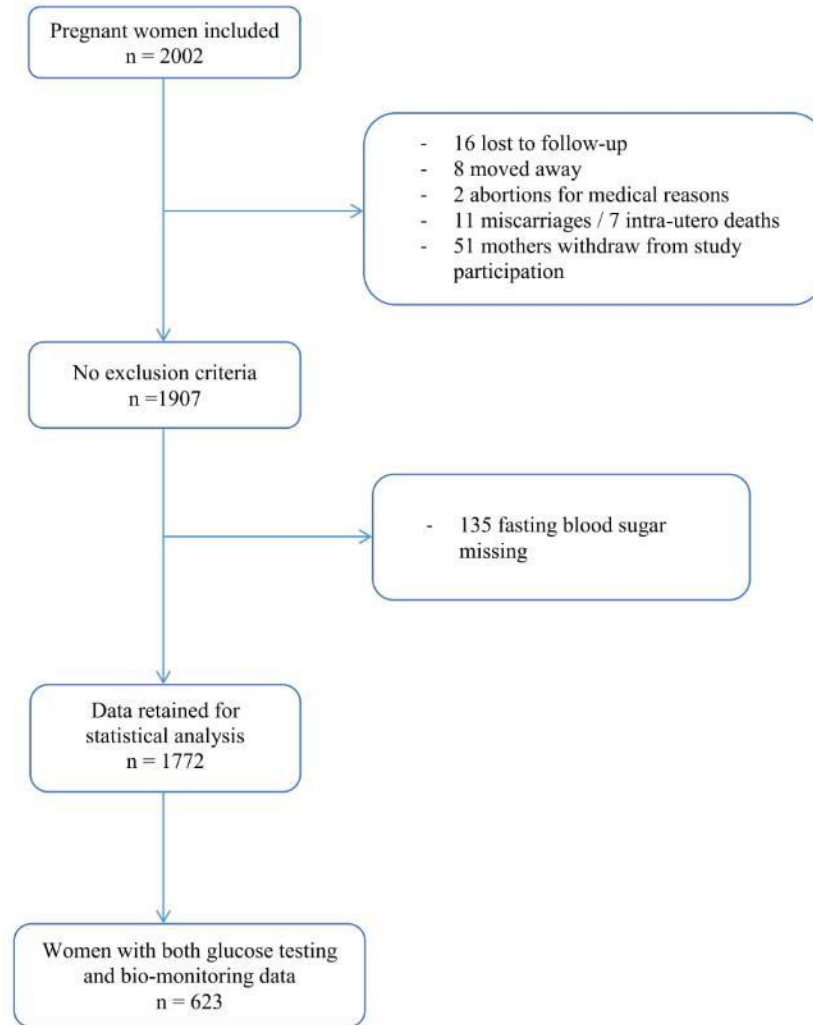


Fig. 1. Flow chart of the study.

section), smoking during pregnancy (yes/no), maternal alcohol use during pregnancy (yes/no), pregnancy induced hypertension (yes/no). Women were classified as having pregnancy induced hypertension based on systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg measured during at least two visits after the 22nd week of gestation, such that the elevated measures could have occurred during or across any of the three periods, at any point after 22 weeks.

2.5. Statistical analyses

Two variables were considered for metal concentrations in the maternal blood: a continuous variable and a categorical variable. The continuous variables were log-natural (ln) transformed because of non-normality of their distribution and their average given as geometric mean. In addition, quartiles of the concentrations of each metal were estimated to describe mothers' exposure to metals according to 4 levels, namely none, light, moderate and high. Other categorical variables were expressed as the number and percentages (%) and continuous variables were expressed as the mean \pm standard deviation (SD). The study population was also compared according to the recruitment centre by applying a chi-squared test (for categorical variables) or a Mann-Whitney *U* test (for continuous variables).

We applied multiple logistic regression analysis to examine the association between each ln metal concentration and GDM or IGT outcomes adjusted for potential confounders. The confounding factors were chosen through review of the literature based on their relationship with exposure and/or outcomes and were tested. Confounders included in our final model were maternal smoking, maternal age, maternal BMI, maternal education level, pregnancy induced hypertension and number of siblings. Models were additionally adjusted for recruitment centre. Analyses were repeated with quartiles of the metal concentrations distribution.

All the statistical analysis was conducted using Stata statistical software 14 (Stata Corp LP, 4905 Lakeway Drive, College Station, Texas 77845, USA) where *P-value* < 0.05 were considered statistically significant.

2.6. Ethical approval

The study was approved by the relevant ethical committees (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale, Le Kremlin Bicêtre University Hospital, and Commission Nationale de l'Informatique et des Libertés), and all participating women gave informed written consent for their own participation and both parents for newborn child after delivery.

3. Results

3.1. Characteristics of the study population and exposure to heavy metals

Of 2002 women recruited into the EDEN cohort, 623 met the inclusion criteria and had outcome data and bio-monitoring data measured (Fig. 1) and data on other used co-variables. The characteristics of the population are given in Table 1. The prevalence of GDM was found 7.06%, while the one for IGT was 10.1%. Study sample of this study differ from sample excluded from the analysis in maternal age, maternal education, and active smoking of the mother (Table S1). In addition, results did not change when adjusted for them in the final model.

In our population, heavy metal concentration of Cd was higher than the normal range ($<0.315 \mu\text{g/L}$) in $>75\%$ of the mothers, however some women had levels higher than the normal of blood Pb ($<50 \mu\text{g/L}$) and Mn ($4\text{--}15 \mu\text{g/L}$) (Table 2). We did not observe any significant difference in the metals between Nancy and Poitiers. While analyzing with Spearman correlation coefficients between ln-transformed metals, only the

Mn was significantly correlated with Pb ($r = 0.124$) or Cd ($r = -0.079$). The correlation between Pb and Cd metals were not found significant (data not shown).

3.2. Associations between heavy metals and gestational diabetes mellitus or impaired glucose tolerance

A statistically significant association was observed between Cd and the combination of GDM and IGT in the crude as well as in the adjusted model (odds-ratio (OR): 1.50, CI: 1.50–1.06 and OR: 1.61, CI: 1.05–2.48, respectively) (Table 3). Associations of borderline significance were observed in crude as well as in adjusted model between Cd and GDM (OR: 1.64, CI: 0.99–2.76 and 1.81, CI: 0.93–3.50 respectively). In addition, a significant association was observed between Pb and GDM in the unadjusted model (OR: 1.83, 95% Confidence Interval (CI): 1.01–3.29) that persisted as a trend after adjusting for confounders (OR: 1.65, CI: 0.82–3.34). However no association was found for IGT or the combination of GDM and IGT for this metal. No statistical significant associations were observed of Mn with GDM.

While taking into account the metal distribution quartiles, a significant increased odd ratio for combined GDM and IGT was observed for Cd in the 4th quartile after adjustment for confounders (OR: 2.33, CI: 1.10–4.92) (Table S2). No statistical evidence of association was observed elsewhere after adjusting for confounders.

Table 1
Characteristics of the study population and by centre.

Characteristics	All n = 623		Poitiers n = 420		Nancy n = 203		P value
	No.	%	No.	%	No.	%	
Maternal age (years)	29.02 ± 4.9						
<25	121	19.5	89	21.2	32	15.7	0.27
25–29	209	33.5	141	33.5	68	33.5	
30–34	206	33.1	130	31.0	76	37.4	
≥35	87	13.9	60	14.3	27	13.4	
Parity							
0	276	44.3	205	48.8	71	34.9	0.003
1	231	37.1	139	33.1	92	45.3	
≥2	116	18.6	76	18.1	40	19.8	
BMI (kg/m ²)	23.01 ± 4.2						
<18.5	50	8.1	34	8.1	16	7.8	0.36
18.5–24.9	405	65.0	264	62.8	141	69.4	
25–30	110	17.6	81	19.3	29	14.4	
>30	58	9.3	41	9.8	17	8.4	
Maternal education							
<2 years after high school	307	49.3	222	52.8	85	41.9	0.06
High school + 2 years	141	22.6	87	20.8	54	26.6	
≥High school + 3 years	167	26.9	105	25.0	62	30.6	
Missing	8	1.2	6	1.4	2	0.9	
Active smoking (cig/day)							
0	483	77.5	315	75.0	168	82.7	0.09
1 to 5	94	15.2	71	16.9	23	11.4	
>5	36	5.7	25	5.9	11	5.4	
Missing	10	1.6	9	2.2	1	0.5	
Passive smoking							
Yes	274	44.0	179	42.6	95	46.8	0.54
No	274	44.0	191	45.5	83	40.8	
Missing	75	12.0	50	11.9	25	12.4	
Alcohol during pregnancy							
Yes	166	26.6	99	23.6	67	33.0	0.04
No	450	72.2	316	75.2	134	66.1	
Missing	7	1.2	5	1.2	2	0.9	
Gestational age	39.1 ± 1.7						
<37 gestational week	37	5.9	33	7.8	4	1.9	0.003
≥37 gestational week	586	94.1	387	92.2	199	98.1	
Employment							
Yes	451	72.4	301	71.7	150	73.9	0.81
No	168	26.9	116	27.6	52	25.6	
Missing	4	0.7	3	0.7	1	0.5	
Caesarean section							
Yes	80	12.8	48	11.4	32	15.7	0.12
No	543	87.2	372	88.6	171	84.3	
Gender newborn							
Males	327	52.5	231	55.0	96	47.3	0.07
Females	296	47.5	189	45.0	107	52.7	
Pregnancy induced hypertension							
Yes	27	4.3	16	3.8	11	5.4	0.35
No	596	95.7	404	96.2	192	94.6	

BMI; body mass index.

4. Discussion

4.1. Main findings

In this French birth cohort, we observed significant associations between maternal concentrations of heavy metals during the pregnancy with GDM or IGT and their combination indicating that the environment the mother encounters during the pregnancy can endanger her health status. More precisely, we found that, after taking into account for potential confounders, Cd was related to both GDM alone and the combination of GDM and IGT and Pb was partially related to GDM.

4.2. Health effects of gestational exposure to selected metals in the literature

Our results confirm and extend previous data by applying a strict methodology and suggest that heavy metals are factors which need to be taken seriously into account and that now is the time to raise this issue.

Cd is a non-essential, toxic heavy metal distributed widely in the environment (Barregard et al., 2013). The principal source of Cd exposure for the general non-smoking population is food (Swaddiwudhipong et al., 2012). In our study women with high blood Cd had higher risk of GDM than those women with low Cd. We observed partial associations in crude as well as in adjusted models between Cd and GDM. To our knowledge only three epidemiological studies recently addressed specifically the association between Cd and GDM (Peng et al., 2015; Romano et al., 2015; Shapiro et al., 2015). However, only a Canadian study (Shapiro et al., 2015) was a prospective birth cohort, while others were either retrospective case-control study (Peng et al., 2015) or a case-cohort (Romano et al., 2015) within prospective studies. A study from China (Peng et al., 2015) showed significant association between meconium Cd and GDM in 3rd and 4th quartile. A USA case-cohort study (Romano et al., 2015) showed that women in highest tertile of urinary Cd had two times the risk of GDM as compared with those in the lowest tertile. However, the Canadian study failed to confirm this finding with Cd measured in blood (Shapiro et al., 2015).

Other epidemiological studies have examined Cd exposure in relation to type 2 diabetes mellitus (Afridi et al., 2013; Barregard et al., 2013, 2014; Chen et al., 2006; Hansen et al., 2017; Liu et al., 2016; Schwartz et al., 2003). Most of them were either cross-sectional (Barregard et al., 2014; Chen et al., 2006; Liu et al., 2016; Schwartz

Table 2
Distribution of metals concentration ($\mu\text{g/L}$) in maternal blood ($n = 623$).

