The Associations Between Bisphenol A and Phthalates, and Measures of Adiposity Among Canadians

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LIST OF ACRONYMS

BMI: Body mass index

BPA: Bisphenol A

BPA-glucuronide: Bisphenol A-glucuronide

BPA-sulfate: Bisphenol A-sulfate

CCHS: Canadian Community Health Study

CDC: Centers for Disease Control

CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas

CHMS: Canadian Health Measures Survey

CI: Confidence interval

CSEP: Canadian Society for Exercise Physiology

DBP: Dibutyl phthalate

DEHP: Di-(2-ethylhexyl) phthalate

DEP: Diethyl phthalate

DNA: Deoxyribonucleic acid

ER: Estrogen receptor

GerES IV: German Environmental Survey IV

GGT: Gamma-glutamyltransferase

GS-MS: Gas chromatography-mass spectrometry

HPLC: High-performance liquid chromatography

ICC: Intraclass correlation coefficient

IOTF: International Obesity Task Force

LOD: Limit of detection

LOQ: Limit of quantification

LS-MS: Liquid chromatography-mass spectrometry

MBP: Mono-n-butyl phthalate

MBzP: Monobenzyl phthalate

MCF-7: Michigan Cancer Foundation-7.

MCMHP: Mono(2-carboxymethylhexyl) phthalate

MEC: Mobile examination centre

MECPP: Mono(2-ethyl-5-carboxypentyl) phthalate

MEHHP: Mono-(2-ethyl-5-hydroxyhexyl) phthalate

MEHP: Mono-(2-ethylhexyl) phthalate

MEOHP: Mono-(2-ethyl-5-oxohexyl) phthalate

MEP: Monoethyl phthalate

NHANES: National Health and Nutrition Examination Survey

NICU: Neonatal intensive care unit

NIH: National Institutes of Health

OR: Odds ratio

PPAR: Peroxisome proliferator-activated receptors.

SAS: Statistical Analysis System

SD: Standard deviation

SD: Standard error

TDI: Tolerable daily intake

t_{max}: Time at which the maximum concentration is achieved

UGT: UDP-glucuronosyltransferase

UGT2B1: UDP-glucuronosyltransferase-2B1

U.S. United States

WC: Waist circumference

WHO: World Health Organization

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ABSTRACT

Bisphenol A (BPA) and phthalates are chemicals found in many consumer products including water bottles, food packaging and cosmetics. Previous research has shown that there is potential for these compounds to contribute to obesity. In this analysis, the Canadian Health Measures Survey was used to investigate possible associations between urinary concentrations of these compounds and measures of adiposity. BPA urine concentrations were found to decrease with age, and significant associations with BMI and waist circumference were found in linear regression in adults. No associations with measures of adiposity were found in logistic regression for adults and significant negative associations were found in children. A similar discrepancy was found for mono-(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate, which were significantly associated with obesity in adults, but showed several significant negative associations in children. Overall, this analysis showed that it is unlikely that BPA and phthalates are contributing to adiposity in the Canadian population.

1. INTRODUCTION

Overweight and obesity remain significant economic and social burdens in Canada. In 2007-09, the prevalence of obesity was 24.3% in men and 23.9% in women, and has increased approximately 10% since 1992[1]. Abdominal obesity has also increased dramatically, from 11.4% in 1981 to 35.6% in 2007-09, with mean waist circumference values increasing by 6.5 cm in men and 10.6 cm in women[2]. It has been estimated that obesity is responsible for 61% to 74% of type 2 diabetes, 14% to 21% of colorectal cancers and 20% of all premature deaths in Canada[3]. Childhood and adolescent obesity has recently been estimated to be 11.7%[4] using the World Health Organization (WHO) cut-offs, an increase from 5% in 1981[5], while 19.8% of Canadians aged 5-17 years are overweight[4]. Children who are obese are much more likely to be obese and to have cardiovascular disease risk factors as adults[6]. Furthermore, childhood obesity is associated with a range of psychological problems such as low self-esteem and behavioural problems[6].

The annual cost of overweight and obesity in Canada was estimated to be $6 billion in 2006, or 4.1% of total health expenditures[7]. Despite the great social and economic burden of obesity in Canada and the western world, its major causes such as sedentary lifestyle and eating energy dense food are difficult to control at the population level. To further understand the large increases in obesity of the last several decades, research has turned to other suspected minor contributors, including endocrine disruptors[8]. In addition to the dramatic changes in lifestyle and diet that western populations have experienced over the last several decades, equally significant changes have occurred in our chemical environment. Endocrine disruptors are chemicals that mimic the action of hormones, such as estrogen or testosterone, by binding to their receptors and eliciting a similar response[9]. Suspected endocrine disruptors include pesticides and industrial

chemicals used in the manufacture of many consumer products such as cosmetics and food containers. Some chemicals such as bisphenol A (BPA) have been detected in approximately 90% of the population[10]. While intimately involved in reproduction and sexual development, research has also shown hormones to be associated with changes in body fat distribution in both men and women[11]. Observed positive associations between urinary concentrations of some endocrine disruptors and obesity have led them to be called “obesogens”[9].

2. LITERATURE REVIEW

2.1 Bisphenol A

BPA is an industrial chemical that has been used in the production of polycarbonate plastics and epoxy resins since the 1950s. BPA gives polycarbonate many desirable qualities such as toughness, transparency and heat resistance, and is used in many consumer products such as toys, drinking containers and eyeglass lenses. Epoxy resins containing BPA are used to line metal food cans, and annual production of BPA is estimated at 6 billion pounds[12].

Research has shown that BPA can migrate from food and beverage containers with repeated washing and use, and legislation has already been passed in Canada and other countries to ban the sale of baby bottles manufactured with BPA. BPA weakly mimics the action of estrogen and has been labeled as an endocrine disruptor[13].

2.1.1 Sources of Exposure

Epoxy resins containing BPA are used to line water pipes, make thermal paper that is used for sales receipts and most notably as coatings on the interior of many food and beverage product containers[12]. Research has found that during the manufacturing process, the polymerization of the resin may be incomplete, and that unreacted epoxy left on the surface may be susceptible to migrate into food that it is in contact with[14].

BPA has been detected in many canned foods in studies conducted in Canada[15], the U.S.[16], the United Kingdom.[17], Belgium[18] and Mexico[19]. For canned foods, the highest concentrations were observed in canned meats and vegetables[20,21], with very little detected in

beverages[17], soup[17] and fruits[18,21]. It is difficult to find consensus among these studies as they contain products listed generically, and similar products from different countries may have varying results. For example, Yoshida et al.[21] studied four different brands of canned corn from the U.S. and Japan and found concentrations between 2.6 µg/can and 11.1 µg/can. Goodson et al.[17] found 11 µg/kg and 42 µg/kg in canned carrots from the United Kingdom and Belgium, respectively. Very high concentrations were found in canned ham from Holland with 377 µg/kg, and corned beef from Brazil with 68 µg/kg[17]. A study of BPA in canned foods on the U.S. market found a broad range of values from different brands of green beans from 16 µg/kg to 500 µg/kg, and refried beans from 12 µg/kg to 680 µg/kg[16].

Cao et al.[15] analyzed the BPA concentration of a variety of canned foods on the Canadian market and found relatively low levels. Canned spaghetti and meatballs had the highest concentrations of BPA with 32 µg/kg (standard deviation (SD): ± 0.75), while mushrooms had the lowest at 5.2 µg/kg (SD: ± 0.85). A great deal of variability was also observed. 15 different canned tuna products produced a range of 9 µg/kg to 534 µg/kg with a median of 55 µg/kg, and 29 condensed soup products varied from a minimum of 4.1 µg/kg to a maximum of 189 µg/kg. The authors showed that the BPA concentrations found in vegetables and soups were higher than in studies from the United Kingdom.[17] and New Zealand[22], although the mean concentration for vegetables was 20 µg/kg, similar to products in the U.S.[16] Potential sources of variation could be differences in laboratory techniques and methods, for example, whether analysis of heterogeneous samples reported the liquid and solid phase concentrations separately, or homogenized them into a single phase.

A survey of the BPA content of canned liquid infant formula in Canada found values ranging from 2.3 µg/kg to 10 µg/kg[23], and a study from the U.S. found similar values with

different brands averaging from 3.97 µg/kg to 8.05 µg/kg[24]. Canned liquid infant formula and polycarbonate baby bottles present a slightly different risk than other products, as infants may be more sensitive to endocrine changes than the general population[25].

Similar to studies from other countries[18], much lower concentrations were observed in a study of canned beverages than canned food products from the Canadian market, with 85% of products analyzed having concentrations less than 1 ng/ml, and a mean of 0.57 ng/ml[26]. This is roughly equivalent to the 0.57 µg/kg for canned food products, and the authors suggested possible differences in the type and amount of coating as reasons[26].

The temperature that cans are heated to and the amount of salt and fat that they contain have both been identified as significantly influencing the migration of BPA. Kang et al.[27] observed that heating cans to 121˚C resulted in notable increases in BPA concentration, but observed similar results whether the duration was 15, 30 or 60 minutes. Furthermore, the authors found significantly greater migration after heating at 121˚C than 105˚C after 30 minutes, suggesting that temperature, but not heating time, affects BPA migration. Similarly, Takao et al.[28] observed much greater migration in cans heated to 100˚C than those heated to 80˚C for 30 minutes, and another study found that heating to 100˚C for as little as 9 minutes can increase BPA migration[29] and that a storage time of 70 days had little effect[29].

Polycarbonate bottles are another consumer product that may be a potent source of BPA exposure. Polycarbonate plastic has many desirable qualities for food applications, such as being heat resistant, lightweight and durable. Polycarbonate is made by polymerizing monomers of BPA and, similar to epoxy resins, heating or exposing polycarbonate to high or low pH may cause hydrolysis, releasing free BPA monomers into food products[14]. Four of the five polycarbonate bottles in a Canadian study had concentrations below 1 ng/ml with a mean of 0.75

ng/ml[30]. Wong et al.[31] analyzed baby bottles from several different countries and found a median concentration of 17.2 mg/kg.

To investigate how repeated use could affect migration, Brede et al.[32] simulated long term use through repetitive dishwashing and brushing and compared BPA migration between these bottles and new bottles. Both sets of bottles were heated at 100˚C for 1 hour, and simulated use significantly increased BPA migration. However, a simulated time period of 169 days did not result in more migration than a period of 51 days[32].

Mountfort et al.[33] performed similar experiments comparing dishwashing and steam sterilization of baby bottles but only for 3, 10 and 20 cycles, finding little to no BPA residue on the bottles and not detecting any in the rinsings or the formula prepared in them between cycles. As Brede et al.[32] used a laboratory dishwasher and generally harsher conditions, the results of Montfort et al.[33], who used a commercial one, may be more relevant to the average household. Maragou et al.[34] also used more relevant conditions and found that 10 cycles of dishwashing at 60˚C or brushing lead to minimal BPA migration.

The tolerable daily intake (TDI) is an "estimate of the amount of a potentially harmful substance (e.g. contaminant) in food or drinking water that can be ingested daily over a lifetime without appreciable health risk".[35] Wong et al.[36] used harsher conditions, incubating a cut portion of 28 polycarbonate baby bottles for 240 hours in either 10% ethanol at 70˚C or corn oil at 100˚C. The authors estimated daily intake to be between 0.012 mg/kg/day and 0.020 mg/kg/day, which comes quite close to the Canadian TDI of 0.025 mg/kg/day[37], although the study was conducted in Singapore. A Canadian study[38] used a much milder temperature of 40˚C and observed migration of 0.11 ng/ml after incubating the bottles in water for eight hours. The migration increased dramatically to 2.39 ng/ml after incubation for 240 hours in 50% ethanol.

A review of BPA migration from polycarbonate bottles identified temperature, contact time and alkalinity as being the main factors affecting BPA release[14]. Using an oil-based food simulant or ethanol as the testing media also increases migration, while distilled water and weak acetic acid less so[14]. Contrary to the results of Brede et al.[32], some studies have found that BPA migration decreases with repeated use, and it has been suggested that there may be residual BPA on the surface of polycarbonate bottles that is released after an initial wash and/or incubation, with little further migration upon repeated cycles[14].

Polycarbonate dental materials have also been identified as a potential source of exposure, where BPA could be released by the heating process used to form polycarbonate dentures[39]. For example, Olea et al.[40] found a wide range (90-931 µg) of BPA concentrations in the saliva of 18 adults one hour after application. However, in a similar experiment, Fung et al.[41] found that BPA was not detectable in saliva beyond three hours after administration of the sealant, and not detectable at all in serum. Variation has been found among different brands of dental sealant[42,43], and two studies found that gargling[43], and saliva removal[42] after administration of the sealant notably reduced BPA concentrations. These studies suggest there might be an initial leeching of BPA after application of the sealant, but not steady exposure over the long term.

Water has been identified as a potential source of BPA exposure as well. A study of drinking water in Ontario detected BPA in 12% of samples surveyed with a median concentration of 2.1 ng/l[44], although a German study found a lower level in drinking water with a range of 0.3 ng/l to 2 ng/l[45]. BPA has been detected in landfills[46], and it has been suggested that leaching from landfills may contribute to BPA levels in the environment and eventually in

drinking water[46]. BPA was found in 41% of 139 streams across the U.S., with a median concentration of 0.14 ng/ml[47].

BPA has also been detected in dust and indoor air, where a study of two locations in the U.S. found BPA in 95% of dust samples with a median concentration of 422 µg/kg[48]. Rudel et al.[49] surveyed 120 homes and found BPA to be present in 86% of house dust samples. A Japanese study found a BPA concentration of 0.51 ng/m^3 in urban outdoor air, and also found seasonal variation, increasing from fall to winter and decreasing from winter to spring[50]. Despite these findings, dust has been estimated to contribute <1% of total BPA exposure[48].

