Influence of environmental exposure to endocrine disruptors on cardiometabolic health

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January 2022

A thesis submitted to McGill University in partial fulfillment of the

requirements of the degree of Doctor of Philosophy

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Abstract

Obesity is a complex endocrine disease closely associated with other cardiometabolic risk factors such as hyperglycemia, dyslipidemia, and hypertension. Together, they increase the risk of diabetes, cardiovascular disease, cancer and premature death. Given the increasing global burden of obesity and metabolic disease, there is an urgent need to understand and intervene on all modifiable factors. Growing evidence suggests that endocrine-disrupting chemicals (EDCs) can disrupt energy and lipid metabolism, influencing obesity as well as cardiometabolic health. Furthermore, rapid infant growth is a well-established risk factor for obesity and could be an early target of EDCs since growth is partly regulated by hormones. This thesis aims to evaluate relationships between exposure to endocrine-disrupting chemicals and cardiometabolic risk factors in both adults and children, focusing on exposures which are ubiquitous in different populations.

For the first objective, I used data from a cross-sectional and nationally representative biomonitoring survey in Canada (the Canadian Health Measures Survey) to investigate whether urinary concentrations of parabens, common preservative agents in cosmetic, personal care, food, and pharmaceutical products, are associated with obesity and cardiometabolic health in adults (n=1137) and children (n=1418). Despite the relatively short half-life of these chemicals, almost all Canadians (92%) had detectable levels of methyl paraben in their urine. The associations with metabolic syndrome appeared to differ by sex: among men, propyl paraben was associated with 40% (95% CI: 3, 90) higher prevalence per 10-fold increase in concentration, while ethyl paraben was associated with 63% (95% CI: 2, 86) lower prevalence among women. Additionally, inverse associations were observed with obesity and high-density lipoprotein among women. No associations were observed among children.

For the second objective, I used data from a birth cohort in South Africa, the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) to examine whether maternal peri-partum serum or urine concentrations of dichlorodiphenyltrichloroethane (DDT) and pyrethroid insecticides used for malaria vector control are associated with adiposity and cardiometabolic health in the children at age 5 years (n=637). Using marginal structural models weighted by the inverse probability of treatment and censoring, I observed that prenatal exposure to pyrethroid insecticides, but not DDT, was associated with lower BMI z-score, waist circumference, and body fat percentage. These findings did not differ by sex.

Finally, for the third objective I examined associations between prenatal exposure to these same insecticides and child growth trajectories from birth through to the age of 5, again using data from VHEMBE (n=751). I applied a flexible mixed-effects growth trajectory model which identified each child's relative size and their age at peak weight velocity and included these parameters as outcomes in marginal structural models weighted by the inverse probability of treatment. This analysis showed that the pyrethroid metabolite *trans*-DCCA was associated with smaller size (-20.9 grams [95% CI: -41.6, -3.2] per 10-fold increase) among boys but not among girls. Associations with *cis*-DBCA and *cis*-DCCA were similar in magnitude but the confidence intervals included the null.

Overall, the findings presented here provide evidence that parabens and pyrethroids may impact cardiometabolic health in adults and children, with different effects in males versus females. Though preservatives and pesticides have their benefits, given the ubiquity of these exposures, their effects on human health should be better understood in order to make informed public health decisions.

Abrégé

L'obésité est une maladie endocrinienne complexe associée à d'autres facteurs de risque cardiométabolique tels que l'hyperglycémie, la dyslipidémie et l'hypertension. Ensemble, ils augmentent le risque de diabète, de maladies cardiovasculaires, de cancer et de décès prématuré. Il est urgent de comprendre et d'intervenir sur tous les facteurs modifiables. De plus en plus d'études suggèrent que les produits chimiques perturbateurs endocriniens (EDC) peuvent altérer le métabolisme énergétique et lipidique, influençant l'obésité ainsi que la santé cardiométabolique. De plus, la croissance rapide du nourrisson est un facteur de risque bien établi d'obésité et pourrait être une cible précoce des perturbateurs endocriniens puisque la croissance est régulée en partie par les hormones. Cette thèse vise à évaluer les relations entre l'exposition aux perturbateurs endocriniens et les facteurs de risque cardiométabolique, en se concentrant sur les expositions omniprésentes dans différentes population.

Pour le premier objectif, j'ai utilisé les données d'une enquête de biosurveillance transversale et représentative à l'échelle nationale au Canada (l'Enquête canadienne sur les mesures de la santé), pour déterminer si les concentrations urinaires de parabènes, des agents de conservation courant sont associés à l'obésité et à la santé cardiométabolique chez les adultes (n=1137) et les enfants (n=1418). Presque tous les Canadiens (92%) avaient des niveaux détectables de méthyl parabène dans leur urine. Les associations avec le syndrome métabolique semblaient différer selon le sexe: chez les hommes, le propyl parabène était associé à une prévalence 40% (95% CI: 3, 90) plus élevée pour une augmentation de concentration d'un facteur 10, tandis que l'éthyl parabène était associé à une prévalence 63% (95% CI: 2, 86) plus faible chez les femmes. De plus, des associations inverses ont été observées avec l'obésité et les lipoprotéines de haute densité chez les femmes.

Pour le deuxième objectif, j'ai utilisé les données d'une cohorte de naissance en Afrique du Sud, le Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) pour examiner si les concentrations sériques ou urinaires périnatales maternelles de dichlorodiphényltrichloroéthane (DDT) et d'insecticides pyréthrinoïdes utilisés contre les vecteurs du paludisme sont associées à l'adiposité et à la santé cardiométabolique chez les enfants de 5 ans (n=637). À l'aide de modèles structurels marginaux pondérés par la probabilité inverse de traitement et de censure, j'ai observé que l'exposition prénatale aux insecticides pyréthrinoïdes, mais pas au DDT, était associée à un score Z d'IMC (Indice de Masse Corporelle), un tour de taille et un pourcentage de graisse corporelle inférieurs.

Enfin, pour le troisième objectif, j'ai examiné les associations entre l'exposition prénatale à ces mêmes insecticides et les trajectoires de croissance de l'enfant de la naissance à l'âge de 5 ans, en utilisant encore les données de VHEMBE (n=751). J'ai appliqué un modèle flexible de trajectoire de croissance à effets mixtes qui a identifié la taille relative de chaque enfant et son âge à la vitesse de poids maximale et a inclus ces paramètres en tant que résultats dans des modèles structurels marginaux, qui ont montrés que le métabolite pyréthrinoïde *trans*-DCCA était associé à une plus petite taille (-20,9 grammes [95% CI: -41,6, -3,2] par augmentation d'unfacteur de 10) chez les garçons mais pas chez les filles.

Dans l'ensemble, les résultats présentés ici suggèrent que les parabènes et les pyréthroïdes pourraient avoir un impact sur la santé cardiométabolique des adultes et des enfants, avec des effets différents chez les hommes et les femmes. Bien que les agents de conservation et les pesticides aient leurs avantages, étant donné l'ubiquité de ces expositions, leurs effets sur la santé humaine devraient être mieux compris afin de prendre des décisions de santé publique éclairées.

List of abbreviations

3-PBA	3-phenoxybenzoic acid
4-F-3-PBA	4-fluoro-3-phenoxybenzoic acid
cis-DBCA	*cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid
cis-DCCA	*cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid
trans-DCCA	*trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid
AIC	Akaike information criterion
APWV	Age at peak weight velocity
BIC	Bayesian information criterion
BMI	Body mass index
CHMS	Canadian Health Measures Survey
CI	Confidence interval
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DDT/E	DDT and DDE
GM	Geometric mean
GPS	Generalized propensity score
HDL	High-density lipoprotein
HPG	Hypothalamic-pituitary-gonadal
IOM	Institute of Medicine
IPTW	Inverse probability of treatment weights
IQR	Interquartile range
LOD	Limit of detection
NHANES	National Health and Nutrition Examination Survey

PR	Prevalence ratio
SD	Standard deviation
SE	Standard error
SITAR	SuperImposition, Translation and Rotation
U.S.	United States
VHEMBE	Venda Health Examination of Mothers, Babies and their Environment

Acknowledgements

My doctoral studies were made possible by the support of the Lloyd-Carr Harris Fellowship from the Faculty of Medicine (2016 – 2018) and a doctoral award from the Fonds de recherche en santé du Québec (2018 – 2021). I also received financial support from a CIHR grant held by Jonathan Chevrier, the Department of EBOH, and the Quebec Inter-university Centre for Social Statistics.

The first of many thanks go to my thesis supervisor, Jonathan Chevrier, for his generous support and guidance throughout my doctoral studies. His unwavering enthusiasm for my work gave me confidence when I needed it most. I was also very fortunate to work with Seungmi Yang and Erica Moodie, who were generous and insightful committee members. Thanks also to all of the faculty, staff, and students who made EBOH such a warm and stimulating environment.

I have benefited greatly over the years from wonderful mentors: Paul Demers, Anne Harris, Victoria Arrandale, and France Labrèche. They are a constant source of support and inspiration. And I am forever grateful to Linn Holness, who gave me my first job in research.

I cannot thank enough my incredible writing buddy and friend, Zoey Cochran, who has accompanied this thesis from the very beginning. It has been such a blessing to share this journey with her. Along the way, I also met my "Paraguayan family" and other dear friends, who made the journey a joyful one.

Finally, a heartfelt thank you to my sister, who is my number one supporter. I dedicate this thesis to our late mother, whose resilience and grace continues to inspire me.

Contribution to original knowledge

Manuscript 1: Evaluates associations between exposure to parabens and the prevalence of obesity and metabolic syndrome in the general Canadian population. This was the first study to examine associations between parabens and metabolic syndrome, along with its components, in a comprehensive assessment of the potential impact of parabens on metabolic health. This is also the first study to investigate associations between parabens and obesity in the Canadian population and is one of the largest studies to date on this topic.

Manuscript 2: This study estimated the causal effects of prenatal exposure to DDT/E and pyrethroid insecticides on child cardiometabolic risk factors, including anthropometrics, measures of adiposity (BMI, waist circumference, and body fat percentage), and blood pressure, at five years of age using inverse probability weighting methods to address confounding and selection bias. The study is also unique in that the population is exposed to these pesticides through the practice of indoor residual spraying (IRS) for malaria vector control.

Manuscript 3: This study investigated whether gestational exposure to insecticides influences child growth trajectories, involving a novel application of the SuperImposition, Translation And Rotation (SITAR) model to the environmental epidemiologic literature. This study also used inverse probability weighting methods to address confounding and selection bias and takes place in a population exposed to these insecticides through indoor residual spraying.

Contribution of authors

Manuscript 1: Kim J and Chevrier J.

JK and JC proposed the current study; JK designed and conducted the analysis, and wrote the manuscript; JC provide feedback. All authors read and approved the final study.

Manuscript 2: Kim J, Yang S, Moodie EEM, Obida M, Bornman R, Eskenazi B, Chevrier J.

JK and JC proposed the current study; JC, RB and BE lead the parent study; SY and EEMM provided analytical guidance and feedback; MO provided field support for the study. All authors read, provided feedback, and approved the final study.

Manuscript 3: Kim J, Yang S, Moodie EEM, Obida M, Bornman R, Eskenazi B, Chevrier J.

JK and JC proposed the current study; JC, RB and BE lead the parent study; JK designed and conducted the analysis, and wrote the manuscript; SY provided substantive and analytical guidance on child growth and the SITAR modell; EEMM provided guidance for the multiple imputation, inverse probability weighting, analysis, and bootstrapping; MO provided field support for the study. All authors read, provided feedback, and approved the final study.

CHAPTER 1. Introduction

Obesity has tripled over the last four decades, resulting in over 670 million adults and 124 million children and adolescents currently classified as obese[1]. This is not just a phenomenon of high-income nations, as 115 million individuals with obesity live in developing countries[1]. The prevalence of overweight among children under the age of 5 in the UN sub-region of Southern Africa is more than twice the global average[2], and obesity among South African children 5 to 19 years of age is 10% among boys and 13% among girls[3], similar to that of their Canadian counterparts (13% in both sexes)[4]. The prevalence of obesity among both men and women in Canada is 27%, but in South Africa, the prevalence diverges greatly by sex (41% among women and 11% among men)[5] and increases markedly with age. In addition, South Africa is undergoing a rapid epidemiologic transition, with average BMI of women and men increasing at a much higher rate (1.8 kg/m^2 and 1.0 kg/m^2 per decade, respectively[6]) than the global average of 0.5 kg/m^2 and 0.4 kg/m^2 per decade[7].

However, excess body weight does not necessarily translate to poor cardiometabolic health outcomes. Compared to individuals who are classified as obese, individuals with metabolic syndrome, which includes a cluster of cardiometabolic risk factors such as abdominal obesity, dyslipidemia, hyperglycemia, insulin resistance, and hypertension, are at higher risk of diabetes, cardiovascular disease, certain cancers and premature death[8]. While one in four Canadians are obese, an estimated one in five have metabolic syndrome. National estimates of metabolic syndrome are not available for South Africa, but the prevalence of diabetes (13%) and hypertension (45%) is high, and diabetes and cerebrovascular diseases are now the second and third leading causes of death in South Africa[5,9,10].

Given the immense burden of obesity and cardiometabolic risk factors, there is an urgent need to understand and intervene on all the modifiable factors that influence them. Although age, genetics, diet and physical activity are major determinants of these conditions, growing evidence suggest that endocrine disrupting chemicals (EDCs), a group of chemicals that can interfere with the human endocrine system, may also play a role by disrupting hormones (for example, estrogen and testosterone) that regulate weight gain and other aspects of cardiometabolic health[11-14]. Much of this epidemiological literature has focused on identifying obesogens, but investigations of metabolic disruption have been sparse for even some of the most ubiquitous EDCs.

1.1. Knowledge gaps

Parabens are a group of widely used preservative agents in cosmetics, food, and pharmaceuticals[15-17], and almost all (93%) Canadians have detectable levels in their urine. These estrogenic and anti-androgenic compounds have been shown to promote differentiation of mesenchymal stem cells into adipocytes[18-20], and increase adiposity in mice[21,22]. However, the epidemiological literature on parabens as obesogens is sparse and inconsistent, with studies of children reporting positive, null, or inverse associations with weight, obesity, or waist circumference in children[23-26], and two studies of adults (one with n=27) reporting null and inverse associations with obesity and waist circumference[27,28]. None of these studies were conducted in Canada; furthermore, despite the potential for parabens to have broader effects on cardiometabolic health through disruption of estrogen and testosterone, and the additional health risks posed by metabolic syndrome relative to obesity, no epidemiological studies have investigated the potential link between paraben exposure and metabolic syndrome in adults or with indicators of metabolic health in children.

Today, exposure to a well-known endocrine disruptor, the insecticide dichlorodiphenyltrichloroethane (DDT), primarily occurs through the practice of indoor residual spraying (IRS), which is a malaria vector control strategy that involves the spraying of insecticides on interior walls, ceilings, rafters, beams and eaves of dwellings[29,30]. The main class of insecticides used for IRS are pyrethroids, which also have endocrine-disrupting properties. However, most epidemiologic studies examining the health effects of these IRS insecticides are conducted in non-IRS populations. In addition to the much higher levels of exposure experienced by inhabitants of sprayed dwellings[31,32], populations residing in IRS areas may be particularly susceptible to the health effects of insecticides due to poverty, malnutrition, and concurrent disease burden. These chemicals can cross the placenta, potentially affecting the developmental programming of the fetus and its long-term health[33,34]. Therefore, studies examining the cardiometabolic health impact of gestational exposure to these insecticides are needed, particularly among IRS populations.

The extant literature on these IRS insecticides also has important methodological limitations. Compared to single time-point measures, growth trajectory modeling which captures dynamic aspects of child growth have been found to better predict cardiometabolic health in later life[35,36], but few studies have investigated these in relation to DDT, and none have done so for pyrethroids. Moreover, only one study used methods to address potential selection bias from loss to follow-up[37], and most used complete-case analysis, further increasing the potential for selection bias[38]. These studies also used confounder selection strategies such as univariate analysis, stepwise approaches, and change-in-estimate which may result in biased estimates and inaccurate confidence intervals[39-41]. These limitations give rise to opportunities to strengthen our understanding of the health impacts of IRS insecticides on cardiometabolic risk factors by implementing advanced epidemiological methods.

1.2. Research Objectives

This dissertation aims to address substantive and methodological gaps in the literature regarding the potential impact of ubiquitous endocrine-disrupting chemicals on cardiometabolic health in Canada and South Africa.

Objective 1: To estimate associations between urinary paraben concentrations, and obesity, metabolic syndrome and its components among Canadian adults and children.
Secondary objective: To examine effect measure modification by sex.

To address Objective 1, I used data from Cycle 4 (2014 – 2015) of the Canadian Health Measures Survey (CHMS) (n=2564), a nationally-representative cross-sectional biomonitoring survey which conducts direct physical assessments and collects biological samples. I examined the relationship between exposure to parabens, based on urinary concentrations, and the prevalence of obesity and metabolic syndrome, as well as BMI and individual components of metabolic syndrome (waist circumference, blood pressure, and serum levels of triglycerides, glucose, and cholesterol). Due to the potential for sexually dimorphic effects of endocrine disruptors, I also investigated effect modification by sex. This Objective is addressed in Chapter 4: Manuscript 1.

Objective 2: To estimate associations between gestational exposure to DDT/E and pyrethroid insecticides on cardiometabolic risk factors (height, weight, BMI, waist circumference, body fat percentage, and blood pressure) among children at five years of age in Limpopo, South Africa

Secondary objective: Examine effect measure modification by sex, household poverty, and maternal energy intake during pregnancy

To address Objective 2, I used data from the Venda Health Examination of Mothers, Babies and Their Environment (VHEMBE), a birth cohort of 751 mothers and their children based in the Limpopo province of South Africa where IRS occurs annually. I examined the relationship between maternal peripartum concentrations of DDT/E in serum and pyrethroid metabolites in urine and a range of cardiometabolic risk factors measured in the children at five years of age (n=637). These risk factors included: weight, height, and BMI z-scores; waist circumference; body fat percentage; and blood pressure. I estimated associations using marginal structural models with inverse probability weights to adjust for confounding and account for selection bias from loss to follow-up. This Objective is addressed in Chapter 5: Manuscript 2.

Objective 3: To estimate associations between of gestational exposure to DDT/E and pyrethroid insecticides on child weight trajectories from birth through five years of age in Limpopo, South Africa

Secondary objective: To examine effect measure modification by sex, household poverty, and maternal energy intake during pregnancy

Objective 3 was also addressed using data from all participants in VHEMBE (n=751). Child weight trajectories using Super-Imposition, Translation, and Rotation (SITAR) were modelled based on over 10,000 child weight measurements taken from birth to five years of age. The estimated SITAR parameters were then modelled as the outcome in relation to maternal peripartum concentrations of DDT/E pyrethroid in marginal structural models accounting for confounding and selection bias. This Objective is addressed in Chapter 6: Manuscript 3.

CHAPTER 2. Literature Review

This literature review will provide a comprehensive overview of the following topics: Section 2.1. will introduce the three groups of EDCs examined in this dissertation: parabens, DDT and its breakdown product dichlorodiphenyldichloroethane (DDE), and pyrethroids; these chemicals have been identified as EDCs, specifically with estrogen- and/or androgen-disrupting properties. Section 2.2. will describe how estrogen and testosterone regulate and influence obesity and cardiometabolic health. Sections 2.3 to 2.5 will summarize the current epidemiologic and animal literature connecting each chemical group with obesity and cardiometabolic health outcomes.

2.1. Endocrine disrupting chemicals (EDCs)

2.1.1. Parabens

Parabens are a group of alkyl esters of para-hydroxybenzoic acid with anti-microbial properties, named according to the alkyl group (e.g. methyl paraben, ethyl paraben, propyl paraben, etc.; see Figure 2.1). They are the most commonly used preservatives in the cosmetic, pharmaceutical, and food industries, providing stability and long shelf-life to products such as shampoos, make-up, lotions, ointments, syrups, jams, baked goods, beverages, and sauces[42].

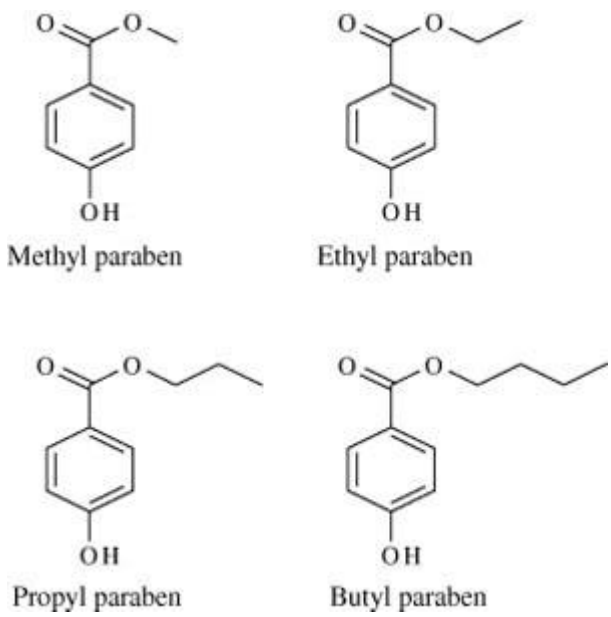


Figure 2.1. Chemical structures of four parabens

Individuals are exposed through dermal contact and/or ingestion of paraben-containing products, with an estimated 66% of daily exposure from the use of cosmetics and personal care products, 33% from pharmaceuticals, and 1% from diet[42]. After absorption, parabens are rapidly metabolized to nonspecific *p*-hydroxybenzoic acid and *p*-hydroxyhippuric acid before being excreted in urine within 72 hours[43,44], with 6 to 17% of the original dose excreted as the parent compound[44]. Urinary concentrations of the parent compound are measured in human biomonitoring studies as specific biomarkers of paraben exposure. Based on US biomonitoring data, total daily exposure to parabens is estimated to be 75 μg/kg per day[45]. As a result of ubiquitous, daily exposure, these compounds are detected at high rates in populations around the world, with detection rates of 98-100% for methyl paraben documented in several countries[45-54].

Several parabens including methyl, ethyl, propyl and butyl parabens bind competitively to estrogen receptors[55-58] and can activate estrogen receptor-dependent pathways[55,59-61]. Estrogen receptor binding affinity appears to increase with the length of the alkyl chain (methyl<ethyl<propyl<butyl) and with the presence of branching (e.g. isobutylparaben) or aromatic rings (e.g. benzylparaben)[58,62-65]. In addition to these estrogenic effects, parabens can also inhibit the activity of estrogen sulfotransferase, the enzyme that inactivates estrogen, and thereby indirectly increase estrogen levels[66]. Parabens also have anti-androgenic properties, as they have been shown to bind to androgen receptors but inhibit androgen signalling[15-17]. Furthermore, butylparaben decreases pubertal testicular expression of the gene encoding aromatase, the enzyme that converts testosterone into estrogen, thus restricting an important source of estrogen in males[67].

2.1.2. Dichlorodiphenyltrichloroethane (DDT)

DDT is an organochlorine insecticide which was used widely during the 1940s and 1950s to manage agricultural and household pests, as well as control the spread of malaria and typhus during World War II[68]. Its insecticidal properties come from their ability to bind to voltage-gated sodium channels in nerve cell membranes, impeding the transmission of nerve impulses, leading to paralysis and death[68]. Following concerns of its impact on the environment, ecosystems, and human health, it was banned in the U.S. and Canada in the 1970s and banned globally in 2001 under the Stockholm Convention on Persistent Organic Pollutants[69]. An exception, however, is made for public health purposes, and it continues to be used for malaria vector control in India and in eight African countries, including South Africa[30] as part of a vector control strategy called indoor residual spraying (IRS) which involves the spraying of insecticides on interior walls, ceilings, rafters, beams and eaves of dwellings[29,30]. In South Africa, DDT is meant to be applied to traditional houses with unpainted or unplastered walls made of mud, clay or wood[29], and one IRS application of DDT is effective for 6 to 12 months[70].

Manufactured DDT is comprised mostly of the active ingredient *p,p'*-DDT (65-80%), the isomer *o,p'*-DDT (15-21%)[71] and their respective breakdown products, *p,p'*- and *o,p'*-dichlorodiphenyldichloroethane (DDE)[68]. These compounds have a half-life of up to 30 years in soil and 150 years in water[68,71], are highly lipophilic, and as such are primarily distributed in lipids throughout the body where they can persist for decades[68]. Dietary exposure can occur from consuming meat, fish, poultry, and dairy products[68,72]. Developing fetuses are exposed *in utero,* as these compounds can also cross the placenta, and postnatal exposure continues through breastmilk, which is a major reservoir of DDT due to its high lipid content[68]. Biomonitoring studies continue to detect the highly persistent main metabolite *p,p'*-DDE in the serum lipids of

over 99% of the general population in both the U.S. and Canada[73,74], despite being banned decades ago in these countries.

DDT binds to and activates estrogen receptors, though with a binding affinity several orders of magnitude lower than that of estradiol[75-77]. *In vitro* and *in vivo* assays have shown that the estrogenicity of *o,p'*-DDT exceeds that of *p,p'*-DDT[75-78]. Both DDT isomers can also bind to the androgen receptor, though the major breakdown product *p,p'*-DDE binds with greater affinity and is the most potent androgen receptor antagonist among the DDT-related compounds[77,79].

2.1.3. Pyrethroids

Pyrethroids are a class of synthetic insecticides based on the chemical structure of pyrethrins, which are naturally occurring insecticidal compounds produced by the Chrysanthemum flower, modified to increase their stability in sunlight and efficacy against insects. Like DDT, pyrethroids target voltage-gated sodium channels in the nervous system, leading to paralysis and death[80]. Unlike DDT, pyrethroids are metabolized rapidly and have half-lives of hours to days in humans[81-83]. Their metabolites can be measured in urine as reliable and specific biomarkers of pyrethroid exposure and include: 3-phenoxybenzoic acid (3-PBA), a metabolite of several pyrethroids including permethrin, cypermethrin, deltamethrin, allethrin, resmethrin and fenvalerate; *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA) and *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*trans*-DCCA), which are the metabolites of the cis and trans isomers of permethrin, cypermethrin and cyfluthrin; 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), a metabolite specific to cyfluthrin; and *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA) which is a metabolite specific to deltamethrin (Table 2.1).

Table 2.1. Metabolites of four common pyrethroid insecticides

Metabolites	Pyrethroid			
	Permethrin	Cypermethrin	Deltamethrin	Cyfluthrin
cis-DBCA			x	
cis-DCCA	x	x		x
trans-DCCA	x	x		x
3-PBA	x	x	x	
4-F-3-PBA				x

Pyrethroids are among the most commonly used insecticides in agricultural and domestic pest-control applications worldwide (e.g. pet shampoos, lice treatments, mosquito repellents)[84]. They are also used extensively for malaria vector control: they are the only approved pesticide class for insecticide-treated bed nets, and the main pesticide class used for indoor residual spraying (IRS)[29,30]. In South Africa, deltamethrin and cypermethrin are used for IRS, except for traditional houses with unpainted or unplastered walls made of mud, clay or wood where DDT is often used instead[29]. After spraying, pyrethroids remain effective on surfaces for up to 10 months[70,85], their longevity aided by the lack of sunlight and external elements which would otherwise contribute to their rapid degradation. Dermal exposure is the main route of exposure in the IRS context, though inhalation exposure and inadvertent ingestion of residues from contaminated hands, uncovered food, or water can also occur. The levels of exposure to IRS applicators or the inhabitants of sprayed homes is not well-documented. In the VHEMBE birth cohort, which takes place in a region of South Africa where IRS occurs annually, all mothers had detectable levels of four pyrethroid metabolites, and median urinary concentrations ranged from 0.23 μg/mL for *cis*-DBCA to 0.71 μg/mL for 3-PBA[86]. Other high-exposure scenarios include occupational exposure, affecting pesticide applicators, farmers, and pesticide manufacturing workers.

In non-IRS populations, domestic use of pesticide products in and around the home can contribute to exposure, though a major source comes from the diet[87,88]. A recent study of 50 North Carolina adults found that among all the food items that participants had consumed over six days

as part of their regular diet, 39% contained detectable residues of at least one pyrethroid, most frequently *cis*-permethrin (17%), bifenthrin (15%), *trans*-permethrin (14%) and deltamethrin (14%). The estimated median of total daily dietary intake doses for participants were 2.7 ng/kg of *cis*-permethrin, 2.1 ng/kg of *trans*-permethrin, 1.6 ng/kg of bifenthrin, and 2.8 ng/kg of deltamethrin[89]. Biomonitoring studies in the U.S. and Canada regularly report high detection frequencies to 3-PBA, *cis*-DCCA, and *trans*-DCCA, with 3-PBA being detected most frequently (70% in the U.S., 99% in Canada)[90,91].

2.2. EDCs as obesogens and metabolic disruptors

The human endocrine system regulates several processes including growth and development, stress response, appetite, and metabolism via a complex set of hormone-mediated feedback and regulatory mechanisms[92]. EDCs are exogenous chemicals that an interfere with these processes by influencing the synthesis, transport, metabolism, binding action, or elimination of hormones[92]. This section describes the cardiometabolic and growth implications of disruptions to the normal function of estrogen and testosterone in the body.

2.2.1. The role of estrogen and testosterone in obesity and metabolic syndrome

In adults, estrogen protects against increased obesity by suppressing appetite and increasing energy expenditure. They suppress appetite by enhancing the effects of satiety hormones such as leptin[93], and by decreasing the effectiveness of hunger hormones such as ghrelin[94]. Additionally, activation of estrogen receptors in the brain results in increased basal energy expenditure[95,96], which is supported by observations of post-menopausal women having lower energy expenditure during exercise and sleep compared to premenopausal women[97,98]. Estrogen can also lead to the conversion of white fat into heat-producing brown fat cells which increases the body's basal energy expenditure[99] and reduces cardiometabolic risk[100].

Estrogen also protects against the cardiometabolic risks associated with obesity. Cardiometabolic risk is not only associated with fat accumulation but also with its distribution in the body. Estrogen favours subcutaneous fat deposition, and is responsible for the pear-shaped female body phenotype, versus visceral (surrounding the internal organs) fat deposition in males. Visceral fat is a source of proinflammatory cytokines that contribute to insulin resistance, as well as free fatty acids that cause increased hepatic glucose production, excess insulin, and other features of the metabolic syndrome[101]. The decline in estrogen at menopause is accompanied by a

shift toward visceral fat deposition and increases in cardiometabolic disease risk; these changes are reversed through estrogen hormone replacement therapy[102].

Testosterone also protects against obesity, particularly in men. Men with physiologic hypogonadism or undergoing androgen deprivation treatment for prostate cancer are at higher risk of developing obesity and associated metabolic disease, including insulin resistance and type 2 diabetes[103-105]. Exogenous testosterone therapy reverses the increased adiposity in hypogonadal men[106-109]. The decline in testosterone with age is also accompanied with increases in body fat mass[110]. The protective effects of testosterone may be partly mediated by estrogen; conversion of testosterone into estrogen contributes to 85% of circulating estrogen in men; the remaining 15% is from testicular production of estrogen.

2.2.2. The role of estrogen and testosterone in child growth and body composition

Growth is influenced by different hormones depending on the period of life.[111] After birth, there is a period in the first few months of life that is characterized by a transient surge in sex hormones, peaking between 1 to 3 months; thus, it has been called "mini-puberty". In boys, there is an increase in testosterone to concentrations similar to those occurring during puberty;[112] in girls, there is an increase in both estrogen and testosterone (although around 30% lower than in boys)[113]. During mini-puberty, linear growth velocity is faster in boys than in girls, which is driven by testosterone[114]. However, relatively little else is known about processes that affect growth and body composition during mini-puberty, thus we may need to draw parallels with puberty.

Sex hormones are known to play an important role during puberty, when they surge in levels and trigger development of sex organs and the linear growth spurt. The pubertal growth spurt is mediated primarily by estrogen, which stimulates growth hormone secretion[115]. Changes in body composition also occur at this time. Testosterone acts synergistically with growth hormone

to reduce fat stores, and increase muscle mass in males[116]. In females, estrogen acts to counteract the lipolytic effects of growth hormone, and thus girls gain significantly more body fat mass during puberty than boys[117].

2.3. Literature Review: Parabens

2.3.1. Parabens and adiposity in humans

The epidemiological literature on parabens as obesogens is sparse and reports mixed findings. Two studies found positive associations between parabens and weight or abdominal circumference among 3 year-old boys; in a French cohort of male babies, maternal gestational urinary concentrations of parabens were not associated with weight from birth to 3 years of age, but methylparaben was associated with greater abdominal circumference at 3 years[23], and in a cross-sectional study of 3-year olds in China, urinary ethyl (but not methyl, propyl or butyl) paraben concentrations were associated with higher weight-for-age z-scores among boys (but not girls)[24]. In contrast, a cross-sectional analysis of children 6 to 19 years of age participating in NHANES found inverse associations between urinary methyl paraben concentrations and prevalence odds of obesity and overweight, as well as waist circumference[28]. Two other studies, including one prospective study, reported null associations; methyl, ethyl, and propyl paraben measured in the urine of 6 to 8 year old girls in the U.S. were not associated with their subsequent weight change until age 15[25], nor were these parabens associated with obesity in a case-control study of Indian children 2 to 14 years of age[26].

Only two studies have examined whether exposure to parabens is associated with adiposity in adults. A small study of 27 adult women in the Czech Republic found no correlations between current BMI and urinary concentrations of methyl and propyl parabens[27]. In contrast, inverse relationships between parabens and several measures of adiposity (obesity, BMI and waist circumference) were observed among adults participating in NHANES, with stronger inverse relationships observed in females[28]. Regarding other measures of cardiometabolic health, one study examined parabens and high blood pressure among adults participating in NHANES, but

found no associations[118]. Prior to the publication of Chapter 4 (Manuscript 1), no studies assessed the relationship between parabens and a comprehensive range of cardiometabolic risk factors in either adults or children.

2.3.2. Parabens and adiposity in animals

Some *in vitro* and animal studies provide some mechanistic evidence for the possible relationship between parabens and adiposity. For instance, parabens can promote the differentiation of mesenchymal stem cells into adipocytes[18-20] rather than cartilage, bone, or muscle cells, though this effect is not always observed[21]; gains in fat mass offset by loss of muscle or bone mass may explain why parabens did not affect overall body weight in most animal studies[22,65,119-122], with only one study observing a decrease in overall body weight in mice exposed to propylparaben[123]. Among the few studies that quantified adiposity, chronic gestational exposure to 20 ug/kg/day of butylparaben increased adipocyte size and total fat mass in the adult female (but not male) offspring[21]. Two other studies administered doses several orders of magnitude higher than the estimated daily human exposure of 75 ug/kg, thus these results may be less relevant to the human health effects; Hu et al. (2016) reported that 100 mg/kg/day of methyl (but not butyl) paraben increased white adipose tissue mass in female mice exposed for 12 weeks since weaning[22], while Boberg et al. (2016) did not observe effects of chronic gestational exposure to 10, 100, or 500 mg/kg/day of butylparaben on fat pad mass in male or female rat offspring at puberty or adulthood[67].