Metals	Abbrev.	Normal range $\mu\text{g/L}$	Limit of detection (LOD)	Percent of detection	Geometric mean (GM)	Minimum	Percentile					Maximum
							5th	25th	50th	75th	95th	
Lead	Pb	<50	0.25	100	16.2	1	6.4	12	17	22	38	80
Cadmium	Cd	<0.315	0.05	100	0.75	0.1	0.2	0.5	0.8	1.2	1.8	9.6
Manganese	Mn	4–15	0.3	100	9.62	1	5	8	10	12	19	53

et al., 2003) or retrospective (Afridi et al., 2013) or case-control (Hansen et al., 2017; Simić et al., 2017) in design, except one study which included limited prospective follow-up after a cross-sectional assessment (Barregard et al., 2013). Despite a limited number of prospective studies, the research has consistently supported the potential association between increasing blood Cd level and type 2 diabetes mellitus. Studies from different populations of the world have also suggested that women and men with type 2 diabetes mellitus have statistically significant higher levels of Cd in urine (Afridi et al., 2008; Barregard et al., 2014; Kolachi et al., 2011; Schwartz et al., 2003), blood (Afridi et al., 2008; Barregard et al., 2014; Hansen et al., 2017; Kolachi et al., 2011) or hair (Afridi et al., 2013; Kolachi et al., 2011).

Pb is a naturally occurring toxic heavy metal found in almost all environmental domains which include soil, water and environment (Cheng et al., 2015; Marx et al., 2016; Romero-Freire et al., 2015). General population is relatively exposed to low concentrations of Pb via cigarette smoking, polluted drinking water, food and air (Augustsson et al., 2015; Mohmand et al., 2015; Pinto et al., 2017). Whereas occupational exposure in welding, smelting, electroplating, painting, battery, pigments, and e-waste recycling workshops are exposed to relatively high concentrations of Pb (Julander et al., 2014; Kazi et al., 2015; Zhang et al., 2016). However, higher Pb exposure may occur through man-made sources, such as in paint around the home, leaded pipes and fixtures that deliver water and soil contaminated with Pb from leaded gasoline. Indeed, in our study some mothers had relatively high levels of blood Pb (i.e. over recommended levels of $5 \mu\text{g/dL}$ as shown in Table 2), without having occupational exposure (data not shown). In unadjusted models, women in our study with high blood Pb had higher risk of GDM than women with low Pb. However, these results were not significant after adjustment for key covariates. Recently, two other epidemiological studies addressed specifically the association between Pb and GDM (Peng et al., 2015; Shapiro et al., 2015). However, they failed in establishing a positive relationship. On the other hand, a retrospective case-control study within a Chinese population found a negatively associated significance in the second and third quartile of Pb (Peng et al., 2015). On the other hand, in Canada, the observed association was not significant (Shapiro et al., 2015). Controversial findings for type 2 diabetes mellitus in life had been shown by previous research as some investigations revealed that blood Pb (Haq et al., 2013), urinary Pb (Afridi et al., 2008) or hair Pb (Afridi et al., 2013) were higher in diabetes as compared to non-diabetes but others reported that blood Pb was not associated with diabetes (Hansen et al., 2017; Moon, 2013),

or even inversely associated with it (Simić et al., 2017). However, study designs differed among the investigations (most were case-control studies) and various biological matrix were used.

Mn is an essential micronutrient required for the activity of metabolism and antioxidant system (Livingstone, 2017). However, the requirements are low and excessive intake or exposure to it can cause neurotoxicity (Hutchens et al., 2017; Pfaller and Bowman, 2017). The association between Mn and GDM was not significant in our study which is consistent with other studies conducted in the general populations (Hansen et al., 2017; Liu et al., 2016; Simić et al., 2017). However, one Korean study revealed that blood manganese levels were negatively associated with increased risk of diabetes (Koh et al., 2014).

4.3. Potential mechanisms

It has been suggested that GDM and Type 2 diabetes mellitus might have common pathogenic pathways (Shaat et al., 2005). GDM is a multifactorial metabolic disorder in which loci interact with environmental factors as well as with family history, changes in life style and behavior (e.g. poor diet, lack of exercise) and obesity (Ehrlich et al., 2016; Romano et al., 2015). Furthermore, the beta cell dysfunction in the islets of Langerhans, impaired insulin secretion and insulin resistance may involve in the pathogenesis of GDM as in type 2 diabetes mellitus (Romano et al., 2015; Wu et al., 2016). It is also hypothesized that the risk of GDM may increase through oxidative stress, up-regulation of inflammatory markers (tumor necrosis factor alpha and interleukin 6), and inhibition of peroxisome proliferator-activated receptor gamma (PPAR- γ) (Wu et al., 2016; Yang et al., 2011). Obesity and insulin resistance are associated with inflammatory factors and play a pivotal role in the development of GDM and type 2 diabetes mellitus (Hotamisligil, 2006). However, studies examining type 2 diabetes versus gestational diabetes are not directly comparable and associations vary across studies.

Another mechanism by which heavy metals like arsenic, Pb and Cd may also increase diabetes risk is through endocrine-disrupting mechanisms (Dyer, 2007; Iavicoli et al., 2009; Tseng, 2004) and by altering methylation patterns of diabetes-related genes (Edwards and Myers, 2007). We did not assess arsenic however, the mechanism of action may be the same for all these metals. However, humans are exposed to multiple toxic substances every day, so that it is hard to know the exact mechanism of action by which each metal causes GDM.

Table 3
Associations (odd ratio and 95% confidence interval) between blood metals^a and gestational diabetes mellitus or impaired glucose tolerance.

Exposure	GDM (44) vs. normal glucose (579)		IGT (63) vs. normal glucose (560)		GDM + IGT (107) vs. normal glucose (516)	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Pb	1.83 (1.01–3.29)[*]	1.65 (0.82–3.34)	0.97 (0.68–1.39)	0.75 (0.49–1.14)	0.99 (0.68–1.43)	0.76 (0.50–1.16)
Cd	1.64 (0.99–2.76) [†]	1.81 (0.93–3.50) [†]	1.19 (0.88–1.61)	1.28 (0.89–1.84)	1.50 (1.06–2.12)[*]	1.61 (1.05–2.48)[*]
Mn	1.41 (0.69–2.91)	0.98 (0.42–2.24)	1.14 (0.73–1.76)	0.92 (0.55–1.54)	0.92 (0.58–1.46)	0.90 (0.53–1.53)

Pb = lead; Cd = cadmium; Mn = manganese; GDM = gestational diabetes mellitus; IGT = impaired glucose tolerance.

Models were adjusted for maternal smoking, maternal age, maternal BMI, maternal education level, pregnancy induced hypertension, number of siblings and recruitment centre.

^a Blood metals were log-natural transformed for normal distribution.

^{*} $P < 0.05$.

[†] $P < 0.10$.

4.4. Strengths and limitations of the study

Our study has strengths as well as limitations. The first strength of our study was the comparability of GDM prevalence with the one from previous studies indicating that in our population selection bias was avoided (Agarwal et al., 2015; Shearrer et al., 2015; Tamayo et al., 2016). Another strength of our study was that exposure of the metals was objectively measured in the mothers' blood during second trimester of the pregnancy. As an additional strength, the diagnosis of GDM was conducted by the physicians, which ensures the quality and we further adjusted for potential confounders to see the associations between metals and GDM. Another strength of our study is the nature of the study design, large and well characterized cohort of pregnant women, which overcomes the limitations of the previous case-control and cross-sectional research. The nature of our study facilitated to exclude the women with diagnosed pre-gestational diabetes mellitus and renal diseases. Blood samples for metals assessment were measured with well validated method of spectrometry and fluorimetry. Structured interviews and medical record abstraction provided us a rich covariate dataset. Lastly, another asset of our study results from the taking into account of confounders of the relationships between exposure to metal and GDM such as maternal age, maternal BMI, tobacco smoking, socioeconomic variables, delivery type.

However, our study also presents limitation. The first limitation of our study is its design: a nested cross-sectional study in a cohort study. Another limitation of our study was that the measurement of heavy metal was only conducted once. It would have been more interesting to study the association between the health related variables and exposure to metals during different points in time. Variation in the exposure of these metals during pregnancy can occur; however it is hypothesized that with continuous exposure and excretion the net exposure remains within a range. As an additional limitation, humans are exposed to a large number of complex mixtures of toxic substances in daily life, so it is hard to see the effect of exposure to a unique metal (Romano et al., 2015). In addition, nowadays the diagnosis of GDM is no longer based on two different tests but on only one test, i.e. 75 g-glucose tolerance with only one value above the limit of fasting, 1 h or 2 h. Whether we underestimated or overestimated the relationship cannot be seen in our study as we had no access to detailed data on glycemia, so the results should be interpreted cautiously. This study design has limitations because the exposure and the health outcome are assessed at the same time, i.e., a nested cross-sectional approach. However, the nested cross-sectional cohort design offers advantages over traditional cross-sectional designs. And indeed, we could determine that we were dealing with isolated GDM, as no mother had a diagnosis of diabetes at 2 years of age of the child.

Overall, further studies are warranted with clinically diagnosed GDM, taking into account potential confounders and measurements of these chemicals at multiple time points during pregnancy. Another limitation of our study is that this cohort was conducted in two cities which differ significantly in many respects and which may not represent general French population. However, in the models we adjusted for centre to take into account the regional specificities and we do not have the ambition to be representative.

5. Conclusion

Our findings add to the growing evidence supporting the role of maternal exposure to heavy toxic metals as a risk factor for GDM, which may be through the involvement of metals in the disruption of glucose uptake and alteration of the related molecular mechanism in glucose regulation.

Acknowledgments

We acknowledge the participating families, midwife research assistants for data collection.

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We acknowledge all funding sources for the EDEN study: Foundation for Medical Research (FRM), National Agency for Research (ANR), National Institute for Research in Public Health (IRESP: TGIR cohorte santé 2008 program), French Ministry of Health (DGS), French Ministry of Research, Inserm Bone and Joint Diseases National Research (PRO-A) and Human Nutrition National Research Programs, Paris-Sud University, Nestlé, French National Institute for Population Health Surveillance (InVS), French National Institute for Health Education (INPES), the European Union FP7 programmes (FP7/2007–2013, HELIX, ESCAPE, ENRIECO, MEDall projects), Diabetes National Research Program (through a collaboration with the French Association of Diabetic Patients (AFD)), French Agency for Environmental Health Safety (now ANSES), Mutuelle Générale de l'Éducation Nationale (MGEN), French National Agency for Food Security, the EU FP7-ENV funded Health and Environment-wide Associations based on Large population Surveys (HEALS) project (N: 603946) and the French-speaking association for the study of diabetes and metabolism (ALFEDIAM). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.11.422>.

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Appendix A. Supplementary data

Exposure to heavy metals during pregnancy related to gestational diabetes mellitus in diabetes-free mothers.

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Table S1. Characteristics of the study population included and excluded in the analysis.

Table S2. Associations (Odd Ratio and 95% Confidence Interval) between and blood metals by quartiles and gestational diabetes mellitus or impaired glucose tolerance.

Table S1. Characteristics of the study population included and excluded in the analysis.