In summary, food is the most important source of BPA exposure in the general population, and exposure occurs mainly through leeching from the linings of food cans and reusable polycarbonate bottles. Studies have shown that canned beverages are a minor to negligible source of exposure. Research suggests that the heating involved in pasteurizing food cans during production, and bottles during cleaning (i.e., using a dish washer) increases leeching into their media. Dental sealants have also been identified as a potential source, although studies have found that there may be an initial release of BPA after application, but not steady exposure over the long term. BPA has also been detected in dust, environmental and drinking water samples but they are likely only a minor source of exposure.

2.1.2 Evidence of Bisphenol A as an Endocrine Disruptor

The earliest modern evidence of the hormonal activity of BPA was found inadvertently by Krishnan et al.[51] when studying whether yeast produce estrogens. The yeast was cultured using distilled water that was autoclaved in polycarbonate flasks. The authors found a substance

in the media that competed with estradiol in binding to estrogen receptors (ERs), and identified it as BPA. The estrogenic activity was subsequently confirmed with a radio receptor assay and a number of functional assays with MCF-7 breast cancer cells. The authors concluded by warning that BPA leeching could be disrupting experiments using polycarbonate flasks and that further research should examine polycarbonate consumer products. The authors found that BPA at concentrations of 25 nM and 50 nM increased proliferation of MCF-7 cells and 10 nM of BPA increased progesterone levels, calculating an affinity for estrogen receptor 1/2000 that of estradiol.

Another study found that the higher concentration of 10 μM was required to induce MCF-7 cell proliferation and calculated a much lower binding affinity of 5000-10000 times lower than estradiol[52]. Gould et al.[53] found that both BPA and estradiol increased uterine peroxidase and progesterone receptor levels when bound to ERα, but that BPA antagonized these effects when they were administered simultaneously. Other studies have also found that BPA exhibits both agonist and antagonist behaviour for ERα[54,55], but acts only as an agonist for ERβ[54]. Furthermore, Kuiper et al.[56] found that BPA binds with approximately six-fold greater affinity to ERβ than ERα. These isoforms are distributed differently among different types of cells and tissues[56], and thus the ER dependent effects of BPA may be variable[54]. This is further complicated by the variety of different promoters and co-activators that are involved in ER activation, which also vary between isoforms and tissues[57].

ERα is known to play a role in the regulation of insulin[58] and adipose tissue[59], and mice missing ERα were found to have insulin resistance and increased numbers of adipocytes in white adipose tissue[60]. Alonso-Magdalena et al.[61] found that estradiol, BPA and diethylstillbestriol, another endocrine disruptor, similarly disrupted the release of calcium ions in pancreatic α-cells,

which signal the secretion of glycogen. An increase in pancreatic β-cell content and hyperinsulinemia[62] was found in mice exposed to either 10 μg/kg of BPA or estradiol for four days. Furthermore, the estrogen antagonist fulvestrant blocked this effect, implying that ERs are involved[62]. Another study found similar effects of estradiol and BPA on the pancreas[63].

Another possible estrogen dependent mechanism is the inhibition of cytochrome P450-dependent aromatase, which converts androgens to estrogens (e.g. estradiol to testosterone) and is highly expressed in adipose tissue stromal cells and preadipocytes[64]. Mice unable to synthesize aromatase showed significantly greater intra-abdominal adipose tissue and elevated insulin levels as compared to controls[65]. Akingbemi et al.[66] and other studies[67-69] have shown reduced aromatase activation in response to BPA exposure.

A range of general metabolic pathology has been observed in rodent studies. Marmugi et al.[70] exposed mice to 5, 50, 500 and 5000 μg/kg/day of BPA for 28 days and observed increased de novo lipogenesis, which is the base rate at which fatty acids are synthesized. Higher amounts of cholesterol esters and triglycerides in the liver were also observed, and importantly, these effects appeared to follow a non-monotonic dose-response curve where effects were more pronounced at lower doses than higher ones.

Several studies have exposed rats perinatally to BPA. When pregnant rats were exposed to 50 μg/kg/day of BPA and their offspring given a high fat diet, severe metabolic syndrome including obesity, dyslipidemia and impaired glucose tolerance were observed[71]. These changes were attenuated in rats given a high fat diet but not exposed to BPA perinatally, and most importantly were not observed in rats exposed to the higher doses of BPA of 250 μg/kg/day to 1250 μg/kg/day, suggesting a non-monotonic dose-response curve[71]. Another study[72] with a similar design exposed pregnant rats to the dramatically lower dose of 0.25 μg/kg/day (1 part per

billion), which is the estimated level of adult dietary intake reported by the Food and Drug Administration. An increase in body weight of perinatally exposed offspring was observed at four weeks, but there were no significant differences in body weight or calorie intake between BPA exposed rats and control rats at seven weeks of age. Additionally, there were no significant differences observed when exposed and control rats were given a high fat diet. Conversely, in another study when pregnant female rats were exposed to 100 µg/kg/day or 1200 µg/kg/day, female offspring of mothers given the lower dose showed increased weight gain into adulthood and this was more pronounced with the lower dose[73]. Another study using a perinatal dose of BPA of 70 µg/kg/day observed significantly increased body weight in both male and female pups which persisted after weaning for females[74]. When pregnant mice were given a relatively low dose of 10 µg/kg/day, weight gain and increased triglycerides were observed four months after delivery compared to non-exposed controls[62].

A series of studies[75,76] also demonstrated very different effects when administering high and low doses of BPA to two-cell mouse embryos. Those exposed to a higher concentration of BPA (100 µM) exhibited slower development to the blastocyst stage, while those exposed to a lower concentrations (1 to 3 nM) exhibited faster development[75,76]. Furthermore, when transferred to and birthed by pseudopregnant mice, the resulting pups exposed to either concentration were significantly heavier than controls at day 21[76].

In summary, BPA exhibits estrogenic activity by binding differentially to estrogen receptors α and β. ERα is known to be involved in insulin and adipose tissue regulation and studies have shown increases in insulin when pancreatic β-cells were exposed to BPA or estradiol. Administering BPA to rodents both peri- and postnatally have produced a range of metabolic effects including weight gain, but also increased cholesterol and triglycerides.

2.1.3 Metabolism and Measurement Variability

After oral administration, BPA has been observed to have a urinary half-life of approximately six hours, and its primary metabolites in humans are BPA-glucuronide, and to a lesser extent BPA-sulfate[77]. A review of BPA biomonitoring studies in animals and humans shows that high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GS-MS) and liquid chromatography-mass spectrometry (LS-MS) are commonly employed as measurement techniques[12]. These methods have many variations and a detailed comparison is beyond the scope of this analysis, but an important commonality among them is the use of enzymatic deconjugation. This involves applying an enzyme that cleaves the glucuronide group from BPA-glucuronide, converting it back to free BPA and is effectively a reverse of human metabolism. This method is likely employed for ease of detection, as one only needs to quantify one analyte when deconjugation is used, as opposed to a mix of free BPA, BPA-glucuronide and possibly BPA-sulfate. Research has shown sharp differences in the estrogenicity of free BPA and BPA-glucuronide[78], and as all of the large biomonitoring datasets (NHANES, CHMS, Songnan Community Study) used this method, there is currently a debate about the legitimacy of total BPA versus free and conjugated measurements[79], which is discussed at length later in this thesis.

Several experiments have found that BPA has efficient first-pass metabolism in rats, where intestinal or hepatic degradation or alternation reduces the amount of the analyte in the blood because it enters systemic circulation[80,81]. This is referred to as first-pass metabolism. BPA-glucuronide is the primary metabolite of BPA, although its portion of total metabolites can vary and another BPA conjugate called BPA-sulfate has been found, albeit in much smaller amounts[81]. Many animal experiments have focused on the liver and small intestine, as the oral

route of exposure is considered the most significant for humans. It has been demonstrated that BPA is glucuronidated in isolated rat liver microsomes by the enzyme UDP-glucuronosyltransferase-2B1 (UGT2B1) which also glucuronidates androgens[82]. Evidence also indicates that significant glucuronidation may occur in the small intestine by a different isoform of the UDP-glucuronosyltransferase (UGT) family[83].

Route of administration appears to be an important factor as a dose administered orally was observed to produce lower plasma levels of free BPA and thus less bioavailability than one given intraneritoneally[81], subcutaneously[81] or intravenously[84]. This may be due to complete glucuronidation via first-pass metabolism[81] of the compound when orally administered, specifically in the proximal intestine[83], and is further evidenced by the larger amount of the conjugated form observed in plasma after oral dosing as compared to the other routes[81]. An oral dose also produced the largest fraction of BPA-glucuronide in plasma, and the smallest of free BPA in feces, also indicating more complete first-pass metabolism[81]. The fecal route appears to be the main pathway of elimination in the rat, although it has been observed that higher oral doses may saturate the main metabolic pathway, resulting in a greater portion being excreted in urine[81]. In male rats, the very large dose of 800 mg/kg used by Knaak & Sullivan[85] resulted in 54-60% of the dose being excreted in feces and 26-31% in urine, compared to the lower dose of 100 µg/kg used by Kurobayashi et al.[86], which resulted in 88% fecal and 7% urinary excretion[86]. Pottenger et al.[81] used doses of 10 mg/kg and 100 mg/kg and found similar results of ~80% elimination in feces and ~15% in urine for male F344 rats, the same breed as Kurobayashi et al. and Pottenger et al. both found that female rats had generally lower fecal and higher urinary excretion than males, suggesting a less complete first pass metabolism. Snyder et al.[80] only used female F344 rats, and observed 50% fecal and 40% urinary elimination compared to Pottenger et

al.[81], with ~69% and ~28% and Kurobayashi et al.[86] with 64% and 35% for fecal and urinary excretion, respectively.

A number of studies have demonstrated the major metabolite of BPA in humans to be BPA-glucuronide[87,88]. After oral administration, BPA is readily absorbed by the gastrointestinal tract and a glucuronic acid group is added to the BPA in the liver via the enzyme UDP-glucuronosyltransferase[87]. The resulting BPA-glucuronide is then rapidly cleared by elimination in urine[87] demonstrating efficient first-pass metabolism. Human biomonitoring studies have shown that a large portion of orally administered BPA is quickly eliminated this way[87], and that total elimination is achieved by 24 hours with an estimated half-life of 6 hours. After orally administering 5 mg of BPA to three men and three women, Volkel et al.[87] observed a urinary half-life of 5.4 hours and that BPA and its metabolites were completely eliminated and recovered in urine ~24 hours after administration. Tsukiokai et al.[88] administered the much lower amount of 0.10 mg and found urinary elimination to be essentially complete at ~5 hours and almost 100% of the sample was recovered in urine as BPA-glucuronide. Tsukiokai et al.[88] also found that BPA reached its peak urinary concentration of 90 ng/ml after 30 minutes, which fell to 26 ng/ml 60 minutes after ingestion. Volkel et al.[87] observed similar results in plasma where concentrations peaked 80 minutes after intake with an initial half-life of 89 minutes, and the administered dose was excreted almost entirely as BPA-glucuronide. Using concentration time courses, Volkel et al.[87] devised a kinetic model with several stages and calculated half-lives of 53 and 17 minutes for the uptake and elimination from plasma, respectively. Both experiments demonstrated efficient first-pass metabolism where all or a majority of the ingested BPA was rapidly absorbed in the digestive tract, converted to BPA-glucuronide in the liver and rapidly eliminated from plasma through urine and feces.

In their discussion, Volkel et al.[87] made several important points in comparing their results to studies in rats. The authors noted that because humans have a higher threshold for biliary or fecal elimination, after BPA-glucuronide is formed in the liver it is released into the bloodstream and, due to high water solubility, rapidly cleared by the kidneys in urine. In rats however, BPA-glucuronide moves from the liver to bile and may be transported back to the gastrointestinal tract via enterohepatic circulation[87]. Oversaturation of the biliary pathway ultimately results in greater retention of BPA, which suggests that levels measured in blood or urine in rats after oral administration may be higher than expected for a comparable dose in humans. Incomplete first-pass metabolism, as well as the much larger doses used in rat studies, may also account for the presence of metabolites such as BPA-sulfate. For example, in their study of rat metabolism, Pottenger et al.[81] administered doses of 10 mg/kg and 100 mg/kg of body weight, ~100 and ~1000 times greater than the dose used by Volkel et al.[87].

Glucuronidation is clearly an important step in the metabolism of BPA, but may not be the rate limiting step in humans. In reference to the high rate of glucuronidation found in isolated rat microsomes as compared to humans, Volkel et al. postulated that transport of BPA from the gastrointestinal tract to central circulation may be the limiting step of these processes, possibly explaining the more complete first-pass metabolism seen in humans than rats[87]. Larger doses might saturate the metabolic pathway, leading to fecal elimination as a hydroxylated metabolite not usually observed at smaller doses[85]. The amount excreted as BPA-glucuronide has been observed to differ between strains of rat, which may be due to differential activity of UDP-glucuronosyltransferase[80], and differences have also been observed between sexes[81,86].