2.3.3. Parabens and sex hormone levels

Animal experiments suggest that the hormone-disrupting effects are not mediated by changes in levels of circulating sex hormones, though they mostly administered doses much higher

than human environmental exposures. Pollock et al. (2017) reported that rats given a single dose of 1 to 9 mg butyl paraben had higher urinary estrogen levels 6 to 10 hours later, but no effect was observed from propyl paraben[124]. Meanwhile, Vo et al. (2010) administered high daily doses (62.5 to 1000 mg/kg per day) of various parabens (including methyl, ethyl, propyl and butyl) for three weeks, but observed no effect on estrogen levels, except for a decrease from 1000 mg/kg of ethyl paraben[65]. Similarly, Kim et al. (2015) reported no effects of isopropyl or isobutyl paraben on estrogen levels in adult rats exposed dermally over a 5 week period[125]. It is therefore likely that the estrogenic effects of parabens are via greater activation of estrogen signaling pathways, rather than increasing endogenous estrogen levels. Evidence regarding impact on testosterone levels is also mixed. Three animal studies demonstrated that exposure to 10 to 1000 mg/kg/day of methyl, propyl, or butyl over 4 to 8 weeks reduced testosterone levels in peri-pubertal rats[119-121,123], though in another study, butyl paraben exposure initially lowered then subsequently raised testosterone levels of rats[119], while a fifth study found no effect on testosterone from methyl or ethyl paraben exposure[122].

There is little evidence that exposure to parabens is associated with changes in sex hormone levels in humans. In a cross-sectional biomonitoring survey in Canada, spot urine concentrations of four parabens (methyl, ethyl, propyl, butyl) were inversely associated with serum levels of estradiol, follicle-stimulating hormone, and luteinizing hormone among girls aged 6 to 17 years (n=382)[126]. However, parabens not associated with serum testosterone in male or female children (6-11 years) or adolescents (12-19 years) in the National Health and Nutrition Examination Survey (NHANES), a national biomonitoring survey in the U.S.[127], and no associations between parabens and serum levels of any reproductive hormones (testosterone, estradiol, sex hormone-binding globulin (SHBC), follicle-stimulating hormone, and luteinizing hormone) were observed among

adult men recruited from an infertility clinic in the U.S. (n=167)[128] or Denmark (n=195)[129]. Finally, a study that took repeated measures at 16-20 weeks and 24-28 weeks gestation among 602 pregnant women in Puerto Rico reported that increases in butyl paraben between these two time points were associated with decreases in SHBG, a protein that transports estrogens and androgens and controls their bioavailability[130], but no associations with testosterone, estradiol, follicle-stimulating hormone, or luteinizing hormone were observed[131].

2.4. Literature Review: DDT/E

2.4.1. DDT/E and adiposity in humans

There is a large epidemiologic literature on the obesogenic properties of DDE. A meta-analysis of seven prospective studies, most of which assessed prenatal exposure from maternal serum in relation to BMI measured in children 4 to 9 years of age, reported 0.13 higher BMI z-scores per natural log increase in DDE concentration[132]. However, multipollutant analyses from the VHEMBE birth cohort suggest that DDT rather than DDE is the obesogenic culprit, with maternal peripartum serum DDT (but not DDE) being positively associated with BMI z-scores at ages 1 and 2 years among girls, but neither were associated with BMI at age 3.5 years[37].

Only VHEMBE and other birth cohorts established during a period of active or recent DDT use had sufficient detection frequencies to investigate relationships between prenatal DDT exposure and child adiposity. Prenatal exposure to DDT was not associated with BMI z-scores from infancy up to the age of 3 years in a cohort of sons in Mexico, nor among 5 year-olds in the U.S. Child Health and Development Study or 7 year-olds in the U.S. Collaborative Perinatal Project[133]. However, in a Spanish birth cohort, the second vs. lowest tertile of maternal DDT was associated with higher BMI and overweight among boys at age 7, and in an agricultural region of California, maternal serum DDT was not associated with adiposity at 7 years, but was positively associated with BMI z-score and waist circumference at 9 and 12 years among boys[134,135]. A follow-up of the U.S. Child Development and Health study reported that maternal serum DDT levels during pregnancy were associated with higher BMI and waist circumference in their daughters at 50 years of age. Overall, there appears to be a more consistent pattern of associations between DDT and adiposity among older children, though associations at younger ages were observed in VHEMBE, possibly explained by the higher levels of DDT in this population.

2.4.2. DDT/E and child growth in humans

A limited number of studies have investigated how prenatal DDT/E exposure affects dynamic measures of child growth. Only one study examined DDT and modelled latent growth trajectories as the outcome; among 249 mother-child pairs in an agricultural region of California, higher maternal DDT and DDE concentrations were associated with a pattern of stable and then increasing BMI after age 5 among boys; however, after confounder adjustment, the association was no longer present[136]. The other studies examined associations between DDE and a change in weight or BMI over two timepoints, with mostly positive findings: in a Spanish birth cohort, DDE was associated with rapid weight gain in the first 6 months (change in weight-for-age z-scores>0.67) and elevated BMI (z-score >= 85% percentile) at 14 months, with similar results when pooled with two other Spanish birth cohorts; however, no such association was observed in a Greek birth cohort[137]; and, in a pooled analysis of seven European birth cohorts (which did not include the aforementioned studies), DDE was associated with greater increases in weight z-scores between birth and 24 months[138]. No studies of DDT and child growth have used flexible mixed-effects models that overcome limitations of interval-based (i.e. change in weight over two timepoints) and grouping-based growth metrics.

2.4.3. DDT/E and adiposity in animals

Most animal studies of DDT exposure found no effects on body weight, including at doses ranging from 0.02 mg/kg to 50 mg/kg[139-146] or up to 200 mg/kg[146-150]. Most studies of prenatal exposure at low-dose (≤2 mg/kg) were null[142-146,151], and only one reported that mice exposed to 2 mg/kg *o,p'*-DDT for seven days during gestation had lower body weight[146]. There were fewer studies of low-dose postnatal exposure, and findings were mixed. The study which evaluated the lowest dose had administered 5.6 µg/kg of a DDT/E mixture to adult male rats once a week for

four weeks, and although no differences in absolute body weight were observed, exposed rats had a lower rate of weight gain on a high fat diet compared to control mice, and their rate of weight loss was higher during the two-week calorie-restriction that followed[152]. In contrast, male mice administered 2 mg/kg of technical grade DDT for five consecutive days followed by once per week for 13 weeks had higher body weight compared to controls[153]. In another study, two weeks of exposure to 0.85 mg/kg of technical grade DDT had no effect on the body weight of male rats[147], while two studies of long-term exposure to *p,p'*-DDT and also found no effects on body weight of rats at lower doses (10 weeks of 0.34-0.37 mg/kg or 3.4-3.8 mg/kg[151]; two years of 0.17-0.21 mg/kg or 1.7-2.2 mg/kg[154]).

However, both of these long-term exposure studies reported decreases in body weight at higher doses: female rats and their female offspring experienced transient decreases in body weight gain at 28 mg/kg, but no effects were observed among male mice or male offspring)[151]; and adult rats exposed daily to 19 mg/kg (males) or 25 mg/kg (females) for two years had lower body weight compared to control rats[154]. In contrast, a study which administered 28 mg/kg of *p,p'*-DDT, *o,p'*-DDT, or *o,p'*-DDE to pregnant mice for five days reported increases in body weight of their adult offspring for all three exposures[155]. Other studies which evaluated DDE alone (not in a mixture with DDT) found no effect on body weight at doses ranging from 0.4 to 100 mg/kg/day[156-159]. At higher doses of up to 200 mg/kg, two studies of DDE[79,158] and several studies of DDT[151,160-162] reported decreases in body weight, which may reflect systemic toxicity rather than effects from endocrine disruption.

2.4.4. DDT/E and other cardiometabolic risk factors in animals

Few animal studies have assessed cardiometabolic outcomes from DDT/E exposure, and only one assessed prenatal exposure. Prenatal exposure to 1.7 mg/kg of DDT did not affect fasting glucose, insulin, cholesterol, or lipid levels in offspring up until 6 months of age[144]; however, in the same experiment, DDT-exposed female offspring who were subsequently fed a high-fat (vs. low-fat) diet for 12 weeks developed elevated cholesterol and triglycerides, impaired glucose tolerance, and insulin resistance, and observed reductions in core temperature and expression of lipolytic and genes linked to insulin resistance in brown adipose tissue suggested that impaired thermogenesis and energy expenditure are possible mechanisms for increased susceptibility of metabolic syndrome in gestationally-exposed adult female offspring[144]. Other studies examined only exposure during adulthood, which may explain the mostly null results in contrast to the positive findings of the prenatal exposure study: adult male rats exposed to a DDT/E mixture of 5.6 µg/kg once a week for four weeks had reduced body temperature, but no changes in serum triglyceride levels[152]; similarly, *p,p'*-DDT did not affect triglyceride or cholesterol levels among adult male rats exposed to 0.1 mg/kg for 12 weeks[163], nor among adult male mice exposed to 1.0 mg/kg for 8 weeks[164]; the latter study also did not observed changes in blood glucose levels.

2.4.5. DDT/E and sex hormone levels

The literature investigating the effects of DDT/E on sex hormone levels and pubertal timing *in vivo* is sparse and inconsistent. Only two animal studies have examined the effects of chronic DDT exposure on estrogen[165]. In an experiment approximating the daily dietary intake of DDT by humans, rats administered 3 µg/kg of *o,p'*-DDT daily since birth had higher estrogen levels during puberty and adulthood compared to unexposed rats, but rats who had additionally been exposed throughout gestation had lower estrogen during puberty[165], suggesting that the timing of exposure

may be a significant factor the ability of DDT to disrupt estrogen levels. Among adult rats exposed for 10 weeks to *p,p'*-DDT at three different doses (approximately 0.34, 3.4 and 25 mg/kg among males and 0.37, 3.8, and 28 mg/kg among females), estradiol levels were lower among female rats in the two highest dose groups, but there was also evidence of systemic toxicity (tremors and death) at these doses[151].

A few additional studies examined effects on testosterone, though no clear patterns have emerged. Concerning DDT, four studies found no effect on testosterone levels at doses ranging from 0.34 to 100 mg/kg[151,166-168], including the study of 10-week exposure described above. In the chronic low-dose experiment described previously, rats exposed to 3 µg/kg of *o,p'*-DDT *in utero* and after birth had lower testosterone during puberty and adulthood; however, rats only exposed postnatally had higher testosterone during puberty, with no differences observed at adulthood[165], again pointing to the significance of prenatal exposure on the long-term endocrine disrupting effects of DDT.

Four studies administered DDE rather than DDT: two studies of short-term post-natal exposure (4 to 5 days) found no effect on testosterone levels[79,169], and two studies of longer-term post-natal exposure (3 to 9 weeks) found decreases in testosterone from relatively high doses of 10, 100, and 200 mg/kg[170,171]. This literature on DDE is therefore limited because none of these studies involve chronic prenatal exposure to environmentally relevant doses.

2.5. Literature Review: Pyrethroids

2.5.1. Pyrethroids and weight in humans

Several studies have investigated the relationship between pyrethroid exposure and weight, but only VHEMBE and two Chinese birth cohorts of slightly smaller sizes (n=454 and n=497) did so in relation to pyrethroid metabolites in addition to 3-PBA, while the three other studies measured only 3-PBA, which is a common metabolite of several pyrethroids and often detected at higher frequencies. Overall, these studies found mostly null associations at birth[172-175], 1 year[176,177], 2 years[177], 3.5 years[37] and 4 years of age[178], though birthweight was inversely related to *cis*-DCCA and positively associated with *trans*-DCCA in a Chinese study of 454 mother-and-child pairs[173]. In addition, three studies examined weight in related to self-reported use of pyrethroids, which was inversely associated with birthweight in two small Polish studies (n=104 and n=377)[179,180], but a large Japanese birth cohort (n=93,718) found no association between mothers' self-reported use of household pesticide products typically containing pyrethroids (e.g. mosquito coils or mats) and child weight at birth or one month[181]. This Japanese study was also the only study to examine pyrethroid exposure in relation to a dynamic measure of weight, reporting no association with infant weight change during the first month after birth. Further epidemiological studies with direct measurement of metabolites other than 3-PBA are needed in order to evaluate which set of, or specific, pyrethroids are associated with weight.

2.5.2. Pyrethroids, adiposity, and other cardiometabolic risk factors in humans

The extant literature on potential impacts of pyrethroids on adiposity and cardiometabolic health is very sparse; only two epidemiologic studies have examined the relationship between exposure to pyrethroids and adiposity in children and no studies have examined outcomes such as

waist circumference, body fat percentage, or blood pressure, though two studies have examined outcomes related to glucose tolerance in highly-exposed occupational settings.

The two studies of child adiposity reported conflicting findings, possibly related to the differences in study design and the lack of measurement of specific pyrethroid metabolites in one of the studies. In the VHEMBE cohort, maternal peripartum urinary concentrations of four pyrethroid metabolites (*cis*-DBCA, *cis*-DCCA, *trans*-DCCA, and 3-PBA) were inversely associated with BMI z-scores at 1, 2, and 3.5 years, particularly among boys[37,177], and in a South Korean birth cohort (n=578), concurrently measured child (but not peripartum maternal) urinary concentrations of 3-PBA were associated with greater BMI z-score at 4 years of age, among girls only[178].

In the two cross-sectional studies of Bolivian pesticide applicators[182] and Chinese pyrethroid factory workers[183], occupational exposure to pyrethroids was linked to higher odds of abnormal glucose regulation. Furthermore, in a 15-year follow-up of adult participants in a national U.S. biomonitoring study (n=2116), individuals with the highest versus lowest tertile of urinary 3-PBA concentrations had three times the risk of cardiovascular disease mortality (HR=3.0, 95% CI: 1.02, 8.80)[184], suggesting that pyrethroids can influence cardiometabolic health. These findings warrant further investigation of the relationship between pyrethroids and a more comprehensive range of cardiometabolic risk factors.

2.5.3. Pyrethroids and weight in animals

In most studies of mice and rats, postnatal[185-192] or gestational exposure[193-195] to pyrethroids ranging from 0.05 to 100 mg/kg did not affect overall body weight, though a few studies observed negative effects on weight. Adult rats administered a chronic low dose (1.28 mg/kg) of deltamethrin for 4 weeks had reduced food intake and less weight gain compared to unexposed

rats[196]; and pregnant mice administered 10 mg/kg deltamethrin for 1 week had lower gestational weight gain, though no effects were observed at lower doses (0.1, 1, 5 mg/kg) nor on fetal weight[195]. Two other studies of prenatal exposure also did not observe effects on offspring weight at low doses: in one study, gestational and lactational exposure of rats to a very high dose (25 mg/kg) of cypermethrin resulted in lower offspring weight from childhood to adulthood (day 7 to 75)[194], but not at lower doses (1 and 10 mg/kg); a similar study of gestational and lactational exposure of mice to low doses (1 and 3 mg/kg) of deltamethrin did not affect offspring body weight measured at 5 months[193]. In summary, the majority of animal studies showed no effect of pyrethroids on weight, and only two studies demonstrated a reduction in weight gain at a relatively low dose (1.28 mg/kg deltamethrin) and the other at a relatively high dose (10 mg/kg deltamethrin). Notably, all animal studies administered doses considerably higher than estimated to occur from normal dietary exposure in humans (2 to 3 μg/kg per day = 0.002 to 0.003 mg/kg per day)[89], therefore the animal evidence is inconclusive for effects on weight at relevant doses.

2.5.4. Pyrethroids and sex hormone levels

Experimental studies of mice exposed to high doses (5 to 100 mg/kg) of the pyrethroids permethrin, cypermethrin or deltamethrin resulted in lower testosterone levels[186,187,189,197-200], although lower doses (1 to 2 mg/kg) resulted in increases in testosterone levels[194,200]. Cross-sectional studies of adolescent and adult men found mostly null associations between urinary metabolite concentrations and serum testosterone levels[201-204], though inverse relationships between *trans*-DCCA and testosterone concentrations[205] as well as free androgen index[206] have been observed. These studies also found positive associations between pyrethroid metabolite concentrations and levels of luteinizing hormone[204,206,207] and follicle-stimulating hormone[206,207], which are positive regulators of sex hormones. Since increases in LH and FSH should result in

increases rather than decreases in testosterone, pyrethroids may be affecting the expression of testosterone through other pathways. For example, animal studies also indicate that chronic exposure to pyrethroids impairs cholesterol synthesis and transport[186,187,189,198,208], likely by inducing oxidative stress in the liver[188,196,209] where pyrethroids are metabolized and cholesterol is synthesized. Cholesterol is a precursor for all steroid hormones including testosterone, as well as other hormones involved in glucose, lipid, and energy metabolism. This may be a more important pathway by which pyrethroids can disrupt these carefully regulated processes and affect cardiometabolic health.

CHAPTER 3. Methodology

This chapter describes two major methodological areas relevant to this dissertation, providing some additional details and context not included in the Manuscripts, for the reader's reference:

- Section 3.1. discusses the measurement of cardiometabolic risk factors other than obesity/BMI, including the modelling of child growth trajectories;
- Section 3.2. describes the use of inverse probability weighting methods to account for confounding and selection bias.

3.1. Measuring cardiometabolic risk factors

3.1.1. Adiposity

Density-based measures are considered the gold standard estimators of body fat composition. These include hydrodensitometry, where a subject is weighed underwater, and air displacement plethysmography, where a subject enters a chamber that measures their volume and weight, and based on the known densities of lean and adipose tissue, their body fat and fat-free mass can be calculated[210]. However, these methods require large, specialized equipment and therefore are impractical for most clinical settings and population-based research. Radiology-based methods such as dual-energy X-ray absorptiometry (DXA), computed tomography (CT), and magnetic resonance imaging are also highly accurate and are commonly used as the reference standard in validation studies of other body fat measures. These methods can be used to further describe the distribution of body fat across specific organs and tissues[210]. However, since these methods involve large, specialized, and expensive equipment, their use is also limited to specific clinical or research settings. Moreover, DXA and CT scans involve small amounts of

ionizing radiation, exposure to which should be minimized in the absence of diagnostic or therapeutic benefits.

Although less accurate than gold standard measures, bioelectrical impedance analysis (BIA) estimates fat and fat-free mass based on the resistance encountered by an electrical current which is passed through the body, since unlike water-rich blood and muscle, body fat is a poor electrical conductor[211]. Portable devices using foot-to-foot BIA are readily available, relatively inexpensive, portable, and easy to use[210]. The BIA device used in Chapter 6 of this dissertation, the Tanita BF-689 (Tanita Corporation, Tokyo, Japan), uses an age- and sex-specific formula to determine body fat percentage based on the raw BIA value. Though these equations are proprietary and therefore unpublished, this device has been validated among children 5 to 11 years of age, showing moderately high correlation with estimates of body fat percentage using DXA (0.786 among boys and 0.764 among girls), though on average it underestimated body fat by 6.75% compared to DXA[212].

Body mass index (BMI), calculated as weight in kilograms divided by height in metres squared, is the most widely used measure of adiposity in population health research[210]. Like other anthropometric measures, it is simple, non-invasive, portable, and inexpensive. It correlates moderately well with gold-standard measures of adiposity such as hydrodensitometry and DXA[213,214]. However, as it is a measure of excess weight rather than excess fat, individuals with high muscle mass and low body fat may incorrectly be characterized as having high adiposity using this measure. Waist circumference, an anthropometric measure of abdominal adiposity, is now considered a critical clinical indicator of cardiometabolic health risks[215], and is strongly associated with cardiovascular disease[216], stroke[217], certain cancers[218], all-cause mortality[219-221] and cardiovascular mortality[222,223].

3.1.2. Metabolic syndrome

Metabolic syndrome is a cluster of cardiometabolic risk factors associated with approximately two-fold increased risk for cardiovascular disease, myocardial infarction, and stroke, and 1.5-fold increased risk for all-cause mortality[8]. The international harmonized consensus definition of metabolic syndrome requires at least three of the following five criteria to be met: abdominal obesity, high triglycerides, low HDL cholesterol, high fasting blood glucose, and high blood pressure[224]. Each criterion is met either by exceeding threshold values (Table 3.1.1) or by the use of medication intended to treat it. For children under 10 years of age, metabolic syndrome cannot be diagnosed[225]; therefore, in this dissertation, other measures were used to capture their cardiometabolic risk.

Table 3.1.1. Criteria in the definition of Metabolic Syndrome[224]

Criterion	Cut-off for males	Cut-off for females
Abdominal obesity	Waist circumference ≥102 cm	Waist circumference ≥88 cm
Low HDL cholesterol	<1.0 mmol/L	<1.3 mmol/L
High serum triglycerides	≥1.7 mmol/L	≥1.7 mmol/L
High fasting blood glucose	≥5.6 mmol/L	≥5.6 mmol/L
High blood pressure	≥130/85 mmHg	≥130/85 mmHg

3.1.3. Child growth

Although weight measured at specific ages remains an important marker of child development, dynamic child growth metrics based on measures taken at two or more time points have been found to predict later metabolic and cardiovascular health better than single time point measures[35,36]. This literature originated with studies linking low birth weight and accelerated childhood growth with higher risk for cardiovascular disease and diabetes in adulthood[226-228], giving rise to what is now called the Developmental Origins of Health and Disease (DOHaD) hypothesis, which posits that exposures in early life program maladaptive responses to the

environment (through epigenetic modifications or changes in tissue development or other biological processes) which predisposes individuals to developing chronic disease in later life[229].

Since then, the literature has consistently demonstrated links between weight gain during early life and the risk of obesity in later childhood, adolescence, and adulthood; a meta-analysis of 17 studies reported that rapid infant weight gain, defined as a change in weight-for-age z-score of ≥0.67 SD during the first two years of life, is associated with 3.7-fold (95% CI: 2.6 – 5.2) increased risk for overweight or obesity at 2 to 46 years[230]. Evidence for a link between childhood weight gain and impaired cardiometabolic health in adulthood is also growing[231-235]. A recent review found that greater weight or BMI gains in infancy increases fat mass and preferentially contributes to central adiposity[231], and several studies have shown associations between greater childhood BMI or weight gain and hypertension in adulthood[233-240], including a pooled analysis of 5 birth cohorts from Brazil, Guatemala, India, the Philippines, and South Africa[240]; associations with lower levels of cardio-protective HDL cholesterol[238], insulin resistance and/or diabetes[233,238,241,242], and metabolic syndome[233] in adulthood have also been observed.

The literature on dynamic measures of child growth, that is, measures of weight, height, or BMI at two or more timepoints, relies primarily on a metric of change between two time points (e.g. weight change from birth to one month, six months, or 24 months), which does not capture the complex nature of growth[243]. Some studies with multiple growth measurements over time use a grouping-based classification based on latent growth patterns[136], which categorizes children into one of a few types of growth patterns; however, the selection of the number and interpretation of the observed latent patterns is inherently subjective, loss of information can occur due to the categorization of the outcome, and observed patterns may not be directly

comparable between studies, which poses challenges for inference[35,244]. Some more sophisticated growth trajectory models which estimate individual-specific growth parameters exist. Flexible mixed-effects models such as SuperImposition, Translation And Rotation (SITAR) models overcome the limitations of simpler models while also allowing for flexibility in the timing and number of measurements for each child. Also, compared to parametric mixed-effects growth models, spline-based SITAR can lead to better model fit[245,246]. Furthermore, unlike other models, the random effects SITAR parameters have a straightforward biological interpretation, comparing the weight (size parameter), as well as the timing (tempo) and velocity (intensity) of the growth spurt, to the average child[247,248].

SuperImposition, Translation And Rotation (SITAR)

SITAR is a flexible mixed effects model which can be used to model weight, height, or BMI trajectories[248]. It fits a natural cubic spline to the population average growth curve (e.g. weight as a function of age), and individual-specific deviations from this mean curve are captured by three normally-distributed random-effects parameters with a mean of zero, where:

$$y_{it} = \alpha_i + h\left(\frac{t - \beta_i}{exp(-c\gamma_i)}\right)$$

a) Size (α) indicates the child's mean weight compared to the average (e.g. in kilograms), representing vertical translation of the weight curve;
b) Tempo (β) indicates the child's age at peak weight velocity compared to the average (e.g. in months), representing horizontal translation of the weight curve; and,
c) Intensity (γ) indicates the child's peak weight velocity relative to population mean, which can be considered as a horizontal stretching/compression of the growth curve (see Figure 1).

Additional model specifications include: number of degrees of freedom of the cubic spline, scale of the y- and x- axes (e.g. untransformed, log-transformed), and which of the random-effects parameters. The best-fitting model can be determined by a number of diagnostics: low Akaike and Bayesian information criterion (adjusted for log-transformation, if necessary), low correlation between random effects, and high variance explained (R^2 generalized to mixed effects models)[249,250]. The complexity of the best-fitting model will depend on the nature of the trajectory as well as the availability and quality of data. For example, frequent measurements may support a more complex model, but relatively infrequent measurements will likely lead to a simpler model even if the true trajectory is more complex, and complex models with too many random effects parameters may not converge. Furthermore, methods exist to identify and remove implausible measurements which may represent errors in data collection[251].

The individual-specific SITAR parameters can be used as predictors of health outcomes in later life, and have been linked to greater childhood weight[252,253], higher fat mass in young adulthood[254], higher BMI and body fat at over 65 years of age[255] and even bone and joint health in adulthood[256,257]. There is also a significant interest in understanding the influences on these growth trajectory parameters themselves; much of this literature has focused on maternal factors such as BMI, gestational weight gain, diabetes, socioeconomic status, education, and smoking[258-261], and breastfeeding[262]. However, no studies of environmental chemical influences on SITAR growth trajectory parameters have been published.

3.2. Inverse probability weighting methods

Inverse probability weighting can be used to adjust for selection bias and confounding, and estimate average causal effects of exposure in the study population using marginal structural models[263,264]. In this method, each subject is weighted based on the probability of the censoring status and exposure (which is often referred to as "treatment" in this literature) they received, creating a pseudo-population in which exposure and baseline covariates are independent of censoring status, and confounders are independent of exposure. When incorporating multiple weights (e.g. for censoring and for treatment), the product of the weights is used. Under the three identifiability assumptions of consistency, exchangeability and positivity, and assuming no misspecification of the models used to estimate the weights, the resulting estimates have a causal interpretation.

Covariate selection and model specification for the inverse probability weights are conducted independent of the outcome, therefore this method overcomes the limitations of confounder selection strategies in traditional multivariable regression models such as univariate analysis, stepwise approaches, and change-in-estimate which can bias estimates and result in inaccurate confidence intervals.[39-41] Additionally, the independence of exposure, censoring, and baseline covariates can be verified in an iterative process until balance is achieved (see Section 4.3.3), meaning that selection bias and confounding from measured covariates has been addressed.

3.2.1. Inverse probability of censoring weights (IPCWs)

Inverse probability of censoring weights (IPCWs) can be derived from a logistic regression model of the probability of being censored, conditional on predictors of censoring status such as the exposure(s) and baseline covariates. The IPCW for each subject is the inverse of the probability of their censoring status received, i.e. $\frac{1-C}{\Pr(C=0|A,Z)} + \frac{C}{\Pr(C=1|A,Z)}$, where C is the censoring status (0, not censored, or 1, censored), A is the exposure, and Z is the vector of baseline covariates. Very low probabilities of not being censored or very high probabilities of censoring can result in large weights, which can increase the variance of the estimates; stabilizing weights with the marginal probabilities of the censoring status received, i.e. $\frac{(1-\mathrm{C})\times \Pr(C=0)}{\Pr(C=0|A,Z)} + \frac{C\times Pr(C=1)}{\Pr(C=1|A,Z)}$, is preferred in order to achieve greater efficiency[263].

At the analysis stage, the weight of censored subjects is set to zero, thereby including in the outcome regression model only subjects for whom follow-up is complete. However, constructing the weights for both censored and uncensored individuals is required in order to assess whether exposures and baseline covariates are successfully balanced after conditioning on the IPCW (see Section 4.3.3).

3.2.2. Inverse probability of treatment weights (IPTWs)

Inverse probability of treatment weights (IPTWs), where treatment refers to exposure status, are derived similarly to IPCWs when the exposure is binary. When the exposure is continuous, the generalized propensity score method can instead be used to model the denominator of the IPTWs[265]. This method estimates the conditional density, rather than probability, of the exposure the subject received, based on the predicted exposure distribution whose parameters are estimated by multivariable linear regression (conditional on confounders).

Similarly, the IPTWs can be stabilized using the marginal exposure density: $\frac{f(A)}{f(A|L)}$, where A is the exposure, and L is the vector of confounders. In addition, L can include predictors of the outcome, which will improve statistical efficiency of the final estimate.

To examine effect modification, the IPTW can instead be stabilized by the exposure density conditional on the effect modifier, i.e. $\frac{f(A|M)}{f(A|L)}$, where M is included in the vector L, and the outcome regression should include terms for the exposure, effect modifier, and their interaction.

3.2.3. Balance assessment of inverse probability weights

A number of diagnostics can be used to evaluate the appropriateness and success of the weights at achieving balance (i.e. similar distributions of covariates) across censoring status or exposure. In other words, these diagnostics can verify that in the weighted sample, censoring is independent of exposure and baseline covariates (thus, addressing selection bias), and that exposure is independent of potential confounders (thus, addressing confounding from measured covariates).

Firstly, the distribution of stabilized weights should be examined for the presence of extreme weights or a mean value that departs from one, which would indicate potential positivity violations or a mis-specified model[263]. Then, standardized differences which compare the means (if continuous) or proportions (if binary) of each covariate across censoring status (for IPCW) or a binary exposure variable (for IPTW) are the main diagnostic tool for assessing balance in the weighted sample[266]; a standardized difference below 0.1 is considered an indication that the covariate is balanced with respect to censoring or exposure. Variance ratios can also be calculated, comparing the variance of the covariate in each censoring or exposure group; by definition,

variance ratios of 1.0 describes a covariate with equal variance in both groups, and a threshold of <2.0 has been suggested to indicate balance.[267] If these diagnostics produce an unsatisfactory result, the models can be adjusted in an iterative process to produce new weights, and persistent extreme weights can be truncated or trimmed[266].

Balance diagnostics in the continuous exposure context

Balance diagnostics based on standardized differences and variance ratios can be adapted to the continuous exposure context by discretizing the exposure variable into quantiles, as in approaches suggested by Hirano and Imbens (2005)[265], Bia and Mattei (2008)[268], and Austin (2019)[269]; in this dissertation, quartiles were used in order to avoid finite sample bias. For each covariate, the standardized differences and variance ratios comparing each quartile to all other quartiles can be calculated, then averaged across the four comparisons, yielding a single mean estimate for each covariate. These mean standardized differences can be expected to vary due to the small sample sizes in each exposure quartile, therefore a threshold of <0.2 has been suggested to indicate balance[269]. Finally, weighted Pearson correlation coefficients can be calculated specifically for continuous covariates in the continuous exposure context, and a threshold of <0.1 can be used to indicate balance[269].

CHAPTER 4. Manuscript 1

4.1. Preface

This chapter contains the first of the three manuscripts in this dissertation examining the potential influence of endocrine-disrupting chemicals on cardiometabolic health.

Parabens are a group of endocrine-disrupting chemicals commonly used as preservative agents in personal care, food, and pharmaceutical products. They are detected in >90% of Canadians, thus their endocrine-disrupting effects could have widespread implications in this population. Despite evidence that they are estrogenic and anti-androgenic, thus affecting hormones which influence adiposity and cardiometabolic health, only two epidemiological studies have examined their associations with obesity, and none have investigated their associations with metabolic syndrome.

This manuscript presents an analysis of urinary concentrations of parabens in relation to the prevalence of obesity and metabolic syndrome in a nationally representative cross-sectional biomonitoring survey in Canada. This manuscript was peer-reviewed and published in *Science of the Total Environment*.

Citation: Kim J and Chevrier J. Exposure to parabens and prevalence of obesity and metabolic syndrome: An analysis of the Canadian Health Measures Survey. *Science of the Total Environment* 2020;713:135116. doi: 10.1016/j.scitotenv.2019.135116.

4.2. Exposure to parabens and prevalence of obesity and metabolic syndrome: An analysis of the Canadian Health Measures Survey

Abstract

Background: Parabens are widely used preservative agents in the cosmetic, food, and pharmaceutical industries. They are estrogenic and anti-androgenic, and thus have the potential to alter the hormonal regulation of energy metabolism, and in turn affect obesity and metabolic health. Compared to obesity alone, having metabolic syndrome (a cluster of cardiometabolic risk factors) further increases the risk of cardiovascular disease, diabetes, and certain cancers. We examined whether exposure to parabens was associated with obesity, metabolic syndrome or its components in the Canadian population.

Methods: Methyl, ethyl, propyl, and butyl paraben concentrations were measured in the urine of 2,564 individuals participating in Cycle 4 (2014 – 2015) of the Canadian Health Measures Survey, a national biomonitoring survey. We assessed associations between specific gravity-corrected log10-transformed paraben concentrations and obesity, metabolic syndrome and its components (waist circumference, HDL cholesterol, triglycerides, fasting blood glucose and blood pressure) via Poisson regression with robust variance estimators for binary outcomes and via linear regression for outcomes expressed continuously. We stratified analyses by age (children aged 3 to 17 years vs. adults aged 18 years and older) and investigated the presence of effect modification by sex.

Results: A 10-fold increase in propyl paraben concentration was associated with a 40% (95% CI: 3, 90) higher prevalence of metabolic syndrome among men, while ethyl paraben was associated with a 63% (95% CI: 2, 86) lower prevalence among women. Among women, methyl paraben was inversely associated with obesity, and methyl, propyl and ethyl parabens were

associated with higher high density lipoprotein (HDL) cholesterol. No associations were observed among children.

Conclusions: This is the first study to report a positive association between parabens and metabolic syndrome in men. Protective associations among women previously reported for obesity were also observed for metabolic syndrome and HDL cholesterol. These results should be confirmed in longitudinal studies.

Introduction

Obesity affects 670 million adults and 125 million children worldwide[1]. Obesity may progress into the metabolic syndrome, a cluster of conditions that includes abdominal obesity, dyslipidemia, hyperglycemia, and hypertension[224]. Compared to obesity alone, metabolic syndrome further raises the risk of developing cardiovascular disease, type 2 diabetes, and certain cancers[8,270]. While one in four Canadian adults are obese[4], approximately one in five have metabolic syndrome[271]. Although age, genetics, diet and physical activity are major determinants of these conditions[224], growing evidence suggests that endocrine disrupting chemicals such as parabens may also play a role by interfering with the hormonal regulation of energy balance[12,272].

Parabens are a group of suspected endocrine disrupting chemicals that are widely used as preservative agents in cosmetics, pharmaceuticals, and food products[273,274]. Environmental exposure to parabens occurs through dermal absorption or ingestion of paraben-containing products; these compounds are then readily metabolized in the liver and excreted in urine within 24 to 48 hours[43,44,275]. Despite this, detection frequencies are above 90% in Canada, Europe and the United States (U.S.)[276], suggesting widespread and frequent exposure to parabens.