Characteristics	Sample of analysis n=623		Samples Excluded for analysis n=1149		P value
	Mean ± SD		Mean ± SD		
	No.	%	No.	%	
Maternal age (years)	29.02 ± 4.9		29.71 ± 4.8		
<25	121	19.5	160	13.9	0.01
25 – 29	209	33.5	414	36.1	
30 – 34	206	33.1	383	33.3	
≥35	87	13.9	192	16.7	
Parity					
0	276	44.3	528	45.9	0.80
1	231	37.1	413	35.9	
≥2	116	18.6	208	18.2	
BMI (kg/m²)	23.01 ± 4.2		23.10 ± 4.4		
<18.5	50	8.1	102	8.9	0.89
18.5 - 24.9	405	65.0	741	64.5	
25 – 30	110	17.6	207	18.0	
>30	58	9.3	99	8.6	
Maternal education					
<2 years after high school	307	49.3	493	42.9	0.01
High school +2 years	141	22.6	248	21.6	
≥ High school +3 years	167	26.9	388	33.8	
Missing	8	1.2	20	1.7	
Active smoking (cig/day)					
0	483	77.5	967	84.2	0.004
1 to 5	94	15.2	127	11.1	
>5	36	5.7	47	4.1	
Missing	10	1.6	8	0.6	
Passive smoking					
Yes	274	44.0	500	43.5	0.97
No	274	44.0	512	44.6	
Missing	75	12.0	137	11.9	
Alcohol during pregnancy					
Yes	166	26.6	311	27.1	0.36
No	450	72.2	832	72.4	
Missing	7	1.2	6	0.5	
Gestational age	39.10 ± 1.7		39.20 ± 1.7		
<37 gestational week	37	5.9	62	5.4	0.63
≥37 gestational week	586	94.1	1087	94.6	
Employment					
Yes	451	72.4	877	76.3	0.15
No	168	26.9	263	22.9	
Missing	4	0.7	9	0.8	
Caesarean section					
Yes	80	12.8	193	16.8	0.02
No	543	87.2	956	83.2	
Gender Newborn					
Males	327	52.5	603	52.5	0.99
Females	296	47.5	546	47.5	

BMI; Body mass index

Table S2. Associations (Odd Ratio and 95% Confidence Interval) between and blood metals ($\mu\text{g/L}$) by quartiles and gestational diabetes mellitus or impaired glucose tolerance.

Exposure	GDM (44) vs. Normal glucose (579)		IGT (63) vs. Normal glucose (560)		GDM + IGT (107) vs. Normal glucose (516)		
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	
Pb	Q1 (1-12)	Reference	Reference	Reference	Reference	Reference	
	Q2 (12-17)	0.87 (0.33-2.28)	0.70 (0.19-2.48)	1.13 (0.63-2.04)	1.08 (0.55-2.12)	0.98 (0.56-1.70)	1.28 (0.65-2.53)
	Q3 (17-22)	0.87 (0.30-2.48)	1.16 (0.34-3.96)	1.19 (0.63-2.25)	0.74 (0.36-1.52)	0.82 (0.44-1.51)	1.07 (0.52-2.18)
	Q4 (22-80)	2.30 (0.97-5.44) [†]	2.25 (0.75-6.77)	1.15 (0.61-2.14)	0.76 (0.38-1.50)	0.92 (0.52-1.64)	0.97 (0.47-2.01)
Cd	Q1 (0.1-0.5)	Reference	Reference	Reference	Reference	Reference	
	Q2 (0.5-0.8)	0.55 (0.20-1.55)	0.62 (0.18-2.09)	1.86 (1.00-3.48)*	1.47 (0.76-2.84)	1.38 (0.79-2.41)	1.93 (0.92-4.06) [†]
	Q3 (0.8-1.2)	0.88 (0.34-2.26)	1.44 (0.38-3.47)	1.57 (0.81-3.03)	1.39 (0.70-2.73)	1.12 (0.62-2.00)	1.92 (0.90-4.10) [†]
	Q4 (1.2-9.6)	1.88 (0.86-4.09)	2.01 (0.73-5.53)	2.17 (1.19-4.03)*	1.49 (0.76-2.92)	1.40 (0.81-2.42)	2.33 (1.10-4.92)*
Mn	Q1 (1-8)	Reference	Reference	Reference	Reference	Reference	
	Q2 (8-10)	0.75 (0.22-2.55)	0.65 (0.15-2.81)	1.73 (0.81-3.71)	1.53 (0.66-3.55)	1.43 (0.67-3.06)	1.68 (0.73-3.85)
	Q3 (10-12)	1.07 (0.39-2.93)	0.82 (0.25-2.65)	1.13 (0.56-2.25)	1.00 (0.47-2.13)	1.13 (0.57-2.24)	1.15 (0.54-2.44)
	Q4 (12-53)	1.69 (0.02-4.91)	1.08 (0.29-4.01)	1.42 (0.66-3.07)	1.16 (0.49-2.18)	1.04 (0.46-2.33)	1.16 (0.47-2.87)

Pb = Lead; Cd = Cadmium; Mn= Manganese; GDM = gestational diabetes mellitus; IGT = impaired glucose tolerance

Models were adjusted for maternal smoking, maternal age, maternal BMI, maternal education level, pregnancy induced hypertension, number of siblings and recruitment centre.

* P<0.05; † P<0.10

3.3 Paper 3: The association between maternal urinary phthalate concentrations and pregnancy induced hypertension: Results from the EDEN Mother-Child Cohort Study.

In our third paper, we show the results on relationships between maternal exposure to phthalates during pregnancy and pregnancy induced hypertension. We have submitted our paper in the journal of Environmental International. We present the details of this paper in the thesis.

The association between maternal urinary phthalate concentrations and pregnancy induced hypertension: Results from the EDEN Mother-Child Cohort Study.

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Running Title: Phthalate metabolites and pregnancy induced hypertension

Key words:

Phthalate, prenatal exposure, endocrine disruptor, urinary biomarkers, mother-child cohort, pregnancy induced hypertension

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Abbreviations:

BMI	Body Mass Index
BBzP	Benzylbutyl phthalate
CI	Confidence Interval
DEHP	di(2-ethylhexyl) phthalate
DBP	Di-n-butyl phthalate
DiBP	di-isobutyl phthalate
DiDP	di-isodecyl phthalate
DiNP	di-isononyl phthalate
HMW	high molecular weight
LOD	limit of detection
LMW	low molecular weight
MEP	Monoethyl phthalate
MBP	Mono-n-butyl phthalate
MiBP	Mono-isobutyl phthalate
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
MBzP	Monobenzyl phthalate
MCOP	Monocarboxy-isooctyl phthalate
MCPP	Mono (3-carboxypropyl) phthalate
MCNP	Monocarboxy-isononyl phthalate
OR	Odds Ratio
SD	Standard Deviation

Abstract

Introduction: Studies have suggested that exposure to endocrine disruptors like phthalates widely used in our daily life (wrapping foods, cosmetics, toys, medical devices, polyvinyl chloride flooring, and building materials) may be associated with elevated blood pressure and increased risk of cardiovascular diseases. Phthalates may cause a pro-inflammatory response and increased oxidative stress and which may be a cause of pregnancy induced hypertension.

Objectives: We evaluated the association between maternal exposure to phthalates during pregnancy and pregnancy induced hypertension.

Methods: 2,002 pregnant women were initially included in the French EDEN mother-child prospective birth cohort study. Eleven phthalates metabolites were quantified in spot maternal urine samples collected between 23rd and 28th week of gestation. The Association of each urinary phthalate metabolite and pregnancy induced hypertension was assessed with adjusted multiple logistic regression analysis.

Results: The study population consisted of 604 women with available data on both pregnancy induced hypertension diagnosis and urine analysis for phthalates. Low molecular weight phthalates metabolites; Monoethyl phthalate (MEP) and Mono-n-butyl phthalate (MBP) were positively associated with pregnancy induced hypertension in crude (Odds Ratio: 1.43 95% Confidence Interval: 1.04-1.96, p-value = 0.02 and 1.48, 1.10-2.01, p-value =0.01) and in adjusted (1.47, 1.01-2.14, p-value = 0.04 and 1.66, 1.11-2.47, p-value = 0.01) models respectively. We observed a borderline association with one of the high molecular weight phthalate metabolite; Mono (3-

carboxypropyl) phthalate (MCP) in crude (1.46, 0.96-2.24, p-value = 0.08) as well as in adjusted model (1.56, 0.93-2.64, p-value = 0.09).

Conclusion: We observed that prenatal exposure to some phthalates which include MEP and MBP, were associated with pregnancy induced hypertension.

Introduction

Studies have suggested that exposure to endocrine disruptors like phthalates widely used in our daily life (wrapping foods, personal care products, cosmetics, toys, medical devices, polyvinyl chloride flooring, and building materials) may be associated with elevated blood pressure and increased risk of cardiovascular diseases (Cantonwine et al. 2016; Trasande et al. 2013). Phthalates are classified into two main groups. Low molecular weight (LMW) phthalates are frequently added to cosmetic products, shampoos, lotions, and other personal care products to preserve scent. Whereas high molecular weight (HMW) phthalates are used as plasticizers of polyvinyl chloride (PVC) because of their flexibility and resistant kinking properties.

Phthalates being ubiquitous, present in the environment. Exposure to phthalates can occur through inhalation, ingestion (including dietary ingestion and incidental ingestion), dermal absorption (including air-to-skin transport), contact with contaminated surface and use of personal care products (Soomro et al. 2018). Phthalates are metabolized into monoester metabolites after exposure and conjugated before being excreted in the urine; hence urinary phthalate metabolites can be used as a biomarkers of exposure (Swan 2008). Studies have revealed that phthalates can also cross the fetoplacental barrier and enter the amniotic fluid and fetal circulation (Ferguson et al. 2015; Li et al. 2013; Swan 2008). Therefore, prenatal and early life are critical in the development of immune system; exposure to toxic pollutants during this period can result in an increased risk of adverse health outcomes during pregnancy as well later in life. However, the exact mechanism through which phthalate exposure may influence cardiovascular health effects is unknown. It is hypothesized that phthalates being endocrine disruptors, affect the metabolism and actions of

androgens, thyroid hormones and cortisol (Boas et al. 2012; Ye et al. 2014). Another mechanism which may cause a pro-inflammatory response, vascular dysfunction and increased oxidative stress (Mathieu-Denoncourt J et al. 2015; Rahmani et al. 2016).

A cross-sectional study in US children and adolescents aged 6-19 years suggested increased urinary phthalate concentrations of HMW phthalate metabolites were associated with higher blood pressure (Trasande et al. 2013). Whereas, another cross-sectional study from Sweden in older adults showed increased serum concentrations of monobenzyl phthalate (MBzP) were associated with several cardiovascular risk factors (Wiberg et al. 2014).

To our knowledge, this is the first prospective population based study. However, there is only one prospective birth cohort study from Cincinnati, USA investigated the relationship between phthalate and pregnancy induced hypertensive diseases (EF Werner et al. 2015).

We hypothesized that increased maternal urinary phthalate concentrations would be associated with increased maternal blood pressure and pregnancy induced hypertension. The aim of this study was to evaluate the association between maternal exposure to phthalates during pregnancy and pregnancy induced hypertension.

Methods

Study population and data collection

The population in this study is a subgroup of the French EDEN (*Etude des Déterminants pré et post natals du développement de la santé de l'Enfant*) mother-child prospective birth cohort (<https://eden.vjf.inserm.fr>). The mothers' recruitment and follow-up procedures, and methodological assumptions have been published in earlier

studies. Briefly, women were given an appointment with a study midwife between the 24th and 28th gestational weeks, during which an interview on life style factors was conducted and biological samples were collected. The information on the mothers and their newborns, including parity, mode of delivery, birth weight, gestational age, season of birth, behavioral and environmental data were collected by the use of questionnaires, interviews and from obstetric and pediatric records. The current study includes all maternal urine samples had been analyzed for metabolites of phthalates as a part of previous studies (Chevrier et al. 2012; Soomro et al. 2018).