Ye et al.[89] assessed the reliability of spot urine measurements of BPA in a group of four men and four women. A smaller sample size was justified in this instance in order to reliably collect spot samples, morning voids and 24-hour collection from each participant, a process that can be costly and time consuming. Spot urine samples were found to vary 70% within the day and 21% between days for the same individuals. Between days, first morning and 24-hour voids expressed more variability than spot samples with 77% and 88%, respectively[89]. Mahalingaiah et al.[90] collected several spot samples from 82 subjects over several years and found that samples collected past 16:00 hours were significantly greater than those collected between 12:00-15:59 hours, though the sample sizes for each time period varied widely. Using data from the National Health and Nutrition Examination Survey (NHANES) 2003-04, Calafat et al.[10] also found notable variability with the time of collection, where samples collected in the morning and evening had significantly greater variations than those collected in the afternoon for adults, but not for children[10]. Despite temporal variability, sensitivity and specificity analysis did demonstrate that a single spot sample from a subject could correctly identify them in the highest tertile of BPA urine concentration[90]. A report conducted by the National Toxicology Program concluded that despite evidence of variability, spot urine samples may still useful to calculate cross-sectional population means[91].

A central issue raised by Volkel et al.[87] is the distinction between free and glucuronidated BPA. Several large studies such as NHANES and the Canadian Health Measures Survey (CHMS) employed a method where the enzyme β-glucuronidase was used to cleave the glucuronide group added in the liver. This method measures total BPA (free and previously conjugated) while only free BPA has demonstrated toxicity in laboratory experiments.

Glucuronidation is a common metabolic pathway for the elimination of xenobiotics such as BPA, but also endogenous compounds such as androgens[92]. UGT2B1 specifically has also been observed to glucuronidate the xenobiotic diethylstilbestrol and opioid compounds and is expressed only in the liver[93]. It has been demonstrated *in vitro* that the gucuronidated BPA has little to no estrogenic activity[78].

Several studies have measured free BPA in urine samples after oral administration with varying methods and limits of quantification (LOQ). With ingestion of 25 µg of BPA, very little free BPA was detected and was determined to account for only 2% of the ingested amount[94]. Another study by the same group[87] did not detect any free BPA in urine samples from six subjects who orally ingested 5 mg, even when large amounts of BPA-glucuronide were detected. Free BPA could only be detected in 10% and 7% of the samples in two biomonitoring datasets of 287 and 203 subjects, respectively[95]. Tsukioka et al.[88] found very low concentrations of free BPA in urine after 5 mg was orally administered to 25 adults. Additionally they found free to total BPA ratios of 2% (0.34-8.1%) and 12% (2.6-29%) in their orally administered and baseline urine samples, respectively. Another study found that among 52 subjects, free BPA could only be detected in 4%[96], and none could be detected in a smaller sample of six subjects[87].

In summary, studies of metabolism have shown that BPA has a urinary half-life of approximately 6 hours and that BPA-glucuronide is its primary metabolite. Measurement of urinary BPA shows moderate variability, although this is likely reduced by large sample sizes. Many biomonitoring studies use a method of detection that does not differentiate free BPA from the BPA-glucuronide, which might not possess any estrogenic activity, and very little free BPA has been detected in studies that measured it.

2.1.4 Human Biomonitoring

Few large surveys have collected biomonitoring data on BPA, most notably NHANES in the U.S. The three NHANES cycles commonly cited in the literature produced somewhat different values (Table 2.1). Geometric means are the preferred representation of urine concentrations as they are more robust to outliers than arithmetic means, which tend to give higher estimates. For example, Melzer et al.[97] calculated the geometric mean for adults who participated in NHANES 2003-04 to be 2.49 ng/ml, but the corresponding arithmetic mean was 4.59 ng/ml. The Songnan Community Study conducted in China had by far the lowest mean of any large study[98] and a sample of Korean adults had by far the highest[99]. Considerable variability has been observed with BPA concentrations, even in large studies such as NHANES where the mean of the 2005-06 cycle was significantly lower than the 2003-04 estimate[100]. As such, the very high mean in the Korean study should be considered with caution, and due consideration to analytical procedures employed.

The InCHIANTI study was started in 2000 to investigate factors contributing to mid- and late-life morbidity in Tuscany, Italy, and urinary BPA was found to have the highest geometric mean of 3.59 ng/ml among large population studies[101]. Wang et al.[98] also used an older sample from the Songnan Community study and found a much lower concentration. Silver et al.[100] found a significant negative association between urinary BPA concentration and age, and significantly higher concentrations in males than females. Interestingly, a negative association with age was also found in the studies of Wang et al.[98] and Galloway et al.[101] who used older population samples and showed that this relationship might extend into old age. In NHANES, urinary BPA was also found to be higher in non-Hispanic Blacks than Mexican Americans and non-Hispanic Whites[10,100]. Unadjusted positive associations with BMI and waist circumference were found in

the InCHIANTI[101] and the Songnan[98] studies, but not in NHANES[100]. Smoking was also investigated and found to be associated with urinary BPA in the NHANES 2003-06, but not in the InCHIANTI or Songnan studies. Similar decreasing trends with age and a higher concentration in men than women were also observed in NHANES[100].

Table 2.1 Urinary bisphenol A concentration in biomonitoring studies.

Study	Dataset (sample size)	Sample description	Geometric mean (95% CI or SD) (ng/ml)
Becker et al. 2009[102]	GerES IV (599)	German children aged 3-14 years	2.66 (2.44, 2.89)
Calafat et al. 2008[10]	NHANES 2003-04 (2,517)	U.S. population aged 6 years or older	2.6 (2.4, 2.9)
Melzer et al. 2010[97]	NHANES 2003-04 (1,455)	U.S. population aged 18-74 years	2.49 (2.20, 2.83)
	NHANES 2005-06 (1,493)		1.79 (1.64, 1.96)
Silver et al. 2011[100]	NHANES 2003-04 (1,364)	U.S. population aged 20 years or older	2.4 (2.1, 2.7)
	NHANES 2005-06 (1,363)		1.7 (1.6, 1.9)
	NHANES 2007-08 (1,662)		2.0 (1.8, 2.1)
	NHANES 2003-08 (4,389)		2.0 (1.9, 2.1)
Galloway et al. 2010[101]	InCHIANTI (715)	Residents of Tuscany aged 20-74 years	3.59 (3.42, 3.77)
Yang et al. 2003[99]	Original data (73)	South Korean adults from Daejon	9.54 (SD 8.32)
Wang et al. 2012[103]	Original data (259)	Chinese children aged 8-15 years	0.40 (0.33, 0.49)
Wang et al. 2012[98]	Songnan Community Study (3,390)	Residents of Songnan Community, Baoshan District, Shanghai, China aged 40 years or over	0.81* (0.47, 1.43)

CI, Confidence interval; GerES IV, The German Environmental Survey IV; NHANES, National Health and Nutrition Examination Survey; SD, Standard deviation. * Type of mean unspecified.

Table 2.2 Urinary bisphenol A concentration adjusted for urine creatinine in biomonitoring studies.

Study	Dataset (sample size)	Sample description	Geometric mean (95% CI) (μg/g creatinine)
Carwile & Michels 2011[104]	NHANES 2003-06 (2,747)	U.S. population aged 18-74 years	2.05 (interquartile range: 1.18, 3.33)
Calafat et al. 2008[10]	NHANES 2003-04 (2,517)	U.S. population aged 6 years or older	2.6 (2.4, 2.8)
Silver at al. 2011[100]	NHANES 2003-04 (1,364)	U.S. population aged 20 years or older	2.4 (2.2, 2.7)
	NHANES 2005-06 (1,363)		1.8 (1.7, 1.9)
	NHANES 2007-08 (1,662)		2.0 (1.9, 2.2)
	NHANES 2003-08 (4,389)		2.1 (2.0, 2.2)
Yang et al. 2003[99]	Original data (73)	South Korean adults from Daejon	8.91

CI, Confidence interval; NHANES, National Health and Nutrition Examination Survey.

The German Environmental Survey IV (GerES IV) studied urinary BPA in German children aged 3-14 years and found a concentration of 2.66 ng/ml (95% confidence interval (CI): 2.44, 2.89)[102], compared to a much lower value of 1.30 ng/ml (95% CI: 1.17, 1.45)[105] in Canadian children aged 6-11 years. Similar to German children, the urinary BPA concentration in U.S. children aged 6-11 years was found to be 3.6 ng/ml (95% CI: 2.9, 4.3)[10] in NHANES 2003-04 and a much lower concentration of 0.45 ng/ml (95% CI: 0.37, 0.55)[103] was observed in a sample of Chinese children aged 8-15 years.

In addition to urine concentration, NHANES data have also been used to estimate daily intake of BPA. It was assumed that BPA intake in a given 24-hour period is equal to excreted BPA in the following 24 hours, and a spot BPA urine concentration multiplied by daily urine output was used as an estimate of intake[106]. This is based on evidence that BPA was almost completely excreted 24-hours after ingestion[87,94], which is a debated finding[107] and discussed at

length elsewhere in this thesis. Sex and age group specific values of daily urine output were drawn from the literature, and the estimated intake was then divided by body weight to yield a standardized measure. Using these methods, the mean BPA intake for the U.S. population was estimated to be 46.8 ng/kg/day (95% CI: 42.6, 51.4)[108] in 2003-04 and 35.1 ng/kg/day (95% CI: 33.3, 37.0)[109] in 2005-06. Similar to the exposure analysis[10,100], BPA intake was higher in men than women, and decreased with age in both cycles[108,109].

Many of the largest and most powerful epidemiological studies on this topic combined multiple cycles of NHANES. Carwile & Michels[104] combined the 2004-05 and 2005-06 cycles and found that adults in the upper three quartiles of urinary BPA concentration were significantly more likely to be obese than those in the lowest quartile as measured by BMI or waist circumference[104]. When stratified by sex, the association weakened and was no longer significant in women. BPA quartiles modeled linearly as indicator variables against continuous BMI produced a non-significant association. The authors concluded that there might be a threshold effect as all three upper quartiles showed a similar strength of association[104]. These results were confirmed in a similar study using NHANES data from 2003-08 where the association was found to be consistent across sex and ethnic/racial groups[110] for both obesity (BMI $\geq$30 kg/m^2) and elevated waist circumference. Urinary BPA concentration was also associated with obesity and elevated waist circumference in a sample of 3,390 Chinese adults[98]. Using data from NHANES, multiple studies have shown a significant positive association between urinary BPA concentration and obesity and elevated waist circumference in children and adolescents[111-113]. Significant positive associations between urinary BPA concentration and metrics of adiposity were also reported in Chinese school children[103,114]. The Health Assessment of Mothers and Children of Salinas (CHAMACOS)[115], a longitudinal cohort study of environmental factors and

children's growth and development, collected urinary BPA measurements during pregnancy and at 5 and 9 years of age. It was observed that prenatal urinary BPA levels were negatively associated with BMI z-score and waist circumference at 9 years of age in girls but not boys[116]. Furthermore, urinary BPA levels at 9 years *were* positively associated with the body parameters in boys but not in girls[116].

Several other studies examined the associations of BPA with a variety of chronic diseases and metabolic syndrome. Using data from NHANES, studies showed significant positive associations of urinary BPA concentration with diabetes[117-119], cardiovascular disease[117,118] and hypertension[119]. Urinary BPA concentration was also positively associated with insulin resistance in a large sample of Chinese adults[98], but not clearly with type 2 diabetes in the same sample[120].

In summary, large biomonitoring studies have shown a range of BPA concentrations in urine for children and adults. Significant positive associations with measures of adiposity have been found for children and adults in NHANES and other studies. Significant positive associations with other chronic conditions such as diabetes, cardiovascular disease and hypertension have also been observed.

2.2 Di-(2-ethylhexyl) Phthalate

Phthalates are a common name for the dialkyl- or alkylarylesters of 1,2-benzenedicarboxylic acid. Low molecular weight compounds such as dibutyl phthalate (DBP) are used in adhesives, inks and cosmetics[121], and high molecular weight compounds such as di-(2-ethylhexyl) phthalate (DEHP) and diisodecyl phthalate are used in polyvinyl chloride (PVC) cables, flooring and roofing[121]. Studies have shown that phthalate exposure is very prevalent[122] and animal studies have shown carcinogenic and teratogenic effects[123], but of particular concern is the anti-androgenic behaviour of some phthalates[124]. Androgens are known to play a role in fat distribution and other metabolic parameters[125], and concern has been raised about how phthalate exposure may reduce levels of testosterone and increase body weight.

2.2.1 Sources of Exposure

In general, DEHP exposure occurs through the use of consumer products such as cosmetics and cleaning products. In a sample of 50 pregnant women, Buckley et al.[126] found that exposure to any DEHP metabolite was significantly associated with using cologne or perfume. A significant positive association was also found between eye shadow, liner or mascara and any DEHP metabolite, as well as several other phthalates such as monobenzyl phthalate (MBzP). Non-significant positive associations were also found with laundry detergent, facial masks, dryer sheets and self-manicures[126].

Food packaging has also been identified as a potential source of exposure. Using a small sample of 20 adults, Rudel et al.[127] conducted a 3-day intervention where participants were given food believed to have negligible phthalate content. The geometric mean of DEHP metabolites

mon-(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) fell by 53-56% during the intervention, and the maxima declined by 93-96%. Notable decreases were not seen for other phthalates such as monoethyl phthalate (MEP) and MBzP, and DEHP metabolites showed a similar decline to BPA, where diet is established as the primary source of exposure[12]. An analysis of retail packed lunches in Japan found that DEHP was the most prevalent phthalate, and one third of the lunches had DEHP levels exceeding the European Union TDI of 1.85 mg for an adult weighing 50 kg[128]. The authors observed a steady increase in the DEHP concentration in chicken during processing, which increased from 0.8 mg/kg to 13.1 mg/kg during cooking, and to 16.9 mg/kg after packaging. Handling foods with PVC gloves was found to significantly increase the DEHP content, especially when sterilized with ethanol[128]. A follow-up study[129] was conducted after DEHP-containing PVC gloves were banned for food preparation in Japan, which found concentrations ~94% less than that of the previous study. A survey of food products on the Belgian market found that DEHP was by far the most abundant phthalate detected, and the highest concentrations were found in fat and oils[130]. This is not surprising as DEHP is a lipophilic compound[131] and the next most potent source was fish and fish products[130].