Evidence from *in vitro* studies shows that parabens can bind to estrogen receptors[55,57-59,63] and inhibit estrogen sulfotransferase, an enzyme that inactivates estrogens[66]. These chemicals also have anti-androgenic effects, reducing testosterone secretion in animals[277,278]. In adult humans, estrogens protect against obesity and metabolic syndrome through a number of mechanisms, including suppressing appetite[94], increasing basal energy expenditure[95,96], converting white fat into healthier brown fat[99,100], and favoring subcutaneous over visceral fat deposition[99]. Both white fat and visceral fat are linked to insulin resistance and dyslipidemia (high triglycerides, high low-density lipoprotein [LDL] cholesterol, and low high-density

lipoprotein [HDL] cholesterol)[279-281]. Testosterone also protects against obesity and metabolic disease in males, due to its conversion into estrogen[103-105]. However, the effects of sex hormones may differ in children and adolescents, relative to adults. In boys, testosterone acts synergistically with growth hormone to reduce fat stores[116], while in girls estrogens counteract the lipolytic effects of growth hormone, leading to increases in body fat mass[117].

Only two studies examined whether exposure to parabens is associated with adiposity in adults: inverse associations with body mass index (BMI) and waist circumference, which were stronger among women than men, were found among U.S. adults participating in the National Health and Nutrition Examination Survey (NHANES)[28] but no associations were found with BMI in a small sample (n=27) of Czech adults[27]. Among children, four studies have examined associations between exposure to parabens and obesity and results have been inconsistent[24-26,28].

Despite the additional risks posed by metabolic syndrome relative to obesity, to our knowledge no human study has investigated the potential link between paraben exposure and metabolic syndrome in adults or with indicators of metabolic health in children. Additionally, no previous study on parabens and obesity has been conducted in Canada. Our objective was therefore to examine whether exposure to parabens is associated with obesity and metabolic syndrome in a large sample of the general Canadian population.

Methods

Data source and study population

The Canadian Health Measures Survey (CHMS) is a national biomonitoring survey conducted by Statistics Canada in collaboration with Health Canada and the Public Health Agency of Canada that includes a sample representative of non-institutionalized Canadians aged 3 to 79 years of age living in the ten provinces[282]. Details of its sampling design and data collection methods have been published previously[91,283,284].

Briefly, the CHMS uses a stratified three-stage sample of one or two respondents from selected dwellings in a selected collection site. An extensive questionnaire is administered during a home visit, followed by an assessment in a mobile clinic where physical measures and biological samples are collected. The survey, which is administered every two years, has a target sample size of approximately 5,700 participants per cycle. In each cycle, selected environmental chemicals are quantified in blood and urine collected from an age-stratified random subsample of approximately 2,500 participants (environmental subsample), while an independent random subsample of approximately 2,500 participants provides fasted blood samples (fasted subsample). Ethics approval for the CHMS was obtained from the Health Canada and Public Health Agency of Canada Research Ethics Boards. Participants older than 14 years of age gave informed written consent; for younger children, a parent or legal guardian provided written consent, and children 6 to 13 years of age provided assent[285].

Among all currently available cycles, parabens were measured in the environmental subsample of Cycle 4 (2014-2015). We excluded pregnant participants, resulting in a total sample size of 2564 (1137 adults and 1418 children) for the analyses involving BMI, waist circumference, HDL cholesterol and blood pressure. Analyses involving fasted outcome

measures (glucose, triglycerides and metabolic syndrome) were conducted among participants included in both the environmental and fasted subsamples (385 adults and 573 children).

Assessment of exposure to parabens

At the clinic visit, spot urine samples were collected, processed, aliquoted, and stored at -30°C until being shipped on dry ice to the reference laboratory for analysis. Concentrations of four parabens (methyl, butyl, ethyl, and propyl paraben) were measured in urine via ultrahigh-performance liquid chromatography-high resolution mass spectrometry[286] at the Western Region Health Canada Laboratory (Burnaby, BC), with detection limits of 1.3, 0.3, 0.9, and 0.3 μg/L, respectively. Urine specific gravity was measured on-site at the mobile clinic with a portable refractometer (Atago PAL-10S, Atago. U.S.A. Inc., Bellevue, WA, USA).

Assessment of obesity and metabolic syndrome

At the mobile clinic visit, height[287], weight[288] and waist circumference[289] of participants were measured using standardized protocols. Blood pressure was taken using an automated oscillometric blood pressure measurement device, and the last five of six measurements were averaged. HDL cholesterol[290] in the serum of all participants, and triglycerides[291] and glucose[292] in venous blood from fasted participants, were quantified at the Health Canada Nutrition Laboratory (Ottawa, ON). At the home visit, medication use in the past month was recorded and coded based on the bottles and containers presented by the participant.

BMI was calculated by dividing body weight in kilograms by height in metres squared. Participants within the fasted subsample were classified as having metabolic syndrome based on the international harmonized consensus definition of meeting at least three of the following five criteria: abdominal obesity, high triglycerides, low HDL cholesterol, high fasting blood glucose, and high blood pressure[224]. Each criterion could be met either by exceeding threshold values

(Table 4.2.1) or by the use of medication intended to treat it. Anatomical Therapeutic Chemical medication codes used to treat each criterion are listed in the Supplementary Table 4.3.1. In children, age- and sex-standardized BMI z-scores were calculated based on World Health Organization 2006 child growth standards[293], which were applied using the igrowup and AnthroPlus STATA macros. Obesity in children was defined as BMI greater than the 95th percentile. Since there is no consensus on whether and how to define metabolic syndrome or on clinically relevant cut-points for each criterion in children, metabolic syndrome was not evaluated but its components were examined as continuous measures[225].

Table 4.2.1. Criteria in the definition of Metabolic Syndrome[224]

Criterion	Cut-off for males	Cut-off for females
Abdominal obesity	Waist circumference ≥102 cm	Waist circumference ≥88 cm
Low HDL cholesterol	<1.0 mmol/L	<1.3 mmol/L
High serum triglycerides	≥1.7 mmol/L	≥1.7 mmol/L
High fasting blood glucose	≥5.6 mmol/L	≥5.6 mmol/L
High blood pressure	≥130/85 mmHg	≥130/85 mmHg

Covariate data

The household questionnaire collected information on a variety of sociodemographic, lifestyle, and nutrition information. Reported total household income was divided by the equivalence scale (square root of the number of household members) to adjust for household size[294], and the highest education level attained by a household member was also determined. Participants were asked how often they consumed 45 food items in the unit of their choosing (times per day, week, month or year), but total caloric, macro or micronutrient intake could not be derived; instead, we created a summary measure of poor diet quality by summing intake of red meat, bacon, hot dogs, fries, chips, and soda (times per year).

At the clinic visit, all participants were given an Actical accelerometer (Phillips – Respironics, OR, USA), which produces an index of physical activity intensity measured in total acceleration "counts" per minute. Total minutes of moderate-to-vigorous physical activity, corresponding to at least 1,500 counts per minute for adults[295] and 1,535 counts per minute for adolescents[296], was calculated for participants with at least four valid days (>10 hours of wear time per day), then averaged over valid days[297].

Statistical analysis

Paraben concentrations (C_{meas}) were corrected (C_{corr}) for urine dilution via specific gravity (SG), based on an adapted formula from Levine and Fahy (1984): $C_{corr} = C_{meas} \times (SG_{mean} - 1)/(SG - 1)$, where the population mean specific gravity (SG_{mean}) is used for standardization. The distribution of paraben concentrations was highly right-skewed. Values were $\log_{10}$-transformed to reduce the influence of outliers. For parabens with detection frequencies above 70%, we imputed values below the limit of detection at random based on a lognormal probability distribution whose parameters were estimated via maximum likelihood estimation[298]. Parabens detected with less than 70% frequency were dichotomized using the detection limit as the cut-off. We also calculated a molar sum of parabens, expressed as propyl paraben[25,28].

Obesity, metabolic syndrome, and each criterion of metabolic syndrome (Table 4.2.1) were modelled as binary outcomes in modified Poisson regression models with robust variance estimators[299]. BMI and each component of metabolic syndrome were also analyzed as continuous outcomes in multivariable linear regression models, excluding participants taking medication used to treat the component (Supplementary Table 4.3.1). We identified the following potential confounders using directed acyclic graphs[300], and included them as co-variates in multivariable regression models: sex (male versus female), adjusted total household

income (continuous), highest household education (high-school or less versus post-secondary diploma/degree), age at clinic visit (continuous), ethnicity (white versus non-white), smoking status (current/former versus never), poor diet index (continuous), and minutes of moderate or vigorous physical activity per day (continuous). Due to the sampling design of the CHMS, the number of degrees of freedom available for multivariable analysis was restricted to eleven[282].

All models were run separately for adults (18 years of age and older) and children (ages 3 to 17 years) due to the possible different effects of parabens during different periods of life. For methyl and propyl paraben, the linearity assumption was examined visually using restricted cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles[301]. The presence of effect measure modification by sex was investigated by including an interaction term between parabens and sex.

Survey weights accounting for the probability of selection into the survey and non-response, along with 500 bootstrap sampling weights for variance estimation, were provided by Statistics Canada. As a sensitivity analysis, the regressions involving metabolic syndrome and fasted triglycerides and fasted glucose were also run using the survey and sampling weights for the fasted subsample, under the recommendation of Statistics Canada (T. Bushnik and A. Mather, personal communication, May 2019). All analyses reported here used the survey and sampling weights specific to the CHMS Cycle 4 subsample in which environmental chemicals were measured, and were conducted in Stata 15 (StataCorp, College Station, TX, USA).

Results

Participant characteristics

After weighting, the study population was balanced with respect to sex. Adult participants had a mean age of 46 years (standard error [SE]: 0.3 years) and children had a mean age of 10 years (SE: 0.1 years). The majority of the population was white (78% of adults and 66% of children). Most adults had a post-secondary education (82%), and just over half of the adults reported being a current or former smoker (Table 4.2.2).

Almost all participants had detectable levels of methyl (92%) and propyl (79%) parabens, and fewer had detectable levels of ethyl (42%) and butyl (19%) parabens. Methyl and propyl paraben were moderately correlated (ρ=0.56, $p < 0.05$) with specific gravity-corrected geometric mean concentrations of 18.5 μg/L and 2.7 μg/L, respectively. Concentrations of methyl paraben were higher and more variable in adults (GM: 21.4 ug/L, interquartile range [IQR]: 109.8 μg/L) than in children (GM: 9.2 ug/L, IQR: 19.0 ug/L); among adults, concentrations were higher and more variable in females (GM: 45.8 μg/L, IQR: 205.2 μg/L) than in men (GM: 10.1 μg/L, IQR: 46.6 μg/L). Similar patterns were observed for propyl paraben (data not shown).

In adults, the prevalence of obesity and metabolic syndrome were 30% (95% CI: 24, 35) and 25% (95% CI: 19, 30), respectively (Table 4.2.2). Among metabolic syndrome components, abdominal obesity (50%; 95% CI: 45, 56) and high triglycerides (38%; 95% CI: 30, 46) were most common. About one in ten children were obese (11%, 95% CI: 8, 14).

Table 4.2.2. Population characteristics

	Adults			Children		
	Binary	*Continuous*		*Binary*	*Continuous*	
	Weighted %	Weighted mean	SE[a]	Weighted %	Weighted mean	SE[a]
Sex, female	50%			51%		
Age (years)		46.2	0.3		10.1	0.1
Adjusted household income ($CAD)		53438.4	2109.0		51246.1	2017.1
Ethnicity, white	78%			66%		
Education, >highschool	82%			86%		
Current or former smoker	52%					
Moderate/vigorous activity (min/day)		16.8	1.4		47.5	2.1
Poor diet consumption (times/year)[b]		444.2	23.4		420.7	13.3
Outcomes						
BMI (kg/m^2)	30%[c]	27.8	0.3	11%[c]	19.2	0.2
Metabolic syndrome[i]	25%					
Waist circumference (cm)	50%[d]	96.2	0.8		66.4	0.5
HDL cholesterol (mmol/L)	31%[e]	1.3	0.02		1.3	0.02
Triglycerides (mmol/L)[i]	38%[f]	1.5	0.04		0.9	0.04
Blood glucose (mmol/L)[i]	14%[g]	5.3	0.1		4.8	0.03
Diastolic blood pressure (mmHg)	31%[h]	113.9	1.1		97.0	0.5
Systolic blood pressure (mmHg)		73.2	0.6		62.8	0.4

[a]Bootstrapped standard error;
[b]Sum of red meat, bacon, hot dogs, fries, chips, and soda;
Binary criteria met by the following thresholds or medication taken to treat it (Supplementary Table 4.3.1):
[c]BMI ≥ 30 kg/m^2 (adults), >95th percentile BMI (children);
[d]Waist circumference ≥102 cm (men), ≥88 cm (women);
[e]HDL cholesterol <1.0 mmol/L (men), <1.3 mmol/L (women);
[f]Triglycerides ≥ 1.7 mmol/L;
[g]Blood glucose ≥5.6 mmol/L;
[h]Blood pressure ≥130/85 mmHg;
[i]Measured in the fasted subsample only

Associations between parabens and obesity and metabolic syndrome among adults

Among adults, all paraben measures were associated with a reduced prevalence of low HDL cholesterol in Poisson models (Figure 4.2.1 and Supplementary Table 4.3.2) and detectable ethyl paraben concentrations were associated with higher HDL cholesterol in linear regression models (Table 4.2.3). After stratification by sex, we observed a 40% (95% CI: 3, 90) higher prevalence of metabolic syndrome among men per 10-fold increase in propyl paraben concentration and slightly higher prevalence for methyl and butyl parabens (Figure 4.2.1, Supplementary Table 4.3.3). Other associations were generally null among men. In women, there was a general pattern of inverse associations between parabens and obesity, metabolic syndrome, waist circumference and HDL cholesterol. For instance, we observed a 38% (95% CI: 14, 56) lower prevalence of obesity and 1.5 kg/m^2 (95% CI: 0.4, 2.7) lower BMI per 10-fold increase in methyl paraben concentration, as well as a 63% (95% CI: 2, 86) lower prevalence of metabolic syndrome for detectable levels of ethyl paraben (Figure 4.2.1 and Supplementary Table 4.3.2). In addition, methyl, propyl, and ethyl parabens were all inversely associated with prevalence of low HDL cholesterol, ranging from a 22% (95% CI: 12, 31) decrease per 10-fold increase in propyl paraben to a 49% (95% CI: 22, 67) decrease for detectable levels of ethyl paraben (Figure 4.2.1 and Supplementary Table 4.3.2). When examined continuously, these parabens were associated with 0.05 to 0.17 mmol/L higher levels of HDL cholesterol (Table 4.2.4).

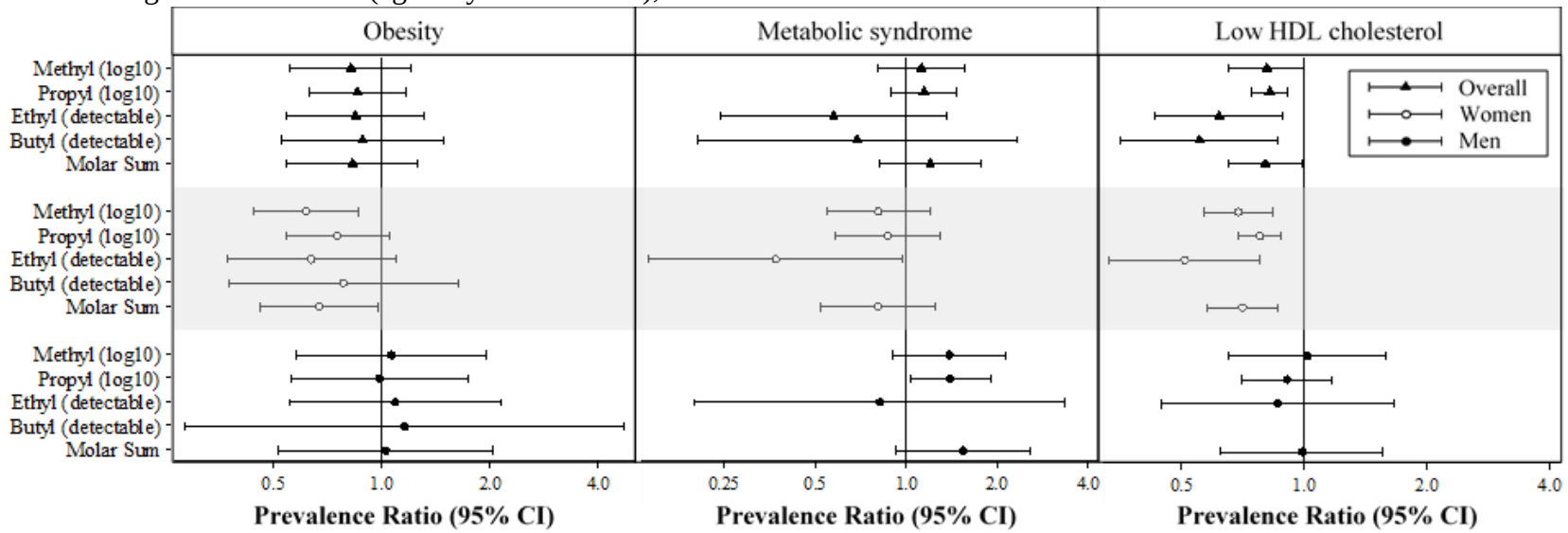
There were no associations with glucose or blood pressure. Sex-specific associations between butyl paraben and fasted binary outcomes (metabolic syndrome, low HDL cholesterol, and high blood glucose) and high triglycerides are not presented because models did not converge due to the low prevalence of detectable butyl paraben and the limited sample size of the

fasted subsample. The use of fasted instead of environmental subsample weights did not qualitatively change these findings (data not shown).

Associations between parabens and obesity and metabolic syndrome among children

Among children, most associations were null (Supplementary Tables 4.3.4 and 4.3.5). However, the general pattern observed for obesity, BMI z-score, waist circumference, and HDL cholesterol were the opposite of those observed in adults: positive trends were observed among girls and negative trends were found in boys. There was statistical evidence of effect measure modification by sex for obesity, but sex-specific estimates were imprecise and crossed the null.

Figure 4.2.1. Associations between urinary paraben concentrations and prevalence of obesity, metabolic syndrome and low HDL cholesterol among Canadian adults (age 18 years and over), 2014 – 2015



Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, smoking status, minutes of moderate or vigorous physical activity per day, and poor diet index; sex-specific estimates for butyl paraben and metabolic syndrome and low HDL cholesterol are not presented because models did not converge. See Supplementary Tables 4.3.2 and 4.3.3 for results in tabular format.

Table 4.2.3. Overall associations between urinary paraben concentrations and BMI and metabolic syndrome components expressed continuously among Canadian adults (age 18 years and over), 2014 – 2015

	BMI (kg/m^2) [β (95% CI)]	**Waist circumference (cm)** [β (95% CI)]	**HDL cholesterol (mmol/L)** [β (95% CI)]	**Triglycerides (mmol/L)** [β (95% CI)]
Methyl (log10)	-0.55 (-1.47, 0.37)	-0.86 (-3.28, 1.55)	0.02 (-0.02, 0.07)	0.08 (-0.11, 0.28)
Propyl (log10)	-0.16 (-1.02, 0.69)	-0.12 (-2.33, 2.09)	0.03 (0.00, 0.07)	0.05 (-0.05, 0.16)
Ethyl (detectable)	-0.70 (-2.17, 0.77)	-1.90 (-5.37, 1.58)	0.01 (0.01, 0.20)[a]	-0.05 (-0.44, 0.35)
Butyl (detectable)	-0.94 (-2.88, 0.99)	-2.25 (-6.54, 2.04)	0.07 (-0.03, 0.17)	0.00 (-0.28, 0.29)
Molar Sum (log10)	-0.42 (-1.54, 0.70)	-0.49 (-3.34, 2.36)	0.02 (-0.02, 0.06)	0.11 (-0.09, 0.31)

	Glucose (mmol/L) [β (95% CI)]	**Systolic blood pressure (mmHg)** [β (95% CI)]	**Diastolic blood pressure (mmHg)** [β (95% CI)]
Methyl (log10)	-0.01 (-0.08, 0.06)	0.39 (-2.12, 2.90)	-0.03 (-1.34, 1.29)
Propyl (log10)	-0.01 (-0.06, 0.04)	0.83 (-1.32, 2.98)	0.09 (-1.14, 1.32)
Ethyl (detectable)	-0.01 (-0.18, 0.17)	0.40 (-2.44, 3.23)	0.75 (-1.54, 3.05)
Butyl (detectable)	-0.02 (-0.19, 0.15)	-2.37 (-8.39, 3.65)	-1.51 (-6.71, 3.70)
Molar Sum (log10)	-0.01 (-0.08, 0.07)	0.28 (-2.18, 2.75)	-0.12 (-1.41, 1.17)

Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, smoking status, minutes of moderate or vigorous physical activity per day, and poor diet index; [a]$p<0.05$

Table 4.2.4. Associations between urinary paraben concentrations and BMI and metabolic syndrome components expressed continuously among Canadian adults (age 18 years and over), stratified by sex, 2014 – 2015

	BMI (kg/m^2)			**Waist circumference (cm)**		
	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]
Methyl (log10)	-1.53 (-2.66, -0.40)[b]	0.42 (-0.69, 1.52)	0.01[c]	-2.92 (-5.87, 0.02)	1.16 (-1.81, 4.14)	0.03[c]
Propyl (log10)	-0.71 (-1.88, 0.45)	0.51 (-0.61, 1.63)	0.11	-1.33 (-4.21, 1.55)	1.37 (-1.62, 4.37)	0.15
Ethyl (detectable)	-2.16 (-3.98, -0.34)[b]	0.84 (-0.87, 2.55)	0.00[c]	-5.34 (-9.35, -1.32)[b]	1.74 (-3.13, 6.61)	0.02[c]
Butyl (detectable)	-1.24 (-3.19, 0.70)	-0.11 (-2.74, 2.52)	0.29	-2.62 (-6.86, 1.62)	-1.22 (-9.40, 6.96)	0.71
Molar Sum (log10)	-1.26 (-2.66, 0.14)	0.47 (-0.92, 1.86)	0.05[c]	-2.25 (-5.59, 1.08)	1.39 (-2.40, 5.18)	0.10[c]
	HDL cholesterol (mmol/L)			**Triglycerides (mmol/L)**		
	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]
Methyl (log10)	0.05 (0.00, 0.11)[b]	-0.01 (-0.06, 0.05)	0.10[c]	0.05 (-0.11, 0.21)	0.05 (0.00, 0.11)[b]	0.64
Propyl (log10)	0.07 (0.02, 0.11)[b]	-0.01 (-0.06, 0.03)	0.01[c]	-0.01 (-0.21, 0.19)	0.07 (0.02, 0.11)[b]	0.51
Ethyl (detectable)	0.17 (0.01, 0.34)[b]	0.03 (-0.07, 0.14)	0.13	-0.31 (-0.79, 0.18)	0.17 (0.01, 0.34)[b]	0.11
Butyl (detectable)	0.06 (-0.07, 0.19)	0.10 (-0.04, 0.25)	0.60	-0.10 (-0.40, 0.20)	0.06 (-0.07, 0.19)	0.12
Molar Sum (log10)	0.05 (-0.01, 0.10)	-0.01 (-0.07, 0.05)	0.14	0.04 (-0.11, 0.20)	0.05 (-0.01, 0.10)	0.32
	Glucose (mmol/L)			**Systolic blood pressure (mmHg)**		
	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]
Methyl (log10)	0.03 (-0.05, 0.12)	-0.04 (-0.16, 0.07)	0.23	-0.43 (-2.64, 1.79)	1.14 (-2.78, 5.06)	0.41
Propyl (log10)	-0.01 (-0.07, 0.06)	-0.01 (-0.09, 0.07)	0.94	0.60 (-2.22, 3.42)	1.11 (-2.07, 4.29)	0.79
Ethyl (detectable)	-0.02 (-0.21, 0.17)	0.01 (-0.25, 0.27)	0.81	0.09 (-4.24, 4.43)	0.70 (-3.80, 5.21)	0.85
Butyl (detectable)	-0.01 (-0.20, 0.19)	-0.08 (-0.34, 0.19)	0.62	-3.44 (-9.46, 2.58)	0.51 (-13.61, 14.64)	0.57
Molar Sum (log10)	0.01 (-0.07, 0.10)	-0.02 (-0.14, 0.09)	0.48	-0.22 (-2.85, 2.40)	0.79 (-3.11, 4.68)	0.62
	Diastolic blood pressure (mmHg)					
	Women [β (95% CI)]	Men [β (95% CI)]	p(int)[a]			
Methyl (log10)	-0.27 (-1.50, 0.96)	0.19 (-1.73, 2.12)	0.60			
Propyl (log10)	-0.30 (-1.59, 0.98)	0.56 (-1.38, 2.50)	0.37			
Ethyl (detectable)	1.56 (-0.93, 4.06)	-0.07 (-3.32, 3.17)	0.33			
Butyl (detectable)	-2.86 (-7.04, 1.32)	2.14 (-13.31, 17.60)	0.50			
Molar Sum (log10)	-0.16 (-1.52, 1.20)	-0.08 (-2.10, 1.94)	0.94			

Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, smoking status, minutes of moderate or vigorous physical activity per day, and poor diet index. [a]p-value for interaction by sex; [b]$p<0.05$; [c]$p<0.1$ for effect measure modification by sex

Discussion

In this national biomonitoring survey, we observed associations between urinary concentrations of propyl paraben and an increased prevalence of metabolic syndrome among men but we found no association with obesity or indicators of metabolic health. We also found inverse associations between paraben concentrations and obesity, BMI, metabolic syndrome, waist circumference, and HDL cholesterol among adult women. While no prior study examined associations between exposure to parabens and metabolic syndrome, the null associations that we found with obesity, BMI and waist circumference among men are consistent with results from a small Czech study[27] in contrast with those from NHANES, a national cross-sectional biomonitoring survey of U.S. residents similar to the CHMS, which observed inverse associations with these outcomes among men[28]. On the other hand, the inverse associations that we report among women are consistent with those from NHANES but contrast with the null results in the Czech population. Other evaluations of BMI as a determinant of paraben exposure found inverse associations overall[51,302,303] and in men[128], or no association in women[304]; however, these analyses did not aim to specifically evaluate associations with adiposity and, as such, did not adjust for important confounders[304].

Our findings among adults are consistent with the anti-androgenic and estrogenic properties of parabens. Lower testosterone has important consequences for estrogen levels in men, since approximately 85% of their circulating estrogens are derived from conversion of testosterone[305]. Estrogens are critical to maintaining body weight and metabolic health; in addition to their anti-obesogenic effects[94-96], they also promote healthier types of fat (brown vs. white, subcutaneous vs. visceral)[99,100], promote vasodilation[306], and protect against dyslipidemia and insulin resistance[93,307]. Therefore, the observed increased prevalence of metabolic syndrome

associated with ethyl paraben among men is consistent with the anti-androgenic properties of parabens. Although no associations with individual components were observed, there were patterns of increased prevalence for high triglycerides, high fasting blood glucose, and high blood pressure among men (Supplementary Table 4.3.3). Furthermore, the observed inverse associations with obesity, metabolic syndrome, and HDL cholesterol among women are also consistent with the protective effects of estrogens against cardiometabolic risk. HDL protects against cardiometabolic disease by delivering excess triglycerides and cholesterol from peripheral tissues to the liver for excretion[308], and by its anti-oxidative and anti-inflammatory properties[309-311]. Oral estrogen replacement therapy in women has been shown to increase HDL cholesterol levels by 5 to 15%[312,313].

While some animal and in vitro studies have found adipogenic effects of parabens, the concentrations capable of inducing these adipogenic effects were high (50-200 μM)[18,314,315] compared to the concentrations measured in human tissues (10-80 nM)[316]. In a study using doses intended to mimic environmental levels of exposure, female rats exposed chronically from birth had lower bodyweight compared to control rats at both adolescence and adulthood[317], a finding which is consistent with our own, and with the epidemiologic literature in general.

The null findings among children fit into a context of a small and inconsistent literature. Ethyl paraben was associated with higher weight z-scores among three year-old Korean boys, but other parabens were not associated with BMI or weight z-scores in either sex[24]. In contrast, parabens were inversely associated with overweight, BMI z-score, and waist circumference among girls but not among boys in NHANES[28]. However, no association was found with obesity among children in India[26] or with weight change in a prospective cohort of girls from three U.S. states[25].

Strengths and limitations

To our knowledge, this is the first study to examine associations between parabens and metabolic syndrome, along with its components, in a comprehensive assessment of the potential impact of parabens on metabolic health. This is also the first study to investigate associations between parabens and obesity in the Canadian population and is one of the largest studies to date on this topic. Very few previous studies have examined associations between parabens and obesity[27,28], waist circumference[28] and blood pressure[118] in adults. The relatively large sample size of the CHMS provided an opportunity to investigate effect modification by sex, which is an important consideration when examining the health effects of chemicals that may affect sex hormones. In addition, the availability of extensive covariate data made it possible to control for several important potential confounders, such as smoking status, dietary intake, and objectively-measured physical activity. The use of survey weights accounted for non-response, reducing the probability of selection bias, and allowing for generalizability of the non-fasted outcome-exposure associations to the Canadian population. In addition, we estimated prevalence ratios rather than prevalence odds ratios; since the binary outcomes are prevalent in this population, odds ratios would overestimate the prevalence ratios.

Our study also has some limitations. Since the CHMS is a cross-sectional survey, we cannot establish firmly whether exposures preceded outcomes and so reverse causality remains possible. For instance, our results could be explained in part by a reduced use of cosmetic or beauty products among obese women. However, we are aware of no empirical evidence supporting this hypothesis. Furthermore, parabens are non-persistent chemicals, and so urinary spot samples represent relatively recent exposure; the half-life of propyl paraben is approximately 3 hours in blood[275], and parabens are excreted in urine within 24 to 48

hours[43,44,275]. However, studies suggest that daily patterns of exposure to parabens may be consistent since moderate (0.42 – 0.62) intraclass correlation coefficients have been reported in adults for repeated spot urine samples over time[303,318].

Conclusion

We observed inverse associations between parabens and obesity, waist circumference, and HDL cholesterol in women, and positive associations with metabolic syndrome in men. These results may have important public health implications given that exposure to parabens is ubiquitous in human populations. However, this being the first study to investigate associations with metabolic syndrome, and given the small number of studies investigating associations with obesity, our results need to be confirmed in other populations, ideally using a longitudinal design and including multiple measurements of parabens during relevant developmental time periods.

4.3. Supplementary Material

Table 4.3.1. Anatomical Therapeutic Chemical (ATC) medication codes for treatment of each criterion[a]

Criterion	**ATC medication codes**
Low HDL cholesterol	"C04AC01" "C04AC03" "C10AB01" "C10AB02" "C10AB04" "C10AB05" "C10AC01" "C10AC02" "C10AX02"
High serum triglycerides	"C04AC01" "C04AC03" "C10AB01" "C10AB02" "C10AB04" "C10AB05" "C10AC01" "C10AC02" "C10AX02" "C10AX06"
High fasting blood glucose	“A10"
High blood pressure	"C02" "C03" "C07" "C08" "C09" "C04AA02" "C04AB01"

[a]ATC codes used in: Statistics Canada. Metabolic syndrome in Canadians, 2009 to 2011. Ottawa, Canada: 2012 (J. Mikedis, personal communication, May 2018)

Table 4.3.2. Associations between urinary paraben concentrations and prevalence of obesity, metabolic syndrome, and its components among Canadian adults (age 18 years and over), 2014 – 2015

	Obesity [PR (95% CI)]	Metabolic syndrome [PR (95% CI)]	Abdominal obesity [PR (95% CI)]	Low HDL cholesterol [PR (95% CI)]
Methyl (log10)	0.82 (0.56, 1.21)	1.13 (0.81, 1.57)	0.94 (0.74, 1.19)	0.81 (0.65, 1.00)[a]
Propyl (log10)	0.86 (0.63, 1.17)	1.14 (0.89, 1.47)	0.99 (0.85, 1.16)	0.82 (0.74, 0.91)[a]
Ethyl (detectable)	0.84 (0.54, 1.31)	0.57 (0.24, 1.36)	0.89 (0.67, 1.18)	0.62 (0.43, 0.89)[a]
Butyl (detectable)	0.89 (0.53, 1.49)	0.69 (0.20, 2.32)	0.91 (0.62, 1.31)	0.55 (0.36, 0.86)[a]
Molar Sum (log10)	0.83 (0.54, 1.26)	1.20 (0.81, 1.78)	0.94 (0.73, 1.20)	0.80 (0.65, 0.98)[a]
	High triglycerides [PR (95% CI)]	High blood glucose [PR (95% CI)]	High blood pressure [PR (95% CI)]	
Methyl (log10)	1.28 (0.90, 1.82)	1.21 (0.81, 1.82)	1.15 (0.93, 1.41)	
Propyl (log10)	1.21 (1.03, 1.42)[a]	1.13 (0.84, 1.53)	1.16 (0.99, 1.36)	
Ethyl (detectable)	1.11 (0.66, 1.87)	0.59 (0.19, 1.83)	1.04 (0.83, 1.30)	
Butyl (detectable)	1.51 (0.75, 3.05)	1.22 (0.05, 31.34)	0.88 (0.46, 1.66)	
Molar Sum (log10)	1.35 (0.93, 1.96)	1.26 (0.77, 2.07)	1.17 (0.97, 1.41)	

Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, smoking status, minutes of moderate or vigorous physical activity per day, and poor diet index; [a]$p<0.05$

Table 4.3.3. Associations between urinary paraben concentrations and prevalence of obesity, metabolic syndrome, and its components among Canadian adults (age 18 years and over), stratified by sex, 2014 – 2015

	Obesity			Metabolic syndrome		
	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]
Methyl (log10)	0.62 (0.44, 0.86)[b]	1.06 (0.58, 1.95)	0.08[c]	0.81 (0.54, 1.21)	1.39 (0.90, 2.15)	0.05[c]
Propyl (log10)	0.75 (0.54, 1.05)	0.99 (0.56, 1.74)	0.41	0.87 (0.58, 1.30)	1.40 (1.03, 1.90)[b]	0.06[c]
Ethyl (detectable)	0.64 (0.37, 1.10)	1.09 (0.55, 2.15)	0.23	0.37 (0.14, 0.98)[b]	0.82 (0.20, 3.36)	0.32
Butyl (detectable)	0.78 (0.37, 1.63)	1.16 (0.28, 4.72)	0.64			
Molar Sum (log10)	0.67 (0.46, 0.97)[b]	1.03 (0.52, 2.04)	0.25	0.81 (0.52, 1.25)	1.54 (0.92, 2.59)	0.05[c]
	Abdominal obesity			Low HDL cholesterol		
	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]
Methyl (log10)	0.88 (0.72, 1.08)	1.03 (0.69, 1.54)	0.34	0.69 (0.57, 0.84)[b]	1.02 (0.65, 1.59)	0.11
Propyl (log10)	0.96 (0.82, 1.12)	1.06 (0.76, 1.47)	0.56	0.78 (0.69, 0.88)[b]	0.91 (0.70, 1.17)	0.32
Ethyl (detectable)	0.88 (0.71, 1.10)	0.90 (0.50, 1.62)	0.95	0.51 (0.33, 0.78)[b]	0.86 (0.45, 1.66)	0.14
Butyl (detectable)	0.92 (0.62, 1.36)	0.85 (0.34, 2.10)	0.85			
Molar Sum (log10)	0.90 (0.73, 1.10)	1.01 (0.62, 1.66)	0.61	0.71 (0.58, 0.86)[b]	0.99 (0.62, 1.56)	0.19
	High triglycerides			High blood glucose		
	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]
Methyl (log10)	1.37 (0.86, 2.19)	1.24 (0.77, 1.98)	0.72	1.47 (0.65, 3.32)	1.16 (0.73, 1.83)	0.55
Propyl (log10)	1.14 (0.79, 1.64)	1.25 (0.86, 1.83)	0.76	1.17 (0.55, 2.47)	1.12 (0.72, 1.74)	0.93
Ethyl (detectable)	0.67 (0.36, 1.25)	1.43 (0.78, 2.62)	0.09[c]	0.55 (0.01, 45.6)	0.61 (0.19, 1.95)	0.96
Butyl (detectable)						
Molar Sum (log10)	1.32 (0.79, 2.21)	1.37 (0.85, 2.19)	0.91	1.34 (0.55, 3.26)	1.24 (0.70, 2.18)	0.86
	High blood pressure					
	Women [PR (95% CI)]	Men [PR (95% CI)]	p(int)[a]			
Methyl (log10)	0.99 (0.73, 1.36)	1.31 (0.94, 1.82)	0.24			
Propyl (log10)	1.06 (0.82, 1.37)	1.27 (0.97, 1.66)	0.37			
Ethyl (detectable)	0.95 (0.57, 1.56)	1.14 (0.84, 1.53)	0.57			
Butyl (detectable)	0.81 (0.41, 1.61)	1.06 (0.43, 2.58)	0.52			
Molar Sum (log10)	1.01 (0.73, 1.39)	1.35 (0.99, 1.85)	0.22			

Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, smoking status, minutes of moderate or vigorous physical activity per day, and poor diet index; estimates for butyl paraben and metabolic syndrome, low HDL cholesterol, high triglycerides, and high blood glucose are not presented because models did not converge.