Maternal blood pressure was measured during routine monthly visits, with the subject in supine position, using a standard mercury sphygmomanometer. One set of measures was taken by the study midwife between 24 and 28 weeks using a different (semi-automated) device, with two measurements averaged to determine the values for that visit.

Measurements taken within each period of gestational trimester (T1, T2, and T3) were averaged to determine the values for systolic blood pressure (SBP) and diastolic blood pressure (DBP) assigned to each period.

Women were classified as having pregnancy induced hypertension based on SBP \geq 140 mmHg and/or DBP \geq 90 mmHg measured during at least two visits after the 22nd week of gestation, such that the elevated measures could have occurred during or across any of the three periods, at any point after 22 weeks. Consequently, no diagnosis of pregnancy induced hypertension was made before 24 weeks. All cases of pregnancy induced hypertension were diagnosed by the physicians.

Urine collection and analysis

Women were invited for a clinical examination and to provide a first morning urine at home before the hospital study visit between the 24th and 28th gestational weeks (second trimester of the pregnancy). If forgotten, the urine sample was collected at the hospital. A polypropylene container (FP40VPS manufactured by Centre Européen de Biotechnologie SA (CEB) in Angers, France) was used. The urine samples were aliquoted and then frozen at -80° C. The analyses of the samples were conducted at the National Center for Environment Health Laboratory at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA) for eleven metabolites of eight phthalates: Diethyl phthalate (DEP), Di-n-butyl phthalate (DBP), Di-isobutyl phthalate (DiBP), Di(2-ethylhexyl) phthalate (DEHP), Benzylbutyl phthalate (BBzP), Di-isononyl phthalate (DiNP), Di-n-octyl phthalate (DOP), and Di-isodecyl phthalate (DiDP).

The targeted phthalate metabolites were monoethyl phthalate (MEP), mono-n-butyl phthalate (MBP), 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-isoctyl phthalate (MCOP), mono(3-carboxypropyl) phthalate (MCP) and monocarboxy-isononyl phthalate (MCNP).

Quantification of the phthalate metabolites concentrations was performed using online solid phase extraction high performance liquid chromatography electrospray ionization isotope dilution tandem mass spectrometry. Creatinine concentrations were also quantified following identical analytical procedure as described previously. (Mortamais et al. 2012) The concentrations of metabolites below the limit of detection (LOD) were replaced by instrumental reading values or by the compound specific lowest

instrumental reading value divided by the square root of two ($LOD/\sqrt{2}$) when the instrumental value found missing. The vast majority of the samples/metabolites (>97%) were above LOD. Two-step standardization was performed to reduce variability in biomarkers urinary concentrations owed to sampling conditions as described previously (Mortamais et al. 2012). There was no significant difference in the concentrations of phthalates in the urines collected at homes compared to those of the urines collected in the hospital.

Other Variables

Information on potential confounders including: gestational age (two-class variable: < 37 gestational week, \geq 37 gestational week), parity, number of siblings (0, 1, 2 \geq 3), maternal age at delivery (< 25 years, 25-29 years, 30 – 34 years, \geq 35 years), pre-pregnancy maternal body mass index (BMI, < 18.5, 18.5-24.9, 25.0-30.0, > 30 kg/m²), maternal educational level (< 2 years after high school, High school +2 years, \geq High school +3 years), household income, city of residence (Nancy / Poitiers), mode of delivery (vaginal, caesarean section), smoking during pregnancy (yes/no), maternal alcohol use during pregnancy (yes/no), maternal physician-diagnosis of pregnancy induced hypertension.

Statistical analysis

Urinary concentrations of phthalate metabolites were natural log (ln) transformed because of non-normality of the distribution. For the purpose of present analysis, MECPP, MEHHP, MEOHP and MEHP were grouped as sum of Σ DEHP (DEHP being their parent compound). We calculated the molar sum of these DEHP metabolites (nanomoles per milliliter) by dividing each metabolite concentration by its molecular weight and then summing the individual concentrations.

The prevalence of pregnancy induced hypertension was computed. We applied multiple logistic regression analysis to examine the association between each phthalate metabolite and Σ DEHP and pregnancy induced hypertension outcomes adjusted for potential confounders. The potential confounders were determined *a priori* from literature review. The confounders included in our final model were maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, and number of siblings. Models were additionally adjusted for recruitment centre. All the statistical analysis was conducted using Stata statistical software 14 (Stata Corp LP, 4905 Lakeway Drive, College Station, Texas 77845, USA) where *p-value* < 0.05 was considered statistically significant.

Ethical Approval

The study was approved by the relevant ethical committees (Comité Consultatif pour la Protection des Personnes dans la Recherche Biomédicale, Le Kremlin Bicêtre University Hospital, and Commission Nationale de l'Informatique et des Libertés), and all participating women gave informed written consent for their own participation and both parents for newborn child after delivery.

Results

Characteristics of study population

Of 2002 women recruited into the EDEN mother child cohort, 604 women with available data on both pregnancy induced hypertension diagnosis and urine analysis for phthalates were included in this study (Figure 1). The majority (98.5%) of these women were educated, 43.5% had less than two years of education after high school while 54.8% had more than that and between 25-34 years of age (29.86 ± 4.8) (Table 1). The average BMI was 23.1 ± 4.5 , while 26.4% were overweight or obese (BMI ≥ 25 kg/m²).

While 13.7% were active smoker and 25% declared some alcohol intake during pregnancy. Four percent had premature newborns, 15.7% had caesarean section at delivery.

The prevalence of pregnancy induced hypertension was found 4.80%. The participants included in this study did not differ in major demographic characteristics except for maternal age, smoking and gestational age (Table S1). However, results did not change when adjusted for them in the final models.

Prenatal exposure to phthalates

Phthalate metabolites were detected in the urine of more than 97% of the mothers. MEP had the highest geometric mean (110.7 $\mu\text{g/L}$), while MCNP had the lowest (1.5 $\mu\text{g/L}$). The limit of detection (LOD) ranged from 0.2 to 0.6 $\mu\text{g/L}$. A summary of detection frequency, as well as geometric means and select percentiles of urinary concentrations of phthalate metabolites are outlined in Table 2.

Spearman correlation coefficients between ln-transformed phthalate metabolites urinary concentrations are shown in Table S2. The majority of biomarkers were positively correlated, except for the correlation between MEP and MiBP, MECPP, MEHHP, MCOP, MCP, or MCNP. All the other biomarkers were significantly correlated with each other (with correlation coefficients ranging from $r = 0.08$, $p < 0.05$ to $r = 0.97$, $p < 0.05$). We also observed that DEHP metabolites were highly correlated with each other ($r \geq 0.77$, $p < 0.05$).

Associations between phthalate metabolites and pregnancy induced hypertension

Low molecular weight phthalates metabolites; MEP and MBP were positively associated with pregnancy induced hypertension in crude (OR: 1.43 95% CI: 1.04-1.96, p-value = 0.02 and OR: 1.48 95% CI: 1.10-2.01, p-value =0.01) and in adjusted (OR: 1.47, 95% CI: 1.01-2.14, p-value = 0.04 and OR: 1.66, 95% CI: 1.11-2.47, p-value = 0.01) models respectively as shown in Table 3. We observed a borderline association with one of the high molecular weight phthalate metabolite; Mono (3-carboxypropyl) phthalate in crude (OR: 1.46, 95% CI: 0.96-2.24, p-value = 0.08) as well as in adjusted model (OR: 1.56, 95% CI: 0.93-2.64, p-value = 0.09). We do not observed associations with other high molecular weight phthalate metabolites with pregnancy induced hypertension.

While taking into account the quartiles, we do not observed association between MEP and pregnancy induced hypertension. However, a significant increased odd ratio for MBP metabolite were observed in the 4th quartile in crude (OR: 3.46, CI: 1.10-10.87, P-value = 0.03) as well as in adjusted for confounders (OR: 5.91 CI: 0.93-37.79, P-value = 0.06) as shown in Table 4. A borderline association for Σ DEHP (OR: 5.45, CI: 0.85-34.84, P-value = 0.07) was observed in the second quartile.

Discussion

In this study, we report an association between maternal prenatal urinary concentration of low molecular weight phthalate metabolites including MEP and MBP and pregnancy induced hypertension. MEP is metabolite of Diethyl phthalate (DEP) used in plasticizer, automotive, cleaning and furnishing products, adhesive, sealants and in cosmetic

products. While MBP is metabolite of Di-n-butyl phthalate (DBP), which is used in diverse range of products such as PVC, paints, printing inks, adhesives, aerosols and cosmetics products: hair spray, a perfume solvent and fixative, skin emollient and a plasticizer in nail polish and finger nail.

Comparison of findings with other published work

To our knowledge, this is the first study to longitudinally assess the phthalate concentrations in relation to pregnancy induced hypertension. However, a prospective birth cohort study from Cincinnati, USA investigated the relationship between phthalate and pregnancy induced hypertensive diseases which includes gestational hypertension, preeclampsia, eclampsia and HELLP syndrome (EF Werner et al. 2015). This study suggested that maternal urinary MBzP concentrations may be associated with increased diastolic blood pressure and risk of pregnancy induced hypertensive diseases. However with did not observed significant association with pregnancy induced hypertension and MBzP phthalate metabolite in our study except a partial significance in the 4th quartile (OR: 2.75, CI: 0.95-7.91, p-value = 0.06). While another study from Boston, USA, investigated the relationship between Bisphenol A and phthalate metabolites and the risk of preeclampsia (Cantonwine et al. 2016). Found a significant association between preeclampsia and MEP and DEHP. We found a significant association between pregnancy induced hypertension and MEP (OR: 1.47, CI: 1.01-2.14, p-value = 0.04) or MBP (OR: 1.66, CI: 1.11-2.47, p-value = 0.01) and a borderline significance in MCP (OR: 1.56, CI: 0.93-2.64, p-value = 0.09).

Other studies on phthalate exposure and blood pressure in children have also shown significant associations (Trasande et al. 2013; Trasande and Attina 2015; Valvi et al. 2015). However, studies from USA (Trasande et al. 2013; Trasande and Attina 2015)

were cross-sectional analysis of a subsample, found increased urinary concentrations of MEP and DEHP were associated with increased blood pressure in children age 6-19 years. Whereas, study from Spain (Valvi et al. 2015) was a population based birth cohort observed that sum of HMW phthalates as well as sum of LMW phthalates were significantly related with lower systolic blood pressure in girls but not in boys at the age of 4 and 7 years. A prospective study on elderly adults from Uppsala, Sweden shown inverse associations between MEP and systolic and diastolic blood pressure (Olsen et al. 2012). Additionally, a cross-sectional study of older adults found increased serum concentrations of MBzP were related to several cardiovascular risk factors (Wiberg et al. 2014).

Potential mechanisms

The exact mechanism is still unclear, however it is likely to be multifactorial in nature and depend on diet, living style, genetic and environmental factors. Since the nutritional imbalance or environmental chemical exposures during pregnancy and development can increase the risk of disease and their effects may be share some common pathways (Barouki et al. 2012). Indeed, hormones, cytokines and nutritional components can both directly activate receptors that stimulate gene expression and also activate or inhibit the enzymes and pathways that are responsible for DNA methylation, chromatin re-modelling and non-coding RNAs which ultimately control gene expression. Thus, epigenetic regulatory pathways are likely sites for effects of both nutrient and environmental toxicant effects during development and potentially also across the lifespan (Barouki et al. 2012).