Certain subsets of the population are at higher risk of phthalate exposure, such as people who frequently require medical procedures such as hemodialysis, blood transfusion and intravenous treatments. Medical tubing and bags used for blood and intravenous liquids are commonly made with PVC, of which DEHP is added as a plasticizer to increase toughness and flexibility[121]. A review of DEHP exposure following treatment with PVC medical devices found a wide range within a given treatment but found maxima of 7.2 mg/kg and 8.0 mg/kg for hemodialysis and blood transfusion, respectively[132].

Particular concerns have been raised regarding the extensive use of DEHP containing devices in neonatal intensive care units (NICUs). Green et al.[133] categorized NICU patients into low, medium and high potential for DEHP exposure given the treatments they were receiving, and found that those in the high exposure group had five times the urinary MEHP concentration as those in the low exposure group. A similar study[134] also measured MEHHP and MEOHP in neonates who were likely to receive an intravenous treatment for more than two weeks. Concentrations varied widely but the ratio of MEHHP/MEOHP remained stable, similar to the results of metabolism studies[135]. The geometric mean MEHP concentration was 100 ng/ml, significantly higher than in a sample of 19 toddlers from California (4.6 ng/ml)[136] and many times higher than 6-11 year olds in the general U.S. population (5.12 ng/ml)[122].

Using data from several different surveys, Meek & Chan[137] calculated estimates of DEHP intake from different sources in the Canadian population. Food was found to be by far the most potent source and accounted for ~90% of DEHP exposure, with ambient air and soil exposures considered negligible. Indoor air was found to be a minor source of exposure with the overall caveat that much of the data were collected in the 1980s and may be out of date or lacking generalizability[137]. House dust has been tested for DEHP metabolites in several studies and the median DEHP levels ranged from 515 mg/kg to 740 mg/kg[138]. One study found no association between levels in house dust and urine, suggesting that house dust may not be an important source of exposure[138].

Specific concern has been given to the safety of toys made of PVC, which children might put in their mouths, causing phthalates to leach from the toys. Experiments on toys made with DEHP have shown that DEHP can leech into a saliva-stimulating medium and that agitation or sucking of the object may increase migration[139].

In summary, the main sources of DEHP exposure are believed to be food packaging and consumer products such as cosmetics. Certain subsets of the population who use DEHP containing medical equipment may be at higher risk of exposure.

2.2.2 Evidence of Di-(2-ethylhexyl) Phthalate and its Metabolites as Endocrine Disruptors

Several studies have demonstrated that DEHP has reproductive toxicity, such as suppressing estradiol[140] and testosterone[141] production, and general reproductive toxicity in rats[142]. Detailed studies of human metabolism have shown that DEHP is quickly metabolized and its bioavailability is likely very low compared to its metabolites[143].

Generally, phthalates show weak to no estrogenic activity, and notably less than other chemicals such as BPA and dichlorodiphenyltrichloroethane[144]. Specifically, MEHP and DEHP showed negligible estrogenic activity in an *in vitro* yeast screen[144], while other phthalates such as butyl benzyl phthalate and DBP were weak xenoestrogens. Another study found that DEHP was a weak agonist of ERα and a weak antagonist of ERβ[145].

Androgens, such as testosterone, are known to play a role in fat regulation and distribution[146]. Androgen receptor-deficient mice have been found to develop obesity at 10 weeks of age[147], and lower endogenous androgens have been observed to predict central adiposity in men[148]. The main function of granulosa cells is to convert androgens to estradiol in response to follicle-stimulating hormone during the menstrual cycle[149]. Lovekamp & Davis[140] exposed isolated rat granulosa cells to 100-200 μM of MEHP and found that estradiol production was reduced in a dose dependent manner. Furthermore, the authors reported a decrease in the aromatase protein, which is the rate limiting enzyme of this process. Both of these observations

were found in another rodent study as well[150]. Similar results have also been observed with cultured human granulosa cells where concentrations of MEHP between 80 µmol/l and 140 µmol/l caused a significant inhibition of estradiol production and a reduction in levels of the aromatase protein[151].

Awal et al.[152] exposed guinea pigs to the very high dose of 2000 mg/kg of MEHP and observed displacement and apoptosis of spermatogenic cells, thin seminiferous epithelia and other reproductive toxicity. Dalgaard et al.[153] orally administered a high dose of 400 mg/kg to Wistar rats and observed general testicular toxicity 12 hours after exposure.

In a sample of Chinese factory workers, Ping et al. observed a significant negative association between MEHP urine concentration and serum testosterone[154]. Approximately half their sample was occupationally exposed to MEHP as demonstrated by a significantly higher geometric mean of 565.7 µg/g creatinine versus 5.7 µg/g creatinine, and the exposed workers had significantly lower serum testosterone than the unexposed workers. In a sample of 180 Taiwanese children followed from birth until 8 years of age, it was found that MEHP levels were significantly correlated with serum progesterone in boys and girls, although levels were not correlated with changes in physical or reproductive development[155].

Hauser et al. conducted several studies[156,157] on semen quality in a sample of men recruited from a fertility clinic and found that MEHP was associated with reduced sperm motility, concentration and morphology, though not in a dose dependent manner. Interestingly, the authors found a higher amount of MEHHP and MEOHP for a given concentration of MEHP resulted in less DNA damage in sperm. They suggest that the reduced toxicity of MEHHP and MEOHP results in a protective effect, and where the further MEHP is metabolized, the lesser the

effects on sperm[157]. In a similar study, urinary MEHP concentration was associated with increased sperm apoptosis in a sample of Chinese men recruited at a fertility clinic[158]. A simple *in vitro* experiment using the androgen receptor found that neither DEHP nor MEHP elicited anti-androgenic behaviour at low concentrations[145].

Desdoits-Lethimonier et al.[141] observed a significant inhibition of testosterone production in human testes explants exposed to DEHP and MEHP. Furthermore, the authors found that DEHP was metabolized by the culture into MEHHP and MEHP, which were likely responsible for its anti-androgenic effect. The authors observed time and dose-dependent effects and suggested that the reduced anti-androgenic effect over time might be due to the conversion of MEHP to MEHHP and MEOHP, of which MEOHP has less hormonal activity[141]. In contrast, a similar study using cultured human fetal testes did not find a reduction in basal or luteinizing hormone induced testosterone production[159].

Stroheker et al.[160] only observed a significant anti-androgenic response at DEHP concentrations ≥100 mg/kg/day in castrated male rats, and the authors established a no observed adverse effect level at 20 mg/kg/day. An *in vitro* test was also performed and found that two unidentified metabolites of MEHP induced stronger anti-androgenic responses than DEHP and MEHP, but testosterone production was not used as an endpoint. Conversely, another study[161] using cultured rat fetal testes found no significant reductions in testosterone after administering DEHP, MEHP or metabolites VI and IX. Borch et al.[162] observed significant anti-androgenic responses in the form of reduced testosterone and Leydig cell effects in in utero exposed rats after MEHP, but only at the very high dose of 300 mg/kg.

In summary, DEHP metabolites have shown anti-androgenic activity in a number of human and animal studies, and androgens are known to play an important role in fat regulation and distribution. There is currently a lack of consensus on which of the three DEHP metabolites mentioned here may be the most bioactive.

2.2.3 Metabolism and Measurement Variability

Studies of human metabolism have shown that after exposure, DEHP is cleaved to its monoester and primary metabolite MEHP. A number of secondary oxidative metabolites are then formed, most notably MEHHP and MEOHP.

Koch et al.[163] gave a single male subject approximately 0.64 mg/kg of DEHP and observed its absorption and elimination in urine and plasma. MEHP was found to have the shortest time to maximum urine concentration, t_{max}, of two hours and a half-life of five hours[164]. MEOHP, MEHHP and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), a minor metabolite, exhibited similar profiles with a t_{max} of 4 hours and a half-life of 10 hours. The authors noted that metabolism took place over distinct phases, where absorption and distribution took place from 4 to 8 hours after intake. The first elimination phase occurred between 8 and 16 hours after administration, whereas a shorter half-life of 2 hours was observed for MEHP, MEOHP, MEHHP and MECPP. In the second elimination phase, the half-life increased to 5 hours for MEHP, 10 hours for MEOHP and MEHHP and 12 to 15 hours for MECPP. Mono(2-carboxymethylhexyl) phthalate (MCMHP), another minor metabolite, exhibited a different metabolic profile reaching local and absolute maxima 8-10 hours and 24 hours after administration with an overall half-life of 24 hours[165]. The amount of each metabolite excreted

relative to one another also changed over time. Overall MEHHP and MECPP had the highest urine concentration, although, due to its longer elimination, MCMHP had the highest urinary concentration 12 hours after administration. MECPP and MCMHP were the metabolites present in the highest concentration 24 hours post-dosing[165]. Although initially present in the highest concentration in urine, MEHP only constituted 7.34% of the dose 44 hours after administration, with the highest portion of 24.7% as MEHHP. The experiment was repeated with lower doses of 4.7 µg/kg and 28.7 µg/kg for which the metabolic profiles were reasonably consistent[164].

Given the relatively fast metabolism of DEHP, measurement of its metabolites may be subject to the same variability as other chemicals in the consumer environment, such as BPA. Fromme et al.[166] collected daily urine samples from 50 men and women over 8 days to investigate the within person variability of phthalates. Age and sex adjusted intraclass correlation coefficients (ICCs) for DEHP metabolites were relatively low at 0.27, 0.21 and 0.22 for MEHP, MEHHP and MEOHP, respectively. ICCs for MECPP and MCMHP were also low at 0.26 and 0.25, and MBzP had the highest ICC of 0.48.

Townsend et al.[167] performed a similar analysis with data from the Nurse's Health Study, which collected several urine samples from participants over the course of several years. DEHP metabolites generally exhibited more within-person than between-person variation, and the within-person variation coefficient for MEHP was very high at 0.93 with an ICC of 0.14 (95% CI: 0.01, 0.67). Other metabolites had more moderate ICCs of ~0.40 and the sum of all DEHP metabolites had an ICC of 0.38 (95% CI: 0.16, 0.65). Limiting the analysis to first morning voids did not appreciably change the results.

Baird et al.[168] studied the variability of samples taken from women trying to get pregnant between 1982 and 1986 and kept in frozen storage. ICCs for DEHP metabolites were again generally low and the ICC for the sum was 0.26 (95% CI: 0.14, 0.41). Meeker et al.[169] conducted one of the largest studies on measurement variability with 269 men and women recruited from a fertility center, with an average of 3.4 urine samples per participant. The authors found even lower ICCs for DEHP metabolites, ranging from 0.13 (95% CI: 0.08, 0.22) for MEHP to 0.15 for MECPP. In a sample of Danish men, MEHP and MEHHP exhibited similar high variability, and unadjusted first morning voids had an ICC of 0.21 for both analytes. The ICCS for the same compounds using 24hour voids were actually lower, and the total of DEHP metabolites had a very low ICC of 0.06 (95% CI: -0.12, 0.30) and 94% of between-person variability. Interestingly, ICCs were higher using spot samples than first morning voids and 24-hour voids. Similar to the other studies, metabolites of DEHP had generally higher variability than other phthalate metabolites. Conversely, much lower variability was observed in a sample of middle aged African American women where the ICC for MEHP was 0.52 (95% CI: 0.32, 0.68), and 0.67 (95% CI: 0.49, 0.79) after creatinine adjustment[170]. This study appears to be an outlier with much lower variability.

Studies have also observed within-day variation for DEHP metabolites. Meeker et al.[169] found a significant association with time of day, where urine concentrations were higher in the early morning (0600-0859h) and the evening (1500-1800h), but lower in the morning and afternoon for MEHP, MEHHP, MEOHP and MECPP. A similar pattern of variability for MEHP was observed in NHANES 1999-00[122]. Meeker et al.[169] also investigated variability by season and found it to be non-significant.

In summary, MEHP, MEHHP and MEOHP are metabolites of DEHP, of which the latter two have a longer urinary half-life. Studies have shown DEHP metabolites to have moderate to high measurement variability in urine.

2.2.4 Human Biomonitoring

There has been debate as to which urinary metabolite is most representative of overall DEHP exposure. In a sample of 62 subjects, Barr et al.[135] found that the ratio of MEHHP/MEOHP was very stable, but the ratios of MEHHP/ MEHP and MEOHP/MEHP had larger standard deviations. The authors suggest that the relative amounts of the secondary metabolites formed during oxidation are similar between individuals, but the extent to which MEHP underdoes oxidation can vary widely. They state that MEHP may be a relevant biomarker for comparisons between studies and when investigating health-related endpoints, but may not be appropriate for comparing to other phthalates, such as MEP, which may be more quickly metabolized and present in lower amounts. They conclude by stating that a combination of urinary levels of the three metabolites MEHP, MEOHP and MEHHP is most representative of overall DEHP exposure. However, a large portion of existing biomonitoring studies only include MEHP (Table 2.3).

Table 2.3 Urinary concentrations (ng/ml) of DEHP metabolites in biomonitoring studies.