[a]p-value for interaction by sex; [b]$p<0.05$; [c]p(interaction)<0.1

Table 4.3.4. Overall associations between urinary paraben concentrations and BMI and metabolic syndrome components expressed continuously among Canadian children (age 3 to 17 years), 2014 – 2015

	BMI z-score [β (95% CI)]	Waist circumference (cm) [β (95% CI)]	HDL cholesterol (mmol/L) [β (95% CI)]	Triglycerides (mmol/L) [β (95% CI)]
Methyl (log10)	0.06 (-0.20, 0.31)	0.02 (-1.94, 1.97)	-0.03 (-0.08, 0.02)	-0.02 (-0.10, 0.07)
Propyl (log10)	0.02 (-0.17, 0.21)	-0.14 (-1.82, 1.55)	-0.02 (-0.06, 0.03)	0.00 (-0.09, 0.09)
Ethyl (detectable)	0.09 (-0.14, 0.31)	1.41 (-0.71, 3.52)	-0.05 (-0.10, 0.01)	-0.10 (-0.21, 0.01)
Butyl (detectable)	0.24 (0.04, 0.44)[a]	0.36 (-2.31, 3.03)	0.00 (-0.08, 0.09)	0.00 (-0.17, 0.16)
Molar Sum (log10)	0.06 (-0.19, 0.31)	-0.05 (-2.05, 1.94)	-0.02 (-0.07, 0.03)	-0.03 (-0.11, 0.06)

	Glucose (mmol/L) [β (95% CI)]	Systolic blood pressure (mmHg) [β (95% CI)]	Diastolic blood pressure (mmHg) [β (95% CI)]
Methyl (log10)	-0.02 (-0.11, 0.07)	-0.92 (-2.59, 0.75)	-0.66 (-2.11, 0.79)
Propyl (log10)	-0.01 (-0.09, 0.06)	-0.52 (-1.74, 0.70)	-0.08 (-1.13, 0.96)
Ethyl (detectable)	-0.12 (-0.34, 0.11)	-0.32 (-2.05, 1.41)	-0.22 (-1.96, 1.51)
Butyl (detectable)	-0.08 (-0.32, 0.15)	1.13 (-2.52, 4.79)	2.33 (-0.89, 5.54)
Molar Sum (log10)	-0.02 (-0.10, 0.07)	-0.73 (-2.40, 0.93)	-0.28 (-1.68, 1.12)

Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, minutes of moderate or vigorous physical activity per day, and poor diet index. [a]$p<0.05$

Table 4.3.5. Associations between urinary paraben concentrations and BMI and metabolic syndrome components expressed continuously among Canadian children (age 3 to 17 years), stratified by sex, 2014 – 2015

	BMI z-score			Waist circumference (cm)		
	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]
Methyl (log10)	0.13 (-0.11, 0.37)	-0.02 (-0.37, 0.33)	0.28	0.60 (-1.75, 2.95)	-0.60 (-2.76, 1.57)	0.22
Propyl (log10)	0.07 (-0.14, 0.29)	-0.05 (-0.29, 0.20)	0.29	0.14 (-2.14, 2.42)	-0.48 (-2.12, 1.15)	0.54
Ethyl (detectable)	0.22 (-0.10, 0.54)	-0.08 (-0.50, 0.35)	0.29	3.11 (-0.14, 6.35)	-0.70 (-3.98, 2.57)	0.12
Butyl (detectable)	0.38 (-0.03, 0.79)	0.03 (-0.45, 0.52)	0.36	1.31 (-2.88, 5.51)	-1.06 (-4.78, 2.66)	0.40
Molar Sum (log10)	0.12 (-0.14, 0.37)	-0.02 (-0.37, 0.34)	0.39	0.41 (-2.08, 2.89)	-0.56 (-2.84, 1.71)	0.40
	HDL cholesterol (mmol/L)			Triglycerides (mmol/L)		
	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]
Methyl (log10)	-0.01 (-0.08, 0.06)	-0.04 (-0.10, 0.02)	0.40	-0.01 (-0.12, 0.09)	-0.02 (-0.15, 0.11)	0.97
Propyl (log10)	0.01 (-0.04, 0.06)	-0.04 (-0.10, 0.02)	0.12	0.02 (-0.06, 0.11)	-0.02 (-0.14, 0.11)	0.45
Ethyl (detectable)	-0.08 (-0.17, 0.00)	-0.01 (-0.07, 0.06)	0.15	-0.08 (-0.27, 0.10)	-0.12 (-0.24, -0.01)[b]	0.74
Butyl (detectable)	-0.03 (-0.14, 0.08)	0.05 (-0.08, 0.18)	0.27	-0.08 (-0.35, 0.19)	0.08 (-0.31, 0.46)	0.55
Molar Sum (log10)	-0.01 (-0.08, 0.06)	-0.03 (-0.09, 0.03)	0.57	-0.02 (-0.11, 0.08)	-0.03 (-0.17, 0.10)	0.82
	Glucose (mmol/L)			Systolic blood pressure (mmHg)		
	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]
Methyl (log10)	-0.04 (-0.17, 0.10)	-0.01 (-0.12, 0.11)	0.70	-1.04 (-4.00, 1.91)	-0.80 (-3.15, 1.56)	0.90
Propyl (log10)	0.01 (-0.11, 0.13)	-0.03 (-0.14, 0.08)	0.65	0.06 (-1.87, 1.98)	-1.25 (-3.00, 0.50)	0.31
Ethyl (detectable)	-0.02 (-0.36, 0.32)	-0.22 (-0.38, -0.06)[a]	0.17	0.38 (-1.28, 2.04)	-1.27 (-4.72, 2.18)	0.36
Butyl (detectable)	-0.21 (-0.64, 0.23)	0.05 (-0.22, 0.32)	0.36	-0.26 (-4.91, 4.39)	3.29 (-0.36, 6.94)	0.13
Molar Sum (log10)	-0.03 (-0.17, 0.11)	0.00 (-0.12, 0.11)	0.77	-0.67 (-3.50, 2.16)	-0.81 (-3.32, 1.70)	0.94
	Diastolic blood pressure (mmHg)					
	Girls [β (95% CI)]	Boys [β (95% CI)]	p(int)[a]			
Methyl (log10)	-0.43 (-3.58, 2.71)	-0.90 (-3.54, 1.73)	0.84			
Propyl (log10)	0.61 (-1.59, 2.80)	-0.95 (-2.84, 0.93)	0.36			
Ethyl (detectable)	1.42 (-0.14, 2.97)	-2.44 (-6.38, 1.51)	0.09[c]			
Butyl (detectable)	1.51 (-3.20, 6.22)	3.59 (0.20, 6.98)[a]	0.43			
Molar Sum (log10)	0.11 (-2.87, 3.08)	-0.72 (-3.47, 2.03)	0.72			

Models adjusted for age, sex, ethnicity, adjusted household income, highest household education, minutes of moderate or vigorous physical activity per day, and poor diet index.

[a]p-value for interaction by sex; [b]$p<0.05$; [c]p(interaction)<0.1

CHAPTER 5. Manuscript 2

5.1. Preface

While Manuscript 1 focuses on the Canadian context, Manuscripts 2 and 3 of this dissertation focus on exposure to endocrine-disrupting chemicals in South Africa. In malaria-endemic regions of South Africa, indoor residual spraying of insecticides on the interior walls and eaves of homes for malaria vector control results in high levels of exposure to DDT and pyrethroid insecticides, which are known endocrine disruptors. Since these compounds can cross the placenta, fetuses may be exposed during critical periods of development and thus have implications for cardiometabolic health in childhood.

This chapter contains Manuscript 2, which examines gestational exposure to DDT/E and pyrethroids in relation to child size (height and weight z-scores), adiposity (BMI z-score, waist circumference, body fat percentage) and blood pressure measured at 5 years of age. This manuscript is in revision at *Environmental Epidemiology*.

5.2. Prenatal exposure to insecticides and child cardiometabolic risk factors in the VHEMBE birth cohort

Abstract

Background: As part of malaria control programs, many countries spray dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides inside dwellings in a practice called indoor residual spraying that results in high levels of exposure to local populations. Gestational exposure to these endocrine- and metabolism-disrupting chemicals may influence child cardiometabolic health.

Methods: We measured the serum concentration of DDT and dichlorodiphenyldichloroethylene (DDE) and urinary concentration of pyrethroid metabolites (*cis*-DBCA, *cis*-DCCA, *trans*-DCCA, 3-PBA) in peripartum samples collected between August 2012 and December 2013 from 637 women participating in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort study based in Limpopo, South Africa. We applied marginal structural models to estimate the relationship between biomarker concentrations and child size (height, weight), adiposity (body mass index (BMI), body fat percentage, waist circumference) and blood pressure at 5 years of age.

Results: Maternal concentrations of all four pyrethroid metabolites were associated with lower adiposity including reduced BMI z-scores, smaller waist circumferences, and decreased body fat percentages. Reductions in BMI z-score were observed only among children of mothers with sufficient energy intake during pregnancy ($\beta_{cis\text{-DCCA, }trans\text{-DCCA}}$=-0.4, 95% confidence interval (CI):-0.7,-0.1; $p_{interaction}$=0.03 and 0.04, respectively) but there was no evidence of effect modification for the other measures of adiposity. Maternal *p,p'*-DDT concentrations were associated with a reduction in body fat percentage (β=-0.4%, 95%CI:-0.8,-0.0).

Conclusions: Gestational exposure to pyrethroids may reduce adiposity in children at 5 years of age.

Introduction

Low- and middle-income countries such as South Africa are experiencing a double-burden of malnutrition characterized by a high prevalence of both under- and over-nutrition. In South Africa, one in four (27%) children under five years of age are stunted, and in some provinces, up to 13% of children are underweight[5]. Concurrently, 13% of children under 5 are overweight[5], more than twice the global average for this age group[2]. Early-life exposure to endocrine-disrupting chemicals may contribute to these patterns by affecting the hormonal regulation of energy, glucose, and lipid metabolism[11,14]. In malaria-endemic regions of South Africa, indoor residual spraying (IRS) of dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides on the interior walls and eaves of homes for malaria vector control results in high levels of exposure to these endocrine-disrupting chemicals.[30-32,319,320] These chemicals can cross the placenta and may interfere with fetal development and have long-term impact on child cardiometabolic health.[14,272]

Pyrethroid insecticides are commonly used for IRS[5] as well as in agriculture and retail products for domestic use. These chemicals have been shown to disrupt androgen signaling[321,322], steroidogenesis[186,187,190], and lipid metabolism in animals and *in vitro*[185,190,193]. Only two epidemiologic studies have examined associations between prenatal exposure to pyrethroids and adiposity in children. In the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) study, conducted in an area of South Africa where IRS is conducted annually, inverse associations were observed between maternal concentrations of pyrethroid metabolites and BMI z-scores at 1, 2, and 3.5 years among boys[37,177], but no associations were found with BMI z-score among South Korean children at age 4 years[178]. It remains unclear whether the associations

observed in VHEMBE persist at older ages, or whether findings from high-income populations such as South Korea are generalizable to IRS populations. Furthermore, to our knowledge, no study has investigated the potential effects of prenatal exposure to pyrethroids on other cardiometabolic risk factors such as waist circumference (a measure of the more metabolically harmful abdominal/visceral fat), body fat percentage, or blood pressure.

DDT is an estrogen agonist and its environmentally-persistent breakdown product dichlorodiphenyldichloroethylene (DDE) is an androgen antagonist[75,77,79]. Epidemiological studies of prenatal exposure to DDT and DDE (DDT/E) have been mixed, reporting positive[134,135,137,177,323-327] or null[37,133,328-333] associations with child adiposity. Cardiometabolic risk factors other than size and adiposity were assessed only in a Greek birth cohort, which found a positive association maternal serum DDE and blood pressure in children at 4 years of age[137]. However, except for VHEMBE, these prior studies did not address potential selection bias from missing covariate data or losses to follow-up of up to 42% of enrolled participants[135], and used confounder selection strategies such as univariate analysis, stepwise approaches, and change-in-estimate which may bias estimates and overestimate precision[39-41]. Furthermore, only VHEMBE occurs in the context of current exposure to DDT from IRS. The objective of this study is therefore to estimate the causal effects of prenatal exposure to DDT/E and pyrethroid insecticides on child cardiometabolic risk factors including anthropometrics, measures of adiposity (including abdominal/visceral fat), and blood pressure at five years of age in a population exposed annually to IRS, using inverse probability weighting methods to address confounding and selection bias.

Methods

Data source

Mothers giving birth at Tshilizidini hospital in South Africa's Limpopo Province were recruited into the VHEMBE study between August 2012 and December 2013. In this region, IRS spraying occurs at the start of the rainy season (October to April). The pyrethroids cypermethrin or deltamethrin are generally sprayed in homes with painted walls, while DDT is generally sprayed in homes with unpainted walls. Eligible women were at least 18 years of age, spoke Tshivenda at home, lived within 20 km of the hospital, intended to remain in the area for at least 2 years, did not have malaria during pregnancy, had contractions at least 5 minutes apart, and delivered a live, singleton infant. Of the 920 women who met eligibility criteria, 752 provided informed consent, completed a baseline questionnaire and provided peripheral blood samples for DDT/E analysis (see Supplementary Figure 5.3.1). Follow-up consisted of a home visit 1 week postpartum and field office visits at 1, 2, 3.5, and 5 years. At the home visit, study staff recorded observations and administered a questionnaire on home materials, pesticide use and storage, and household assets. Follow-up field office visits included extensive questionnaires on various demographic and health information, biological sample collection as well as physical assessments of both the mother and child. Of the 640 mother-and-child pairs who attended the 5-year visit (88% retention, excluding 24 child deaths), physical assessments were completed for 637 of the children. Of these, 628 mothers had provided sufficient urine volume for pyrethroid metabolite analysis. Ethics approval for the VHEMBE study was obtained from McGill University, the University of Pretoria, Tshilidzini Hospital, the Limpopo Department of Health and Social Development, and the University of California, Berkeley.

Maternal serum DDT/E and urinary pyrethroid metabolite concentrations

Maternal blood and urine samples were collected in Tshilidzini hospital at the time of delivery, and were processed immediately after collection and stored at -80°C until shipment on dry ice to analytical laboratories. Maternal serum concentrations of DDT/E isomers (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE) were measured by the Emory University Environmental Health Laboratory (Atlanta, USA) using gas chromatography-tandem mass spectrometry.[334] Maternal urine concentrations of the following pyrethroid metabolites were measured by the Institut National de Santé Publique du Québec (Québec City, Canada) using gas chromatography-tandem mass spectrometry[335]: *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA), *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA), *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*trans*-DCCA), 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). Urine specific gravity was measured with a portable refractometer (Atago PAL-10S; Tokyo, Japan) and total serum lipid concentrations were estimated based on total cholesterol and triglyceride levels measured by standard enzymatic methods (Roche Chemicals; Indianapolis, USA)[336].

One 3-PBA measurement did not meet quality control standards and was discarded. Due to low quantification frequencies, 4-F-3-PBA (8%) and *o,p'*-DDE (16%) were excluded from further analyses. For the other analytes, concentrations below the limits of detection (LOD) were imputed at random based on log-normal probability distributions whose parameters were estimated via maximum likelihood[298]. Values between the LOD and limit of quantification (LOQ) were assigned the machine-read values. Pyrethroid metabolite concentrations were

specific gravity (SG)-corrected for urine dilution and expressed in µg/L[337]. DDT/E were corrected for serum lipid content and expressed in ng/g lipid.

Child cardiometabolic risk factors (size, adiposity, and blood pressure)

At the 5-year visit, trained staff measured child weight to the nearest 0.01kg and body fat percentage to the nearest 0.1% (via bioelectrical impedance) using a Tanita Children's Body Fat Monitor BF-689 (Arlington Heights, USA)[212]. Child standing height using a Charder HM200P stadiometer (Taichung, Taiwan), waist circumference using a measuring tape, and blood pressure using an OMRON oscillometric device (Lake Forest, USA) were measured in triplicate and then averaged, following U.S. National Health and Nutrition Examination Survey protocols[338]. Age- and sex- standardized z-scores for height, weight, and BMI were calculated using the WHO's *igrowup*[339] and *who2007*[340] Stata macros, which implement the 2006-2007 WHO child growth standards[293].

Covariates

At the baseline and follow-up visits, study staff administered questionnaires to mothers or primary caregivers on potential confounders and conducted anthropomorphic assessments. Maternal post-delivery weight was measured using a Beurer PS06 scale (Ulm, Germany) and height was measured in triplicate using a Charder HM200P stadiometer (Taichung, Taiwan), then averaged.

The baseline questionnaire collected data on sociodemographic characteristics (e.g. date of birth, marital status, household income and household size), diet and lifestyle (e.g. food frequency, alcohol and smoking during pregnancy), and health. Mothers also reported their occupational and domestic use of pesticides, as well as the presence of agricultural workers in the household. Based on Statistics South Africa guidelines, households earning less than 386 Rands/person/month were

defined as living with food poverty.[52] Food insecurity was defined as two or more affirmative responses to the US National Center for Health Statistics' Six-Item Food Security Scale.[53] Mothers' daily total energy intake was estimated based on a locally-validated quantitative food frequency questionnaire[54] by a South African nutritionist using the FoodFinder3 software (SouthAfrica Medical Research Council/WAMTechnology CC). The Institute of Medicine-recommended total daily energy intake for mothers in late pregnancy was calculated based on their age (years), height (meters), and post-partum weight (kg) since pre-pregnancy weight was not available: 4.184kJ/cal×[452+354−(6.91×age)+1.27×(9.36×weight)+726×height]; energy intake below this threshold was classified as insufficient.[55,56] Mothers' HIV status during pregnancy was ascertained from self-report or use of antiretroviral drugs indicated in medical records.

In order to capture socioeconomic status in this region where much of the economy is informal[5,37], a family wealth index was constructed based on South Africa Demographic and Health Survey methodology, using data from the baseline questionnaire and the 1-week home visit (questionnaire and staff observations)[37]. Duration of exclusive and non-exclusive breastfeeding was calculated based on responses from questionnaires administered at the 1-week and 1, 2, and 3.5-years. We also constructed a child diet diversity score to explore potential confounding by child dietary intake[37]. The score was calculated as the total number of different food groups (e.g. fruit, vegetables, meat, chicken, fish, milk, or eggs) eaten in the past month by the child based on maternal report at 3.5-years.

Statistical analysis

The relation between a 10-fold increase in maternal lipid-corrected serum DDT/E or specific gravity-corrected urinary pyrethroid metabolite concentrations on each anthropometric and cardiometabolic health outcome were estimated using marginal structural models with inverse

probability weights constructed from the product of two weights: inverse probability of censoring weights (IPCWs) to account for potential selection bias due to loss to follow-up[263]; and, inverse probability of treatment weights (IPTWs) to control for confounding[265,346]. Under the three identifiability assumptions of consistency, exchangeability and positivity, and assuming no misspecification of the models used to estimate the weights, the resulting estimates have a causal interpretation.

Further details on the construction of the weights are provided in Supplementary Material Section 5.3.2. Briefly, we used logistic regression to estimate the probability of the censoring status of each subject (i.e. completed the 5-year visit vs. lost to follow-up), conditional on predictors of censoring identified using directed acyclic graphs (DAGs) and constructed IPCWs based on the inverse of these probabilities and stabilized the weights with the marginal probability of the censoring status received[263]. Then, excluding censored individuals, we constructed IPTWs based on the generalized propensity score (GPS) method for each exposure, using multivariable linear regression to estimate the density function conditional on potential predictors of the outcomes and confounders of exposure-outcome relationships identified using the DAG (Supplementary Figure 5.3.2)[265,346].

The following covariates were included in both IPCW and IPTW models: child sex (boy/girl); household food poverty (yes/no), food insecurity (yes/no), and wealth index (continuous); maternal age (years, continuous), height (metres, continuous), post-delivery weight (kg, continuous), education (high school vs. no high school), marital status (married or living-as-married vs. not married), energy intake during pregnancy (insufficient/sufficient), alcohol use during pregnancy (yes/no), HIV status at delivery (positive/negative), duration of exclusive breastfeeding (months, continuous), and parity (continuous). In the IPCW models, we also

included gestational age (preterm vs. not preterm) and DDT/E and pyrethroid metabolite concentrations. All analyte concentrations were $\log_{10}$-transformed to reduce the influence of outliers, resulting in estimates of effect per 10-fold increase in concentration.

Inverse probability weighting accounts for selection bias and confounding by creating a pseudo-population in which censoring is independent of exposure and covariates and exposure is independent of confounders[263]. This can be verified by assessing, in the weighted sample, whether exposure and covariates are equally distributed (i.e. balanced) between censored and uncensored individuals, and whether the distribution of confounders is balanced at different levels of exposure. For this purpose, we conducted the following recommended diagnostics[266,269]: i) standardized differences, to compare proportions or means across (exposure or censorship) categories; ii) correlations, to evaluate associations between continuous covariates and the continuous exposures, and iii) variance ratios, to compare variability across (exposure or censorship) categories. Following published guidelines, variables with standardized differences below 0.2 when comparing across exposure quartiles (accounting for additional variability expected from small sample sizes)[269], below 0.1 when comparing across censoring status, and correlations below 0.1, were considered to be balanced[266,269]. Variance ratios of 1.0 describes a covariate which has equal variance across exposure categories, and a threshold of <2.0 has been suggested to indicate balance[267]. Further details on the inverse probability weights and balance assessment are provided elsewhere[347] and in Supplementary Material Section 5.3.2.

In order to account for the small amount of missing covariate values (181 of 11,265; 1.6% missingness), we conducted multiple imputation by chained equations with imputation models including all participants enrolled at baseline (n=751). In the imputation models, we included all outcomes, exposures and covariates identified above and generated 10 imputed datasets[38] (see

Supplementary Material Section 5.3.3 for additional details). Since endocrine disruptors may differentially affect boys and girls[34,99], and effects on cardiometabolic risk factors may differ based on socioeconomic and nutritional context[37], we also investigated effect measure modification by child sex, food poverty, and maternal energy intake during pregnancy by including cross-product terms in models. We used a threshold of $p<0.1$ to indicate statistical evidence of effect modification. We constructed 95% confidence intervals (CIs) from bootstrapping the entire procedure (multiple imputation, estimation of IPCW and IPTW and outcome regressions) 500 times[348,349]. All analyses were conducted using Stata 14 (StataCorp, College Station, TX).

Results

Participant characteristics

All VHEMBE mothers (n=637) were Black Africans. At delivery, the average age of mothers was 26.4 years, and just under half were married (46%) and had a high school education (43%) (Table 5.2.1). Most households lived below the South African food poverty line (61%), and many were food insecure (42%). The prevalence of HIV infection among mothers was 12% at delivery. Half of the children were female (49%) and 12% were preterm (<37 weeks gestational age at birth). One quarter of the children were born small-for-gestational age (<10th percentile) and 7% had a low birthweight (<2500g)[350]. The median duration of exclusive breastfeeding without introduction of water or solids was short (2.3 months), though breastfeeding continued for longer (median=16.1 months) (Table 5.2.1).

DDT/E and pyrethroids were detected in virtually all participants. DDT/E concentrations varied greatly, with up to a 100,000-fold difference in exposure (Table 5.2.2). The pyrethroid metabolites *cis*-DCCA, *trans*-DCCA, and 3-PBA were highly correlated with each other

(Pearson's *r*=0.83 to 0.87) but were only moderately correlated with *cis*-DBCA (*r*=0.33 to 0.53), and were not correlated with DDT/E (*r*=-0.02 to 0.04). Isomers of DDT/E were highly intercorrelated (*r*=0.69 to 0.85). Few mothers (6%) reported that IRS had been sprayed in their home during their pregnancy, and one-third (33%) were aware that IRS was used in their village. Occupational exposure to pesticides was infrequent, with 7% of mothers reporting use of pesticides at work during pregnancy, and 7% of households included an agricultural worker. Domestic use of pesticides was more frequent, with mothers reporting use of pesticides in the yard (13%) and indoors (32%).

Inverse probability weights and covariate balance diagnostics

The mean of each set of inverse probability weights was 1.00 for all models and no extreme weights were observed, suggesting that the positivity assumption was not violated (range=0.22-3.07; Supplementary Table 5.3.1). Inverse probability weighting achieved covariate balance, with all mean absolute standardized differences being below 0.2, all correlations being below 0.1 and all variance ratios being about 1.0, indicating that confounding by measured variables was controlled. Balance diagnostics for *trans*-DCCA are shown in Figure 5.2.1 for illustration purposes; diagnostics for other analytes are shown in Supplementary Figures 5.3.4 to 5.3.6.

Effects of gestational pyrethroid exposure on child cardiometabolic risk factors at 5 years of age

Overall, maternal concentrations of all pyrethroid metabolites were associated with reduced BMI z-score, waist circumference and body fat percentage in the children. Magnitudes were relatively consistent across metabolites, with an approximately 0.2 decrease in BMI z-score (e.g. $\beta_{cis\text{-DBCA}}$=-0.18, 95%CI:-0.33,-0.03), 0.6 to 0.9 cm smaller waist circumference (e.g. $\beta_{cis\text{-DBCA}}$=-0.57, 95%CI:-1.09,-0.06), and 0.7 to 0.8% reduced body fat percentage (e.g. $\beta_{cis\text{-DBCA}}$=-

0.75, 95%CI:-1.34, -0.17) per 10-fold higher concentration of each metabolite (Table 5.2.3). Pyrethroids were not associated with child height or weight z-scores or blood pressure overall (Table 5.2.3).

Inverse associations between pyrethroid metabolites and adiposity were observed only among children whose mothers had sufficient energy intake during pregnancy. In this subgroup, *cis*-DCCA (β=-0.43, 95%CI:-0.73,-0.14) and *trans*-DCCA (β=-0.40, 95%CI:-0.67,-0.12) were each associated with lower BMI z-score, with p-values for interaction (p_{inter}) of 0.03 and 0.05, respectively, as well as lower body fat percentage ($\beta_{cis\text{-}DCCA}$=-1.30, 95%CI:-2.37,-0.24; $\beta_{trans\text{-}DBCA}$=-1.32, 95%CI:-2.27,-0.37), though evidence of effect modification for this outcome was weaker (p_{inter}=0.12 and 0.15, respectively; Table 5.2.4). Inverse associations between pyrethroid metabolites and BMI z-scores also tended to be stronger among children from non-poor households relative to those from poor households, especially for *cis*-DBCA (β=-0.36, 95%CI:-0.61,-0.11; p_{inter}=0.08) and to a lesser extent 3-PBA (β=-0.40, 95%CI:-0.73,-0.06; p_{inter}=0.13) (Table 5.2.5). Associations between pyrethroids and other outcomes did not vary by maternal energy intake (Table 5.2.4, Supplementary Table 5.3.2) or poverty (Table 5.2.5, Supplementary Table 5.3.3).

When we investigated effect modification by child sex, *trans*-DCCA concentrations were associated with higher height z-score among girls (β=0.23, 95%CI:0.05,0.41) but not among boys (β=-0.06, 95%CI:-0.25,0.14; p_{inter}=0.04), and associations with measures of adiposity or blood pressure did not vary by sex (Table 5.2.6, Supplementary Table 5.3.4).

Effects of gestational DDT/E exposure on child cardiometabolic risk factors at 5 years of age

We observed a 0.39% (95%CI:-0.76,-0.02) reduction in body fat percentage per 10-fold higher *p,p'*-DDT concentration. Estimates of similar magnitude were observed for *o,p'*-DDT and

p,p'-DDE, though confidence intervals included the null (Table 5.2.3). In analyses examining effect modification by child sex, greater reductions in body fat percentage were observed among boys relative to girls for all three analytes, but evidence of effect modification was limited (p_{inter} of 0.12 to 0.20; Table 5.2.6).

Table 5.2.1. Characteristics of VHEMBE participants who completed the 5-year visit, Limpopo, South Africa (n = 637)

Baseline maternal characteristics		
Age, years (mean, ±SD)	26.4	±6.2
Height, cm (mean, ±SD)	158.1	±6.9
Post-delivery weight, kg (mean, ±SD)	69.1	±13.8
Post-delivery BMI, kg/m^2 (mean, ±SD)	27.7	±5.5
Married or living-as-married (n, %)	296	46%
High school diploma (n, %)	276	43%
Nulliparous (n, %)	272	43%
Insufficient energy intake during pregnancy[a] (n, %)	427	68%
Any alcohol during pregnancy (n, %)	37	6%
HIV positive (n, %)	79	12%
Baseline household sociodemographic characteristics		
Food poverty[b] (n, %)	388	61%
Food insecurity[c] (n, %)	267	42%
Child characteristics		
Female sex (n, %)	313	49%
Low birthweight, <2500g (n, %)	47	7%
Preterm birth, <37 weeks (n, %)	79	12%
Any breastfeeding, months (mean, ±SD)	16.1	±7.0
Exclusive breastfeeding, months (mean, ±SD)	2.3	±1.9

Abbreviations: BMI, body mass index; SD, standard deviation.

[a]Below the Institute of Medicine recommended total daily caloric intake for mothers in late pregnancy.[344]

[b]Below the food poverty line of 386 Rand/person/month.[341]

[c]Two or more affirmative response to the US National Center for Health Statistics' Six-Item Food Security Scale.[342]

Table 5.2.2. Distribution of maternal peripartum serum DDT/E (ng/g lipid) and urinary pyrethroid metabolite (μg/L, specific gravity-corrected) concentrations among VHEMBE study participants, Limpopo, South Africa

	n	≥ LOD[a], %	≥ LOQ[b], %	Geom Mean	GSD	Min	Percentiles			Max
							25	50	75	
o,p′-DDT	637	90.7%	45.1%	9.22	4.57	<LOD	3.58	7.73	23.19	2029.27
p,p′-DDT	637	98.1%	90.7%	71.02	6.57	<LOD	19.79	60.70	263.12	15027.56
p,p′-DDE	637	100%	97.5%	295.24	4.75	3.98	94.40	256.53	860.66	22613.43
cis-DBCA	628	100%	99.6%	0.34	3.06	0.02	0.15	0.32	0.74	13.39
cis-DCCA	628	100%	99.9%	0.47	2.54	0.05	0.26	0.45	0.80	209.49
trans-DCCA	628	100%	99.6%	0.55	3.03	0.03	0.25	0.53	1.04	268.95
3-PBA	627	100%	100%	1.10	2.36	0.10	0.65	1.03	1.84	88.22

Abbreviations: Geo mean, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; LOQ, limit of quantification

[a]LODs: 0.01 ng/mL (*o,p′*-DDT and *p,p′*-DDT), 0.03 ng/mL (*p,p′*-DDE), 0.0025 μg/L (*cis*-DBCA), 0.0045 μg/L (*cis*-DCCA), 0.0038 μg/L (*trans*-DCCA), and 0.0047 μg/L (3-PBA).

[b]LOQs: 0.05 ng/mL (*o,p′*-DDT and *p,p′*-DDT), 0.15 ng/mL (*p,p′*-DDE), 0.0082 μg/L (*cis*-DBCA), 0.015 μg/L (*cis*-DCCA), 0.013 μg/L (*trans*-DCCA), and 0.016 μg/L (3-PBA).

Table 5.2.3. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations and cardiometabolic risk factors among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

		Height z-score β (95% CI)	**Weight z-score** β (95% CI)	**BMI z-score** β (95% CI)	**Fat percentage, %** β (95% CI)	**Waist circumference, cm** β (95% CI)	**Systolic blood pressure, mmHg** β (95% CI)	**Diastolic blood pressure, mmHg** β (95% CI)
DDT/E	*o,p'*-DDT	0.07 (-0.03, 0.17)	0.09 (-0.01, 0.18)	0.06 (-0.06, 0.17)	-0.24 (-0.75, 0.26)	0.09 (-0.25, 0.42)	0.35 (-0.97, 1.67)	0.49 (-0.77, 1.74)
	p,p'-DDT	-0.00 (-0.10, 0.09)	0.02 (-0.07, 0.11)	0.02 (-0.06, 0.11)	-0.39 (-0.76, -0.02)[a]	-0.02 (-0.34, 0.29)	0.04 (-0.88, 0.97)	0.22 (-0.71, 1.14)
	p,p'-DDE	0.10 (-0.01, 0.21)	0.10 (-0.01, 0.20)	0.05 (-0.06, 0.15)	-0.28 (-0.70, 0.14)	0.24 (-0.14, 0.62)	0.07 (-1.01, 1.14)	-0.00 (-1.08, 1.07)
Pyrethroid metabolites	*cis*-DBCA	0.02 (-0.11, 0.15)	-0.11 (-0.24, 0.02)	-0.18 (-0.33, -0.03)[a]	-0.75 (-1.34, -0.17)[a]	-0.57 (-1.09, -0.06)[a]	-0.19 (-1.69, 1.32)	-0.12 (-1.75, 1.50)
	cis-DCCA	0.03 (-0.14, 0.19)	-0.10 (-0.26, 0.05)	-0.19 (-0.34, -0.03)[a]	-0.65 (-1.26, -0.04)[a]	-0.88 (-1.45, -0.30)[a]	0.24 (-1.45, 1.93)	-0.65 (-2.66, 1.37)
	trans-DCCA	0.09 (-0.03, 0.21)	-0.06 (-0.19, 0.06)	-0.17 (-0.32, -0.03)[a]	-0.78 (-1.28, -0.27)[a]	-0.58 (-1.07, -0.10)[a]	0.06 (-1.27, 1.39)	-1.30 (-2.77, 0.16)
	3-PBA	0.03 (-0.15, 0.21)	-0.10 (-0.27, 0.07)	-0.18 (-0.37, 0.00)	-0.75 (-1.44, -0.05)[a]	-0.84 (-1.50, -0.17)[a]	0.15 (-1.76, 2.07)	-0.98 (-3.07, 1.10)

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

[a]95% CI excludes the null.