Epidemiological evidence indicates that exposure to phthalates during in utero is associated with increased blood pressure in childhood (Trasande et al. 2013). Studies

have revealed that the risk of pregnancy induced hypertension may increase through oxidative stress, up-regulation of inflammatory markers (tumor necrosis factor alpha and interleukin 6), and inhibition of peroxisome proliferator-activated receptor gamma (Rahmani et al. 2016; Sutherland et al. 2014; Usuda and Kanda 2014). The possible mechanism is may be the phthalates mediated deregulation of blood pressure is reduced endothelium-derived nitric oxide production, which is mainly produced by endothelial nitric oxide synthase, one of the key vessel relation factor in regulation of vascular tone (Lee et al. 2016). In addition to the transcriptional regulation, endothelial nitric oxide synthase activity can be modulated by kinase-dependent phosphorylation at various sites.

Strengths and limitations

This study has several strengths and limitations. The study design of our study is a nested cross-sectional in a population based prospective cohort, which has nutritional, environmental, biological and social factors data starting during the prenatal life are a strength of our study, which allowed us to control for potential confounders and to assess exposure before the health outcomes. A nested cross-sectional cohort design offers advantages over the traditional cross-sectional designs. The diagnosis of pregnancy induced hypertension was conducted by physicians, which ensures the quality and we further adjusted for potential confounders to see the associations. The values of the exposure biomarkers were standardized for urine sampling conditions to reduce subject variability (Mortamais et al. 2012). However, we were limited to one urine sample per mother during the second trimester of pregnancy, thus we were unable to assess the variability in concentrations of each metabolite across the pregnancy. But it is also not clearly known at what point(s) in gestation the mothers are having higher risk of exposures. Consistency in urinary concentrations during

pregnancy has been observed for some environmental exposure biomarkers and other studies have suggested that phthalate biomarkers are relatively stable for a period of weeks to months (Hauser et al. 2004; Teitelbaum et al. 2008). Other studies have also used second trimester maternal urinary concentration for biomarkers (Gascon et al. 2015; Whyatt et al. 2014). However, increasing the number of urine samples collected would have provided us a more accurate estimate of the average exposure during the gestation period. Finally, we also adjusted for many potential confounders; however residual confounding (if any) cannot be discarded.

Creatinine correction is most often used for urinary biomarkers of phthalates, phenols, and pesticides. There are limitations to the usage of creatinine to normalize for urine dilution; different investigators have used specific gravity as an alternative of creatinine to adjust phthalate urinary biomarkers for urine dilution (AC Just et al. 2012; Whyatt et al. 2014), but we did not have specific gravity measurements. Nevertheless, specific gravity is extremely correlated with creatinine (Barr et al. 2005) and consequently it is not doubtless that we overcorrected for urine dilution. Regarding exposure assessment, it might be important to explore other techniques for assessment of the exposure, particularly those that may offer a more exhaustive and coordinated picture of the individual environment, for instance by assessing particular items use in conjunction with indoor air levels and additionally biomarkers of exposure. Another limitation of our study is that this cohort was conducted in two cities which differ in many respect and which may not represent general French population. However, in the models we adjusted for centre to take into account regional specificities.

The exposures we studied are relatively prevalent, and some biomarker urinary concentrations approach those with significant effects in experimental models. In a

healthy cohort such as ours, effects of hormonally active environmental exposures may be small, yet a continuous exposure can affect adversely.

Conclusion

We observed that prenatal exposure to some phthalates which include MEP and MBP, were associated with pregnancy induced hypertension. Our data provide new suggestive evidence that prenatal exposure to certain phthalates may play a role in the development of pregnancy induced hypertension.

Table 1. Characteristics of the study population and by centre.

Characteristics	All n=604		Poitiers n= 347		Nancy n= 257		P value
	Mean \pm SD		No.	%	No.	%	
	No.	%					
Maternal age (years)	29.86 \pm 4.8						
<25	79	13.0	48	13.8	31	12.0	0.8
25 – 29	218	36.1	123	35.5	95	37.0	
30 – 34	205	34.0	120	34.6	85	33.1	
\geq 35	102	16.9	56	16.1	46	17.9	
Parity							
0	269	44.5	174	50.1	95	37.0	0.005
1	222	36.8	117	33.7	110	42.8	
\geq 2	113	18.7	56	16.2	52	20.2	
BMI (kg/m²)	23.1 \pm 4.5						
<18.5	57	9.4	27	7.9	30	11.7	0.4
18.5 – 24.9	379	62.9	217	62.5	162	63.0	
25 – 30	110	18.3	68	19.6	42	16.3	
>30	49	8.1	30	8.6	19	7.4	
Missing	9	1.5	5	1.4	4	1.6	
Maternal education							
<2 years after high school	263	43.5	172	49.6	91	35.4	0.004
High school +2 years	132	21.9	72	20.7	60	23.3	
\geq High school +3 years	199	32.9	98	28.3	101	39.4	
Missing	10	1.7	5	1.4	5	1.9	
Active smoking (cig/day)							
0	519	86.0	295	85.0	224	87.2	0.6
1 to 5	60	9.9	39	11.2	21	8.1	
>5	23	3.8	12	3.5	11	4.3	
Missing	2	0.3	1	0.3	1	0.4	
Passive smoking							
Yes	192	31.8	127	36.6	65	25.3	0.01
No	405	67.1	216	62.3	189	73.5	
Missing	7	1.1	4	1.1	3	1.2	
Alcohol during pregnancy							
Yes	151	25.0	72	20.8	79	30.7	0.01
No	450	74.5	274	78.9	176	68.5	
Missing	3	0.5	1	0.3	2	0.8	
Gestational age	39.4 \pm 1.4						
<37 gestational week	24	4.0	13	3.8	11	4.3	0.7
\geq 37 gestational week	580	96.0	334	96.2	246	95.7	
Employment							
Yes	458	75.9	256	73.8	202	78.6	0.3
No	142	23.5	89	25.6	53	20.6	
Missing	4	0.6	2	0.6	2	0.8	
Caesarean section							
Yes	95	15.7	47	13.6	48	18.7	0.08
No	509	84.3	300	86.4	209	81.3	
Season of urine sample collection							
Winter	189	31.3	101	29.1	88	34.2	0.12

Spring	146	24.2	89	25.6	57	22.2	
Summer	129	21.3	83	23.9	46	17.9	
Autumn	140	23.2	74	21.4	66	25.7	
Time of sampling							
Midnight – 7:59	310	51.3	128	36.9	183	71.2	
8:00 - 11:59	149	24.7	76	21.9	72	28.0	0.00001
12:00 - 19:00	5	0.8	4	1.1	1	0.4	
Missing	140	23.2	139	40.1	1	0.4	

BMI; Body mass index

Figure 1: Flow chart of the study.

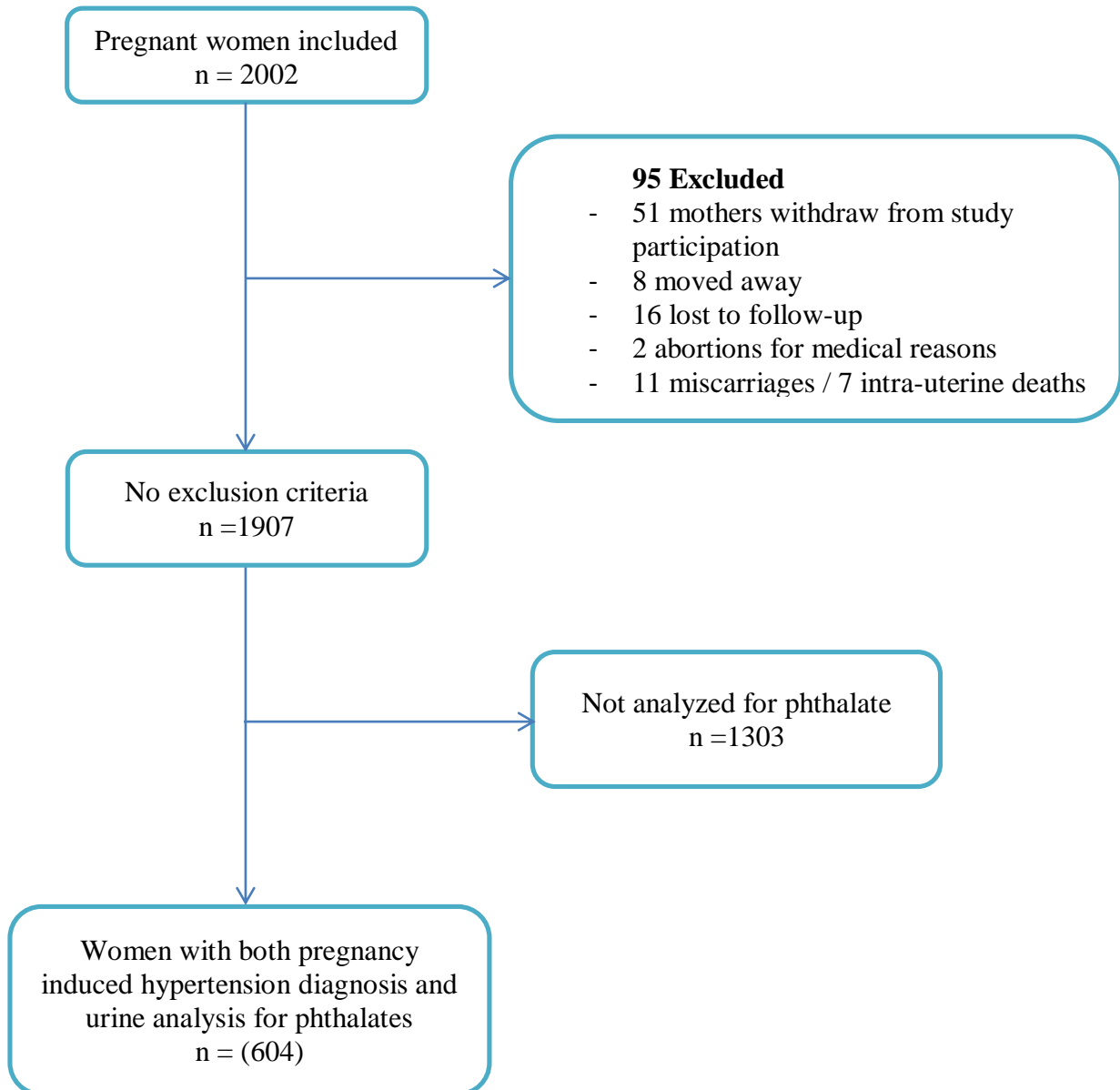


Table 2. Distribution of phthalate metabolites concentration ($\mu\text{g/L}$) in maternal prenatal urine ($n = 604$).