Study	MEHP	MEHHP	MEOHP	MECPP	MCMHP	Sample	Method
Koch et al. 2003[163]	10.3	46.8	36.5	NA	NA	85 German subjects aged 7-64 years	Median
Fromme et al. 2007[166]	Women: 4 Men: 5.1	Women: 17.5 Men: 22.7	Women: 14.4 Men: 15.9	Women: 25.6 Men: 24.4	Women: 9.1 Men: 10.2	50 German subjects aged 14-60 years	Median
Blount et al. 2000[171]	3.0	NA	NA	NA	NA	298 subjects aged 20-60 years NHANES III 1988-1994	Geometric mean
Silva et al. 2004[122]	3.2	NA	NA	NA	NA	2,541 subjects aged 6+ years NHANES 1999-2000	Geometric mean
Barr et al. 2003[135]	4.5	35.9	28.3	NA	NA	50 American subjects	Median
Kato et al. 2003[172]	<LOD	17.4	15.6	NA	NA	176 American subjects	Median
Pruess et al. 2005[173]	9.8	47.5	39.7	85.5	36.6	19 German subjects	Median
Wittassek et al. 2007[174]	7.6	21	16.7	26.9	8.7	634 German subjects aged 20-29	Median
Becker et al. 2009[102]	6.4	47.9	37.0	62.5	20.8	599 German children aged 3-14 years	Geometric mean

DEHP, di(2-ethylhexyl) phthalate; LOD, limit of detection; MCMHP, mono[2-(carboxymethyl)hexyl] phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, 2-ethyl-5-hydroxy-hexyl phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, 2-ethyl-5-oxyhexyl phthalate; NA, not available; NHANES, National Health and Nutrition Examination Survey.

Table 2.4 Proportions of DEHP metabolites excreted in urine in biomonitoring studies.

Study	MEHP	MEHHP	MEOHP	MECPP	MCMHP	Method	Sample
Silva et al. 2004[122]	6.6%	29.7%	15.3%	31.8%	10.3%	Fraction of 8 DEHP metabolites measured	2,541 subjects aged 6+ years NHANES 1999-2000
Koch et al. 2005[164]	5.9%	23.3%	15.0%	18.5%	4.2%	Mean fraction of 4.7, 28.7 and 650 µg/kg applied doses	1 adult male

DEHP, di(2-ethylhexyl) phthalate; MCMHP, mono[2-(carboxymethyl)hexyl] phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, 2-ethyl-5-hydroxy-hexyl phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, 2-ethyl-5-oxyhexyl phthalate; NHANES, National Health and Nutrition Examination Survey.

The highest concentration of MEHP was found by Koch et al.[163] in 85 subjects aged 7-64 years. A high concentration of MEHP was also observed by Pruess et al.[173] who also found the highest concentrations of MEHHP and MEOHP, however the study included only 19 subjects. In another German study, Fromme et al.[166] observed levels roughly half of those found by Pruess et al.[173] and Koch et al.[175], and similar urine concentrations among men and women. Results from NHANES were similar, showing relatively low urinary concentrations of MEHP[122]. Two smaller studies of U.S. subjects found somewhat disparate levels, and the levels of MEHP were below the limit of detection. The largest German study[102] observed a relatively low level of BPA in urine. In NHANES 1999-00[122], urine concentration was higher among women than men, and significantly higher in children than adolescents or adults as measured by MEHP.

In summary, it appears best to use concentrations of all three DEHP metabolites as biomarkers for DEHP exposure, although MEHP is suitable when MEHHP and MEOHP are not

available. A range of urinary concentrations for adults and children have been found in NHANES and several German studies.

2.3 Measures of Adiposity

The four different measures of adiposity used in this analysis are BMI, waist circumference, waist-to-hip ratio and skinfold thickness. The utility of a measure of adiposity is often determined by its ability to predict future metabolic and cardiovascular disease. Data from NHANES[176], the Framingham Study[177], and the U.S. Nurses Health Study[178] have been used to demonstrate that waist circumference is most associated with these risks.

However, measures of abdominal obesity and BMI are generally considered complementary in terms of assessing the risk of associated morbidity[179-182]. The use of a waist circumference measure *within* BMI categories is recommended by the Health Canada[183] and the U.S. National Institutes of Health (NIH)[184] and has been found to have more utility than either measure alone. Furthermore, Janssen et al.[185] have shown that while waist circumference may be better than BMI alone, BMI is still significantly predictive of associated morbidity when waist circumference is stratified by high and low risk. It has also been found that BMI and waist circumference independently contribute to the prediction of non-abdominal, abdominal subcutaneous, and visceral fat[186]. With regard to different measures of abdominal obesity, waist circumference has been found to be superior to waist-to-hip ratio and waist-to-height ratio in a Canadian study of cardiovascular disease risk factors[187].

Three different scales of general childhood obesity were used in this thesis. The Centers for Disease Control (CDC) BMI thresholds created in 2000 are based on growth curves derived from

national survey data collected between 1963 and 1994[188]. The International Obesity Task Force (IOTF) developed sex and age specific BMI cut-offs based on international survey data that are meant to be childhood equivalents of the commonly used adult thresholds for overweight and obesity[189] of 25kg/m^2 and 30kg/m^2, respectively. Similar to the CDC method, the WHO used international survey data to create BMI-for-age growth charts meant to represent children raised in ideal conditions and a "gold standard" in growth rates[190]. The IOTF system uses specific BMI values as thresholds, whereas the CDC method designates obesity as being at or above the 95th percentile and overweight as being at or above the 85th percentile and below the 95th. Similar to the CDC method, the WHO system designates obesity as having a BMI more than two standard deviations from the mean and overweight between one and two standard deviations, which approximate to the 97.7th and 84th percentile, respectively. The IOTF and CDC methods are descriptive in that they are based on a specific reference population or populations, while the WHO system is prescriptive in that it was design to represent a general standard of childhood BMI status[191].

3. RESEARCH OBJECTIVES

The purpose of this study was to explore the associations of BPA and phthalate urine concentrations with various measures of adiposity. This current study used urinary BPA, MEHP, MEHHP, and MEOHP concentrations in relation to BMI, waist circumference, waist-to-hip ratio and skinfold measurements.

3.1. The Study Hypothesis

The hypothesis is that urinary BPA and phthalate concentrations are associated with adiposity, and more strongly associated with overall adiposity measures such as BMI than measures of central obesity such as waist circumference and waist-to-hip ratio, given that the former are more sensitive to general increases in adiposity as opposed to greater adiposity in the abdominal region only.

3.2 The Study Objectives

The objectives of the study were:

1. To determine the levels of BPA and phthalate concentrations in urine in the Canadian population; and
2. To investigate the associations of BPA and phthalate urine concentrations with measures of adiposity.

4. SIGNIFIANCE OF THE THIS STUDY

Several studies have previously investigated the association between BPA urine concentration and adiposity; however, this is the first using a nationally representative Canadian sample. A number of previous studies have shown urinary BPA concentration to be associated with BMI, waist circumference and metabolic disorders such as type 2 diabetes, but research has also shown that urinary BPA can vary widely between populations and these results are not generalizable to the Canadian population.

Research on associations between phthalate urine concentration and measures of adiposity has been sparser, but some significant positive associations have been found[192,193]. This current study uses data from a large and nationally representative Canadian sample, and provides estimates of BPA and phthalate urine concentrations, as well as giving insight into their potential relationship with adiposity.

5. STUDY DESIGN

This study used data from the CHMS, a nationally representative survey designed to study the health of Canadians through direct physical measurements such as blood pressure, body weight and physical fitness. The CMHS is a repeated cross-sectional survey conducted by Statistics Canada in partnership with Health Canada and the Public Health Agency of Canada. Two cycles of the CHMS were used in this analysis. Cycle 1 (2007-2009) included 5,604 subjects aged 6 to 79 years with an overall response rate of 51.7%[194] and cycle 2 (2009-2011) included 6,395 subjects aged 3 to 79 years with an overall response rate of 55.5%[195].

The goal of the survey is to create baseline national data, which can be used for assessing the burdens that several conditions place on the Canadian population such as obesity, cardiovascular disease and concentrations of environmental chemicals in blood and urine. The survey also seeks to investigate associations between risk factors and health outcomes, and assess emerging public health issues.

The first 2 cycles of the CHMS used a household interview to collect information on nutrition, smoking and alcohol use, physical activity, current health status and demographic and socioeconomic variables. The questionnaire was designed for computer-assisted personal interviewing, which guides a subject through the interview process and customizes further questions based on answers already given. For direct physical measures, one day to six weeks later, participants visited a mobile examination centre (MEC), where measures of height, weight, waist and hip circumference, skinfolds, physical fitness, blood pressure were taken and blood and urine samples collected. The MEC component of the survey also included a clinical questionnaire, which collected age and sex data that was coded as separate variables from the age and sex data collected from the household questionnaire.

The sampling procedure employed divided Canada into 257 potential collection sites, which were geographic areas with a population of at least 10,000 and were situated so that urban and rural participants travelled a maximum distance of 50 and 100 kilometres, respectively. Potential collection sites did not cross census metropolitan boundaries. These collections sites were then stratified into the five regions of British Columbia, the Prairies (Alberta, Manitoba Saskatchewan and Yellowknife), Ontario, Quebec and the Atlantic provinces (Newfoundland and Labrador, Prince Edwards Island, Nova Scotia and New Brunswick). Based on the constraints of using the MEC (constant relocation), the number of collections sites used was

limited to 15 in cycle 1 and 18 in cycle 2. Sites were distributed to the five regions relative to population size and then selected based on proximity to a census metropolitan area. Cycles 1 and 2 of the CHMS covered approximately 96% of their target Canadian population. Notable exclusions included persons living on Aboriginal reserves, full-time members of the Canadian Forces, institutionalized persons and residents of certain remote regions. A comprehensive description of the design, methodology, data collection and ethical issues of the CHMS can be found elsewhere[194,195]. The survey also included an environmental urine module where the urine concentration of various chemicals was measured, such as pesticides, BPA and pthalates[196].

6. METHODS

The details of the CHMS human biomonitoring, anthropometry and other methods have been documented previously[194,195]. Salient aspects of each of the variables used in this study are summarized below.

6.1 Anthropometry

Physical measures were collected in a customized MEC. Standing height was measured only for participants who were able to stand and a self-reported height was used for all others (n=25). Participants were asked to stand with their feet together, head and back against the wall, head in the Frankfort plane while a measurement was made after a deep inspiration using a fixed stadiometer (Proscale 200, Accurate Technology Inc., Fletcher, NC). Height was measured in duplicate to the nearest 0.01 cm.

Weight was measured on a digital scale (Mettler Toledo scale with Panther Plus digit readout, Mettler Toledo Canada, Mississauga) to the closest 0.1 kg after subjects removed their shoes, any heavy accessories and emptied their pockets. Waist circumference was measured directly on the skin to the closest 0.1 cm using an inelastic tape (Gullick tape measure). In 2007-09, the WHO protocol was used where the measure is taken at the mid-point between the highest point of the iliac crest and the last floating rib, and the measure was taken at the end of a normal expiration. Another popular method was the NIH protocol[197], which uses the highest point of the iliac crest as the landmark. The 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children[198] endorsed the use of the NIH protocol as it uses boney reference points which may be easier for people for self-measure. As such, both protocols were included in the CHMS in 2009-11.

Hip circumference was used to calculate waist-to-hip ratio and was measured at the maximum protuberance of the hips or buttocks region using an inelastic tape (Gullick tape measure) to the nearest 0.1 cm. Skinfolds of the tricep, bicep, subscapular, iliac crest and medial calf were measured twice to the nearest 0.2 mm and the mean was calculated. If the values were more than 0.4 mm apart, a third measurement was conducted and the mean of the two measurements closest in value used, unless they are equidistant in which case a mean was calculated using all three values. The mean of each measure was then summed to create an overall skinfold measure. Skinfold measurements were not taken on participants with a BMI $\geq$30 kg/m^2.

All previously described measures were based on the protocols from the Canadian Standardized Test of Fitness 3rd Edition[199]. Standing height and weight were used to calculate

BMI based on the following equation: BMI = weight(kg)/(height(m))^2; and waist-to-hip ratio was determined by dividing waist circumference by hip circumference.

6.2 Biomonitoring

Urine collection was conducted in the MEC. Approximately 60 ml of urine was collected mid-stream from all respondents in CHMS 2007-09. In CMHS 2009-11, urine was collected using the first-catch method[195].

Urine samples were aliquoted into 20 ml tubes and frozen for shipping and storage. The urine samples were analyzed at the Centre de toxicology du Quebec of L'Institut national de sante publique du Quebec. To prepare the samples for BPA detection, they were first thawed overnight, an internal standard of BPA added, which was then hydrolyzed with β-glucuronidase and derivatized with pentafluorobenzyl bromide. The derivatized products were extracted and analyzed by gas chromatography (Agilent 6890 or 7890 Gas Chromatograph, Agilent, Santa Clara, CA) coupled to mass spectrometry (Quattro Micro-GC, Waters Corporation, Milford, MA). This procedure measures the free and conjugated forms of BPA together, giving the concentration of total BPA in the sample. A blank was used to determine potential contamination which was then subtracted from each final value.

For the measurement of phthalates, urine samples were first spiked with an internal standard and hydrolyzed using β-glucuronidase in an ammonium acetate buffer. The samples were then loaded onto strong anion-exchange solid phase extraction columns and eluted using 2% formic acid in an acetonitrile solution. The resulting sample was evaporated under nitrogen,

reconstituted in 0.2 ml of water and analyzed by ultra-performance liquid chromatography followed by mass spectrometry.

Urine creatinine was measured using the colorimetric end-point Jaffe method[200,201]. A solution of sodium picrate reacts with creatinine to form a red compound, which is detected by its absorbance on an autoanalyzer (Hitachi 917 chemistry autoanalyer, Roche, Basel, Switzerland).