Table 5.2.4. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations and adiposity, by maternal energy intake sufficiency, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	BMI z-score			**Fat percentage, %**			**Waist circumference, cm**		
	Sufficient β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**	**Sufficient** β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**	**Sufficient** β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**
o,p′-DDT	-0.02 (-0.25, 0.20)	0.08 (-0.04, 0.21)	0.43	-0.80 (-1.65, 0.06)	-0.04 (-0.62, 0.55)	0.15	0.01 (-0.80, 0.82)	0.11 (-0.26, 0.48)	0.83
p,p′-DDT	-0.07 (-0.28, 0.15)	0.06 (-0.03, 0.15)	0.30	-0.81 (-1.70, 0.08)	-0.23 (-0.60, 0.14)	0.24	-0.15 (-0.92, 0.62)	0.02 (-0.31, 0.35)	0.70
p,p′-DDE	-0.07 (-0.33, 0.18)	0.10 (-0.01, 0.21)	0.24	-0.88 (-1.90, 0.14)	-0.05 (-0.47, 0.37)	0.15	-0.01 (-0.91, 0.89)	0.32 (-0.04, 0.68)	0.50
cis-DBCA	-0.17 (-0.48, 0.13)	-0.19 (-0.37, -0.01)	0.90	-1.06 (-2.09, -0.03)[a]	-0.62 (-1.32, 0.07)	0.49	-0.39 (-1.43, 0.65)	-0.71 (-1.32, -0.11)[a]	0.60
cis-DCCA	-0.43 (-0.73, -0.14)[a]	-0.05 (-0.23, 0.13)	0.03[b]	-1.30 (-2.37, -0.24)[a]	-0.31 (-1.01, 0.40)	0.12	-0.97 (-1.94, 0.00)	-0.83 (-1.54, -0.12)[a]	0.82
trans-DCCA	-0.40 (-0.67, -0.12)[a]	-0.06 (-0.23, 0.10)	0.04[b]	-1.32 (-2.27, -0.37)[a]	-0.51 (-1.10, 0.08)	0.15	-0.58 (-1.48, 0.32)	-0.58 (-1.15, 0.00)	1.00
3-PBA	-0.39 (-0.78, 0.00)	-0.10 (-0.30, 0.11)	0.19	-1.35 (-2.74, 0.03)	-0.53 (-1.35, 0.30)	0.31	-0.71 (-1.96, 0.54)	-0.94 (-1.72, -0.16)[a]	0.75

Abbreviations: CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

[a]95% CI excludes the null.

[b]p-value for interaction<0.1.

Table 5.2.5. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations and adiposity, by household food poverty status, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	BMI z-score			**Fat percentage, %**			**Waist circumference, cm**		
	Non-poor β (95% CI)	**Poor** β (95% CI)	**p_{inter}**	**Non-poor** β (95% CI)	**Poor** β (95% CI)	**p_{inter}**	**Non-poor** β (95% CI)	**Poor** β (95% CI)	**p_{inter}**
o,p'-DDT	0.10 (-0.04, 0.25)	0.03 (-0.13, 0.19)	0.52	-0.13 (-0.77, 0.51)	-0.30 (-1.00, 0.41)	0.75	0.14 (-0.36, 0.63)	0.05 (-0.40, 0.50)	0.80
p,p'-DDT	0.06 (-0.08, 0.19)	0.01 (-0.11, 0.14)	0.67	-0.07 (-0.63, 0.49)	-0.54 (-1.04, -0.04)[a]	0.23	0.14 (-0.26, 0.54)	-0.09 (-0.54, 0.36)	0.46
p,p'-DDE	-0.02 (-0.17, 0.14)	0.09 (-0.07, 0.24)	0.39	-0.24 (-0.87, 0.40)	-0.29 (-0.91, 0.33)	0.91	0.18 (-0.35, 0.71)	0.28 (-0.25, 0.81)	0.80
cis-DBCA	-0.36 (-0.61, -0.11)[a]	-0.07 (-0.27, 0.12)	0.08[b]	-1.10 (-2.06, -0.13)[a]	-0.55 (-1.31, 0.20)	0.40	-0.96 (-1.78, -0.13)[a]	-0.35 (-1.00, 0.31)	0.25
cis-DCCA	-0.27 (-0.53, -0.01)[a]	-0.14 (-0.34, 0.06)	0.44	-0.29 (-1.35, 0.76)	-0.85 (-1.65, -0.05)[a]	0.42	-0.90 (-1.82, 0.02)	-0.86 (-1.58, -0.14)[a]	0.94
trans-DCCA	-0.30 (-0.56, -0.03)[a]	-0.12 (-0.30, 0.07)	0.28	-0.73 (-1.74, 0.28)	-0.78 (-1.40, -0.16)[a]	0.93	-0.73 (-1.62, 0.16)	-0.51 (-1.11, 0.08)	0.69
3-PBA	-0.40 (-0.73, -0.06)[a]	-0.08 (-0.31, 0.15)	0.13	-0.79 (-2.09, 0.51)	-0.71 (-1.57, 0.15)	0.92	-0.98 (-2.10, 0.13)	-0.76 (-1.58, 0.05)	0.75

Abbreviations: CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

[a]95% CI excludes the null.

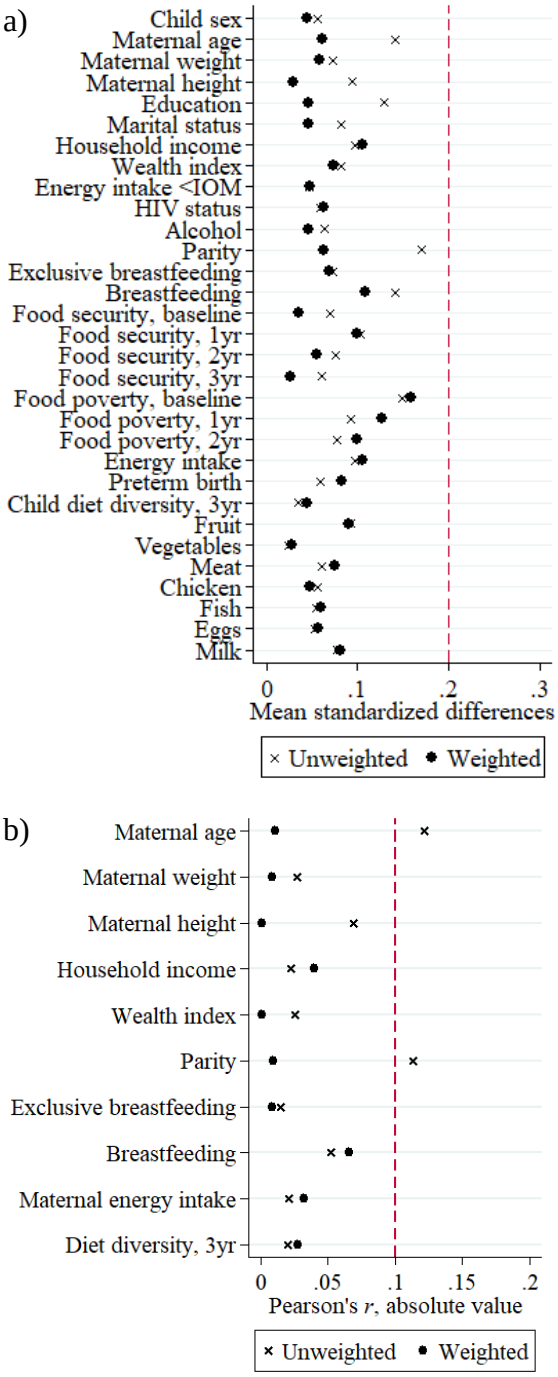
[b]p-value for interaction <0.1.

Table 5.2.6. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations on adiposity, by sex, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	BMI z-score			**Fat percentage, %**			**Waist circumference, cm**		
	Boys β (95% CI)	**Girls** β (95% CI)	p_{inter}	**Boys** β (95% CI)	**Girls** β (95% CI)	p_{inter}	**Boys** β (95% CI)	**Girls** β (95% CI)	p_{inter}
o,p′-DDT	-0.03 (-0.16, 0.11)	0.13 (-0.05, 0.31)	0.20	-0.65 (-1.15, -0.14)[a]	0.14 (-0.67, 0.95)	0.12	-0.11 (-0.50, 0.28)	0.29 (-0.29, 0.86)	0.27
p,p′-DDT	-0.02 (-0.13, 0.10)	0.07 (-0.07, 0.21)	0.36	-0.63 (-1.08, -0.18)[a]	-0.10 (-0.71, 0.52)	0.20	-0.04 (-0.35, 0.27)	0.09 (-0.45, 0.63)	0.70
p,p′-DDE	-0.01 (-0.15, 0.13)	0.10 (-0.07, 0.28)	0.35	-0.64 (-1.12, -0.17)[a]	0.10 (-0.67, 0.87)	0.13	0.06 (-0.35, 0.48)	0.44 (-0.22, 1.11)	0.35
cis-DBCA	-0.15 (-0.35, 0.04)	-0.20 (-0.43, 0.04)	0.79	-0.43 (-1.10, 0.23)	-1.03 (-1.97, -0.09)[a]	0.31	-0.29 (-0.88, 0.29)	-0.81 (-1.66, 0.03)	0.32
cis-DCCA	-0.15 (-0.37, 0.07)	-0.22 (-0.43, -0.01)[a]	0.62	-0.14 (-0.94, 0.67)	-1.13 (-2.05, -0.21)[a]	0.11	-0.71 (-1.48, 0.06)	-1.05 (-1.87, -0.22)[a]	0.55
trans-DCCA	-0.16 (-0.36, 0.04)	-0.19 (-0.40, 0.01)	0.79	-0.40 (-1.08, 0.28)	-1.16 (-1.98, -0.34)[a]	0.17	-0.58 (-1.20, 0.04)	-0.60 (-1.34, 0.14)	0.97
3-PBA	-0.15 (-0.41, 0.11)	-0.23 (-0.48, 0.02)	0.65	-0.28 (-1.10, 0.54)	-1.22 (-2.31, -0.12)[a]	0.19	-0.46 (-1.25, 0.34)	-1.20 (-2.20, -0.20)[a]	0.24

Abbreviations: CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.
[a]95% CI excludes the null.

Figure 5.2.1. Balance diagnostics for the final inverse probability weight for *trans*-DCCA before (×) and after (•) weighting: a) mean standardized differences across exposure quartiles and b) correlations with continuous potential confounders



Discussion

Main findings and interpretation

We found that higher maternal urine concentrations of *cis*-DBCA, *cis*-DCCA, *trans*-DCCA and 3-PBA were inversely associated multiple measures of adiposity (BMI z-score, waist circumference and body fat percentage) among 5-year-old South African children participating in the VHEMBE study. These results are consistent with inverse associations with BMI and/or weight-for-height z-scores reported among VHEMBE children at 1, 2 and 3.5 years[37,177]. While these previous reports suggested that associations were more pronounced among boys, in the present study we did not find evidence of effect modification by sex. In the only other study to examine gestational pyrethroid exposure and adiposity, maternal urine concentrations of 3-PBA were not associated with BMI z-score in a slightly smaller sample (n=478) of South Korean children at 4 years of age[178]; however, because the investigators adjusted for potential mediators including gestational age and birthweight, the reported estimates may have been biased towards the null[173,179,180].

Similar to our findings at age 3.5 years[37], we observed larger reductions in BMI z-score from pyrethroid exposure among children whose mothers had sufficient energy intake during pregnancy and no effect in children of mothers with insufficient intake. It is possible that children who are in an energy-poor environment may have reached a physiological minimum that prevents them from losing additional fat mass. Some experimental data supports our findings: mice chronically exposed to cypermethrin during puberty had lower body fat percentage and triglyceride levels compared to unexposed mice[190], but no effect on body fat was observed among permethrin-exposed mice who were fed a low-fat diet[191,192]. However, evidence

for effect modification by energy intake was weaker for other adiposity measures and we found no evidence of effect modification by food poverty.

The exact mechanism of a possible anti-adipogenic effect of pyrethroids is unclear. Animal studies indicate that chronic exposure to pyrethroids such as permethrin and cypermethrin and deltamethrin induces changes in energy metabolism, including upregulation of pyruvate kinase[190], an enzyme involved in glycolysis[351]; uncoupling protein 2[190] and peroxisome proliferator-activated receptor alpha[185,190], which promote lipid breakdown[352,353]; and hormone-sensitive lipase[190], whose main function is to mobilize stored fats[354]. In addition, animal studies show that exposure to pyrethroids increases serum testosterone, a hormone with known anti-adipogenic effects[194,200].

In contrast to the literature which suggests an adipogenic effect of prenatal exposure to DDT/E, we found that *p,p'*-DDT was associated with a slight reduction in body fat percentage overall; however, the estimated magnitude was small and no associations were observed with the other isomers or other measures of adiposity. We therefore cannot exclude the possibility that this finding may be due to chance. We previously reported positive associations between maternal peripartum DDT concentrations and BMI z-score at ages 1 and 2 years among girls[177], but not at 3.5 years, in VHEMBE[37]. Evidence from other birth cohorts is mixed, with some reporting greater overweight, BMI, and/or waist circumference in boys at ages 6.5, 9, and 12 years[134,135,327], and increased BMI and waist circumference among daughters at 50 years of age[355], while other studies found no associations with adiposity at ages ranging from infancy to 20 years[133,330-332].

Strengths and Limitations

Our study presents several improvements over the existing literature. Importantly, other than VHEMBE[37] no prior studies used methods to address potential selection bias from loss to follow-up. In the present study, we noted imbalances for several variables when comparing participants lost to follow-up to those retained at the 5-year visit (Supplementary Figure 5.3.3); if outcomes were also related to loss to follow-up, this would create conditions for selection bias to arise. In addition, many studies used complete-case analysis in lieu of imputing missing covariate data, further increasing the potential for selection bias[38], and used confounder selection strategies such as univariate analysis, stepwise approaches, and change-in-estimate which may bias estimates and result in inaccurate confidence intervals[39-41].

In the current analysis, we applied inverse-probability weighting methods[263]. Though unmeasured confounding remains possible, these methods also allowed us to verify that exposures and measured confounders were balanced between censored and uncensored participants and across the exposure range after weighting. We also used multiple imputation to address the small amount of missing covariate data and used bootstrapping to calculate accurate confidence intervals for our effect estimates. Nevertheless, residual confounding or chance could explain our study findings, which rely on additional untestable assumptions, such as consistency and correct model specification.

We investigated multiple measures of adiposity, each capturing slightly different aspects of body composition and together providing a more detailed portrait of child health. While BMI is the most commonly used metric, one of its major disadvantages is that it does not distinguish between lean and fat mass[210]. Body fat percentage was measured using a bioelectrical impedance device validated in children[356], and waist circumference measures abdominal fat which is more

strongly linked to poor cardiometabolic health[357,358]. The agreement across all three measures lends greater confidence to our overall finding that pyrethroids may reduce adiposity, whereas findings with only a single measure may point to specific aspects of body composition or reflect chance findings.

In contrast to other studies investigating DDT and/or pyrethroids in an agricultural setting or in the context of historical widespread use, a major contribution of the VHEMBE study is that it takes place in the current indoor residual spraying context, addressing a key knowledge gap on the potential unintended health effects of this practice. Notably, all VHEMBE participants have detectable levels of *cis*-DBCA, a metabolite specific to deltamethrin which is the pyrethroid most commonly used for indoor residual spraying in South Africa, and we were therefore uniquely able to report on associations with child cardiometabolic risk factors. This said, pyrethroids are also commonly used in agriculture and retail products and so part of the exposure to VHEMBE participants may originate from these sources as well.

A limitation of this study is that exposure to pyrethroids was assessed based on a single measurement around the time of delivery, which may have introduced non-differential measurement error and may thus have attenuated our effect estimates. However, the reliability of spot urine concentrations of pyrethroid metabolites in representing longer term exposure may vary by population and context; intraclass correlation coefficients of 0.85 in Poland and 0.21 in the U.S have been reported[90,91]. In the context of IRS, elevated exposure to inhabitants may persist for months from repeated contact with contaminated surfaces, bedding, furniture, and stored food, especially since the pyrethroids used for IRS remain effective for up to 10 months, and the lack of direct sunlight and external elements indoors slows their degradation[92,93]. Furthermore, indicators of regular pesticide use, such as the presence of pesticide storage containers and self-reported use

of pesticides in the yard were associated with higher pyrethroid metabolite concentrations among VHEMBE mothers, suggesting that a single measurement may be representative of longer-term exposure in the VHEMBE population[8,89].

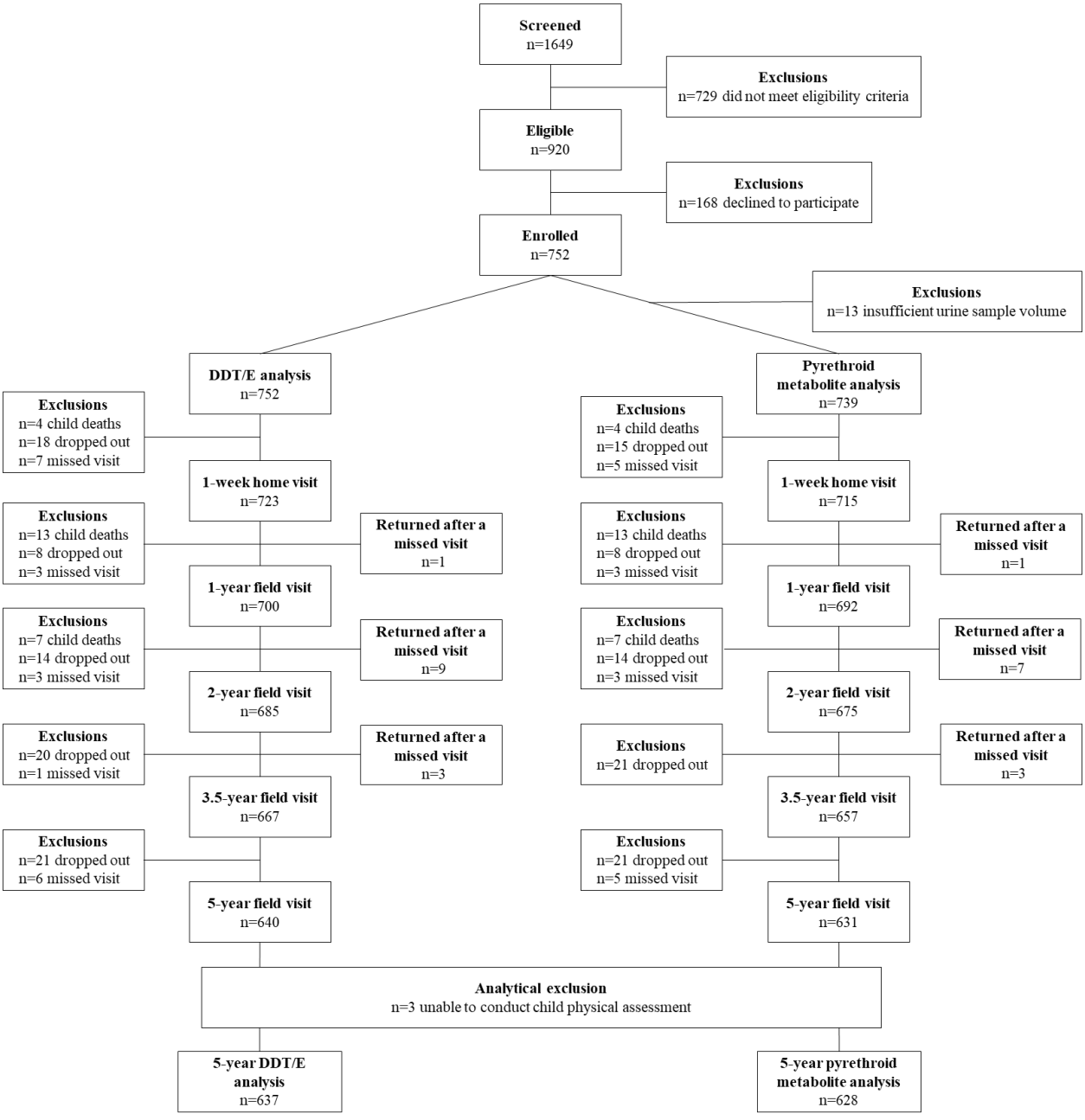
Conclusions

This study finds that prenatal exposure to pyrethroids may be related to reduced adiposity in children at five years of age. Such depletion of fat stores may be most detrimental in nutrient-poor environments. Future studies should investigate whether these associations persist later in childhood and consider evaluating relations with growth trajectories, which may better predict cardiometabolic risk[35,36].

5.3. Supplementary Material

5.3.1. Flow diagram of participants in the VHEMBE study

Figure 5.3.1. Flow diagram of participants in the VHEMBE study



5.3.2. Inverse probability weights for censoring and treatment

Two types of inverse probability weights were constructed: inverse probability of censoring weights (IPCWs), to address selection bias from loss to follow-up from baseline to the 5-year study visit; and inverse probability of treatment weights (IPTWs) for each exposure, to address confounding.

We used a directed acyclic graph (Figure 5.3.2) to identify the following potential confounders of the exposure-outcome relationship and predictors of censoring and outcomes to be included in both the IPTW and IPCW models: child sex (boy/girl); household food poverty (yes/no), food insecurity (yes/no), and wealth index (continuous); maternal age (years, continuous), height (metres, continuous), post-delivery weight (kg, continuous), education (high school vs. no high school), marital status (married or living-as-married vs. not married), energy intake during pregnancy (insufficient/sufficient), alcohol use during pregnancy (yes/no), HIV status (positive/negative), duration of exclusive breastfeeding (months, continuous), and parity (continuous). In the IPCW models, we also included gestational age (preterm vs. not preterm) and DDT/E and pyrethroid metabolite concentrations.

For the IPCWs, we first used logistic regression to estimate the probability of the censoring status of each subject (i.e. completed the 5-year visit vs. lost to follow-up), conditional on all exposures and covariates identified above[263]. We constructed IPCWs based on the inverse of these probabilities and stabilized the weights with the marginal probability of the censoring status received[263].

Then, excluding censored individuals, we constructed IPTWs based on the generalized propensity score (GPS) method[265,346] for each exposure, using multivariable linear regression to

estimate the exposure density function conditional on the covariates identified above. We then generated stabilized IPTWs with the GPS in the denominator and the marginal exposure density in the numerator[263]. To investigate effect measure modification by child sex, food poverty, and energy intake during pregnancy, we generated IPTWs that were instead stabilized by the exposure density conditional on the effect modifier.

The final inverse-probability weights used in the marginal structural models (i.e. outcome regressions) were the product of the IPCWs and IPTWs.

Balance diagnostics for inverse probability weights

We assessed covariate balance for the IPCWs and the final inverse probability weights (IPCW × IPTW)[266,269]. For the IPCWs, we calculated standardized differences and variance ratios for each covariate, comparing participants at the 5-year visit to those lost to follow-up (Figure 5.3.3). Modelling death and dropout as two separate censoring mechanisms led to poor balance of the IPCW for deaths due to finite sample bias (24 deaths, 3%), therefore, only a single IPCW model was used.

For the final sets of inverse probability weights, we assessed balance using three metrics:

i) Pearson correlation coefficients between the exposure and each continuous covariate (Figure 5.3.4)

ii) Standardized differences comparing all covariates across each quartile of exposure versus all other quartiles. Their absolute values were then averaged across the four comparisons (Figure 5.3.5). Quartiles of exposure were used to avoid small cell sizes and finite sample bias when assessing standardized differences.

iii) Variance ratios comparing the variance of all covariates across each quartile of exposure versus all other quartiles, which were then averaged across the four comparisons (Figure 5.3.6).

Following published guidelines, variables with mean absolute standardized differences below 0.2 when comparing across exposure quartiles (accounting for additional variability expected from small sample sizes)[269], below 0.1 when comparing across censoring status, and correlations below 0.1, were considered to be balanced[266,269]. Variance ratios of 1.0 describes a covariate which has equal variance across exposure categories, and a threshold of <2.0 has been suggested to indicate balance[267].

Figure 5.3.2. Directed acyclic graph of the relationship between maternal peripartum DDT/E and pyrethroid metabolite concentrations and child size, adiposity and cardiometabolic health

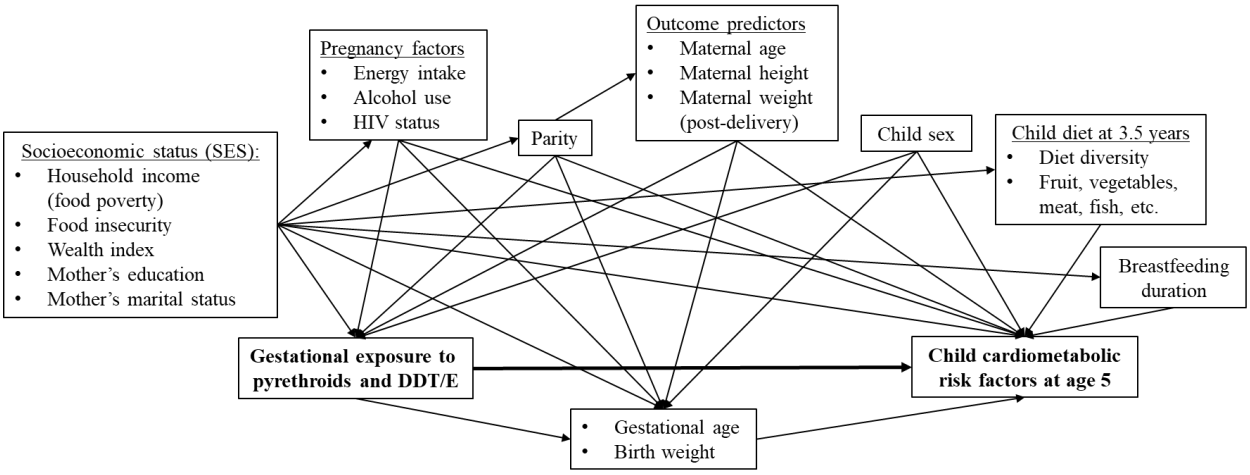
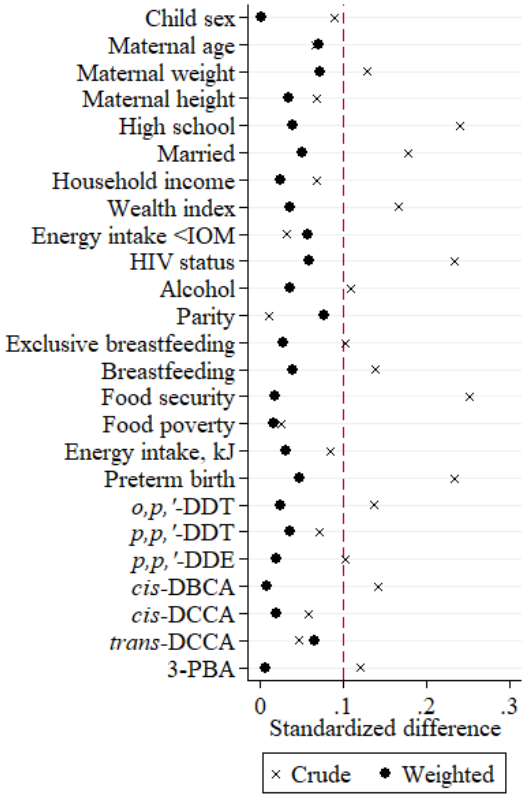


Figure 5.3.3. Balance diagnostics comparing participants at the 5-year visit to those lost to follow-up, before (×) and after (•) IPCW-weighting

a) Absolute standardized differences



b) Variance ratios

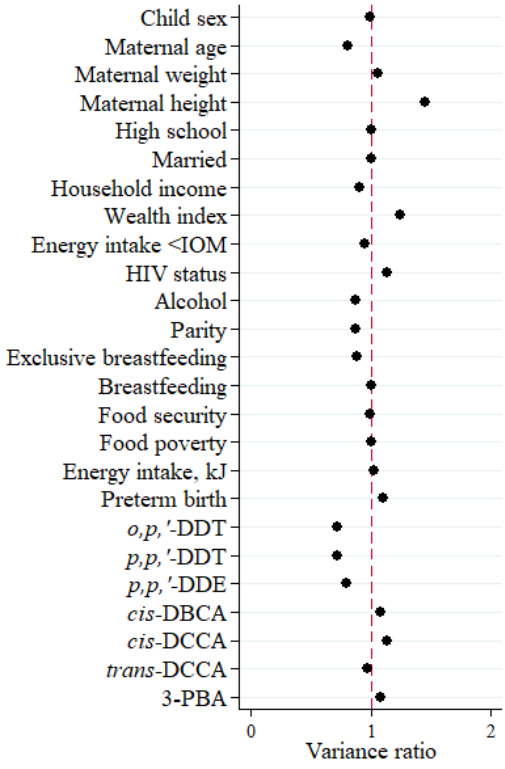


Table 5.3.1. Distribution of the final inverse probability weights for the overall model and models investigating effect modification

Exposure	Model	n	Mean	SD	Min	Max
p,p'-DDE	Overall	637	1.00	0.11	0.87	1.90
	Child sex	637	1.00	0.26	0.27	2.74
	Food poverty	637	1.00	0.25	0.32	2.95
	Energy intake	637	1.00	0.26	0.27	2.75
o,p'-DDT	Overall	637	1.00	0.25	0.29	2.63
	Child sex	637	1.00	0.23	0.21	3.83
	Food poverty	637	1.00	0.20	0.24	2.94
	Energy intake	637	1.00	0.23	0.21	3.90
p,p'-DDT	Overall	637	1.00	0.23	0.21	3.79
	Child sex	637	1.00	0.22	0.36	2.34
	Food poverty	637	1.00	0.18	0.37	1.82
	Energy intake	637	1.00	0.22	0.36	2.34
cis-DBCA	Overall	637	1.00	0.21	0.37	2.57
	Child sex	628	1.00	0.20	0.35	2.21
	Food poverty	628	1.00	0.20	0.32	2.29
	Energy intake	628	1.00	0.20	0.36	2.17
cis-DCCA	Overall	628	1.00	0.20	0.44	2.09
	Child sex	628	1.00	0.21	0.32	2.49
	Food poverty	628	1.00	0.21	0.33	2.62
	Energy intake	628	1.00	0.22	0.30	2.75
trans-DCCA	Overall	628	1.00	0.21	0.31	2.45
	Child sex	628	1.00	0.21	0.38	2.15
	Food poverty	628	1.00	0.21	0.39	2.11
	Energy intake	628	1.00	0.20	0.32	2.50
3-PBA	Overall	628	1.00	0.21	0.39	2.13
	Child sex	627	1.00	0.24	0.45	3.13
	Food poverty	627	1.00	0.23	0.42	2.98
	Energy intake	627	1.00	0.24	0.41	2.89

Abbreviations: SD, standard deviation; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Figure 5.3.4. Correlations between each exposure and continuous potential confounders, before (×) and after (•) inverse probability weighting

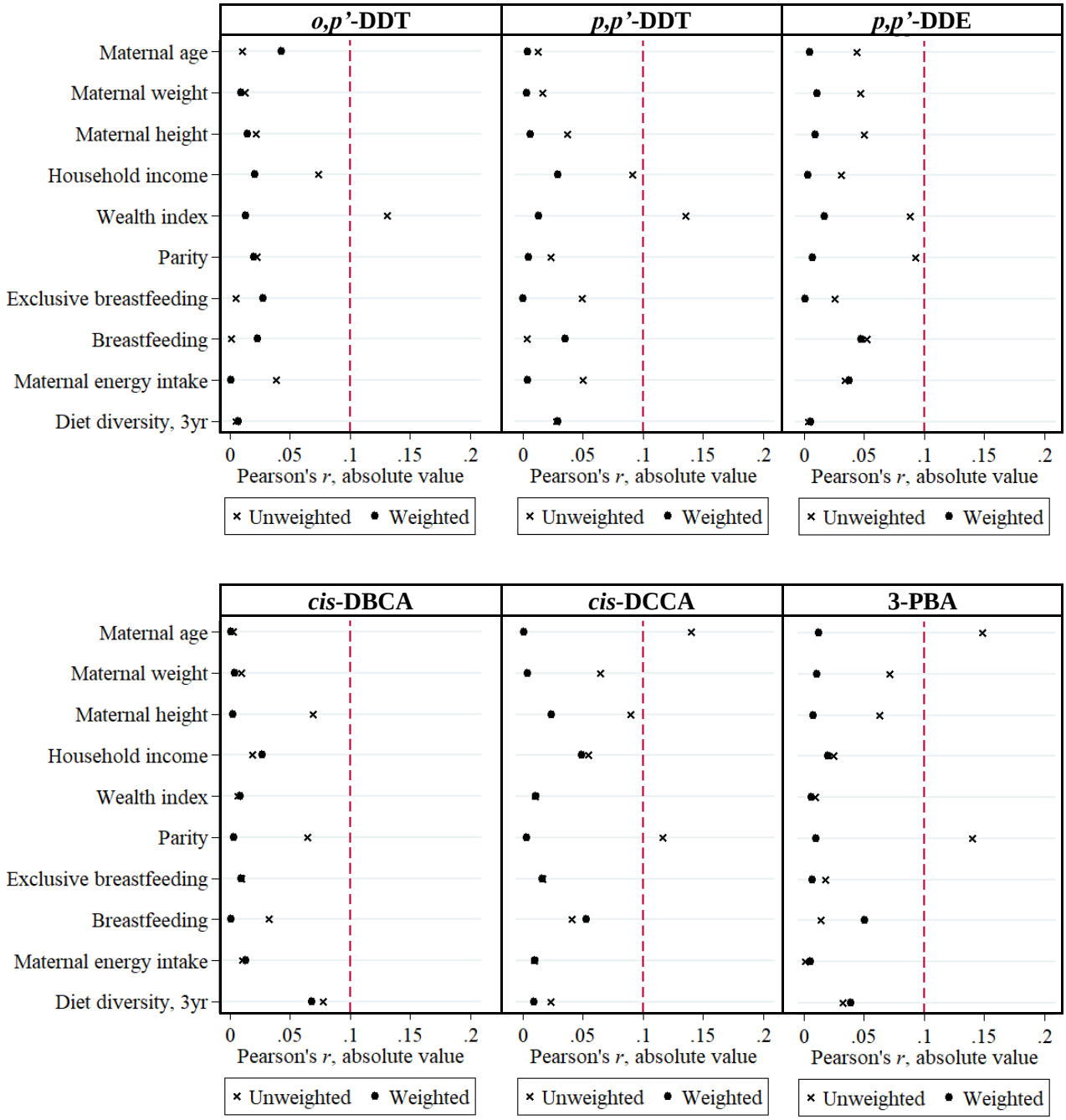


Figure 5.3.5. Absolute standardized differences of all potential confounders, averaged across exposure quartiles, before (×) and after (•) inverse probability weighting