Parent compound	Abbrev.	Phthalate metabolites	Abbrev.	LOD ($\mu\text{g/L}$)	%>LOD	Geometric mean (GM)	Minimum	Percentile					Maximum
								5th	25th	50th	75th	95th	
Diethyl phthalate	(DEP)	Monoethyl phthalate ($\mu\text{g/L}$)	MEP	0.6	100	110.7	7.9	21	51	96	195	714	4093.4
Di-n-butyl phthalate	(DBP)	Mono-n-butyl phthalate ($\mu\text{g/L}$)	MBP	0.2	100	51.6	2.8	12	28	43	73	438	2418.8
Di-isobutyl phthalate	(DiBP)	Mono-isobutyl phthalate ($\mu\text{g/L}$)	MiBP	0.2	100	42.7	1.5	12	25	39	69	166	689.9
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethyl-5-carboxypentyl) phthalate ($\mu\text{g/L}$)	MECPP	0.2	100	40.5	2.6	12	25	38	62	160	2640.5
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethyl-5-hydroxyhexyl) phthalate ($\mu\text{g/L}$)	MEHHP	0.2	100	26.9	1.7	6.8	16	27	44	103	2103.8
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethyl-5-oxohexyl) phthalate ($\mu\text{g/L}$)	MEOHP	0.2	99.7	22.1	0.6	5.6	14	23	36	84	1441.4
Di(2-ethylhexyl) phthalate	(DEHP)	Mono(2-ethylhexyl) phthalate ($\mu\text{g/L}$)	MEHP	0.5	98	7.2	0.1	1.6	4.4	7.7	15	35	292.3
Benzylbutyl phthalate	(BBzP)	Monobenzyl phthalate ($\mu\text{g/L}$)	MBzP	0.3	100	19.7	1.6	4.5	11	18	33	104	970.4
Di-isononyl phthalate	(DiNP)	Monocarboxy-isoocetyl phthalate ($\mu\text{g/L}$)	MCOP	0.2	99	4.1	0.1	1.2	2.4	3.9	6.5	19	375.3
Di-n-octyl phthalate	(DOP)	Mono(3-carboxypropyl) phthalate ($\mu\text{g/L}$)	MCPP	0.2	100	2.1	0.1	0.7	1.3	1.9	3.4	9.1	78.7
Di-isodecyl phthalate	(DiDP)	Monocarboxy-isononyl phthalate ($\mu\text{g/L}$)	MCNP	0.2	97.5	1.5	0.1	0.5	0.8	1.2	2.2	9.5	686.8

Table 3. Association (Odd Ratio and 95% CI) between maternal urinary concentrations of phthalate metabolites and pregnancy induced hypertension.

Phthalate metabolite	Unadjusted			Adjusted ^a		
	OR	95% CI	P-Value	OR	95% CI	P-Value
MEP	1.43	1.04-1.96*	0.02	1.47	1.01-2.14*	0.04
MBP	1.48	1.10-2.01*	0.01	1.66	1.11-2.47*	0.01
MiBP	1.27	0.81-1.99	0.28	1.22	0.69-2.16	0.49
MECPP	1.26	0.82-1.95	0.29	1.22	0.68-2.21	0.49
MEHHP	1.21	0.79-1.83	0.37	1.16	0.67-1.99	0.59
MEOHP	1.19	0.78-1.83	0.41	1.12	0.64-1.95	0.69
MEHP	0.94	0.66-1.33	0.72	0.84	0.54-1.34	0.48
MBzP	1.31	0.91-1.89	0.15	1.08	0.66-1.78	0.75
MCOP	0.81	0.51-1.29	0.38	0.99	0.58-1.69	0.98
MCP	1.46	0.96-2.24[†]	0.08	1.56	0.93-2.64[†]	0.09
MCNP	1.05	0.72-1.52	0.81	1.24	0.80-1.92	0.33
ΣDEHP	1.03	0.93-1.15	0.56	1.01	0.88-1.17	0.85

^aOR adjusted for maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, number of siblings, and recruitment centre

MEP = Monoethyl phthalate; MBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; 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; MBzP = Monobenzyl phthalate; MCOP = Monocarboxy-isoocetyl phthalate; MCP = Mono (3-carboxypropyl) phthalate; MCNP = Monocarboxy-isononyl phthalate; DEHP = di(2-ethylhexyl) phthalate.

*P<0.05

[†]P<0.10

Table 4. Association (Odd Ratio and 95% CI) between maternal urinary concentrations of phthalate metabolites and pregnancy induced hypertension by quartiles.

Phthalate metabolites		Quartiles			
		Q1 (Reference)	Q2 OR (95% CI) P-value	Q3 OR (95% CI) P-value	Q4 OR (95% CI) P-value
MEP	Crude	1	1.63 (0.52-5.11) 0.39	0.98 (0.28-3.52) 0.99	2.29 (0.78-6.77) 0.13
	Adjusted	1	0.99 (0.19-5.12) 0.98	0.63 (0.09-4.24) 0.63	1.24 (0.26-5.92) 0.78
MBP	Crude	1	1.25 (0.33-4.78) 0.73	1.78 (0.51-6.23) 0.36	3.46 (1.10-10.87) 0.03*
	Adjusted	1	1.78 (0.25-12.77) 0.56	2.76 (0.37-20.40) 0.31	5.91 (0.93-37.79) 0.06†
MiBP	Crude	1	2.75 (0.72-10.61) 0.14	3.49 (0.94-12.97) 0.06†	2.75 (0.72-10.61) 0.14
	Adjusted	1	0.46 (0.07-3.08) 0.42	2.43 (0.49-11.98) 0.27	1.92 (0.35-10.46) 0.44
MECPP	Crude	1	1.41 (0.44-4.57) 0.55	2.07 (0.69-6.21) 0.19	1.41 (0.44-4.57) 0.55
	Adjusted	1	7.90 (0.83-75.43) 0.07†	6.09 (0.62-59.70) 0.12	6.47 (0.66-63.11) 0.11
MEHHP	Crude	1	1.85 (0.60-5.65) 0.28	1.85 (0.60-5.65) 0.28	1.20 (0.36-4.04) 0.75
	Adjusted	1	2.95 (0.51-17.06) 0.22	2.46 (0.40-15.14) 0.33	2.29 (0.33-15.91) 0.39
MEOHP	Crude	1	4.25 (1.17-15.41) 0.02*	2.75 (0.71-10.61) 0.14	2.04 (0.50-8.31) 0.31
	Adjusted	1	16.08 (1.63-158.12) 0.01*	3.33 (0.26-42.47) 0.35	5.72 (0.52-62.95) 0.15
MEHP	Crude	1	0.58 (0.21-1.65) 0.30	0.79 (0.30-2.05) 0.62	0.48 (0.16-1.44) 0.19
	Adjusted	1	0.40 (0.07-2.22) 0.29	0.84 (0.19-3.62) 0.81	0.52 (0.09-3.08) 0.47
MBzP	Crude	1	1.42 (0.44-4.57) 0.55	0.79 (0.21-3.01) 0.73	2.75 (0.95-7.91) 0.06†
	Adjusted	1	0.81 (0.15-4.21) 0.80	0.50 (0.08-3.11) 0.46	1.79 (0.39-8.25) 0.45
MCOP	Crude	1	0.99 (0.36-2.74) 0.99	0.86 (0.31-2.45) 0.79	0.74 (0.25-2.18) 0.58
	Adjusted	1	3.99 (0.70-22.68) 0.11	3.41 (0.49-23.49) 0.21	2.42 (0.36-16.17) 0.35
MCPP	Crude	1	1.41 (0.44-4.57) 0.55	1.41 (0.44-4.57) 0.55	2.07 (0.69-6.21) 0.19
	Adjusted	1	1.89 (0.32-10.71) 0.48	0.95 (0.13-6.53) 0.95	2.49 (0.54-11.52) 0.24
MCNP	Crude	1	0.99 (0.31-3.17) 0.98	1.17 (0.38-3.58) 0.77	1.71 (0.61-4.84) 0.31
	Adjusted	1	1.11 (0.19-6.51) 0.90	1.30 (0.24-6.92) 0.75	1.71 (0.32-9.11) 0.52
ΣDEHP	Crude	1	1.63 (0.52-5.11) 0.39	2.07 (0.69-6.21) 0.19	1.21 (0.36-4.04) 0.75
	Adjusted	1	5.45 (0.85-34.84) 0.07†	3.39 (0.52-22.15) 0.20	3.18 (0.45-22.41) 0.24

OR adjusted for maternal smoking, maternal age, maternal BMI, maternal education level, gestational age, number of siblings, and recruitment centre

MEP = Monoethyl phthalate; MBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; 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; MBzP = Monobenzyl phthalate; MCOP = Monocarboxy-isooctyl phthalate; MCPP = Mono (3-carboxypropyl) phthalate; MCNP = Monocarboxy-isononyl phthalate; DEHP = di(2-ethylhexyl) phthalate.

*P<0.05

†P<0.10

Supplementary Material:

The association between maternal urinary phthalate concentrations and pregnancy induced hypertension: Results from the EDEN mother-child cohort study.

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Table S1. Characteristics of the study population included and excluded in the analysis.

Table S2. Spearman correlation between ln-transformed standardized urinary concentrations of phthalate metabolites.

Table S1. Characteristics of the study population included and excluded in the analysis.

Characteristics	Sample of analysis n=604		Samples Excluded for analysis n=1303		P value
	Mean ± SD		Mean ± SD		
	No.	%	No.	%	
Maternal age (years)	29.8±4.8		29.5±4.7		
<25	79	13.0	190	14.6	0.04
25 – 29	218	36.1	412	31.6	
30 – 34	205	34.0	421	32.3	
≥35	102	16.9	280	21.5	
Parity					
0	269	44.5	590	45.3	0.9
1	222	36.8	474	36.4	
≥2	113	18.7	239	18.3	
BMI (kg/m²)	23.1±4.5		23.3±4.7		
<18.5	57	9.4	138	10.6	0.9
18.5 - 24.9	379	62.9	804	61.7	
25 – 30	110	18.3	228	17.5	
>30	49	8.1	110	8.4	
Missing	9	1.5	23	1.8	
Maternal education					
<2 years after high school	263	43.5	641	49.2	0.11
High school +2 years	132	21.9	266	20.4	
≥ High school +3 years	199	32.9	371	28.5	
Missing	10	1.7	25	1.9	
Active smoking (cig/day)					
0	519	86.0	1049	80.5	0.02
1 to 5	60	9.9	167	12.8	
>5	23	3.8	71	5.5	
Missing	2	0.3	16	1.2	
Passive smoking					
Yes	192	31.8	562	43.1	0.00001
No	405	67.1	707	54.3	
Missing	7	1.1	34	2.6	
Alcohol during pregnancy					
Yes	151	25.0	337	25.9	0.04
No	450	74.5	940	72.2	
Missing	3	0.5	26	1.9	
Gestational age	39.4±1.4		39.1±1.8		
<37 gestational week	24	4.0	82	6.3	0.04
≥37 gestational week	580	96.0	1221	93.7	
Employment					
Yes	458	75.9	929	71.3	0.02
No	142	23.5	347	26.6	
Missing	4	0.6	27	2.1	
Caesarean section					
Yes	95	15.7	187	14.4	0.4
No	509	84.3	1116	85.6	

BMI; Body mass index

Table S2. Spearman correlation between ln-transformed standardized[‡] urinary concentrations of phthalate metabolites.

	MBP	MiBP	MECPP	MEHHP	MEOHP	MEHP	MBzP	MCOP	M CPP	MCNP
MEP	0.13*	0.03	0.04	0.06	0.08*	0.08*	0.10*	0.07	0.02	0.01
MBP		0.37*	0.26*	0.31*	0.32*	0.24*	0.40*	0.13*	0.62*	0.13*
MiBP			0.26*	0.29*	0.30*	0.27*	0.38*	0.20*	0.24*	0.11*
MECPP				0.90*	0.92*	0.77*	0.31*	0.36*	0.31*	0.27*
MEHHP					0.97*	0.83*	0.36*	0.33*	0.35*	0.25*
MEOHP						0.84*	0.38*	0.32*	0.36*	0.24*
MEHP							0.31*	0.25*	0.27*	0.19*
MBzP								0.19*	0.28*	0.17*
MCOP									0.28*	0.42*
M CPP										0.33*

MEP = Monoethyl phthalate; MBP = Mono-n-butyl phthalate; MiBP = Mono-isobutyl phthalate; 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; MBzP = Monobenzyl phthalate; MCOP = Monocarboxy-isoctyl phthalate; M CPP = Mono(3-carboxypropyl) phthalate; MCNP = Monocarboxy-isononyl phthalate

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

* P<0.05

4. DISCUSSIONS

In this discussion, we first summarize the main results reported in our papers and compare them with previous research studies. Then we discuss the strengths and limitations of this PhD work. Finally, we present our conclusions based on this work.