6.3 Exclusion Criteria

Subjects were excluded from analysis if BPA or a DEHP metabolite was not measured. Subjects missing creatinine values were also excluded. Subjects were excluded if they were missing information for BMI, waist circumference or waist-to-hip ratio. A subsample of participants who wore activity monitors to collect direct measurements of physical activity and sedentary behavior was used in this analysis. Participants were excluded from the activity monitor sample if they had less than 4 days of wearing the monitor for 10 hours or more. Participants were excluded from the skinfold sample if they had a BMI ≥30 kg/m^2, or were missing any of the 5 skinfold measures. The CHMS data used in this thesis was accessed through Statistics Canada's Research Data Centre Program which only releases unweighted data if the counts are above 30. As the unweighted number of excluded subjects for certain samples was below 30, the unweighted counts could not be released.

6.4 Bisphenol A Sample

CHMS 2007-09 conducted BPA measurements on all participants in the main sample, although CHMS 2009-11 only performed BPA analysis on participants in the environmental urine subsample. Respondents attending the MEC were randomly assigned to the environmental urine subsample. The goal of the sampling in 2009-11 was to include 250 participants of each sex for the 3-5, 6-11 and 12-19 year-old age groups, and 500 respondents of each sex for the 20-79 year-old age group with a total of 2,500 participants. There were 4,713 adults included in the BPA analysis, 3,619 from CHMS 2007-09 and 1,094 from CHMS 2009-2011. There were 2,705 children included in the BPA analysis, 1,799 from CHMS 2007-09 and 906 from CHMS 2009-11. There were 3,770 adults and 2,136 children included in the BPA activity monitor sample.

The skinfold sample included all those in the original BPA sample who had valid measurements for all five skinfold sites. The skinfold sample excluded those with a BMI $\geq$30 kg/m^2. The adult and child skinfold samples had 3,493 and 2,596 participants, respectively.

6.5 Phthalate Samples

In adults, phthalate measurements were limited to those aged 49 years or under. All respondents aged 6 to 19 years were selected for this measure. Adults aged 20 to 49 years were selected if they lived in a dwelling where no one had previously been selected (e.g., a child from the dwelling), with a desired total of 3,000 participants.

The MEHP adult sample included 2,037 participants. The MEHHP and MEOHP adult samples were identical and included 2,056 participants. A joint sample of 2,037 adult participants was used only for the regression analysis with metabolite ratios. The MEHP child

sample included 2,702 participants. The MEHHP and MEOHP child samples were identical and included 2,719 participants.

6.6 Main Variables

6.6.1 Chemical Concentrations in Urine

The primary independent variables in this study were BPA and phthalate concentration in urine (ng/ml), and BPA and phthalate concentration in urine divided by urine creatinine concentration (µg/g creatinine). For CHMS 2007-09, BPA concentration had to be rounded to two significant digits to match CHMS 2009-11 and reflect measurement error as directed by CHMS documentation. Urine creatinine was converted from mmol/l to g/l by dividing by 8.84 g/mmol and rounding to two significant digits. Those under the limit of detection (LOD) were assigned half the LOD as directed by CHMS documentation (Table 6.3).

6.6.2 Anthropometric Measures and Skinfold Measures

The anthropometric measures used in this analysis were BMI (kg/m^2), waist circumference (cm) and waist-to-hip ratio. Obesity for adults was defined as having a BMI ≥30 kg/m^2. Men and women with a waist circumference ≥102 cm and 88 cm, respectively, were counted as having a high risk waist circumference[202]. Men and women with a waist circumference ≥94 cm and 80 cm, respectively, but <102 cm and 88 cm were counted as having a medium risk waist circumference, and all others as low risk[202]. Men and women with a waist-

to-hip ratio ≥0.85 and 0.90, respectively, were counted as having a high waist-to-hip ratio and all others were classified as low[203].

For children, the BMI norms of the CDC and IOTF protocols were already coded in the survey. The WHO protocol was also used via a macro downloaded from the WHO website. For all three protocols children were divided into obese and non-obese groups. Skinfold measures were only used in linear regression and the following measures were used: tricep, bicep, subscapular, iliac crest, medial calf and a combined skinfold measure.

6.7 Important Covariates

Urine creatinine was an important covariate in this analysis as it is used to correct for urine volume[204]. There is relevant criticism of this method[204] and others have used specific gravity instead; however only urine creatinine was available for this analysis. Using the Akaike criteria, Ye et al.[205] found that using urine creatinine adjustment was superior to no correction at all. They found similar fits for both creatinine adjusted concentrations and using it as a covariate, but it has been suggested that using an adjusted concentration was inferior as it provides a single β coefficient, and associations with the outcome variable cannot be interpreted separately[204]. This does not present as much of a problem in descriptive statistics, so adjusted concentrations were used. Several previous studies have used urine creatinine specifically as a covariate[104,113,117].

Other covariates used were sex, age, education, race, smoking status, physical activity and sedentary behaviour. Some of these such as sex and age have been shown to be associated with urinary BPA concentration[100], while others such as smoking[206], race[207], physical activity[208] and education[207] are more known to be associated with adiposity.

The age and sex recorded on the clinical questionnaire, as opposed to in the household survey, were used. Race was divided into White and non-White. Smoking status had three categories: current smokers, ex-smokers and never smokers. Current smokers included those scored as daily, occasional (former daily) or always occasional smokers. Ex-smokers included former daily and former occasional smokers, and never smokers included those scored as never smokers.

In the BPA sample, adult education was divided into two categories: with and without post-secondary education. In the phthalate sample, this coding was too disproportionate, so adult education was divided into: with and without post-secondary graduation. For children, the highest level of household education was used and was divided into two groups: with and without post-secondary graduation.

Physical activity was divided into active and inactive groups based on an index variable derived from an estimate of daily energy expenditure[209]. Active participants included those scored as active or moderately inactive and inactive ones included those scored as inactive. Children ≤11 years had a different physical activity variable which measured the amount of hours per week they participated in physical activities. Those below the median were counted as being inactive while those at the median or higher were counted as being active.

Study participants were divided into low sedentary and high sedentary categories. Those who reported ≤24 hours of sedentary behaviour in the past three months were counted as low sedentary, while those with 25 hours or more were counted as high sedentary. Children ≤11 years had a different variable which measured daily hours of sedentary behaviour: those lower than the median were counted as low sedentary, while those at or higher than the median were counted as high sedentary.

6.7.1 Activity Monitor Sample

The activity monitor sample included different variables for physical activity and sedentary behaviour. The Canadian Society for Exercise Physiology standard[196] of ≥150 minutes per week of moderate-to-vigorous physical activity (MVPA) in bouts of at least 10 minutes was used for adults. The corresponding standard for children is 60 minutes of MVPA a day[196]. Very few children achieved this standard in the current study, so we used the cut-point of at least 5 days of 60 minutes of MVPA. An important caveat is that subjects have different numbers of valid days of wear, creating an unequal opportunity to achieve this standard, although ~65% of subjects had at least 6 valid days. In linear regression, the probability of accumulating 60 minutes of MVPA 6 days a week was used as described in Colley et al.[210], with the Statistical Analysis System (SAS) code obtained from Statistics Canada. For both adults and children, no established standards of sedentary behaviour exist, and we grouped the participants into two categories: high sedentary (mean or higher) and low sedentary (less than the mean).

6.7.2 Missing Values

Missing values were imputed as the mode for categorical variables and the mean for continuous variables (Table 6.1).

Table 6.1 Imputed values for covariates.

Variable	Imputed value
Race	White
Physical activity	Adults: Inactive Children aged 6-11 years: 2 hours/week Children aged 12-17 years: Inactive
Physical activity linear	1.3 (daily energy expenditure)
Sedentary behaviour	Adults: 45 or more hours in the past 3 months Children aged 6-11 years: 2 hours/day Children aged 12-17 years: 20-24 hours past 3 months
Smoking	Adults: Never smoked
Education	Adults: Post-secondary graduation Children: Post-secondary graduation

6.8 Statistical Analysis

6.8.1 Combining of cycles

Procedures for combining the 2007-09 and 2009-11 cycles of CHMS were drawn from the instruction manual provided by Statistics Canada[211]. The common age range between the cycles was 6-79 years. Combined samples were first stratified by their combined weighting variable (Table 6.2). The cycle specific weight variables for BPA were different, as BPA analysis was performed on the entire main sample in CHMS 2007-09 but only on the environmental urine subsample in CHMS 2009-11. Note that while some of the names of the combined sample and cycle specific variables are the same, these are different variables obtained from different sets of weights. In CHMS 2007-09, the BPA values needed to be adjusted to two significant digits to match CHMS 2009-11, and were done so using SAS code obtained from the instruction manual[211].

Urine creatinine adjusted BPA values for CHMS 2009-11 were then recalculated and rounded to two significant digits. Urine creatinine values were first converted from mmol/l to g/l

by multiplying by 8.82 g/mmol as directed by the manual. For both BPA and phthalates, measurements below the LOD were assigned a value equal to half the limit of detection (Table 6.3).

Table 6.2 Cycle-specific and combined sample weighting variables in CHMS 2007-11.

Sample	Cycle specific weight variable	Combined sample weight variable
Bisphenol A	2007-09: WGT_FULL 2009-11: WGT_EU	WGT_EU
Phthalates	2007-09: WGT_PHTH 2009-11: WGT_EU	WGT_PHTH

CHMS, Canadian Health Measures Survey.

Table 6.3 Limits of detection for bisphenol A and phthalates used for imputation in CMHS 2007-11.

Chemical	CHMS 2007-09	CHMS 2009-11
Bisphenol A	0.2 ng/ml	0.2 ng/ml
MEHP	0.2 ng/ml	0.41 ng/ml
MEOHP	0.2 ng/ml	0.12 ng/ml
MEHHP	0.4 ng/ml	0.38 ng/ml

CHMS, Canadian Health Measures Survey; MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

6.8.2 Descriptive Statistics

The geometric mean of urinary BPA concentration was calculated for each cycle and stratified by the variables described above. A single combined sample was used for the phthalates explored as they had smaller sample sizes and more variability between cycles. Only sex and age were included in the phthalate tables, while the BPA tables included sex, age, obesity, elevated waist circumference, elevated waist-to-hip ratio, education, physical activity, sedentary behaviour and smoking. All descriptive analyses were repeated for the creatinine adjusted BPA and phthalate values. The numbers of participants shown in the descriptive tables

were calculated using standardized weights, and standardized weights were calculated by dividing the regular weight variable by the mean of the weights in the sample.

6.8.3 Regression Analysis

Basic linear regression was used to determine whether BPA or phthalates differed significantly by any of the characteristics explored in the descriptive tables. Log-transformed BPA or phthalate concentrations, were modeled as the dependent variable, and the characteristic of interest as the only independent variable. The p-value for the total sample represents the difference in geometric means between the 2007-09 and 2009-11 cycles.

Multiple logistic regression was used to examine the association between urinary BPA and phthalate concentrations and general obesity, an elevated waist circumference and an elevated waist-to-hip ratio. Participants were divided into quartiles based on their BPA concentration, the second, third and fourth quartiles were compared to the first quartile and statistical significance was tested accordingly. The estimates were first adjusted by sex, age and urine creatinine, while the fully adjusted adult model also included race, education, smoking status, physical activity and sedentary behaviour. The fully adjusted child model also included race, highest level of household education, physical activity and sedentary behaviour. As CHMS 2007-11 had different variables of physical activity and sedentary behaviour in the 6-11 and 12-17 year-old age groups, the fully adjusted model for children was stratified by age.

Multiple linear regression analysis was conducted for continuous outcomes. BPA and phthalate concentrations were log-transformed to create a more normal distribution. Linear regression included an indicator variable for BPA or phthalate quartile as the primary

independent variable. The estimates were first adjusted by sex, age and urine creatinine, while the fully adjusted adult model also included race, education, smoking status, physical activity and sedentary behaviour. Similar to the logistic analysis, the fully adjusted model for children was stratified by age, and also included race, highest level of household education, physical activity and sedentary behaviour. All regression analyses were conducted using the bootstrap technique with 500 replicates to control for the sampling structure of the CHMS and achieve proper variance.

6.8.4 Activity Monitor Sample

For adults, the amount MVPA in bouts of 10 minutes was summed up over the number of valid days per participant. This value was divided by the number of days, and multiplied by seven to create a theoretical weekly total based on the number of days they had. The normal total was used for participants with 7 days of wear. For children, the number of valid days where they achieved 60 minutes or more of MVPA was summed. As no standard existed for sedentary behaviour that required calculating a theoretical weekly total, the average minutes of sedentary time over the number of days for a given participant was used. All analyses were conducted with SAS 9.3 or 9.4 with $\alpha = 0.05$.

7. BISPHENOL A

7.1 Results

7.1.1 Adult Sample

The geometric means of urinary BPA were 1.13 ng/ml (95% CI: 1.07, 1.20) in 2007-09 (Table 7.1) and 1.20 ng/ml (95% CI: 1.09, 1.32) in 2009-11 (Table 7.2). In both cycles the mean urinary BPA concentration was greater among men than women, though this difference was only significant in 2007-09. The 18-29 year-old age group had the highest urinary BPA level in 2007-09 and the 30-39 year-old age group had the highest level in 2009-11. The mean BPA concentration generally decreased with age in both cycles and the 18-29 year-old age group in 2007-09 had significantly greater urinary BPA levels than most other age groups.