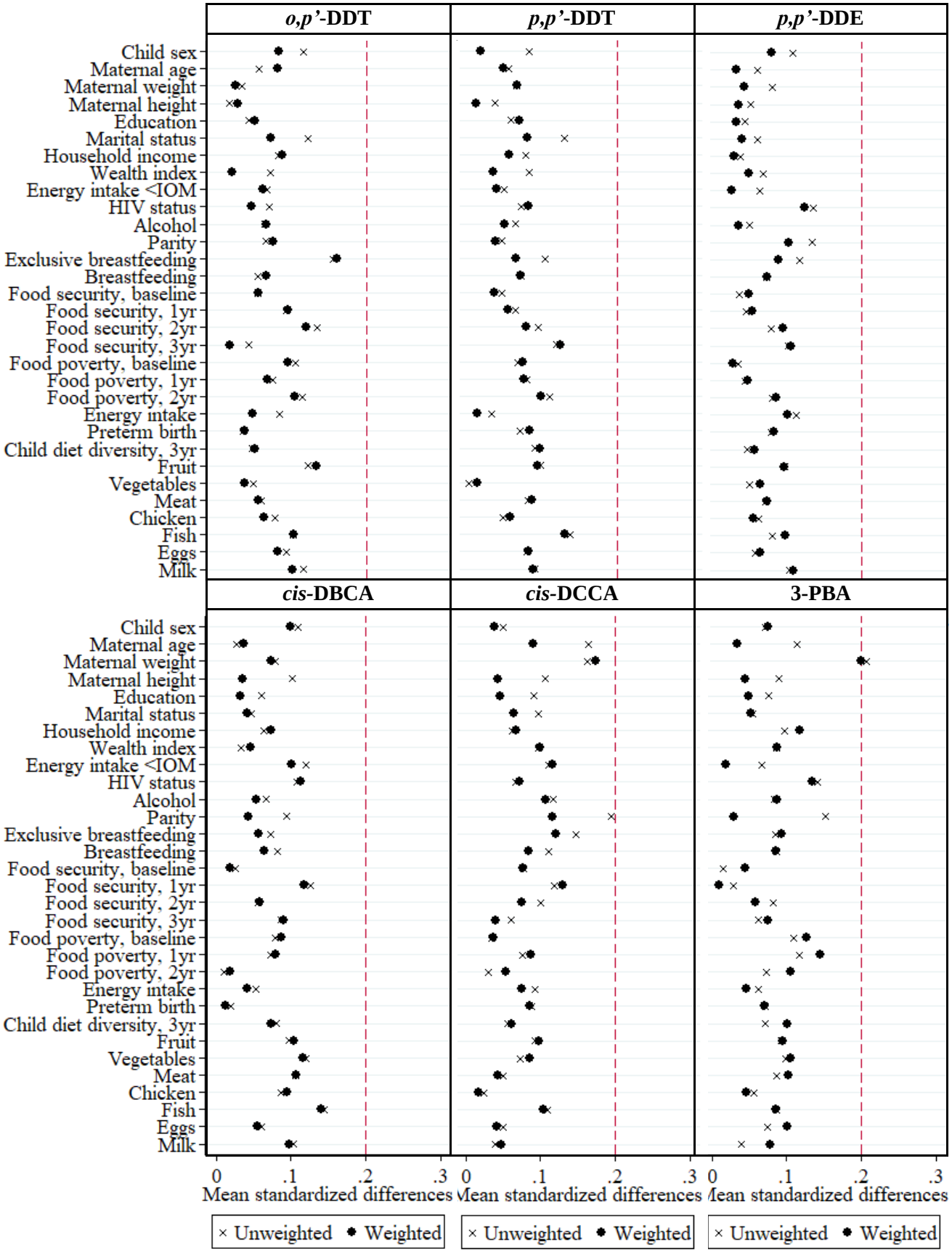
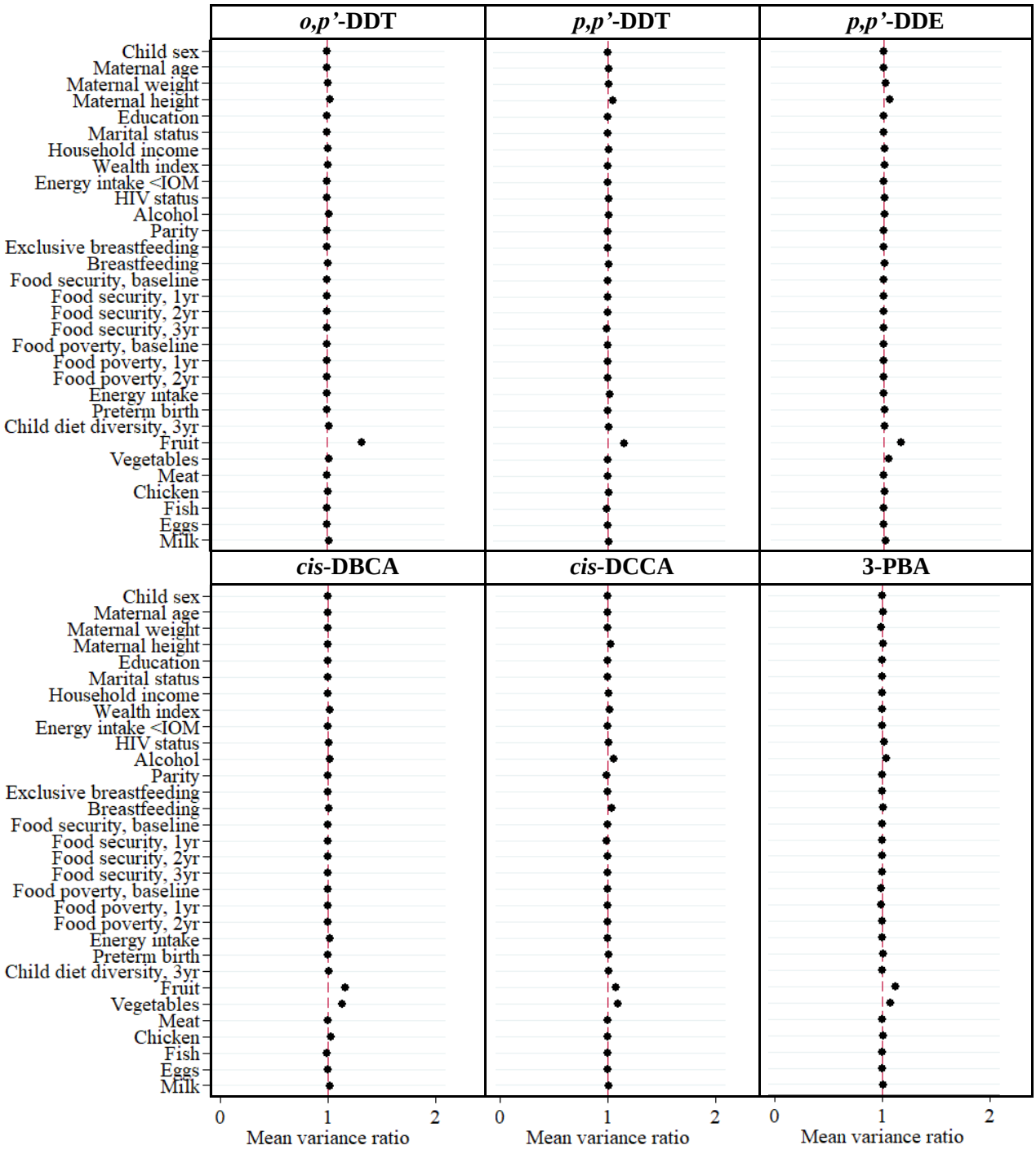


Figure 5.3.6. Variance ratios for all potential confounders, averaged across exposure quartiles, after inverse probability weighting



5.3.3. Multiple imputation by chained equations

We conducted multiple imputation by chained equations using the mi suite of commands in Stata version 14.2 (StataCorp, College Station, TX). Continuous variables were imputed using predictive mean matching and binary variables were imputed using logistic regression. We used a burn-in period of 10 iterations and generated 10 imputed datasets[38].

We included in the imputation models all outcomes, exposures and covariates identified in Section 2.5. (Statistical analysis), with the exception of two derived variables (food poverty and insufficient maternal energy intake) and included the component variables in the imputation models instead. Specifically, we derived missing values of food poverty from imputed total household income, and derived missing values of insufficient maternal energy intake from imputed maternal age, height, post-delivery weight, and energy intake during pregnancy.

We also included auxiliary variables in the imputation models to improve prediction of total household income (auxiliary variable: food poverty at the 1-year study visit) and exclusive breastfeeding (auxiliary variable: total breastfeeding duration)[38].

Finally, we also included variables representing the interaction between the exposures and effect modifiers (sex, food poverty, food insecurity, maternal energy intake sufficiency)[360]. However, to address issues of collinearity and to reduce the number of terms added to the imputation models, we only included interaction terms for three of the seven exposures (*p,p'*-DDT, *cis*-DBCA and *cis*-DCCA) and three of the four effect modifiers (sex, food poverty, and maternal energy intake sufficiency). As stated in our Results section, correlations were high between congeners of DDT/E (Pearson's r= 0.69 to 0.85) and between the pyrethroid metabolites *cis*-DCCA, *trans*-DCCA, and 3-PBA (r= 0.83 to 0.88), therefore the analyte that was most strongly correlated with the other two analytes within the group was selected (*p,p'*-DDT and *cis*-DCCA,

respectively); *cis*-DBCA was not correlated with the other pyrethroid metabolites. Among the effect modifiers, food poverty and food insecurity were highly associated with each other ($p<0.001$), therefore only interaction terms with food poverty were created.

5.3.4. Effect modification of prenatal insecticide exposure on child size and blood pressure

Table 5.3.2. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations and size and blood pressure, by maternal energy intake sufficiency, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	Height z-score			**Weight z-score**		
	Sufficient β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**	**Sufficient** β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**
o,p′-DDT (ng/g lipid)	0.22 (0.01, 0.43)[a]	0.02 (-0.10, 0.14)	0.10	0.13 (-0.09, 0.35)	0.07 (-0.04, 0.18)	0.65
p,p′-DDT (ng/g lipid)	0.09 (-0.11, 0.29)	-0.04 (-0.15, 0.07)	0.26	0.02 (-0.22, 0.25)	0.02 (-0.08, 0.11)	0.99
p,p′-DDE (ng/g lipid)	0.21 (0.01, 0.41)[a]	0.05 (-0.09, 0.19)	0.19	0.09 (-0.15, 0.33)	0.10 (-0.01, 0.20)	0.94
cis-DBCA (µg/L)	0.11 (-0.13, 0.36)	-0.04 (-0.21, 0.13)	0.34	-0.03 (-0.29, 0.23)	-0.16 (-0.32, 0.00)	0.42
cis-DCCA (µg/L)	0.09 (-0.14, 0.33)	-0.01 (-0.24, 0.23)	0.57	-0.23 (-0.46, 0.01)	-0.04 (-0.24, 0.17)	0.24
trans-DCCA (µg/L)	0.21 (-0.01, 0.42)	0.03 (-0.13, 0.19)	0.22	-0.13 (-0.36, 0.10)	-0.03 (-0.18, 0.12)	0.46
3-PBA (µg/L)	0.24 (-0.07, 0.55)	-0.07 (-0.30, 0.15)	0.10	-0.10 (-0.38, 0.19)	-0.12 (-0.32, 0.09)	0.91
	Systolic blood pressure, mmHg			**Diastolic blood pressure, mmHg**		
	Sufficient β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**	**Sufficient** β (95% CI)	**Insufficient** β (95% CI)	**p_{inter}**
o,p′-DDT (ng/g lipid)	0.04 (-1.61, 1.69)	0.47 (-1.27, 2.21)	0.73	-0.39 (-2.27, 1.50)	0.83 (-0.74, 2.39)	0.34
p,p′-DDT (ng/g lipid)	-0.19 (-1.53, 1.16)	0.14 (-1.06, 1.33)	0.73	-0.89 (-2.52, 0.73)	0.66 (-0.47, 1.79)	0.13
p,p′-DDE (ng/g lipid)	0.22 (-1.39, 1.84)	0.02 (-1.41, 1.44)	0.85	-0.48 (-2.34, 1.39)	0.27 (-1.09, 1.62)	0.54
cis-DBCA (µg/L)	1.55 (-0.96, 4.05)	-1.06 (-2.81, 0.70)	0.09[b]	1.41 (-1.80, 4.62)	-0.92 (-2.67, 0.82)	0.20
cis-DCCA (µg/L)	0.48 (-2.38, 3.33)	0.12 (-2.08, 2.32)	0.85	-0.82 (-4.10, 2.47)	-0.52 (-3.04, 1.99)	0.89
trans-DCCA (µg/L)	0.74 (-1.39, 2.87)	-0.27 (-2.03, 1.49)	0.49	-1.11 (-3.47, 1.26)	-1.41 (-3.33, 0.51)	0.85
3-PBA (µg/L)	1.89 (-1.69, 5.47)	-0.59 (-2.88, 1.70)	0.26	1.13 (-3.41, 5.67)	-1.81 (-4.19, 0.57)	0.27

Abbreviations: CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

[a]95% CI excludes the null;

[b]p-value for interaction<0.1.

Table 5.3.3. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations and size and blood pressure, by household food poverty status, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	Height z-score			**Weight z-score**		
	Non-poor β (95% CI)	**Poor** β (95% CI)	**p_{inter}**	**Non-poor** β (95% CI)	**Poor** β (95% CI)	**p_{inter}**
o,p'-DDT (ng/g lipid)	0.02 (-0.15, 0.19)	0.10 (-0.02, 0.22)	0.45	0.08 (-0.07, 0.23)	0.09 (-0.04, 0.22)	0.93
p,p'-DDT (ng/g lipid)	0.04 (-0.11, 0.19)	-0.03 (-0.15, 0.09)	0.48	0.06 (-0.06, 0.19)	-0.00 (-0.13, 0.12)	0.47
p,p'-DDE (ng/g lipid)	0.14 (-0.05, 0.32)	0.08 (-0.06, 0.22)	0.60	0.08 (-0.08, 0.23)	0.11 (-0.04, 0.26)	0.77
cis-DBCA (µg/L)	0.01 (-0.25, 0.28)	0.03 (-0.13, 0.19)	0.94	-0.24 (-0.46, -0.01)	-0.03 (-0.20, 0.14)	0.17
cis-DCCA (µg/L)	-0.08 (-0.38, 0.22)	0.09 (-0.12, 0.30)	0.37	-0.24 (-0.52, 0.03)	-0.02 (-0.22, 0.17)	0.22
trans-DCCA (µg/L)	0.02 (-0.24, 0.28)	0.12 (-0.04, 0.27)	0.54	-0.20 (-0.44, 0.05)	0.00 (-0.16, 0.16)	0.20
3-PBA (µg/L)	0.07 (-0.27, 0.41)	0.02 (-0.20, 0.24)	0.81	-0.23 (-0.55, 0.08)	-0.04 (-0.25, 0.18)	0.33
	Systolic blood pressure, mmHg			**Diastolic blood pressure, mmHg**		
	Non-poor β (95% CI)	**Poor** β (95% CI)	**p_{inter}**	**Non-poor** β (95% CI)	**Poor** β (95% CI)	**p_{inter}**
o,p'-DDT (ng/g lipid)	-0.92 (-2.39, 0.54)	1.04 (-0.75, 2.83)	0.08[a]	1.01 (-0.96, 2.99)	0.12 (-1.46, 1.70)	0.49
p,p'-DDT (ng/g lipid)	-0.30 (-1.43, 0.82)	0.32 (-0.98, 1.62)	0.46	0.90 (-0.49, 2.29)	-0.20 (-1.38, 0.98)	0.24
p,p'-DDE (ng/g lipid)	-0.62 (-2.08, 0.84)	0.47 (-0.96, 1.89)	0.28	0.90 (-0.86, 2.67)	-0.51 (-1.81, 0.80)	0.21
cis-DBCA (µg/L)	-0.55 (-2.92, 1.82)	0.01 (-1.94, 1.97)	0.72	0.33 (-1.90, 2.57)	-0.45 (-2.72, 1.83)	0.64
cis-DCCA (µg/L)	0.54 (-2.03, 3.11)	0.04 (-2.21, 2.29)	0.78	-1.08 (-3.57, 1.41)	-0.42 (-3.23, 2.40)	0.73
trans-DCCA (µg/L)	0.11 (-2.07, 2.28)	-0.02 (-1.76, 1.72)	0.93	-1.30 (-3.53, 0.93)	-1.32 (-3.20, 0.57)	0.99
3-PBA (µg/L)	0.08 (-2.96, 3.13)	0.18 (-2.31, 2.68)	0.96	-1.03 (-4.05, 1.99)	-0.95 (-3.76, 1.85)	0.97

Abbreviations: CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

[a]p-value for interaction<0.1.

Table 5.3.4. Relations between a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations and size and blood pressure, by sex, among 5-year-old children participating in the VHEMBE study, Limpopo, South Africa

	Height z-score			**Weight z-score**		
	Boys	**Girls**	**p_{inter}**	**Boys**	**Girls**	**p_{inter}**
	β (95% CI)	β (95% CI)		β (95% CI)	β (95% CI)	
o,p'-DDT (ng/g lipid)	0.07 (-0.08, 0.21)	0.08 (-0.06, 0.22)	0.93	0.03 (-0.09, 0.15)	0.13 (-0.02, 0.29)	0.33
p,p'-DDT (ng/g lipid)	0.07 (-0.05, 0.18)	-0.05 (-0.18, 0.09)	0.20	0.04 (-0.06, 0.13)	0.02 (-0.12, 0.16)	0.88
p,p'-DDE (ng/g lipid)	0.11 (-0.03, 0.25)	0.10 (-0.07, 0.26)	0.91	0.07 (-0.06, 0.20)	0.13 (-0.04, 0.31)	0.60
cis-DBCA (µg/L)	0.06 (-0.14, 0.27)	-0.00 (-0.19, 0.18)	0.65	-0.06 (-0.23, 0.11)	-0.14 (-0.34, 0.07)	0.57
cis-DCCA (µg/L)	-0.15 (-0.40, 0.10)	0.18 (-0.05, 0.41)	0.07[b]	-0.19 (-0.41, 0.03)	-0.04 (-0.26, 0.19)	0.34
trans-DCCA (µg/L)	-0.06 (-0.25, 0.14)	0.23 (0.05, 0.41)[a]	0.04[b]	-0.14 (-0.32, 0.03)	0.02 (-0.17, 0.20)	0.24
3-PBA (µg/L)	0.01 (-0.25, 0.26)	0.04 (-0.20, 0.29)	0.85	-0.09 (-0.31, 0.13)	-0.13 (-0.37, 0.10)	0.79
	Systolic blood pressure, mmHg			**Diastolic blood pressure, mmHg**		
	Boys	**Girls**	**p_{inter}**	**Boys**	**Girls**	**p_{inter}**
	β (95% CI)	β (95% CI)		β (95% CI)	β (95% CI)	
o,p'-DDT (ng/g lipid)	-0.39 (-2.03, 1.25)	1.07 (-0.83, 2.97)	0.26	-0.53 (-2.16, 1.10)	1.50 (-0.41, 3.41)	0.11
p,p'-DDT (ng/g lipid)	-0.00 (-1.24, 1.23)	0.18 (-1.13, 1.49)	0.84	0.17 (-0.95, 1.29)	0.35 (-1.05, 1.75)	0.84
p,p'-DDE (ng/g lipid)	0.19 (-1.16, 1.54)	-0.05 (-1.65, 1.54)	0.82	0.05 (-1.28, 1.38)	-0.09 (-1.73, 1.55)	0.89
cis-DBCA (µg/L)	-1.22 (-3.38, 0.94)	0.90 (-1.06, 2.85)	0.15	-0.09 (-2.25, 2.06)	0.02 (-2.48, 2.51)	0.95
cis-DCCA (µg/L)	-0.44 (-2.72, 1.84)	0.71 (-1.90, 3.32)	0.52	-1.88 (-4.25, 0.48)	0.35 (-2.87, 3.58)	0.26
trans-DCCA (µg/L)	-0.44 (-2.28, 1.41)	0.52 (-1.51, 2.56)	0.50	-1.65 (-3.77, 0.46)	-1.00 (-3.18, 1.19)	0.68
3-PBA (µg/L)	-1.36 (-3.78, 1.06)	1.51 (-1.39, 4.42)	0.14	-2.35 (-4.85, 0.16)	0.19 (-3.12, 3.49)	0.24

Abbreviations: CI, confidence interval; p_{inter}, p-value for interaction; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

[a]95% CI excludes the null.

[b]p-value for interaction<0.1.

CHAPTER 6. Manuscript 3

6.1. Preface

In Manuscript 2, gestational exposure to pyrethroids were associated with lower adiposity (BMI z-score, waist circumference, and body fat percentage) in the children at 5 years of age. As described in Chapter 3, although weight measured at specific ages remains an important marker of child development, child growth trajectories may be better indicators of later metabolic and cardiovascular health. Therefore, using data from the same VHEMBE birth cohort, the manuscript presented in this chapter examines the potential influence of gestational exposure to DDT/E and pyrethroid insecticides on weight trajectories from birth to 5 years of age. This manuscript has been accepted at *Epidemiology*.

6.2. Prenatal exposure to insecticides and child weight trajectories in South Africa: the VHEMBE birth cohort

Abstract

Background: Dichlorodiphenyltrichloroethane (DDT) or pyrethroid insecticides are sprayed inside dwellings for malaria vector control, resulting in high levels of exposure to millions of people, including pregnant women. These chemicals disrupt endocrine function and may affect child growth. Prior studies have yielded inconsistent results but few have investigated growth trajectories.

Methods: We investigated associations between gestational insecticide exposure and child growth trajectories in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort of 751 children born between 2012 and 2013 in Limpopo, South Africa. We modelled child weight trajectories from birth to 5 years using SuperImposition, Translation And Rotation (SITAR) based on child weight measurements taken at follow-up visits and abstracted from medical records. We then estimated associations between peripartum maternal concentrations of serum DDT, dichlorodiphenyldichloroethylene (DDE), or urinary pyrethroid metabolites and SITAR parameters (size and tempo) using marginal structural models.

Results: A 10-fold increase in maternal concentrations of the pyrethroid metabolite *trans*-DCCA was associated with 20.9g (95%CI: -40.1, -1.6) smaller size (lower weight) among boys while no association was found among girls (β =9.2g (95%CI: -14.04, 32.45); $p_{interaction}$=0.07). Results also suggested that pyrethroids may be associated with earlier tempo (age at peak weight velocity) but confidence intervals included the null. No associations with DDT or DDE were observed.

Conclusion: Inverse associations between pyrethroids and weight trajectory parameters among boys may reflect disruption of androgen pathways. These findings are consistent with our previous research in this population and support the endocrine-disrupting potential of pyrethroids in humans.

Introduction

Child growth is a global metric for development and wellbeing, and an important indicator of current and future health status. While slow growth can predict poor health outcomes[361,362], accelerated growth may increase the risk of obesity and cardiometabolic disease later in life[240,363]. Aside from genetics and nutrition, exposure to endocrine-disrupting chemicals may influence child growth by interfering with sex hormones involved in critical periods of rapid growth during hypothalamic-pituitary-gonadal (HPG) axis activation[111]. Each year, millions of individuals, including pregnant women, are exposed to high levels of such chemicals from indoor residual spraying (IRS), a malaria control method which consists of the application of insecticides on the interior walls of dwellings[30-32,320].

Pyrethroids, commonly used in agriculture and commercial pest control products globally and the most frequently used class of insecticides for IRS, disrupt androgen function[15,30,321,364,365]. However, few human studies have investigated their potential impact on child weight. In the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE), a birth cohort conducted in Limpopo, South Africa where IRS occurs annually, maternal urinary pyrethroid metabolite levels were not associated with weight at birth,[86] but were inversely associated with weight z-scores among boys at 1, 2, and 3.5 years[37,177,366]. In a Chinese birth cohort, the sum of three maternal pyrethroid metabolite concentrations was inversely associated with weight at birth[173]. However, other studies found no associations with weight at birth[174], 1 year,[176] and 4 years of age[178].

The insecticide dichlorodiphenyltrichloroethane (DDT) is a well-established estrogen agonist[75,77]. Though it is allowed for public health uses including IRS[30], it was otherwise banned in Western countries since the 1970s and internationally since 2001, leading to low detection

frequencies in most populations. Detection frequencies were sufficient to investigate associations between gestational exposure to DDT and postnatal child weight in only two cohorts (VHEMBE and a U.S. study initiated before DDT was banned), but several more studies have investigated DDT's more persistent, anti-androgenic breakdown product dichlorodiphenyldichloroethylene (DDE). In VHEMBE, maternal serum DDT but not DDE was associated with increased weight among girls at birth[86], 1 and 2 years[177], but no associations were observed at 3.5 years[37], and in the U.S. study, neither were associated with child weight at birth or 5 years[328]. Similarly, maternal serum DDE levels were not associated with weight measured from birth to 12 months in a Mexican birth cohort[329], but other studies reported positive associations with offspring weight at older ages[325,367,368].

Although weight measured at specific ages remains an important marker of child development, dynamic child growth metrics based on measures taken at two or more time points have been found to predict later metabolic and cardiovascular health better than single time point measures[35,36]. For example, rapid infant weight gain has been linked to: a 3.7-fold (95%CI: 2.6, 5.2) increased risk for overweight or obesity (based on a meta-analysis of 17 studies)[230], central adiposity[231], hypertension[233-240], lower HDL cholesterol[238], insulin resistance and/or diabetes[233,238,241,242], and metabolic syndome[233] in adolescence or adulthood. However, the few prior studies that investigated associations between exposure to pyrethroids or DDT/E and dynamic growth metrics primarily relied on changes between two time points (e.g. birth to one month, six months, or 24 months), which does not capture the complex nature of growth dynamics[136-138,181,324,326]. One study applied a grouping-based classification based on latent growth patterns[136]; however, these methods pose challenges for inference: subjective selection of the number and interpretation of observed patterns, loss of information from categorization of the

outcome, and lack of comparability of observed patterns between studies. SuperImposition, Translation And Rotation (SITAR) models, which have been used widely in the perinatal epidemiologic literature[243,245,246,369] overcome these limitations and estimate three biologically interpretable parameters: overall weight or other growth metric over time (size), and the timing (tempo) and velocity (intensity) of the growth spurt[248].

Here, we seek to estimate the effect of gestational exposure to DDT/E and pyrethroid insecticides on child weight trajectories from birth through five years of age, in a population residing in an area where these IRS insecticides are sprayed annually. This study presents a novel application of SITAR to investigate environmental chemical influences on child weight trajectories.

Methods

Data source

The VHEMBE study recruited mothers giving birth between August 2012 and December 2013 at Tshilizidini hospital in the Vhembe district of Limpopo, South Africa. Eligible women were at least 18 years of age, spoke Tshivenda at home, lived within 20 km of the hospital, intended to remain in the area for at least 2 years, did not have malaria during pregnancy, had contractions at least 5 minutes apart, and delivered a live, singleton infant. Study staff approached 1,649 mothers, 920 of whom met eligibility criteria. Of the eligible women, 752 provided informed consent; baseline questionnaire and peripheral blood samples for DDT/E analysis were available from 751 mothers, 738 of whom provided sufficient urine samples for pyrethroid analysis. Follow-up continued with a home visit 1 week postpartum and field office visits were completed at 1, 2, 3.5, and 5 years, with visit-over-visit retention rates of 96-99% after excluding child deaths.

All participating mothers provided informed consent. Ethics approval for the VHEMBE study was obtained from McGill University (Montreal, Quebec, Canada), the University of Pretoria (Pretoria, Gauteng, South Africa), Tshilidzini Hospital (Thohoyandou, Limpopo, South Africa), the Limpopo Department of Health and Social Development (Polokwane, Limpopo, South Africa), and the University of California, Berkeley (Berkeley, California, USA).

Exposure measurement: maternal serum DDT/E and urinary pyrethroid metabolites

Maternal urine and venous blood samples collected at delivery were processed immediately after collection and stored at -80°C until shipment to analytical laboratories on dry ice. Maternal serum concentrations of DDT/E isomers (*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, and *p,p'*-DDE) were measured using gas chromatography-tandem mass spectrometry by the Emory University Environmental Health Laboratory (Atlanta, Georgia, USA)[334]. Total serum lipid concentrations

were estimated based on total cholesterol and triglycerides which were measured by standard enzymatic methods (Roche Chemicals, Indianapolis, USA)[336]. Maternal urine concentrations of the following five pyrethroid metabolites were measured using gas chromatography-tandem mass spectrometry by the Institut National de Santé Publique du Québec (Quebec City, Quebec, Canada)[335]: *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DBCA), *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*cis*-DCCA), *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid (*trans*-DCCA), 3-phenoxybenzoic acid (3-PBA) and 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA). Urine specific gravity was measured with a portable refractometer (Atago PAL-10S; Tokyo, Japan). Limits of detection (LOD) were: 0.01ng/mL (*o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE), 0.03ng/mL (*p,p′*-DDE), 0.0025µg/L (*cis*-DBCA), 0.0045µg/L (*cis*-DCCA), 0.0038µg/L (*tran*s-DCCA), 0.0047µg/L (3-PBA), and 0.005µg/L (4-F-3-PBA). Limits of quantification (LOQ) were: 0.05ng/mL (*o,p′*-DDT, *p,p′*-DDT, *o,p′*-DDE), 0.15ng/mL (*p,p′*-DDE), 0.0082µg/L (cis-DBCA), 0.015µg/L (cis-DCCA), 0.013µg/L (trans-DCCA), 0.016µg/L (3-PBA), and 0.011µg/L (4-F-3-PBA).

Urinary concentrations of *o,p'*-DDE and 4-F-3-PBA were above the LOQ in only 16% and 8% of samples, respectively, and thus were excluded from further analyses. For the other analytes, concentrations below the LOD were imputed at random based on log-normal probability distributions whose parameters were estimated via maximum likelihood methods[298]. Machine-read values were used for values between the LOD and the LOQ. DDT and DDE were corrected for serum lipid content and expressed in ng/g lipid. Pyrethroid metabolite concentrations (C_{meas}) were corrected (C_{corr}) for urine dilution via specific gravity (SG), based on the formula from Levine and Fahy [337]: $C_{corr} = C_{meas} \times (1.024-1)/(SG-1)$.

Outcome measurement: child weight

Child weight was measured by trained study staff to the nearest 10 grams at the 1- and 2-year visits using a pediatric digital scale (Tanita BD-590; Tokyo, Japan), and at the 3.5- and 5-year visits using a standard digital scale (Tanita HD-351; Tokyo, Japan); a single measure was taken at each time point based on high reliability during testing. In addition, birthweight and body weight measurements recorded at the hospital, during well-child appointments, and other clinic visits were abstracted from medical records by a registered nurse, including the child's age in weeks or months when measurements were taken.

Covariates from maternal questionnaires and anthropometrics

Trained bilingual (Tshivenda and English) local study staff administered questionnaires at baseline and follow-up field office visits to collect sociodemographic, nutrition, and health information (Table 6.2.1). At baseline, mothers reported their age, marital status, total household income, and total household size. Food poverty was defined as earning less than 386 Rands/person/month based on Statistics South Africa guidelines[341]. Food insecurity was defined as two or more affirmative responses to the US National Center for Health Statistics' Six-Item Food Security Scale[342]. Daily total energy intake in kilojoules (kJ) was estimated using the FoodFinder 3 software (SouthAfrica Medical Research Council/WAMTechnology CC) based on a quantitative food frequency questionnaire designed by a South African nutritionist and validated in the local population[343]. Insufficient energy intake during pregnancy was defined according to the Institute of Medicine (IOM)-recommended total daily caloric intake for high-activity mothers in the third trimester, which was calculated based on their age, height, and post-delivery weight[344,345]. Maternal HIV status during pregnancy was ascertained from self-report or medical records indicating use of antiretroviral drugs, which were abstracted by registered nurses on the

study team. In addition, mothers' post-delivery weight was measured using a Beurer PS06 scale (Ulm, Germany) and height was measured in triplicate using a Charder HM200P stadiometer (Taichung, Taiwan), then averaged.

Information on duration of breastfeeding were obtained from the 1-week and 1-, 2-, and 3.5-year questionnaires. A family wealth index was constructed based on data obtained from the 1-week home visit (questionnaire and staff observations), following South Africa's Demographic and Health Surveys methodology in order to capture socioeconomic status in this region where much of the economy is informal[5,37].

To explore potential confounding by child dietary intake, we constructed a child diet diversity score reflecting the number of different food groups eaten, as reported by mothers at the 3.5-year visit[345].

Statistical analysis

Child weight trajectories estimated using SITAR

After removing 22 outliers (> 3 standard deviations from expected values conditional on preceding measurements)[251], we modelled child weight measurements from the study visits and medical records (n=13,489; median: 12 measurements per child, interquartile range: 4 to 24) using the `sitar` package (version 1.1.1) in R (version 3.6.1)[249,370]. SITAR fits a natural cubic spline to the average population growth curve (in this case, weight in kilograms versus age in months) from which individual deviations are captured by three random effect parameters[248], where:

$$y_{it} = a_i + h\left(\frac{t - b_i}{exp(-c_i)}\right)$$

a) Size (*a*) indicates the child's mean weight compared to the average (in kilograms), representing vertical translation of the weight curve;
b) Tempo (*b*) indicates the child's age at peak weight velocity compared to the average (in months), representing horizontal translation of the weight curve; and
c) Intensity (*c*) indicates the child's growth rate compared to the average (expressed as a fraction).

After fitting candidate models including all children, as well as models stratified by sex, the random effects parameters estimated by the best-fitting SITAR model were used as outcomes in marginal structural models as described below (see Supplementary Material Section 6.3.1 for details).

Inverse probability of treatment weight (IPTW) construction and balance assessment

To control for confounding, we constructed stabilized inverse probability of treatment weights (IPTW) based on the generalized propensity score (GPS) method[265,346]. To construct the GPS, we used multivariable linear regression to estimate the conditional density of each participant's exposure. The lipid- or specific gravity- corrected exposure concentrations were $\log_{10}$-transformed to reduce the influence of outliers. We included the following potential confounders and predictors of the outcomes as the independent variables, which were identified based on a directed acyclic graph (Supplementary Figure 6.3.2): child sex (boy/girl); household food poverty (yes/no), food insecurity (yes/no), and wealth index (continuous); maternal age (years, continuous), height (metres, continuous), post-delivery weight (kg, continuous), education (high school vs. no high school), marital status (married or living-as-married vs. not married), energy intake during pregnancy (insufficient/sufficient), alcohol use during pregnancy (yes/no), HIV

status (positive/negative), duration of exclusive breastfeeding (months, continuous), and parity (continuous).