4.1 Exposure to phthalates and the development of eczema in children

In our first paper, we reported an association between maternal prenatal urinary concentration of metabolites of DiBP and DiNP and eczema development in offspring preschool boys followed-up between birth and 5 years of age. We observed that the metabolites of DiBP and DiNP were significantly associated with both phenotypes of eczema i.e., early onset and late onset. Our study also shows a significant association between prenatal exposure to various phthalates and allergic eczema in boys at the age of 5 years based on IgE levels. The metabolites of DBP, DiBP and DEHP were significantly associated with ever eczema in IgE sensitized boys and borderline significance was associated with MBzP, the major BBzP metabolite.

The table 3 shows epidemiological studies on relationships between exposure to phthalates and development of eczema in children.

Table 3. Epidemiological studies on relationships between exposure to phthalates and development of eczema in children.

Reference	Study design	Study population	Exposure	Results
(Shi et al. 2018)	Cross sectional	434 children, age 5-10 years	MMP, MEP, MiBP, MnBP, MECPP, MEHHP, MEOHP, MEHP, MBzP	significant associations for MEHHP, MEOHP and MBzP
(Berger et al. 2018)	Birth cohort	392 children, age 7 years	MEP, MnBP, MiBP	Non-significant associations
(Gascon et al. 2015)	Birth cohort	437 children at 6 months, 421 at 14 months, 385 at 4 years, 361 at 7 years and atopy at age 4years in 175 children	MBzP, MEP, MiBP, MnBP and DEHP including its metabolites; MEHP, MEHHP, MEOHP, MECPP.	Non-significant associations
(Smit et al. 2015)	Birth cohort	1024 children, age 5-9 years	DEHP and DiNP metabolites	AOR (95% CI) DiNP Current eczema, 0.71 (0.52-0.96) ; DiNP ever eczema, 0.90 (0.74-1.09)
(Stelmach et al. 2015)	Birth cohort	147 children, age 2 years	DEP, DiBP, DnBP, BBzP, DEHP, DiNP, DOP	Non-significant association for eczema, but significant association for food allergy

				AOR (95% CI) 4.17 (1.17-17.89)
(Callesen et al. 2014)	Case-control	269 controls and 171 cases, age 3-5 years	MEP, MnBP, MiBP, MBzP, MEHP, MEHHP, MEOHP, MECPP	Q4 vs. Q1, AOR (95% CI) MEP, 2.27 (1.12 – 4.62)
(Ait Bamai et al. 2014)	Cross-sectional	122 children, age 2 years	DMP, DEP, DiBP, DnBP, BBzP, DEHP, DiNP, DEHA	AOR (95% CI) DiBP, medium 11.95 (1.37-104.00), high 15.03 (1.91-117.99) AOR (95% CI) BBzP, medium 4.02 (1.01-16.03), high 6.55 (1.70-25.29)
(A Just et al. 2012)	Birth cohort	407 children, age 2, 3 and 5 years	MBzP	Relative risk, (95% CI) 1.52 (1.21-1.91)
(Bornehag et al. 2004b)	Nested case-control	198 cases and 202 controls, age 3 – 8 years	DEP, DIBP, DnBP, BBzP, DEHP, DINP	Q4 vs. Q1, AOR (95% CI) BBzP, 2.56 (1.24 – 5.32)

Other epidemiological studies from china (Shi et al. 2018), Japan (Ait Bamai et al. 2014; Ait Bamai et al. 2016), Sweden (Bornehag et al. 2004b), USA (A Just et al. 2012), Taiwan (Hsu et al. 2012), Korea (Kim et al. 2017), Greenland and Ukraine (Smit et al. 2015), and Poland (Stelmach et al. 2015) have reported a potential relationship between phthalates exposure and eczema related symptoms in children. However, most of these studies used a case-control (Bornehag et al. 2004a; Bornehag et al. 2004b; Bornehag et al. 2005; Bornehag et al. 2006) or cross sectional (Ait Bamai et al. 2014; Hoppin et al. 2013; Hsu et al. 2012) design or relied on phthalate levels in dust as a marker of phthalate exposure (Ait Bamai et al. 2014; Bornehag et al. 2005; Kolarik et al. 2008; Langer et al. 2014).

A recent cross-sectional study of 434 children aged 5-10 years of age from Shanghai, China, morning urine samples were collected from children, and found a significant associations for MEHHP, MEOHP and MBzP (Shi et al. 2018). However, two other recent studies from Salinas Valley, California (Berger et al. 2018; Berger et al. 2019) did not observed an associations between prenatal phthalate exposure and eczema in 7 years old children. In a birth cohort study from New York City, MBzP, the major BBzP metabolite, was associated with the development of eczema by 5 years of age in 407 children (A Just et al. 2012). In a Swedish study, an association between BBzP levels in dust and atopic dermatitis was observed in cross-sectional data (Bornehag et al. 2004a). While in a birth cohort from Greenland and Ukraine with 1,024 mother-child pairs, oxidative metabolites of DiNP in maternal serum were negatively associated with current eczema as well as with ever eczema in boys and girls from Greenland (Smit et al. 2015). A Danish study in children between 3 and 5 years of age

found a relationship between atopic dermatitis and MEP in urine, but only in the fourth quartile, and no association with MiBP, a DiBP metabolite, while other metabolites of DiNP were not investigated (Callesen et al. 2014).

A Japanese study (Ait Bamai et al. 2016) found the daily phthalate intakes estimated from urinary metabolites were higher in children than their parents. For infants and toddlers, one of the most important phthalate intake sources is floor dust. Young children ingest up to 10 times more house dust than adults, and are therefore more vulnerable to exposure to phthalates because of their hand-to-mouth behavior on the flooring. House dust is also well known to be a main exposure source to allergens and other EDCs for infants. Similar to indoor air concentrations, levels of phthalates in settled dust have been associated with PVC building materials (Ait Bamai et al. 2016; Bornehag et al. 2005). Similarly, a Taiwanese study (Hsu et al. 2012) among 101 children between 3 and 9 years of age, BBzP levels in house dust were associated with eczema and asthma examined the home environment along with bio-monitoring data. They also noted that exposure to indoor dust-borne BBzP, DBP, and the metabolites MBP and MEHP in urine from children were associated with increased risk of having eczema, asthma and related symptoms.

4.2 Health effects of gestational exposure to selected heavy metals

In our second paper, we observed significant associations between maternal concentrations of heavy metals during the pregnancy with GDM or IGT and their combination indicating that the environment the mother encounters during the pregnancy can endanger her health status. More precisely, we found that, after taking into account for potential confounders, Cd was related to both GDM alone and the combination of GDM and IGT and Pb was partially related to GDM.

Cd is a toxic heavy metal distributed widely in the environment (Barregard et al. 2013). The principal source of Cd exposure for the general non-smoking population is food (Swaddiwudhipong et al. 2012). Occupational exposure limits to Cd have been lowered 50-100 fold in the last few decades as the toxic effects of Cd were better understood (Wallace et al. 2015). Three recent studies from China (Liu et al. 2018; Peng S et al. 2015; Xing et al. 2018) showed significant association between urine Cd or meconium Cd and GDM. A USA case-cohort study (Romano et al. 2015) showed that women in highest tertile of urinary Cd had two times the risk of GDM as compared with those in the lowest tertile. However, the Canadian birth cohort study did not observed significant results to confirm this finding with Cd measured in blood (Shapiro et al. 2015).

Table 4 shows epidemiological studies on relationships between exposure to Cd and gestational diabetes mellitus.

Table 4. Epidemiological studies on relationships between exposure to Cd, Pb, Mn and gestational diabetes mellitus.

Reference	Study design	Study population	Exposure	Results
(Oguri et al. 2019)	Cross-sectional	16,955 pregnant women	Cd, Pb	No significant association
(Liu et al. 2018)	Birth cohort	2026 pregnant women	Cd	The risk ratios (RRs) of GDM for the middle tertile: 1.04 (95% CI: 0.74, 1.44), and 1.36 (95% CI: 0.98, 1.90) for the top tertile.
(Xing et al. 2018)	Birth cohort	6837 pregnant women	Cd	The relative risk (RR) 1.16; (95% CI: 1.03, 1.33) increase in risk of GDM
(Shapiro et al. 2015)	Birth Cohort	1274	Pb, Cd, As, Hg	No significant association with Cd or Pb
(Peng S et al. 2015)	Case-control	1359	Pb, Cd, As, Hg, Cr	Cd, AOR 16.87 (4.19-67.86) in 3 rd quartile and AOR 11.95 (2.97-48.04) in 4 th quartile Pb, a negative association in 2 nd and 3 rd quartile 0.37 (0.16-0.86) and 0.16 (0.06-0.44), respectively

Other epidemiological studies from different parts of the world examined Cd exposure in relation to type 2 diabetes mellitus (Afridi et al. 2013; Barregard et al. 2013; Barregard L et al. 2014; Chen et al. 2006; Hansen et al. 2017; Liu et al. 2016; Schwartz et al. 2003). Most of them were either cross-sectional or retrospective (Afridi et al. 2013) or case-control (Hansen et al. 2017; Simić et al. 2017) in design, except one study which included limited prospective follow-up after a cross-sectional assessment (Barregard et al. 2013). Despite a limited number of prospective studies, the research has consistently supported the potential association between increasing blood Cd level and type 2 diabetes mellitus. Studies from different populations of the world have also suggested that women and men with type 2 diabetes mellitus have statistically significant higher levels of Cd in urine (Afridi et al. 2008; Barregard L et al. 2014; Kolachi et al. 2011; Schwartz et al. 2003), blood (Afridi et al. 2008; Barregard L et al. 2014; Hansen et al. 2017; Kolachi et al. 2011) or hair (Afridi et al. 2013; Kolachi et al. 2011).

Pb is another toxic heavy metal and a ubiquitous environmental contaminant (Cheng et al. 2015; Dyer 2007; Marx et al. 2016; Romero-Freire et al. 2015). Occupational exposure can occur through painting, battery, pigments, welding, smelting, electroplating, and e-waste recycling workshops are exposed to relatively high concentrations of Pb (Julander et al. 2014; Kazi et al. 2015; Zhang et al. 2016). However, general population is usually exposed to low levels of Pb concentrations via polluted drinking water, cigarette smoking, food and air (Augustsson et al. 2015; Mohmand et al. 2015; Pinto et al. 2017). Higher Pb exposure may occur through man-made sources, such as in paint around the home, leaded pipes and fixtures that deliver water and soil contaminated with Pb from leaded gasoline.

Recently, other epidemiological studies addressed specifically the association between Pb and GDM (Oguri et al. 2019; Peng S et al. 2015; Shapiro et al. 2015). Table 4 shows epidemiological studies on relationships between exposure to Pb and gestational diabetes mellitus. A retrospective case-control study within a Chinese population found a negatively associated significance in the second and third quartile of Pb (Peng S et al. 2015). On the other hand, in Canadian study, the observed association was not significant (Shapiro et al. 2015).

Controversial findings for type 2 diabetes mellitus in life had been shown by previous research as some investigations revealed that blood Pb (Haq et al. 2013), urinary Pb (Afridi et al. 2008) or hair Pb (Afridi et al. 2013) were higher in diabetes as compared to non-diabetes but others reported that blood Pb was not associated with diabetes (Hansen et al. 2017; Moon 2013), or even inversely associated with it (Simić et al. 2017). However, study designs differed among the investigations (most were case control studies) and various biological matrix were used.