Urinary BPA was higher among overweight or obese subjects than normal or underweight subjects in both cycles, and these differences were more pronounced in 2007-09. BPA urine concentration was similar among waist circumference and waist-to-hip ratio categories in both cycles. Urinary BPA level increased with smoking frequency but the association was non-significant.

The urine creatinine adjusted geometric means were 1.37 µg/g (95% CI: 1.30, 1.45) in 2007-09 (Table 7.3) and 1.16 µg/g (95% CI: 1.08, 1.25) for 2009-11 (Table 7.4). Women had significantly higher levels in both cycles, but associations with age and smoking were attenuated. There was a significant difference in the creatinine adjustment means between cycles.

Table 7.1 Geometric mean (95% CI) of urinary bisphenol A concentration in Canadians aged 18 years or older in CHMS 2007-09 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (ng/ml)	P-value
Total	3,619	1.13 (1.07, 1.20)	0.3460**
Sex			
Men	1,803 (49.8)	1.29 (1.23, 1.36)	Reference
Women	1,816 (50.2)	0.99 (0.90, 1.10)	<0.0001
Age (years)			
18-29	754 (20.8)	1.48 (1.21, 1.82)	Reference
30-39	666 (18.4)	1.28 (1.15, 1.43)	0.2578
40-49	840 (23.2)	0.97 (0.89, 1.06)	0.0002
50-59	621 (17.2)	1.15 (1.03, 1.27)	0.0070
60-69	476 (13.1)	0.95 (0.83, 1.09)	0.0017
70+	262 (7.2)	0.81 (0.66, 0.99)	<0.0001
BMI category			
Under or normal weight	1,419 (39.2)	1.04 (0.92, 1.18)	Reference
Overweight	1,343 (37.1)	1.20 (1.12, 1.28)	0.0690
Obese	858 (23.7)	1.18 (1.05, 1.34)	0.1047
Waist circumference			
Low	1,533 (42.4)	1.18 (1.04, 1.33)	Reference
Medium	810 (22.4)	1.06 (0.96, 1.18)	0.3382
High	1,276 (35.3)	1.12 (1.03, 1.19)	0.4899
Waist-to-hip ratio			
Low	1,711 (47.3)	1.16 (1.06, 1.26)	Reference
High	1,908 (52.7)	1.11 (1.03, 1.19)	0.4009
Education			
Low	1,115 (30.2)	1.15 (1.05, 1.27)	0.6084
High	2,504 (69.2)	1.12 (1.05, 1.20)	Reference
Physical activity			
Active	744 (20.5)	1.09 (1.02, 1.16)	Reference
Moderately inactive	903 (25.0)	1.21 (1.09, 1.35)	0.1325
Inactive	1,972 (54.5)	1.11 (1.03, 1.19)	0.7460
Sedentary behaviour			
Low	1,604 (44.3)	1.17 (1.09, 1.26)	Reference
High	2,015 (55.7)	1.10 (1.02, 1.18)	0.1118
Race			
White	2,990 (82.6)	1.17 (1.09, 1.25)	0.0706
Non-white	629 (17.4)	0.97 (0.80, 1.17)	Reference
Smoking			
Daily or occasional	780 (21.5)	1.26 (1.06, 1.49)	0.0746
Former daily or occasional	1,078 (29.8)	1.15 (1.07, 1.24)	0.0728
Never	1,761 (48.7)	1.07 (1.03, 1.14)	Reference

BMI, Body mass index; CHMS, Canadian Health Measures Survey; CI, confidence interval.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.2 Geometric mean (95% CI) of urinary bisphenol A concentration in Canadians aged 18 years or older in CHMS 2009-11 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (ng/ml)	P-value
Total	1,094	1.20 (1.09, 1.32)	0.3460**
Sex			
Men	538 (49.2)	1.25 (1.08, 1.45)	Reference
Women	556 (50.8)	1.15 (1.01, 1.32)	0.4552
Age (years)			
18-29	241 (22.1)	1.25 (0.93, 1.68)	Reference
30-39	180 (16.4)	1.39 (1.13, 1.71)	0.5948
40-49	213 (19.5)	1.19 (0.88, 1.61)	0.8394
50-59	223 (20.4)	1.20 (0.90, 1.59)	0.8643
60-69	155 (14.1)	1.02 (0.79, 1.31)	0.2486
70+	82 (7.5)	1.08 (0.82, 1.43)	0.5450
BMI category			
Under or normal weight	436 (39.8)	1.10 (0.91, 1.34)	Reference
Overweight	372 (34.0)	1.21 (1.00, 1.47)	0.4745
Obese	286 (26.1)	1.34 (1.06, 1.69)	0.2792
Waist circumference			
Low	485 (44.4)	1.18 (0.96, 1.44)	Reference
Medium	232 (21.2)	1.22 (1.05, 1.43)	0.7986
High	377 (34.5)	1.22 (1.01, 1.47)	0.8481
Waist-to-hip ratio			
Low	441 (40.3)	1.25 (1.02, 1.54)	Reference
High	653 (59.7)	1.22 (1.01, 1.33)	0.6296
Education			
Low	293 (26.8)	1.14 (0.96, 1.36)	0.5280
High	801 (73.2)	1.22 (1.09, 1.37)	Reference
Physical activity			
Active	232 (21.2)	1.42 (1.15, 1.76)	Reference
Moderately inactive	261 (23.8)	1.14 (0.89, 1.46)	0.2463
Inactive	601 (55.0)	1.15 (1.01, 1.31)	0.0883
Sedentary behaviour			
Low	423 (38.7)	1.18 (0.99, 1.39)	Reference
High	671 (61.3)	1.22 (1.05, 1.41)	0.7904
Race			
White	896 (81.9)	1.16 (1.05, 1.29)	Reference
Non-white	198 (18.0)	1.39 (1.06, 1.83)	0.3014
Smoking			
Daily or occasional	246 (22.5)	1.44 (1.12, 1.86)	0.1028
Former daily or occasional	268 (24.5)	1.17 (1.01, 1.35)	0.7480
Never	580 (53.0)	1.13 (0.97, 1.31)	Reference

BMI, Body mass index; CHMS, Canadian Health Measures Survey; CI, confidence interval.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.3 Geometric mean (95% CI) of urinary bisphenol A concentration adjusted for urine creatinine in Canadians aged 18 years or older in CHMS 2007-09 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (µg/g)	P-value
Total	3,619	1.37 (1.30, 1.45)	0.0013**
Sex			
Men	1,803 (49.8)	1.24 (1.16, 1.33)	Reference
Women	1,816 (50.2)	1.51 (1.42, 1.62)	<0.0001
Age (years)			
18-29	754 (20.8)	1.45 (1.31, 1.61)	Reference
30-39	666 (18.4)	1.52 (1.36, 1.71)	0.6045
40-49	840 (23.2)	1.22 (1.09, 1.36)	0.0264
50-59	621 (17.2)	1.49 (1.29, 1.71)	0.6745
60-69	476 (13.1)	1.29 (1.11, 1.47)	0.2093
70+	262 (7.2)	1.23 (1.08, 1.40)	0.0016
BMI category			
Under or normal weight	1,418 (39.2)	1.37 (1.23, 1.53)	Reference
Overweight	1,343 (37.1)	1.35 (1.27, 1.44)	0.8016
Obese	858 (23.7)	1.42 (1.33, 1.49)	0.6714
Waist circumference			
Low	1,533 (42.4)	1.38 (1.25, 1.52)	Reference
Medium	810 (22.4)	1.33 (1.19, 1.47)	0.6390
High	1,276 (35.3)	1.40 (1.30, 1.49)	0.8058
Waist-to-hip ratio			
Low	1,711 (47.3)	1.42 (1.31, 1.53)	Reference
High	1,908 (52.7)	1.33 (1.24, 1.43)	0.1864
Education			
Low	1,115 (30.8)	1.26 (1.10, 1.45)	0.7059
High	2,504 (69.2)	1.38 (1.28, 1.47)	Reference
Physical activity			
Active	744 (20.5)	1.35 (1.21, 1.50)	Reference
Moderately inactive	903 (25.0)	1.42 (1.30, 1.54)	0.4675
Inactive	1,972 (54.5)	1.36 (1.28, 1.45)	0.8490
Sedentary behaviour			
Low	1,604 (44.3)	1.43 (1.34, 1.53)	Reference
High	2,015 (55.7)	1.33 (1.24, 1.43)	0.0649
Race			
White	2,990 (82.6)	1.40 (1.32, 1.50)	Reference
Non-white	629 (17.4)	1.23 (1.06, 1.43)	0.0892
Smoking			
Daily or occasional	780 (21.5)	1.41 (1.24, 1.61)	0.4306
Former daily or occasional	1,078 (29.8)	1.41 (1.30, 1.54)	0.2431
Never	1,761 (48.7)	1.33 (1.24, 1.43)	Reference

BMI, Body mass index; CHMS, Canadian Health Measures Survey; CI, confidence interval.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.4 Geometric mean (95% CI) of urinary bisphenol A concentration adjusted for urine creatinine in Canadians aged 18 years or older in CHMS 2009-11 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (µg/g)	P-value
Total	1,094	1.16 (1.08, 1.25)	0.0013**
Sex			
Men	538 (49.2)	1.03 (0.92, 1.2)	Reference
Women	556 (50.8)	1.31 (1.17, 1.46)	0.0050
Age (years)			
18-29	241 (22.1)	0.97 (0.76, 1.25)	Reference
30-39	180 (16.4)	1.28 (1.02, 1.61)	0.1042
40-49	213 (19.5)	1.05 (0.79, 1.41)	0.7194
50-59	223 (20.4)	1.38 (1.08, 1.76)	0.0410
60-69	155 (14.1)	1.19 (0.94, 1.51)	0.2610
70+	82 (7.5)	1.22 (0.97, 1.54)	0.2165
BMI category			
Under or normal weight	436 (39.8)	1.13 (0.97, 1.34)	Reference
Overweight	372 (34.0)	1.12 (0.92, 1.35)	0.8858
Obese	286 (26.1)	1.26 (1.13, 1.42)	0.3498
Waist circumference			
Low	485 (44.4)	1.13 (0.97, 1.31)	Reference
Med	232 (21.2)	1.20 (0.95, 1.51)	0.6887
High	377 (34.4)	1.18 (1.08, 1.29)	0.6563
Waist-to-hip ratio			
Low	441 (40.3)	1.14 (0.99, 1.31)	Reference
High	653 (59.7)	1.18 (1.08, 1.28)	0.6925
Education			
Low	293 (26.8)	1.18 (1.02, 1.37)	0.8594
High	801 (73.2)	1.16 (1.03, 1.30)	Reference
Physical activity			
Active	232 (21.2)	1.31 (1.15, 1.50)	0.0081
Moderately inactive	261 (23.8)	1.25 (1.03, 1.53)	0.6864
Inactive	601 (55.0)	1.07 (0.97, 1.19)	Reference
Sedentary behaviour			
Low	423 (38.7)	1.11 (0.99, 1.26)	Reference
High	671 (61.3)	1.19 (1.06, 1.34)	0.4739
Race			
White	896 (81.9)	1.16 (1.07, 1.26)	Reference
Non-white	198 (18.1)	1.17 (0.94, 1.46)	0.9397
Smoking			
Daily or occasional	246 (22.5)	1.26 (1.06, 1.51)	0.1360
Former daily or occasional	268 (24.5)	1.37 (1.20, 1.57)	0.0049
Never	570 (53.0)	1.04 (0.91, 1.19)	Reference

BMI, Body mass index; CHMS, Canadian Health Measures Survey; CI, confidence interval.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.5 Odds ratios (95% CI) for the 2nd, 3rd and 4th bisphenol A quartiles as compared to the 1st quartile for various adiposity measures.

	Model 1*		Model 2**	
	Odds Ratio (95% CI)	P-value	Odds Ratio (95% CI)	P-value
BMI ≥ 30kg/m^2				
Quartile 4	1.32 (0.82, 2.12)	0.2618	1.37 (0.87, 2.17)	0.2130
Quartile 3	1.02 (0.67, 1.54)	0.9402	1.01 (0.70, 1.73)	0.8150
Quartile 2	0.96 (0.64, 1.44)	0.8531	0.96 (0.60, 1.53)	0.7629
Quartile 1	1.00	Reference	1.00	Reference
Elevated waist circumference				
Quartile 4	1.01 (0.64, 1.59)	0.9656	1.07 (0.70, 1.65)	0.7482
Quartile 3	0.85 (0.53, 1.36)	0.4954	0.91 (0.56, 1.47)	0.7008
Quartile 2	0.93 (0.62, 1.42)	0.7459	0.95 (0.61, 1.48)	0.8194
Quartile 1	1.00	Reference	1.00	Reference
Elevated waist-to-hip ratio				
Quartile 4	1.00 (0.67, 1.50)	0.9872	1.00 (0.68, 1.47)	0.9869
Quartile 3	1.45 (0.88, 2.38)	0.1438	1.44 (0.88, 2.38)	0.1487
Quartile 2	0.88 (0.57, 1.36)	0.5693	0.84 (0.53, 1.31)	0.4397
Quartile 1	1.00	Reference	1.00	Reference

BMI, Body mass index; CI, confidence interval.
* Adjusted for urine creatinine, age and sex.
** Adjusted for race, education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Logistic regression analysis showed that obesity (BMI ≥30 m/kg^2) was not significantly associated with urinary BPA level (Table 7.5). The odds ratio for obesity in the 4th BPA quartile versus the 1st quartile was 1.37 (95% CI: 0.87, 2.17) in the fully adjusted model. Odds ratios decreased from the 4th to the 2nd quartile in all models and values for the 2nd and 3rd quartiles were close to null. No association was apparent when modeling elevated waist circumference as the dependent variable (Table 7.5).