Applying IPTWs generates a pseudo-population in which exposure is independent of the measured confounders[263]. To confirm this, we assessed whether the distribution of covariates was similar, or "balanced", across the exposure range in the IPTW-weighted sample by calculating correlations between each exposure and continuous covariate, as well as the absolute standardized difference of covariates in each exposure quartile versus all other exposure quartiles[265]. As recommended by Austin (2018), we considered correlations below 0.1 and absolute standardized differences below 0.2 as indicating balance[269]. We also calculated the average of variance ratios for all covariates across exposure quartiles, using a threshold of 2.0 to indicate balance[267]. Following best practices for specifying propensity score models[266], we considered log-transformations and machine learning methods in an iterative process to improve balance, resulting in the $\log_2$-transformation of all continuous covariates except for the wealth index. Further details on constructing the IPTW and balance assessment are provided in Supplementary Material Section 6.3.3.

Estimating effects of prenatal insecticide exposure on child weight trajectory parameters

The effects of a 10-fold increase in maternal lipid-corrected serum DDT/E or specific gravity-corrected urinary pyrethroid metabolite concentrations on each estimated child-specific random-effects SITAR parameter were estimated based on marginal structural models with IPTW. Under the four assumptions of consistency, exchangeability, positivity, and no misspecification of the propensity score model, marginal structural models generate effect estimates that have a causal interpretation[263].

Since these insecticides disrupt sex hormones and sex-specific effects on child weight have been reported[37,86,177], we conducted secondary analyses investigating effect modification by child sex. In addition, since socioeconomic status has often been found to modify the health effects of environmental exposures[86,371-376], and previous analyses in VHEMBE pointed to undernutrition as a possible explanation[37,345,377], we also investigated potential effect modification by food poverty, food insecurity, and maternal energy intake during pregnancy. This was done by including an interaction term between the effect modifier and the exposure, using the threshold of $p<0.1$ to indicate statistical evidence of effect measure modification. Effect modifiers were also taken into account in the IPTW for each analysis (see Supplementary Material Section 6.3.3 for more details).

We imputed missing covariate values using multiple imputation by chained equations[38] (see Supplementary Material Section 6.3.2) and constructed 95% confidence intervals by bootstrapping the multiple imputation, estimation of IPTW and outcome regressions 500 times[348,349]. SITAR parameters were treated as fixed parameters in this analysis. All analyses other than SITAR were conducted using Stata version 14.2 (StataCorp, College Station, TX).

Results

Participant characteristics

The average age of mothers participating in the study was 26.4 years (Table 6.2.1). All were Black Africans, and just over half were unmarried (52%), had less than a high school diploma (55%), and lived below the South African food poverty line (61%). Many lived in food insecure households (44%). The prevalence of HIV among mothers was 14%. Half of the children were female (49%), 24% were small-for-gestational age (<10^{th} percentile) at birth and 14% were born preterm (<37 weeks gestational age at birth). The median duration of exclusive breastfeeding without introduction of water or solids was short (1.5 months), though breastfeeding continued for longer (median=16 months; Table 6.2.1).

Virtually all participants had detectable levels of DDT/E and pyrethroid metabolites (Table 6.2.2). Correlations were high between congeners of DDT/E (Pearson's r=0.69 to 0.85) and between the pyrethroid metabolites *cis*-DCCA, *trans*-DCCA, and 3-PBA (r=0.83 to 0.88). However, *cis*-DBCA was only moderately correlated with the other pyrethroid metabolites (r=0.32 to 0.51), and pyrethroid metabolites were not correlated with DDT/E (r=-0.03 to 0.07).

Table 6.2.1. Selected characteristics of VHEMBE study participants, Limpopo, South Africa (n = 751)

	n		
Maternal baseline characteristics			
Age, years (mean, ±SD)	751	26.4	±6.3
Height, cm (mean, ±SD)	739	158.2	±6.8
Weight, kg (mean, ±SD)	740	68.8	±13.7
BMI, kg/m^2 (mean, ±SD)	735	27.5	±5.4
Married or living-as-married (freq, %)	751	359	48%
High school diploma (freq, %)	751	339	45%
Nulliparous (freq, %)	751	326	43%
Insufficient energy intake during pregnancy[a] (freq, %)	735	444	68%
Ever smoker (freq, %)	751	6	1%
Any alcohol during pregnancy (freq, %)	751	69	5%
HIV positive (freq, %)	751	103	14%
Household sociodemographic characteristics			
Food poverty[b] (freq, %)	748	460	61%
Food insecurity[c] (freq, %)	750	329	44%
Child characteristics			
Child sex, female (freq, %)	751	364	48%
Low birthweight, <2500g (freq, %)	750	63	8%
Preterm birth, <37 weeks (freq, %)	751	103	14%
Small for gestational age, $<10^{th}$ percentile (freq, %)	750	182	24%
Any breastfeeding, months (mean, ±SD)	695	15.9	±7.0
Exclusive breastfeeding, months (mean, ±SD)	702	2.3	±1.9

Abbreviations: BMI, body mass index; freq, frequency; SD, standard deviation.
[a]Below the Institute of Medicine (IOM) recommended total daily caloric intake for mothers in late pregnancy [344].
[b]Below the food poverty line of 386 Rand/person/month in South Africa [341].
[c]Two or more affirmative response to the US National Center for Health Statistics' Six-Item Food Security Scale [342].

Table 6.2.2. Distribution of maternal peripartum serum DDT/E (ng/g lipid) and urinary pyrethroid metabolites (μg/L, specific gravity-corrected) concentrations among VHEMBE study participants, Limpopo, South Africa (n=751)

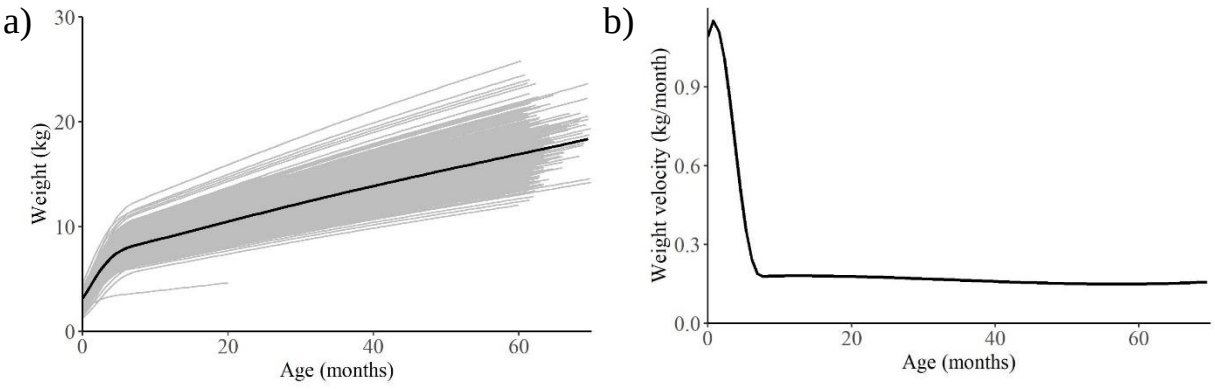
						Percentiles			
	n	**%>LOD**	**%>LOQ**	**GM (SD)**	**Min**	**25**	**50**	**75**	**Max**
DDT/E									
o,p′-DDT	751	90.6%	43.2%	8.93 (4.63)	0.14	3.40	7.13	22.65	2029.27
p,p′-DDT	751	98.0%	90.7%	69.55 (6.67)	0.14	18.95	55.26	260.99	15027.56
p,p′-DDE	751	100%	97.2%	287.91 (4.84)	3.98	91.79	242.18	878.89	26301.26
Pyrethroid metabolites									
cis-DBCA	738	100%	99.6%	0.35 (3.02)	0.02	0.16	0.33	0.74	13.39
cis-DCCA	738	100%	99.9%	0.48 (2.55)	0.05	0.26	0.46	0.79	209.49
trans-DCCA	738	100%	99.6%	0.55 (3.07)	0.03	0.26	0.53	1.05	268.95
3-PBA	737	100%	100%	1.12 (2.38)	0.10	0.66	1.05	1.84	102.38

Abbreviations: DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid; GM, geometric mean; LOD, limit of detection; LOQ, limit of quantification; SD, standard deviation.

Child weight trajectories estimated using SITAR

SITAR models with all three random effects did not converge. The best-fitting SITAR models in the overall cohort, girls only, and boys only were all based on log-transformed weight, three degrees of freedom in the population average spline, and random effect parameters for size and tempo (Supplementary Tables 6.3.1-3). Since the overall and sex-specific models had similar fit (based on variance explained), correlations between random effects, and shape of the weight trajectory (Supplementary Figure 6.3.1), the overall model was selected as the final model for parsimony. The fitted weight trajectories (weight vs. age and weight velocity vs. age) are shown in Figure 6.2.1. The estimated average age at peak weight velocity was just under one month (26.6 days, standard deviation=12.2).

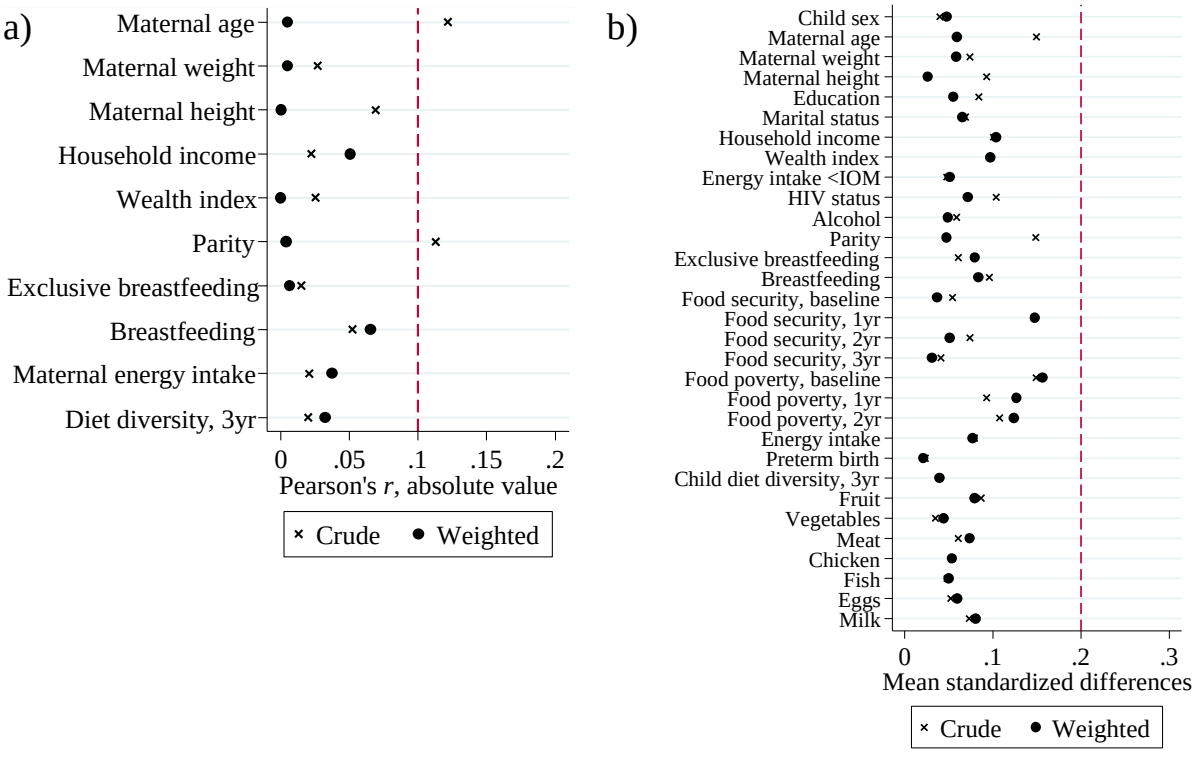
Figure 6.2.1. SITAR-modelled weight trajectories of VHEMBE children from birth to five years, based on all available weight measurements (study visits and medical records).
a) Predicted population average (black line) and child-specific weight trajectories (grey lines), kg vs. month.
b) Predicted population average weight velocity (first derivative of the population average weight trajectory), kg/month vs. month.



Inverse-probability of treatment weights (IPTW)

The distribution and diagnostics of the IPTW for each exposure are shown in Supplementary Material Section 6.3.3. The mean of the stabilized IPTW was 1.00 and no extreme weights were observed (Supplementary Table 6.3.4). As an example, the balance diagnostics for the *trans*-DCCA weight are shown in Figure 6.2.2; the diagnostics for all other exposures can be found in Supplementary Figures 6.3.3 and 6.3.4. For each IPTW, all correlations between exposure and continuous covariates were below 0.1 (Figure 6.2.2a, Supplementary Figure 6.3.3) and all standardized differences were below 0.2 (Figure 6.2.2b, Supplementary Figure 6.3.4), indicating that balance was achieved[378].

Figure 6.2.2. Balance diagnostics for the inverse-probability of treatment weight (IPTW) for trans-DCCA
a) Correlations between $\log_{10}$(*trans*-DCCA) concentrations and continuous covariates, before (×) and after (•) IPTW-weighting; correlations below 0.1 indicate balance.
b) Standardized differences of all covariates compared across $\log_{10}$(trans-DCCA) quartiles and averaged, before (×) and after (•) IPTW-weighting; standardized differences below 0.2 indicate balance.



Effects of prenatal insecticide exposure on child weight trajectory parameters

We found that higher maternal concentrations of multiple pyrethroid metabolites were associated with smaller size and tempo parameters, corresponding to lower average weight from birth to 5 years and earlier age at peak weight velocity, respectively; however, the confidence intervals for all estimates crossed the null (Table 6.2.3). For instance, a 10-fold increase in *cis*-DBCA was associated with 13.4g smaller size (95% CI: -29.3, 2.5) and 1.8-day earlier tempo (95% CI: -3.7, 0.1).

However, in analyses examining effect modification by sex, we found that 10-fold higher maternal concentrations of *trans*-DCCA were associated with 20.9g (95%CI: -40.1, -1.6) smaller size among boys only (p-value for interaction, p_{inter}=0.07). Other pyrethroid metabolites, including *cis*-DCCA and *cis*-DBCA were also associated with smaller size among boys but confidence intervals included the null and statistical evidence of effect modification by sex was limited (p_{inter}=0.13 for *cis*-DCCA and 0.41 for *cis*-DBCA; Table 6.2.4). Associations between pyrethroids and size among girls were weak, with estimates ranging from -6.7g (95%CI: -30.0, 16.7) for *cis*-DBCA to 10.3g (95%CI: -19.3, 40.0) for 3-PBA.

We did not observe associations between DDT/E and size or tempo parameters, overall or by sex (Tables 6.2.3 and 6.2.4). Moreover, we did not observe effect modification by food poverty, food insecurity or maternal energy intake during pregnancy for any associations between maternal insecticide concentrations and these child weight trajectory parameters (Supplementary Tables 6.3.5-6.3.7).

Table 6.2.3. Effects of a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations on birth to 5-year weight trajectory parameters among children participating in the VHEMBE study, Limpopo, South Africa

	n	Size (g) β (95% CI)	Tempo (days) β (95% CI)
o,p′-DDT	751	1.5 (-10.2, 13.2)	-0.4 (-1.7, 1.0)
p,p′-DDT	751	2.9 (-6.5, 12.3)	-0.4 (-1.5, 0.6)
p,p′-DDE	751	6.5 (-5.1, 18.1)	-0.3 (-1.6, 1.0)
cis-DBCA	738	-13.4 (-29.3, 2.5)	-1.8 (-3.7, 0.1)
cis-DCCA	738	-7.6 (-26.5, 11.3)	-1.9 (-4.0, 0.3)
trans-DCCA	738	-7.2 (-21.1, 6.7)	-1.4 (-3.2, 0.4)
3-PBA	737	-0.2 (-18.6, 18.2)	-2.2 (-4.8, 0.5)

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; cis-DBCA, cis-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; cis-DCCA, cis-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; trans-DCCA, trans-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Table 6.2.4. Effects of a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations on birth to 5-year weight trajectory parameters, by child sex

	n	Boys β (95% CI)	Girls β (95% CI)	p-value, interaction
Size (grams)				
o,p′-DDT	751	-6.7 (-22.1, 8.8)	12.1 (-7.2, 31.4)	0.15
p,p′-DDT	751	-4.4 (-17.8, 9.0)	10.6 (-4.0, 25.3)	0.15
p,p′-DDE	751	1.1 (-14.6, 16.8)	12.4 (-6.5, 31.4)	0.39
cis-DBCA	738	-20.3 (-41.7, 1.2)	-6.7 (-30.0, 16.7)	0.41
cis-DCCA	738	-22.8 (-50.7, 5.1)	9.3 (-18.7, 37.2)	0.13
trans-DCCA	738	-20.9 (-40.1, -1.6)	9.2 (-14.0, 32.5)	0.07[b]
3-PBA	737	-11.7 (-38.8, 15.4)	10.3 (-19.3, 40.0)	0.31
Tempo (days)				
o,p′-DDT	751	0.4 (-1.4, 2.2)	-1.1 (-3.2, 1.0)	0.31
p,p′-DDT	751	-0.1 (-1.6, 1.4)	-0.9 (-2.4, 0.7)	0.50
p,p′-DDE	751	0.8 (-0.9, 2.5)	-1.4 (-3.5, 0.6)	0.12
cis-DBCA	738	-1.7 (-4.4, 1.1)	-1.8 (-4.6, 1.0)	0.95
cis-DCCA	738	-2.5 (-5.2, 0.3)	-1.2 (-4.7, 2.2)	0.59
trans-DCCA	738	-2.0 (-4.1, 0.2)	-0.7 (-3.7, 2.3)	0.50
3-PBA	737	-1.7 (-5.1, 1.7)	-2.7 (-6.9, 1.5)	0.72

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.
[a]95% CI excludes the null.
[b]p-value for interaction <0.1.

Discussion

Main findings and interpretation

Results suggests that the pyrethroid metabolite *trans*-DCCA, and to a lesser extent *cis*-DBCA and *cis*-DCCA, are associated with lower average weight among boys between birth and 5 years of age. Findings are consistent with previous VHEMBE studies based on weight measured at 1, 2, and 3.5 years[37,177,366], though no associations were observed with birthweight[86]. Similarly, two Chinese birth cohorts did not observe associations between *cis*- or *trans*-DCCA and weight at birth (n=454)[173] or 1 year (n=497)[176]. This suggests that the potential effect of pyrethroid metabolites on child growth may not be evident at birth, and the lower exposures and statistical power in the latter study may explain the null finding at 1 year. The only study to investigate a dynamic growth metric did not find an association between self-reported use of pyrethroid-containing products and weight change during the first month[181]; however, non-differential exposure misclassification may have contributed to this null finding. Among the few animal studies, exposure to cypermethrin, an IRS insecticide used in the VHEMBE area which metabolizes into *cis/trans*-DCCA and 3-PBA, resulted in lower weight from childhood to adulthood among rat offspring[194]. However, two studies of prenatal exposure to deltamethrin, which is also used in IRS and metabolizes into *cis*-DBCA and 3-PBA, found no effect on mouse offspring weight at birth[195] nor in adulthood[193].

Our results also suggest that prenatal exposure to pyrethroid insecticides may be associated with earlier age at peak weight velocity, though confidence intervals crossed the null. The timing of peak weight velocity identified by the SITAR model is consistent with minipuberty, a period of HPG axis activation occurring in the first few months of life characterized by surges in gonadotropins and sex hormones, and is accompanied by a growth spurt driven by testosterone in

boys and estrogen in girls[111,112,114,379]. Thus, if pyrethroid exposure does affect growth and the timing of peak weight velocity, one possible mechanism would be through disruption of this endocrine axis. Several experimental studies have shown that exposure to pyrethroids lowers testosterone levels in mice[186,187,189,197-200], though two studies reported increases[194,200]. Furthermore, there is some animal and human evidence that pyrethroids disrupt the timing of puberty, which is another period of HPG activation[207,364,380,381]. The potential link between gestational exposure to pyrethroids, disruption of the HPG axis, and growth merits further investigation.

We did not find evidence that prenatal exposure to DDT/E were associated with postnatal growth within the first five years, which is consistent with the literature investigating weight at single time points. The literature concerning dynamic growth metrics is mixed, and not directly comparable due to differences in outcome measures. Only one study measured maternal serum DDT and reported an association with a latent growth pattern of stable and then increasing BMI after age 5 among boys in an agricultural region of California (n=249), but this was no longer present after confounder adjustment[136]. Larger studies of interval-based metrics have reported associations between DDE and weight gain in the first 6 months (three pooled Spanish birth cohorts, n=1285)[326] and 24 months (five pooled European birth cohorts, n=1791)[138], while a Greek birth cohort of similar size to VHEMBE (n=689) with much lower DDE exposure did not observe associations with weight gain from birth to 6 months[137]. The present study is potentially underpowered to detect these associations.

Limitations and Strengths

A limitation of this analysis is the measurement of exposure at a single timepoint to represent gestational exposure to pyrethroids, which have a biological half-life measured in hours or days[382]. However, intraclass correlation coefficients for repeated spot urine measurements of pyrethroid metabolites have been found to vary from 0.21 in the U.S to 0.85 in Poland, suggesting that the reliability of a single measurement may vary from one population to another[383,384]. Furthermore, the pyrethroids used for IRS have been designed to be stable in the environment, and remain effective throughout the rainy season for up to 10 months, aided by protection indoors from direct sunlight and external elements which would otherwise lead to their rapid degradation[70,85]; therefore, elevated exposure to inhabitants may persist for months from repeated contact with contaminated surfaces, bedding, furniture, and stored food. Moreover, indicators of regular pesticide use, such as storing pesticide containers on the homestead and self-reported use of pesticides in the yard, were associated with higher pyrethroid metabolite concentrations among VHEMBE mothers[32,359], suggesting that a single measurement may be representative of longer-term exposure in the VHEMBE population.

While numerous studies have investigated environmental chemical influences on child weight measured at single time-points, very few have been conducted on child growth trajectories[136,385-388]. This is, to the best of our knowledge, the first study of prenatal environmental chemical influences on child growth trajectories estimated by SITAR, a method which overcomes limitations of other measures. For example, interval-based metrics can vary widely in the timepoints selected (e.g. birth to 6 months vs. 12 months), the measure being compared (e.g. absolute vs. standardized weight), and whether it is expressed in absolute vs. relative terms and continuously vs. categorically[243]; and grouping-based methods result in loss of information due to

categorization, and the number and interpretation of the observed growth patterns is subjective[35,244]. SITAR parameters have a straightforward biological interpretation, comparing the weight (size parameter) as well as the timing (tempo) and velocity (intensity) of the growth spurt to the average child[247,248], while accounting for non-linear growth patterns and allowing for flexibility in the timing and number of measurements for each child. Also, compared to parametric mixed-effects growth models, spline-based SITAR can lead to better model fit[245,246].

Another methodological advantage of this study is the use of IPTW, which allowed us to verify that all measured potential confounders identified *a priori* were balanced across exposure quartiles. We also verified the balance of post-exposure covariates not included in the GPS such as preterm birth, household food poverty at follow-up visits and child's dietary diversity and intake of fruit, vegetables, meat, and fish at 3.5 years (Figure 1, eFigures 2.2 and 2.3). Confirming covariate balance presents an important advantage over multivariable regression adjustment used in most of the existing literature. We also took advantage of extensive questionnaire data to control for a wide variety of potential confounders.

Conclusions

This study demonstrated a novel application of SITAR growth trajectory modelling to examine the influence of environmental chemicals on growth. Our results suggest that prenatal exposure to pyrethroid insecticides may suppress growth among boys, which may reflect disruption of androgens critical to physical development in early infancy. Evidence was strongest for *trans*-DCCA, a metabolite of several pyrethroids including cypermethrin, which is used for IRS as well as in commercial insecticides. Implications of our findings may thus go beyond that of IRS. The worldwide use of pyrethroids in agricultural and domestic applications has increased over the past two decades as the main replacement for banned organophosphate pesticides,

reaching a global market value of 3 billion USD[389]. However, given the sparsity of the existing literature, further studies are needed to confirm our findings, especially among populations from IRS or other highly-exposed settings.

6.3. Supplementary Material

6.3.1. Selecting the best-fitting SITAR model

Prior to fitting the SITAR models, we used the conditional weights approach to identify implausible weight measurements[251] and removed 22 outlier measurements greater than three standard deviations from expected values conditional on preceding measurements; this is an improvement over traditional methods that identify outliers based on cross-sectional population distributions.

As stated in the manuscript, the weight trajectories of each child were modelled using SuperImposition, Translation And Rotation (SITAR)[248]. This model fits a natural cubic spline to the average population growth curve (in this case, weight in kilograms versus age in months). Individual deviations from this mean curve are captured by three random-effects parameters, where:

$$y_{it} = \alpha_i + h\left(\frac{t - \beta_i}{exp(-c\gamma_i)}\right)$$

a) Size (α) indicates the child's mean weight compared to the average (in kilograms), representing vertical translation of the weight curve;

b) Tempo (β) indicates the child's age at peak weight velocity compared to the average (in months), representing horizontal translation of the weight curve; and,

c) Intensity (γ) indicates the child's growth rate compared to the average (expressed as a fraction).

To identify the best-fitting SITAR model, we ran candidate SITAR models with all possible combinations of the following specifications, in the overall cohort and by sex:

i) all three random effects, size and tempo only, or size and intensity only [3 options]

ii) untransformed or log-transformed child weight [2 options]

iii) three to five degrees of freedom for the population average spline [3 options].

We evaluated all candidate SITAR models based on the following criteria: low Akaike information criterion and Bayesian information criterion adjusted for log-transformation (aAIC, aBIC), low correlation between random effects, and high variance explained measured by R^2 generalized to mixed effects models[249]. The diagnostics for all converged models are shown in Tables 6.3.1 (overall), 6.3.2 (girls), and 6.3.3 (boys).

Results

Models with all three random effects did not converge. Among models with two random effects, those with log-transformed weight performed better compared to models based on untransformed weight (Tables 6.3.1 to 6.3.3). The model based on log-transformed weight, random effects for α (size) and β (tempo) random effects, and 3 degrees of freedom for the population average spline explained the highest proportion of variance in the entire cohort as well as in girls only and boys only, therefore these were considered the best-fitting models (Tables 6.3.1 to 6.3.3). The best-fitting overall and sex-specific models had similar variance explained (75% overall, 76% girls only, 74% boys only; see Tables 6.3.1 to 6.3.3), similar correlations between random effects (0.44, 0.44, and 0.47, respectively; see Tables 6.3.1 to 6.3.3), and similar fitted weight velocity curves (see Figure 6.3.1); therefore, the more parsimonious overall model was selected as the final model.

Table 6.3.1. Diagnostics for converged SITAR models fit on the entire cohort. The colour scale highlights better metrics in green (lower AIC, lower BIC, higher variance explained, lower correlation between random effects), and worse metrics in red (higher AIC, higher BIC, lower variance explained, higher correlation between random effects).

Random effects	Weight variable	df	Adjusted AIC	Adjusted BIC	Variance explained	Correlation
Size (α), tempo (β)	Weight, kg	4	32,651	32,725	66	0.95
Size (α), tempo (β)	Log(weight)	3	30,788	30,855	75	0.44
Size (α), tempo (β)	Log(weight)	4	30,650	30,724	69	0.44
Size (α), tempo (β)	Log(weight)	5	30,645	30,727	67	0.44
Size (α), intensity (γ)	Weight, kg	4	32,178	32,252	68	0.91
Size (α), intensity (γ)	Weight, kg	5	32,027	32,109	68	0.91
Size (α), intensity (γ)	Log(weight)	4	30,775	30,849	69	0.36
Size (α), intensity (γ)	Log(weight)	5	30,748	30,830	66	0.37

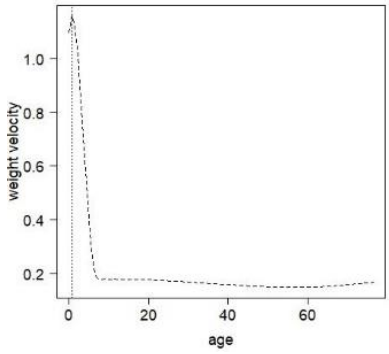
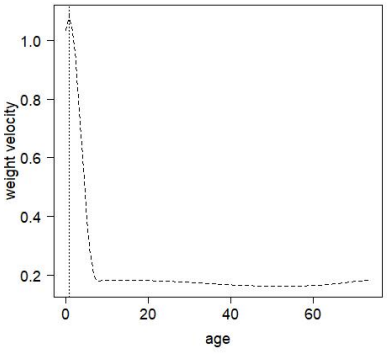
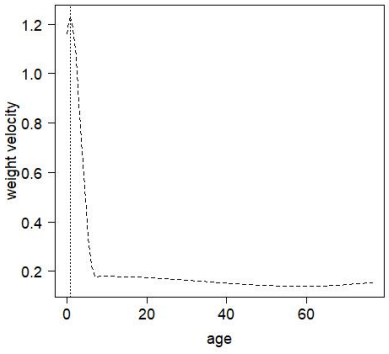
Table 6.3.2. Diagnostics for converged SITAR models fit on girls only.

Random effects	Weight variable	df	Adjusted AIC	Adjusted BIC	Variance explained	Correlation
Size (α), tempo (β)	Weight, kg	3	16092	16153	67	0.95
Size (α), tempo (β)	Weight, kg	4	16048	16115	66	0.96
Size (α), tempo (β)	Weight, kg	5	16012	16086	66	0.96
Size (α), tempo (β)	Log(weight)	3	14523	14583	76	0.44
Size (α), tempo (β)	Log(weight)	4	14461	14528	71	0.43
Size (α), tempo (β)	Log(weight)	5	14455	14529	69	0.43
Size (α), intensity (γ)	Weight, kg	3	15891	15952	69	0.88
Size (α), intensity (γ)	Weight, kg	4	15538	15605	70	0.9
Size (α), intensity (γ)	Weight, kg	5	15451	15525	70	0.91
Size (α), intensity (γ)	Log(weight)	4	14508	14575	70	0.35
Size (α), intensity (γ)	Log(weight)	5	14502	14576	69	0.35

Table 6.3.3. Diagnostics for converged SITAR models fit on boys only.

Random effects	Weight variable	df	Adjusted AIC	Adjusted BIC	Variance explained	Correlation
Size (α), tempo (β)	Weight, kg	3	16396	16457	67	0.96
Size (α), tempo (β)	Weight, kg	4	16369	16437	65	0.96
Size (α), tempo (β)	Weight, kg	5	16346	16421	65	0.95
Size (α), tempo (β)	Log(weight)	3	16053	16114	74	0.47
Size (α), tempo (β)	Log(weight)	5	15979	16054	64	0.46
Size (α), intensity (γ)	Weight, kg	3	17158	17219	63	0.89
Size (α), intensity (γ)	Weight, kg	4	16304	16372	66	0.93
Size (α), intensity (γ)	Weight, kg	5	16255	16330	66	0.92
Size (α), intensity (γ)	Log(weight)	4	16055	16123	66	0.40
Size (α), intensity (γ)	Log(weight)	5	16033	16108	63	0.40

Figure 6.3.1. Plots of weight velocity (kg/month) vs. age (months) using the best-fitting SITAR models for the entire cohort, girls only, and boys only. All models were based on log-transformed weight, random effects for α (size) and β (tempo) random effects, and 3 degrees of freedom for the population average spline. The dashed vertical line indicates the age at peak weight velocity.

Entire cohort	Girls	Boys
		

6.3.2. Multiple imputation by chained equations

We conducted multiple imputation by chained equations using the mi suite of commands in Stata version 14.2 (StataCorp, College Station, TX). Continuous variables were imputed using predictive mean matching and binary variables were imputed using logistic regression. We used a burn-in period of 10 iterations and generated 10 imputed datasets[38].

We included in the imputation models all outcomes, exposures and covariates identified in the Statistical Analysis Section ii of the paper, with the exception of two derived variables (food poverty and insufficient maternal energy intake) and included the component variables in the imputation models instead. Specifically, we derived missing values of food poverty from imputed total household income, and derived missing values of insufficient maternal energy intake from imputed maternal age, height, post-delivery weight, and energy intake during pregnancy. We also included auxiliary variables in the imputation models to improve prediction of total household income (auxiliary variable: food poverty at the 1-year study visit) and exclusive breastfeeding (auxiliary variable: total breastfeeding duration)[38].

Finally, we also included variables representing the interaction between the exposures and effect modifiers (sex, food poverty, food insecurity, maternal energy intake sufficiency)[360]. However, to address issues of collinearity and to reduce the number of terms added to the imputation models, we only included interaction terms for three of the seven exposures (*p,p'*-DDT, *cis*-DBCA and *cis*-DCCA) and three of the four effect modifiers (sex, food poverty, and maternal energy intake sufficiency). As stated in our Results section, correlations were high between congeners of DDT/E (Pearson's r= 0.69 to 0.85) and between the pyrethroid metabolites *cis*-DCCA, *trans*-DCCA, and 3-PBA (r= 0.83 to 0.88), therefore the analyte that was most correlated with the other two analytes within the group was selected (*p,p'*-DDT and *cis*-DCCA, respectively);

cis-DBCA was not correlated with the other pyrethroid metabolites. Among the effect modifiers, food poverty and food insecurity were highly associated with each other ($p<0.001$), therefore only interaction terms with food poverty were created.

6.3.3. Inverse-probability of treatment weights (IPTW)

Methods

First, we constructed generalized propensity scores (GPS) for each continuous exposure A, by estimating the conditional density function f[A|L], where L is the vector of covariates. To do so we used multivariable linear regression, with L including the following potential confounders and predictors of the outcomes identified based on a directed acyclic graph (Figure 6.3.2): child sex (boy/girl); household food poverty (yes/no), food insecurity (yes/no), and wealth index (continuous); maternal age (years, continuous), height (metres, continuous), post-delivery weight (kg, continuous), education (high school vs. no high school), marital status (married vs. not married), energy intake during pregnancy (insufficient/sufficient), alcohol use during pregnancy (yes/no), HIV status (positive/negative), duration of exclusive breastfeeding (months, continuous), and parity (continuous).

We then generated stabilized inverse-probability of treatment weights (IPTW) with the GPS as the denominator and the marginal exposure density f[A] as the numerator[263]. When the GPS is correctly specified, marginal structural models using stabilized IPTW weights $\frac{f[A]}{f[A|L]}$ result in asymptotically unbiased estimators for the true causal effect[264,390]. To investigate effect measure modification by child sex, food poverty, and energy intake during pregnancy, we fit marginal structural models using IPTW that were stabilized by using the conditional exposure density f[A|M] as the numerator, where M is the effect modifier. Because the GPS already included all effect modifiers M (i.e. M was a subset of L), the GPS was used as the denominator.