Mn is a naturally occurring element and an essential nutrient that plays a role in bone mineralization, protein and energy metabolism, metabolic regulation and antioxidant system (Livingstone 2017). However, the requirements are low and excessive intake or exposure to it can cause neurotoxicity (Hutchens et al. 2017; Pfalzer and Bowman 2017). The association between Mn and GDM was not significant in our study which is consistent with other studies conducted in the general populations (Hansen et al. 2017; Liu et al. 2016; Simić et al. 2017). However, one Korean study revealed that blood manganese levels were negatively associated with increased risk of diabetes (Koh et al. 2014).

4.3 Exposure to phthalates and pregnancy induced hypertension

In our third study, we report an association between maternal prenatal urinary concentration of low molecular weight phthalate metabolites including MEP and MBP and pregnancy induced hypertension. We found a significant association between pregnancy induced hypertension and MEP (OR: 1.47, CI: 1.01-2.14, p-value = 0.04) or MBP (OR: 1.66, CI: 1.11-2.47, p-value = 0.01) and a borderline significance in MCP (OR: 1.56, CI: 0.93-2.64, p-value = 0.09).

A very limited number of studies have been conducted previously. A prospective birth cohort study from Cincinnati, USA investigated the relationship between phthalate and pregnancy induced hypertensive diseases which includes gestational hypertension, preeclampsia, eclampsia and HELLP syndrome (E Werner et al. 2015). This study findings shows that maternal urinary MBZP concentrations may be associated with increased diastolic blood pressure and risk of pregnancy induced hypertensive diseases. While in another study from Boston, USA, the relationship between Bisphenol A and phthalate metabolites and the risk of preeclampsia (Cantonwine et al. 2016), and reported a significant association between preeclampsia and MEP and DEHP was observed.

Few other studies on phthalate exposure and blood pressure in children have also shown significant associations (Trasande and Attina TM 2015; Trasande L et al. 2013; Valvi et al. 2015). However, studies from USA (Trasande and Attina TM 2015; Trasande L et al. 2013) were cross-sectional analysis of a subsample, found increased urinary concentrations of MEP and DEHP were associated with increased blood pressure in children age 6-19 years. Whereas, study from Spain (Valvi et al. 2015) was a population based birth cohort observed that sum of HMW phthalates as well as sum

of LMW phthalates were significantly related with lower systolic blood pressure in girls but not in boys at the age of 4 and 7 years. A prospective study on elderly adults from Uppsala, Sweden shown inverse associations between MEP and systolic and diastolic blood pressure (Olsen et al. 2012). While, in another Swedish cross-sectional study in which the older adults were found with increased serum concentrations of MBzP were related to several cardiovascular risk factors (Wiberg et al. 2014).

4.5 Strengths and limitations

Strengths

1. A key strength of our studies are the use of a prospective cohort study with nutritional, environmental, biological and social factors starting during the prenatal life are a strength of our study, which allowed us to control for potential confounders and to assess exposure before the health outcomes. Hence large sample size and was drawn from the general population.
2. We used predesigned questionnaires according to The International Study of Asthma and Allergies in Childhood (ISAAC), structured interviews and medical record abstraction provided us a rich covariate dataset.
3. To reduce subject variability, values of the phthalate exposure biomarkers were standardized for urine sampling conditions (Mortamais et al. 2012).
4. Exposure of the phthalates and metals were objectively measured in the mothers' urine and blood during second trimester of the pregnancy.
5. The quantification of the phthalate metabolites concentrations were performed by using on-line solid phase extraction high performance liquid chromatography electrospray ionization isotope dilution tandem mass spectrometry.
6. Blood samples for metals assessment were measured with well validated method of spectrometry and fluorimetry.
7. The diagnosis of GDM and pregnancy induced hypertension were conducted by the physicians, which ensures the quality and we further adjusted for potential confounders to see the associations.

8. The nature of our second and third study facilitated to exclude the women with diagnosed pre-gestational diabetes mellitus, renal diseases and hypertension. And indeed, we could determine that we were dealing with isolated GDM, as no mother had a diagnosis of diabetes at 2 years of age of the child.

Limitations

1. This cohort was conducted in only two cities of France, which may not represent general French population. However, in the models we adjusted for centre to take into account the regional specificities.
2. The study designs of our second and third study were nested cross-sectional within a cohort. However a nested cross-sectional cohort design offers advantages over traditional cross-sectional designs. Further, we can determine that we are dealing with isolated GDM or pregnancy induced hypertensive mothers, as no mother had a diagnosis of diabetes or hypertension at two years of age of the child.
3. The measurement of phthalates and heavy metals was conducted only once. We were limited to one urine sample per mother during the second trimester of pregnancy, thus we were unable to assess the variability in concentrations of each phthalate metabolite and heavy metal across the pregnancy. It would have been more interesting to study the association between the health related variables and exposure to phthalates and metals during different points in time. However it is not clearly known that at which point(s) in gestation the mother or infants are at higher risk of exposure.

4. Humans are exposed to a large number of complex mixtures of toxic substances in daily life, so it is hard to see the effect of exposure to a unique phthalate or metal.
5. The diagnosis of GDM is no longer based on two different tests but on only one test, i.e. 75 g-glucose tolerance with only one value above the limit of fasting, 1 h or 2 h. Whether we underestimated or overestimated the relationship cannot be seen in our second study as we had no access to detailed data on glycemia, so the results should be interpreted cautiously.
6. In our first study, we considered only male offspring because the initial purpose was to see the effects of phthalates on malformations of male genitalia (Philippat et al. 2012). Although considering only males limits the generalization of our results, it is not a source of bias. However, from a statistical point of view, focusing on one sex is another way to increase the study accuracy when the sample size is limited.
7. Creatinine was used to normalize urine dilution, other studies have used specific gravity as an alternative to phthalates to adjust phthalate urinary biomarkers. However, studies have also shown that the specific gravity is highly correlated with creatinine.
8. The questionnaire data were self-reported and thus could have been subject to misclassification; in addition, recall bias was a possibility that might in part account for the high prevalence of eczema in our study population.

4.5 Conclusion

This work is based on data from a French Mother-Child prospective cohort study. We wrote three papers, which are the basis of this PhD work. It has contributed to the critical issues in the area of maternal and child health, which are not studied much previously. These findings are important for public health because humans are continuously exposed to these EDCs (phthalates and heavy metals) chemicals in their everyday life.

In summary, in our first paper, we observed strong associations between phthalates measures during the second trimester of pregnancy and childhood eczema, which was quite strong. The prevalence of early onset and late onset eczema was 15.7% and 14.7%, respectively. We observed strong statistically significant associations between both early and late onset eczema and metabolites of two phthalates in particular; DiBP and DiNP. Among IgE sensitized boys, metabolites of DBP and DiBP were also significantly associated with ever eczema.

In our second paper, the metals namely Pb, Cd, and Mn were measured in blood during second trimester of the pregnancy. We observed the prevalence of GDM and IGT were 7.1% and 10.1% respectively. The Cd was statistically significant to GDM or IGT in the model adjusted for confounders, while Pb to GDM was found at borderline significance.

In our third paper, the prevalence of pregnancy induced hypertension was found 4.80%. We found strong associations between phthalate measures during the second trimester of pregnancy and pregnancy induced hypertension. We observed that low

molecular weight phthalate metabolites: MEP and MBP were significantly related to pregnancy induced hypertension.

Our results add new and important information to the growing number of studies that emphasize and providing suggestive evidence on the relationship between EDCs (phthalates and heavy metals we studied) and maternal and children health, that prenatal exposure to certain phthalates and heavy toxic metals may play a role in the development of GDM and pregnancy induced hypertension during pregnancy and later eczema in early childhood. Our results raise concerns on the impact of phthalates and heavy metals that persist long term in the environment on maternal and child health, which opens actions for prevention. Our data also indicates that policy can predict exposure. Minimizing exposure to these EDCs can reduce the disease burden.

Future research focusing on larger populations and addressing multiple exposures assessed at multiple points during pre-natally and post-natally are required to provide more evidence on possible contributions of emerging pollutants to study the role of phthalates and heavy metals.

5. FUTURE RESEARCH

1. Although direct causal links between exposures to EDCs and disease states in humans are difficult to draw, results from basic research and epidemiological studies make it clear that more screening for exposures and targeting at risk groups is a high priority.
2. The studies in this thesis, we utilized environmental and biological samples to characterize exposure to phthalates and heavy metals and assessed the associations between prenatal exposure and risk of GDM, pregnancy induced hypertension during pregnancy and eczema in children during early childhood. Future research will bridge across these areas of exposure science, statistical methods and health outcomes to better understand the role of EDCs.
3. In addition to enhancing research in these areas is an important and effective approach is prevention of disease. Chemical policies at local, state, and national levels, as well as globally, need to be formulated, financed, and implemented to ensure the best public health. By communicating these priorities to basic and clinical researchers, physicians, community advocates, and the public at large, we are hopeful that early identification and intervention will be facilitated.
4. A further dimension to consider in future research is multiple exposures of hormonally active agents such as these. In terms of prevention, exposure to these chemicals can be avoided if the contents of products are already known or listed on the products; unfortunately, they are often not listed on the labels because of being non-active ingredients.

5. In long term the exposome approach and Environment-Wide Associations Study (EWAS) is applied for better understanding. This strategy is currently going-on based on large population surveys globally under Pregnancy And Childhood Epigenetics (PACE) project, for which EPAR team is one of the main participants (Figure 6).

Genes, Environment, and Health Initiative

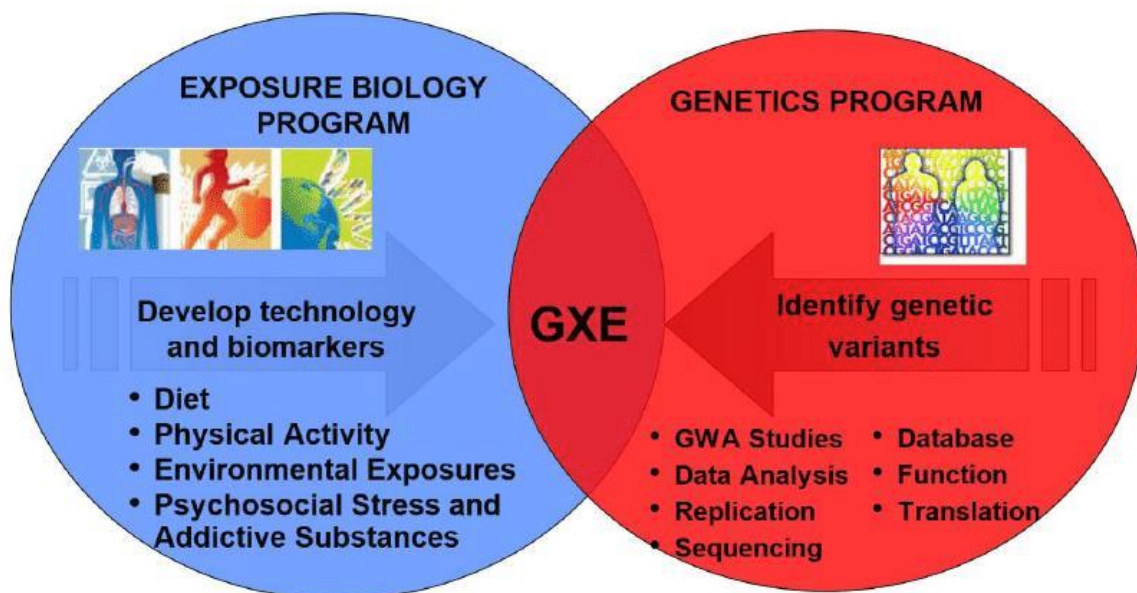


Figure 6. EXPOSOME approach and the Environment-Wide Associations Study (EWAS) in relation to eczema in children, GDM, pregnancy induced hypertension (From David M. Balshaw, making the case for advancing the EWAS).

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