Balance diagnostics

We assessed covariate balance before and after weighting by the IPTW for each exposure using three metrics:

iv) Pearson correlation coefficients between the exposure and each continuous covariate, with those below 0.1 indicating balance[269].

v) Standardized differences comparing all covariates across each quartile of exposure versus all other quartiles, which were then averaged across the four comparisons. Quartiles of exposure were used to avoid small cell sizes and finite sample bias when assessing standardized differences. A threshold of 0.20 for standardized differences has been suggested to indicate balance when conducting balance diagnostics for the GPS[269].

vi) Variance ratios comparing the variance of all covariates across each quartile of exposure versus all other quartiles, which were then averaged across the four comparisons. By definition, a variance ratio of 1.0 describes a covariate which has equal variance across exposure categories, and a threshold of <2.0 has been suggested to indicate balance[267].

Balance was improved through an iterative process. For instance, balance improved for all continuous covariates after $\log_2$-transformation, except for the wealth index, which is a normally-distributed variable and thus was kept un-transformed. In an attempt to further improve balance, we used machine learning algorithms to define our GPS among the candidate confounders and outcome predictors that we identified *a priori*. Using SuperLearner, which creates a weighted average of models fit using different machine learning algorithms and evaluated using cross-validation[391], resulted in overfitting and positivity violations indicated by stabilized weights with a mean not equal to 1 (data not shown) and furthermore did not improve balance diagnostics[392]. Therefore, we proceeded with our more parsimonious original model.

Figure 6.3.2. Directed acyclic graph of the relationship between gestational exposure to insecticides and child weight trajectory

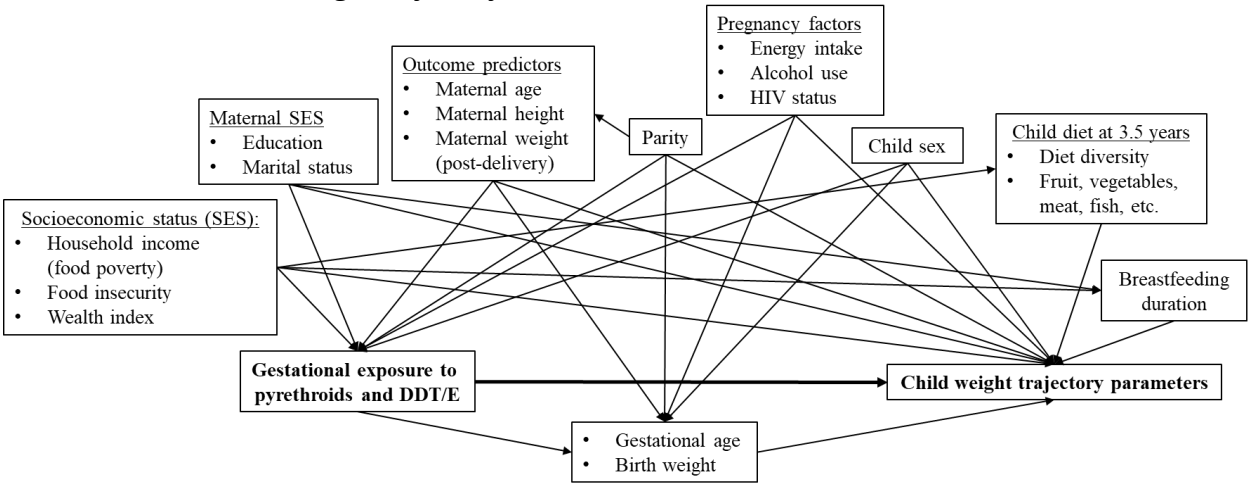
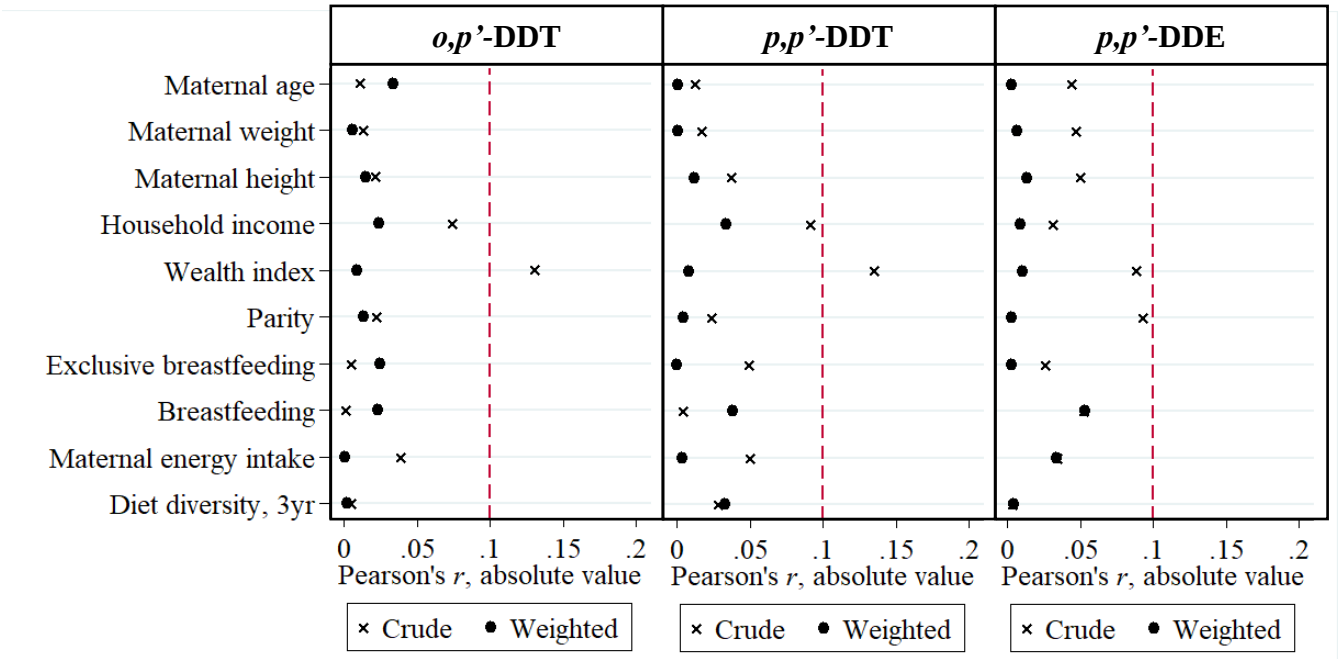


Table 6.3.4. Distribution of stabilized inverse probability of treatment weights (IPTW) for each exposure, in overall and effect modification models

Exposure	Model	n	Mean	SD	Min	Max
o,p'-DDT	Overall	751	1.00	0.18	0.27	2.68
	Child sex	751	1.00	0.16	0.22	2.26
	Food poverty	751	1.00	0.17	0.30	2.41
	Food insecurity	751	1.00	0.18	0.29	2.54
	Energy intake	751	1.00	0.18	0.27	2.67
p,p'-DDT	Overall	751	1.00	0.20	0.36	2.29
	Child sex	751	1.00	0.18	0.31	1.97
	Food poverty	751	1.00	0.19	0.40	2.32
	Food insecurity	751	1.00	0.20	0.39	2.45
	Energy intake	751	1.00	0.20	0.38	2.42
p,p'-DDE	Overall	751	1.00	0.20	0.39	2.00
	Child sex	751	1.00	0.20	0.36	2.10
	Food poverty	751	1.00	0.20	0.41	1.92
	Food insecurity	751	1.00	0.20	0.38	2.06
	Energy intake	751	1.00	0.19	0.43	1.90
cis-DBCA	Overall	738	1.00	0.14	0.43	1.76
	Child sex	738	1.00	0.13	0.40	1.85
	Food poverty	738	1.00	0.13	0.46	1.83
	Food insecurity	738	1.00	0.14	0.43	1.78
	Energy intake	738	1.00	0.13	0.41	2.12
cis-DCCA	Overall	738	1.00	0.18	0.29	2.57
	Child sex	738	1.00	0.19	0.30	2.59
	Food poverty	738	1.00	0.19	0.29	2.61
	Food insecurity	738	1.00	0.19	0.27	2.73
	Energy intake	738	1.00	0.19	0.29	2.55
trans-DCCA	Overall	738	1.00	0.17	0.38	2.17
	Child sex	738	1.00	0.17	0.39	2.15
	Food poverty	738	1.00	0.17	0.35	2.11
	Food insecurity	738	1.00	0.16	0.29	1.99
	Energy intake	738	1.00	0.17	0.41	2.30
3-PBA	Overall	737	1.00	0.25	0.18	3.83
	Child sex	737	1.00	0.24	0.22	3.62
	Food poverty	737	1.00	0.25	0.17	3.76
	Food insecurity	737	1.00	0.24	0.15	3.52
	Energy intake	737	1.00	0.24	0.16	3.47

Figure 6.3.3. Correlations between each exposure and continuous potential confounders, before (×) and after (●) IPTW-weighting



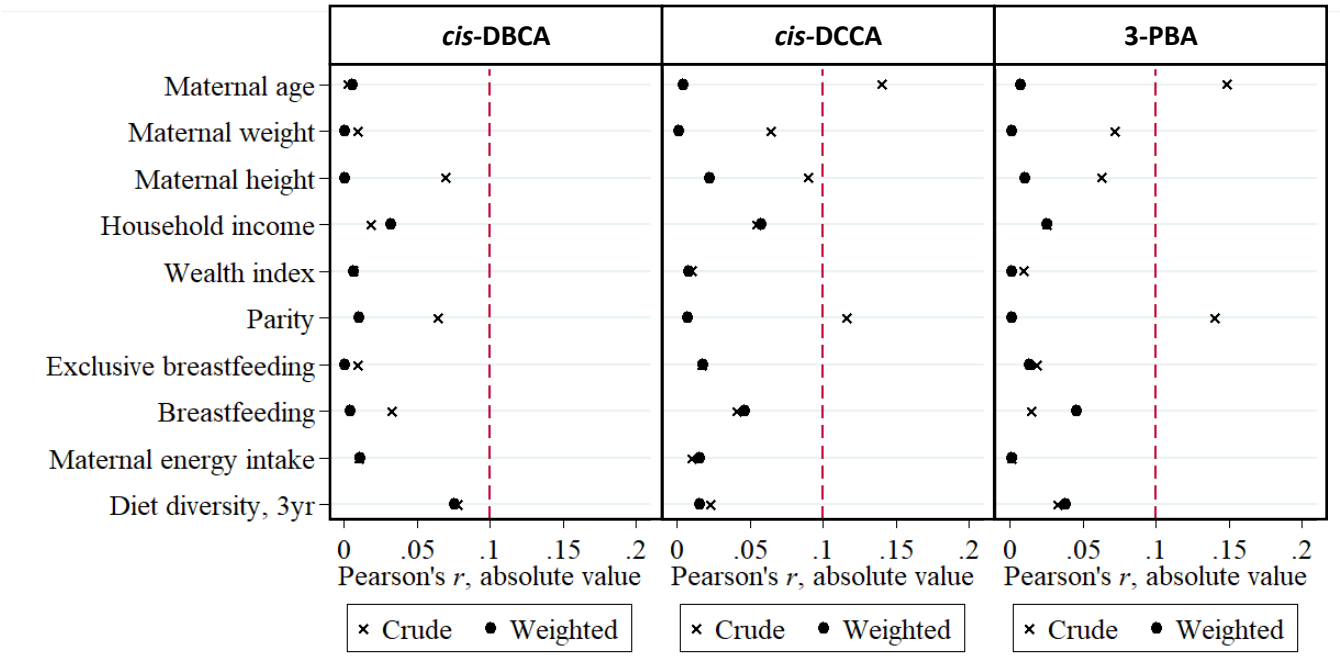
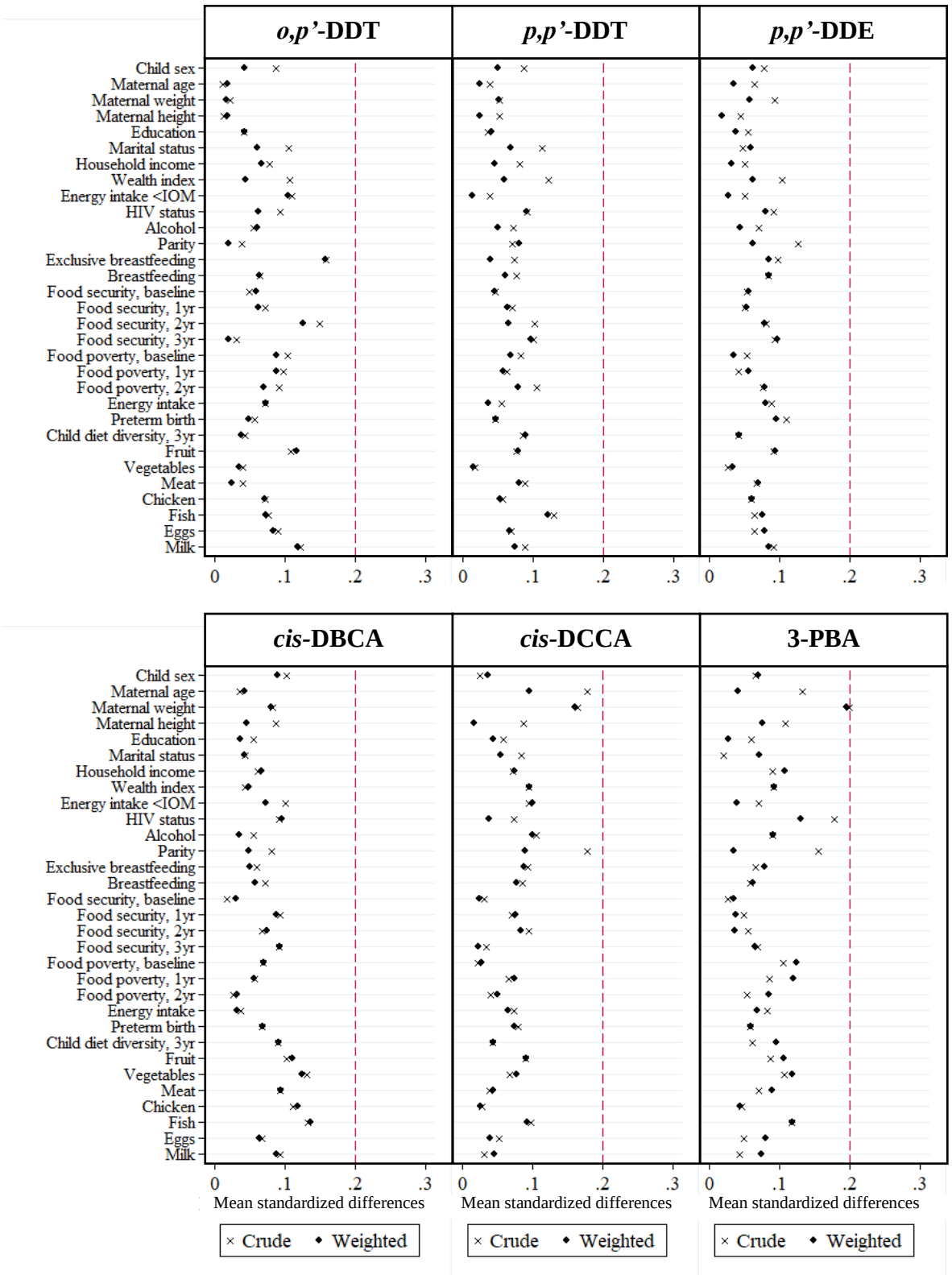


Figure 6.3.4. Standardized differences of all potential confounders, averaged across exposure quartiles, before (×) and after (•) IPTW-weighting.



6.3.4. Outcome regressions: effect measure modification by food poverty, food insecurity, and maternal energy intake during pregnancy

Table 6.3.5. Effects of a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (µg/L) concentrations on birth to 5-year weight trajectory parameters, by food poverty status among children participating in the VHEMBE study, Limpopo, South Africa.

	n	No food poverty β (95% CI)	Food poverty β (95% CI)	p-value, interaction
Size (grams)				
o,p′-DDT	751	1.9 (-18.8, 22.6)	1.8 (-13.2, 16.8)	0.99
p,p′-DDT	751	7.0 (-9.2, 23.2)	1.9 (-10.3, 14.1)	0.63
p,p′-DDE	751	6.9 (-13.9, 27.8)	6.7 (-8.7, 22.0)	0.99
cis-DBCA	738	-23.8 (-52.6, 5.0)	-7.6 (-27.6, 12.4)	0.38
cis-DCCA	738	-12.6 (-47.1, 22.0)	-4.0 (-27.8, 19.8)	0.70
trans-DCCA	738	-11.4 (-37.6, 14.9)	-4.8 (-23.3, 13.6)	0.70
3-PBA	737	1.6 (-35.2, 38.4)	0.4 (-23.1, 24.0)	0.96
Tempo (days)				
o,p′-DDT	751	-0.3 (-2.7, 2.1)	-0.3 (-1.9, 1.3)	1.00
p,p′-DDT	751	-0.4 (-2.1, 1.3)	-0.3 (-1.6, 1.0)	0.93
p,p′-DDE	751	-0.1 (-2.4, 2.2)	-0.3 (-1.9, 1.3)	0.92
cis-DBCA	738	-1.0 (-4.6, 2.6)	-2.2 (-4.5, 0.1)	0.59
cis-DCCA	738	-2.5 (-5.2, 0.2)	-1.4 (-4.5, 1.6)	0.62
trans-DCCA	738	-1.4 (-4.0, 1.2)	-1.4 (-3.9, 1.1)	0.99
3-PBA	737	-2.6 (-6.3, 1.1)	-1.8 (-5.3, 1.7)	0.76

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Table 6.3.6. Effects of a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (μg/L) concentrations on birth to 5-year weight trajectory parameters, by food insecurity status among children participating in the VHEMBE study, Limpopo, South Africa.

	n	Food secure β (95% CI)	Food insecure β (95% CI)	p-value, interaction
Size (grams)				
o,p′-DDT	751	2.8 (-14.4, 20.0)	0.4 (-17.6, 18.4)	0.86
p,p′-DDT	751	-1.2 (-14.5, 12.1)	8.2 (-6.9, 23.2)	0.38
p,p′-DDE	751	0.0 (-15.8, 15.9)	14.7 (-5.5, 34.9)	0.29
cis-DBCA	738	-16.1 (-38.2, 5.9)	-9.4 (-34.0, 15.2)	0.70
cis-DCCA	738	-6.6 (-33.4, 20.2)	-7.3 (-34.1, 19.4)	0.97
trans-DCCA	738	-4.5 (-24.8, 15.9)	-9.5 (-30.7, 11.8)	0.75
3-PBA	737	2.9 (-21.7, 27.5)	-3.1 (-31.7, 25.6)	0.76
Tempo (days)				
o,p′-DDT	751	-0.2 (-2.1, 1.7)	-0.5 (-2.4, 1.4)	0.84
p,p′-DDT	751	-0.6 (-2.0, 0.7)	-0.1 (-1.5, 1.4)	0.56
p,p′-DDE	751	-0.5 (-2.3, 1.3)	0.1 (-1.8, 1.9)	0.64
cis-DBCA	738	-1.1 (-3.9, 1.6)	-2.6 (-5.3, 0.2)	0.48
cis-DCCA	738	-3.2 (-5.9,-0.5)	-0.2 (-3.6, 3.2)	0.18
trans-DCCA	738	-2.0 (-4.3, 0.3)	-0.7 (-3.5, 2.2)	0.47
3-PBA	737	-3.2 (-6.5, 0.1)	-1.1 (-5.0, 2.9)	0.41

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

Table 6.3.7. Effects of a 10-fold increase in maternal peripartum DDT/E (ng/g lipid) or pyrethroid metabolite (μg/L) concentrations on weight trajectory parameters, by maternal energy intake sufficiency during pregnancy among children participating in the VHEMBE study, Limpopo, South Africa.

	n	Sufficient β (95% CI)	Insufficient β (95% CI)	p-value, interaction
Size (grams)				
o,p′-DDT	751	14.2 (-11.0, 39.5)	-3.2 (-17.2, 10.9)	0.25
p,p′-DDT	751	14.3 (-7.3, 35.9)	-2.3 (-13.0, 8.4)	0.19
p,p′-DDE	751	16.2 (-10.1, 42.6)	1.9 (-11.0, 14.8)	0.35
cis-DBCA	738	-12.7 (-41.5, 16.0)	-13.9 (-34.8, 7.1)	0.95
cis-DCCA	738	-22.3 (-53.5, 9.0)	0.8 (-24.5, 26.1)	0.28
trans-DCCA	738	-13.5 (-37.9, 11.0)	-3.8 (-22.6, 15.0)	0.56
3-PBA	737	-17.0 (-48.0, 14.0)	7.0 (-17.0, 31.1)	0.24
Tempo (days)				
o,p′-DDT	751	-0.4 (-2.9, 2.1)	-0.3 (-2.0, 1.3)	0.98
p,p′-DDT	751	0.0 (-1.8, 1.8)	-0.6 (-1.9, 0.7)	0.59
p,p′-DDE	751	0.5 (-1.5, 2.5)	-0.6 (-2.2, 1.1)	0.41
cis-DBCA	738	-3.1 (-6.3, 0.1)	-1.1 (-3.5, 1.4)	0.34
cis-DCCA	738	-2.3 (-5.3, 0.8)	-1.6 (-4.4, 1.2)	0.76
trans-DCCA	738	-2.1 (-5.0, 0.8)	-1.0 (-3.3, 1.2)	0.55
3-PBA	737	-2.0 (-6.1, 2.1)	-2.3 (-5.5, 0.9)	0.90

Abbreviations: CI, confidence interval; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; *cis*-DBCA, *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *cis*-DCCA, *cis*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2,-dicholorvinyl)-2,2-dimethyl-cyclopropane carboxylic acid; 3-PBA, 3-phenoxybenzoic acid.

CHAPTER 7. Discussion

7.1. Summary of Findings

This dissertation examined the hypothesis that some endocrine disrupting chemicals can also act as so-called "metabolic disruptors"[14], thus affecting cardiometabolic outcomes beyond obesity. My goal was to address substantive gaps in knowledge concerning ubiquitous EDCs in each of two contexts, Canada and South Africa, while also addressing methodological limitations in the extant literature. Overall, my findings suggest that parabens may indeed have an impact on the cardiometabolic health outcomes other than obesity and also highlight the importance of examining sexually dimorphic effects of endocrine-disrupting chemicals.

In Chapter 4 (Manuscript 1), I investigated associations between urinary paraben concentrations and the prevalence of obesity and, as a novel contribution to this literature, metabolic syndrome and its individual components (waist circumference, triglycerides, cholesterol, glucose, and blood pressure). For the dichotomous outcomes, I estimated prevalence ratios using modified Poison regression with robust variance estimators, which is preferable to prevalence odds ratios when the outcome is common. The results suggested that parabens are associated with detrimental cardiometabolic health among adult men (specifically, higher prevalence of metabolic syndrome) but are inversely associated with obesity and HDL cholesterol among adult women. A U.S. study with similar design and adjustment for confounders had observed inverse associations with obesity overall, but more pronounced in women[28]. These dimorphic effects could reflect the relative importance of the estrogenic and anti-androgenic effects of parabens between the two sexes, but further research is needed to understand the underlying mechanisms.

In Chapter 5 (Manuscript 2), I investigated associations between gestational exposure to DDT/E and pyrethroid insecticides on various cardiometabolic risk factors (child size, adiposity and blood pressure) among children 5 years of age in the VHEMBE birth cohort, which is based in a malaria endemic region of South Africa where indoor residual spraying occurs annually; this study setting is a unique and much-needed contribution to this literature, as most investigations of the health effects of these insecticides takes place outside of the IRS context. In addition, I controlled for confounding and selection bias from loss to follow-up using marginal structural models. All four pyrethroid metabolites examined were associated with three measures of adiposity: BMI z-score, waist circumference, and body fat percentage, and the inverse associations between *cis/trans*-DCCA and BMI z-score and fat percentage were more pronounced among children whose mothers had sufficient energy intake during pregnancy. We had also observed this pattern of effect modification in associations between pyrethroids and thinness at 3.5 years; a possible explanation for this somewhat counterintuitive finding is that the children in a low-nutrient environment (for which maternal energy intake is a proxy) have less fat to lose, and thus the effect is obscured in this group.

In Manuscript 3, I modelled the VHEMBE children's weight trajectories from birth to 5 years of age and investigated whether maternal peripartum DDT/E or pyrethroid concentrations were associated with the estimated growth parameters (size and tempo). I found that *trans*-DCCA was associated with smaller size (i.e. lower average weight) among boys but not girls, with evidence of effect modification by sex; decreases in size associated with *cis*-DBCA and *cis*-DCCA were similar in magnitude, but the confidence intervals included the null. In addition, overall findings suggested that pyrethroids may be associated with earlier age at peak weight velocity, but estimates crossed the null. This was the first study to examine associations between pyrethroids

and growth trajectories. Notably, the timing of the infant growth spurt observed coincided with minipuberty, a period of activation of the endocrine HPG axis characterized by surges in sex hormones and rapid infant growth; further research is needed to determine whether this is could be a potential target of endocrine-disrupting effects, and to understand its possible role in widely documented associations between infant growth and subsequent adiposity[393-399] and cardiometabolic health[36,235,238,242,400].

7.2. Strengths and Limitations

Overall, this dissertation makes important contributions to our understanding of ubiquitous EDCs as metabolic disruptors by investigating cardiometabolic health outcomes beyond obesity. I assessed outcomes such as metabolic syndrome and its individual components (waist circumference, triglycerides, glucose, HDL cholesterol, and blood pressure), body fat percentage measured by bioelectrical impedance, and child growth trajectories which have been linked to long-term cardiometabolic health outcomes more strongly than single timepoint measures. Another major strength is the examination of EDCs in two very different settings: Canada and South Africa. Much of the research on the health effects of EDCs is conducted in high-income countries, but many more studies are needed to understand the impact in low- and middle-income countries where the exposure context can differ dramatically. For example, VHEMBE uniquely takes place in a setting where IRS occurs annually, median pyrethroid concentrations are two to four times higher than for Canadian women of similar age (20 to 39 years)[32] and nearly 70% of mothers do not meet IOM guidelines for energy intake during pregnancy. Importantly, *cis*-DBCA, the metabolite specific to deltamethrin which is used in IRS in South Africa, is infrequently measured or detected in other studies[173,176,178], therefore VHEMBE addresses a key gap in the literature on the health effects of this particular insecticide used for IRS.

In addition to addressing these substantive gaps in knowledge, this dissertation also has methodological strengths. Both the CHMS and VHEMBE collected information on an extensive list of potential confounders, including smoking status, dietary intake, and accelerometer-based physical activity (CHMS, Manuscript 1) and socioeconomic status based on both income and assets, maternal weight, smoking or alcohol use during pregnancy, and breastfeeding (VHEMBE, Manuscripts 2 and 3), allowing for the adjustment of confounders which were lacking in many previous studies. In addition, in Manuscripts 2 and 3, I included all *a priori* identified potential confounders in the generalized propensity score models used to generate the inverse-probability weights, which overcame the need for confounder selection strategies used by other studies which are known to result in biased estimates and inaccurate confidence intervals intervals[39-41]; furthermore, I verified the balance of all measured potential confounders, as well as post-exposure covariates (preterm birth, household food poverty at follow-up visits, and child dietary intake at 3.5 years) not included in the generalized propensity scores, providing empirical evidence that these variables did not confound the effect estimates. In Manuscript 2, which included only a subset of VHEMBE participants who were followed up to the 5-year visit, I also incorporated inverse-probability of censoring weights to adjust for selection bias from loss to follow-up, which was also a limitation of the extant literature, and in Manuscripts 2 and 3, I used multiple imputation by chained equations to address missing covariate data, minimizing the potential for selection bias.

This work also has some limitations. Selection bias in the CHMS is mitigated by the sampling and survey weights which accounted for total and partial (completed household survey but not clinic visit) non-response, but variables such as environmental chemical levels were not taken into account when deriving the survey weights, therefore selection bias from differential participation with respect to exposure level remains a possibility. Additionally, due to the cross-

sectional nature of the CHMS biomonitoring survey, reverse causation cannot be ruled out as a contributor to the findings of Manuscript 1. In VHEMBE, potential selection bias remains from eligible mothers who refused to participate in the study (18%), and basic demographic information collected on these mothers could be used to develop additional inverse-probability of censoring weights to address this potential source of selection bias in future analyses.

In both the CHMS and VHEMBE, exposure was ascertained from a single measurement. While DDT and DDE may represent longer-term exposure due to their long half-lives (6 and 10 years, respectively), parabens and pyrethroids are non-persistent chemicals whose urinary concentrations represent recent exposure; parabens are excreted in urine within 1 to 2 days[43,44,275] and pyrethroids are metabolized excreted within 3 days[81-83]. However, daily patterns of exposure to paraben-containing products may result in consistent exposure with moderate intraclass correlation coefficients of 0.42 to 0.71 reported for repeated spot urine samples[303,318,401], and since IRS insecticides are designed to be effective throughout the rainy season, elevated exposure to pyrethroids may persist for weeks or months from repeated contact with contaminated surfaces, bedding, furniture, and stored food.

Finally, Manuscripts 2 and 3 estimated causal effects using marginal structural models, which rely on some key assumptions[402]: i) that the models used to derive the inverse probability weights were correctly specified; ii) that the consistency assumption holds (i.e. the observed outcome for each individual is equal to their expected counterfactual outcome); iii) that there is no unmeasured confounding (exchangeability); and, iv) that each subject has a non-zero conditional exposure probability (no positivity violation i.e. the denominator of the weights is non-zero). Though the first three assumptions are untestable, we did observe mean weights of 1.0 without extreme values, suggesting that positivity violations did not occur.

7.3. Public Health Significance

By 60 to 79 years of age, approximately 40% of Canadians meet the criteria for metabolic syndrome, posing a substantial threat to their cardiometabolic health[403]. My findings suggest that exposure to parabens is associated with higher prevalence of metabolic syndrome among Canadian men, but lower prevalence of obesity among women. However, as this study is the first to report associations between parabens and metabolic syndrome, additional epidemiological studies are needed to confirm these findings. In particular, longitudinal studies with multiple measures of parabens are needed to confirm the temporality of these associations. While almost all Canadian men have detectable levels of methyl (89%) and propyl (73%) paraben, concentrations vary widely; the 10th to 90th percentile of methyl paraben concentration among Canadian men represents a 100-fold difference[404]. Studies investigating the determinants of paraben exposure may provide insight on how to minimize exposure to this ubiquitous preservative.

In March 2020, as part of Canada's Chemical Management Plan, Health Canada, along with Environment and Climate Change Canada, proposed the addition of four parabens (methyl, propyl, butyl, and *iso*-butyl) to the List of Toxic Substances in Schedule 1 of the Canadian Environmental Protection Act[405]. The proposed actions included: introducing restrictions on the use of parabens in cosmetic products, and reducing acceptable concentrations of parabens in non-prescription drugs and natural health products regulated under the Food and Drugs Act. Although not yet adopted, such restrictions would be a step towards minimizing exposure while maintaining the preservative activity of parabens necessary for the safe use of these products.

Additionally, my findings from the VHEMBE birth cohort suggest that gestational exposure to pyrethroids may influence growth during early childhood, reducing adiposity overall at age 5 and reducing weight trajectories from birth to 5 years of age among boys. This potential

disruption of energy and lipid metabolism may be particularly detrimental for children in a nutrient-poor environment; however, these findings should be replicated in a similar setting. Impeded child growth, particularly within the first 1000 days of life during which critical periods of development occur, can have lifelong consequences on health and well-being[406,407], as well as being an important cause of child morbidity and mortality[361,408-410]. At a population level, this can have significant consequences for countries in terms of healthcare costs and economic growth[411]. In malaria-endemic areas, child growth is already impeded by poverty, malnutrition, and disease; exposure to high concentrations of pyrethroid insecticides may also play a role in exacerbating this public health problem.

In VHEMBE, the range of exposure in the population greatly exceeded the exposure contrast for which effects were estimated (10-fold increases). Exposure mitigation strategies to reduce exposure to IRS insecticides without reducing the efficacy of malaria prevention exist, but only 22 to 74% of inhabitants of sprayed villages in Limpopo, South Africa reported using the WHO-recommended strategies of removing bedding, water, and food from the house during spraying[320,412]. Moreover, the use of IRS has declined over the past decade, with only 2% of the global population at risk of malaria currently protected by IRS[30], in favour of pyrethroid insecticide-treated bed nets which have lower potential for human exposure and are more effective and cost-effective than IRS[413,414]. However, as pyrethroids are now widely used in agricultural and domestic applications around the world, their health effects should continue to be investigated, including the potential for long-term impacts on cardiometabolic heath.

7.4. Future Research

There are several opportunities for furthering our understanding of the potential effects that these endocrine-disrupting chemicals have on our cardiometabolic health. Continued follow-up of

the VHEMBE children should be carried out to see if associations with reduced adiposity remain, or if new associations appear at older ages. In addition, adiposity rebound, which is the rise in BMI observed after infancy, typically between 3 and 7 years of age[415], is a very useful outcome to measure as it is strongly associated with measures of poor cardiometabolic health in adolescence and adulthood, including metabolic syndrome[416,417]. Similarly, the timing of puberty has been linked to cardiometabolic risk, including cardiovascular disease and mortality[418-421]; pubertal timing can be estimated based on the development of secondary sexual characteristics (Tanner staging)[422,423] or based on SITAR[424], with height measurements taken at annual intervals sufficient to estimate the timing and intensity of the pubertal growth spurt[425]. Investigating the influence of gestational exposure to DDT/E and pyrethroid insecticides on these outcomes would be a novel and exciting avenue of research into their potential effects on cardiometabolic health.

Exposure to these endocrine-disrupting chemicals occurs in the context of hundreds of other chemical exposures, some of which may also have endocrine-disrupting effects. In recent years there has been a growing interest in estimating the joint health effects of environmental chemical mixtures, highlighted in a 2015 workshop held by the U.S. National Institute of Environmental Health Sciences[426]. Going beyond traditional approaches such as shrinkage, variable selection, principal component or factor analysis, newer methods have been specifically developed to address high dimensionality and correlation in environmental mixtures while identifying the main drivers of health effects. These include: weighted quantile sum regression[427] and Bayesian kernal machine regression[428], which accounts for non-linear exposure-response relationships as well as interaction. More recently, methods combining exposure mixtures analyses with causal inference have also been developed (e.g. quantile g-computation[429]). Furthermore, exposures occur throughout the life course, so implementing studies with multiple measures of

prenatal and postnatal exposure would be able to inform us of the relevant or critical time windows of exposure. Methods such as multiple informant models[430] or distributed lag models[431] could be used to jointly estimate associations over various exposure windows, with recent extensions combining these methods with mixtures approaches (e.g. distributed lag models + weighted quantile sum regression)[432]. The limiting factor for such studies is not the lack of statistical methods; rather, such studies will require considerable coordinated effort and pooling of resources in order to quantify a large number of exposures multiple times over the life course. However, without this, we may be underestimating the total impact of environmental chemical exposure on human health.

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