Modeling waist-to-hip ratio did not produce any significant associations and the odds ratios for the 2nd and 4th quartile comparisons were close to the null (Table 7.5). Age, and white race were significant in the models for all three measures, and female sex was also significant in

the elevated waist circumference and waist-to-hip ratio models. Both measures of abdominal obesity showed weaker positive associations with urinary BPA than did general obesity.

Log-transformed BPA was significantly positively associated with all three measures of adiposity (Table 7.6). Similar to the logistic regression models, the most significant covariates were age and white race, but smoking, physical activity and sedentary behaviour were also significant in the linear model. Modeling BPA quartile linearly produced similar positive associations although did not achieve significance with waist-to-hip ratio (Table 7.7). Similar to the logistic results, female sex was significant in the elevated waist circumference and waist-to-hip ratio models.

Log-transformed BPA was also significantly associated with the iliac crest and sum of all skinfold measurements in linear regression (Table 7.8). Modeling triceps skinfold as the dependent variable was just below significance (Table 7.8).

Table 7.6 Log-transformed urinary bisphenol A concentration modeled linearly against measures of adiposity.

	Model 1*		Model 2**	
Dependent variable	β coefficient	P-value	β coefficient	P-value
Body mass index	0.3551	0.0023	0.3835	0.0005
Waist circumference	1.0476	0.0017	1.1219	0.0006
Waist-to-hip ratio	0.0054	0.0396	0.0056	0.0318

* Adjusted for urine creatinine, age and sex.
** Adjusted for race education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table 7.7 Urinary bisphenol A quartile modeled linearly against measures of adiposity.

Dependent variable	β coefficient*	P-value
Body mass index	0.3716	0.0027
Waist circumference	0.9552	0.0143
Waist-to-hip ratio	0.0043	0.1451

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

Table 7.8 Log-transformed urinary bisphenol A concentration modeled linearly against skinfold measurements.

Dependent variable	β coefficient*	P-value
Triceps	0.2589	0.0541
Biceps	0.1485	0.1040
Subscapular	0.3209	0.1343
Iliac crest	0.0667	0.0037
Medial calf	0.1338	0.2686
Sum of skinfolds	1.5285	0.0183

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

The activity monitor subsample generally produced lower odds ratios than the main sample (Table 7.9), where the odds of obesity in the 4th quartile as compared to the 1st were 1.14 (95% CI: 0.63, 2.07). Odds ratios for waist circumference were at or below the null. Waist-to-hip ratio showed a modest positive association in the 2nd quartile comparison, although the other odds ratios were close to the null. Generally the results of the activity monitor subsample were similar to the main sample but showed weaker associations.

Table 7.9 Activity monitor sample odds ratios (95% CI) for various adiposity measures in the 2nd, 3rd and 4th bisphenol A quartiles as compared to the 1st quartile.

	Odds Ratio (95% CI)*	P-value
BMI $\geq$ 30kg/m^2		
Quartile 4	1.14 (0.63, 2.07)	0.6634
Quartile 3	0.94 (0.54, 1.63)	0.8130
Quartile 2	1.03 (0.56, 1.89)	0.9304
Quartile 1	1.00	Reference
Elevated waist circumference		
Quartile 4	0.90 (0.53, 1.51)	0.6819
Quartile 3	0.69 (0.38, 1.24)	0.2170
Quartile 2	1.00 (0.57, 1.76)	0.9963
Quartile 1	1.00	Reference
Elevated waist-to-hip ratio		
Quartile 4	0.99 (0.65, 1.51)	0.9657
Quartile 3	1.35 (0.80, 2.28)	0.2566
Quartile 2	0.93 (0.57, 1.52)	0.7816
Quartile 1	1.00	Reference

BMI, Body mass index; CI, confidence interval.

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

7.1.2 Child Sample

The geometric means of urinary BPA concentration among subjects aged 6-17 years were 1.34 ng/ml (95% CI: 1.20, 1.49) in 2007-09 and 1.33 ng/ml (95% CI: 1.15, 1.54) in 2009-11 (Tables 7.10-7.11). Urinary BPA was higher in girls in 2007-09, and boys in 2009-11. BPA urine concentration did not significantly vary across any of the characteristics explored, and even weak associations were not consistent between cycles. After adjustment for urine creatinine the geometric means were 1.56 µg/g (95% CI: 1.42, 1.72) and 1.21 µg/g (95% CI: 1.05, 1.39) for CHMS 2007-09 and 2009-11 (Tables 7.12-7.13), respectively, which were significantly different. Significant negative associations with age in both cycles were seen after creatinine adjustment.

Table 7.10 Geometric mean (95% CI) of urinary bisphenol A concentration in Canadians aged 6-17 years in CHMS 2007-09 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (ng/ml)	P-value
Total	1,799	1.34 (1.20, 1.49)	0.8078**
Sex			
Boys	952 (52.9)	1.30 (1.13, 1.51)	Reference
Girls	847 (47.1)	1.38 (1.24, 1.54)	0.4366
Age (years)			
6-11	833 (46.3)	1.31 (1.18, 1.44)	Reference
12-17	966 (53.7)	1.37 (1.19, 1.57)	0.3434
CDC BMI category			
Under or normal weight	1,333 (74.1)	1.34 (1.21, 1.49)	Reference
Overweight	230 (12.8)	1.31 (1.08, 1.59)	0.7349
Obese	236 (13.1)	1.34 (1.01, 1.77)	0.9602
IOTF BMI category			
Normal weight	1,347 (74.9)	1.34 (1.21, 1.49)	Reference
Overweight	295 (16.4)	1.28 (1.10, 1.48)	0.3859
Obese	158 (8.8)	1.43 (0.98, 2.10)	0.6995
WHO BMI category			
Under or normal weight	1,241 (69.0)	1.34 (1.21, 1.49)	Reference
Overweight	309 (17.1)	1.33 (1.07, 1.66)	0.9359
Obese	249 (13.8)	1.33 (1.04, 1.70)	0.9117
Race			
White	1,401 (77.9)	1.36 (1.24, 1.49)	Reference
Non-white	398 (22.1)	1.27 (0.96, 1.68)	0.6076
Physical activity aged 12+ years			
Active	740 (76.6)	1.40 (1.15, 1.71)	Reference
Inactive	226 (23.4)	1.28 (0.94, 1.74)	0.6597
Physical activity aged 6-11 years			
Active	278 (33.4)	1.20 (1.06, 1.35)	Reference
Inactive	555 (66.6)	1.36 (1.23, 1.50)	0.0077
Sedentary behaviour aged 12+ years			
High	412 (42.6)	1.40 (1.24, 1.59)	0.6721
Low	555 (57.4)	1.35 (1.13, 1.62)	Reference
Sedentary behaviour aged 6-11 years			
High	286 (34.3)	1.32 (1.16, 1.49)	0.8681
Low	547 (65.7)	1.30 (1.14, 1.48)	Reference

BMI, Body mass index; CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task Force; WHO, World Health Organization.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.11 Geometric mean (95% CI) of urinary bisphenol A concentration in Canadians aged 6-17 years in CHMS 2009-11 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean	P-value
Total	906	1.31 (1.12, 1.53)	0.8078**
Sex			
Boys	476 (52.5)	1.37 (1.10, 1.70)	Reference
Girls	430 (47.5)	1.24 (1.05, 1.46)	0.3900
Age (years)			
6-11	415 (45.8)	1.35 (1.11, 1.64)	Reference
12-17	491 (54.2)	1.27 (1.05, 1.55)	0.6102
CDC BMI category			
Under or normal weight	665 (73.4)	1.34 (1.14, 1.56)	
Overweight	135 (14.9)	1.31 (0.89, 1.93)	0.9235
Obese	106 (11.7)	1.14 (0.87, 1.51)	0.2270
IOTF BMI category			
Normal weight	670 (73.9)	1.34 (1.14, 1.57)	Reference
Overweight	164 (18.1)	1.22 (0.88, 1.70)	0.5546
Obese	72 (8.0)	1.22 (0.77, 1.92)	0.6276
WHO BMI category			
Under or normal weight	621 (68.5)	1.33 (1.12, 1.57)	Reference
Overweight	180 (19.9)	1.35 (0.99, 1.83)	0.9101
Obese	105 (11.6)	1.14 (0.86, 1.50)	0.2576
Race			
White	654 (72.1)	1.34 (1.14, 1.57)	Reference
Non-white	252 (27.9)	1.24 (0.90, 1.71)	0.6340
Physical activity aged 12+ years			
Active	481 (53.0)	1.28 (1.02, 1.61)	Reference
Inactive	425 (47.0)	1.24 (0.95, 1.63)	0.8388
Physical activity aged 6-11 years			
Active	367 (74.6)	1.51 (1.06, 2.16)	Reference
Inactive	125 (25.4)	1.30 (1.02, 1.64)	0.4625
Sedentary behaviour aged 12+ years			
High	418 (46.2)	1.32 (0.97, 1.81)	0.6517
Low	488 (53.8)	1.21 (0.97, 1.51)	Reference
Sedentary behaviour aged 6-11 years			
High	283 (57.7)	1.17 (0.81, 1.70)	0.2800
Low	208 (42.3)	1.45 (1.17, 1.79)	Reference

BMI, Body mass index; CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task Force; WHO, World Health Organization.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.12 Geometric mean (95% CI) of urinary bisphenol A concentration adjusted for urine creatinine in Canadians aged 6-17 years in CHMS 2007-09 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (µg/g)	P-value
Total	1,799	1.56 (1.42, 1.72)	0.0050**
Sex			
Boys	952 (52.9)	1.47 (1.29, 1.66)	Reference
Girls	847 (47.1)	1.67 (1.53, 1.83)	0.0193
Age (years)			
6-11	833 (46.3)	2.00 (1.82, 2.21)	Reference
12-17	966 (53.7)	1.26 (1.13, 1.41)	<0.0001
CDC BMI category			
Under or normal weight	1,333 (74.1)	1.57 (1.42, 1.75)	Reference
Overweight	230 (12.8)	1.57 (1.29, 1.91)	0.9915
Obese	236 (13.1)	1.47 (1.15, 1.89)	0.6237
IOTF BMI category			
Normal weight	1,347 (74.9)	1.58 (1.43, 1.76)	Reference
Overweight	295 (16.4)	1.50 (1.27, 1.76)	0.3764
Obese	158 (8.8)	1.49 (1.01, 2.18)	0.7291
WHO BMI category			
Under or normal weight	1,241 (69.0)	1.56 (1.40, 1.73)	Reference
Overweight	309 (17.1)	1.64 (1.37, 1.96)	0.4931
Obese	249 (13.8)	1.48 (1.16, 1.89)	0.7051
Race			
White	1,401 (77.9)	1.58 (1.46, 1.72)	Reference
Non-white	398 (22.1)	1.49 (1.21, 1.84)	0.5454
Physical activity aged 12+ years			
Active	740 (76.6)	1.29 (1.09, 1.52)	Reference
Inactive	226 (23.4)	1.17 (1.00, 1.38)	0.5541
Physical activity aged 6-11 years			
Active	278 (33.4)	1.88 (1.66, 2.12)	Reference
Inactive	555 (66.6)	2.07 (1.86, 2.29)	0.0916
Sedentary behaviour aged 12+ years			
High	412 (42.6)	1.20 (1.05, 1.37)	0.3520
Low	555 (57.4)	1.31 (1.13, 1.52)	Reference
Sedentary behaviour aged 6-11 years			
High	286 (34.3)	2.00 (1.79, 2.22)	0.9635
Low	547 (65.7)	2.00 (1.75, 2.30)	Reference

BMI, Body mass index; CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task Force; WHO, World Health Organization.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 7.13 Geometric mean (95% CI) of urinary bisphenol A concentration adjusted for urine creatinine in Canadians aged 6-17 years in CHMS 2009-11 stratified by various characteristics.

Characteristic	N (%)*	Geometric Mean (µg/g)	P-value
Total	906	1.20 (1.04, 1.40)	0.0050**
Sex			
Boys	476 (52.5)	1.20 (0.98, 1.46)	Reference
Girls	430 (47.5)	1.21 (1.04, 1.41)	0.8923
Age (years)			
6-11	415 (45.8)	1.56 (1.29, 1.87)	Reference
12-17	491 (54.2)	0.97 (0.82, 1.15)	<0.0001
CDC BMI category			
Under or normal weight	665 (73.4)	1.21 (1.05, 1.39)	Reference
Overweight	135 (14.9)	1.21 (0.84, 1.74)	0.9897
Obese	106 (11.7)	1.16 (0.86, 1.57)	0.7437
IOTF BMI category			
Normal weight	670 (73.9)	1.22 (1.06, 1.40)	Reference
Overweight	164 (18.1)	1.17 (0.87, 1.58)	0.7774
Obese	72.3 (8.0)	1.16 (0.71, 1.92)	0.8279
WHO BMI category			
Under or normal weight	621 (68.5)	1.20 (1.04, 1.40)	Reference
Overweight	180 (19.9)	1.23 (0.93, 1.64)	0.8417
Obese	105 (11.6)	1.16 (0.86, 1.58)	0.8063
Race			
White	654 (72.1)	1.22 (1.06, 1.41)	Reference
Non-white	252 (27.9)	1.16 (0.89, 1.50)	0.6223
Physical activity aged 12+ years			
Active	481 (53.0)	0.96 (0.80, 1.15)	Reference
Inactive	425 (47.0)	1.00 (0.81, 1.24)	0.6678
Physical activity aged 6-11 years			
Active	367 (74.6)	1.73 (1.26, 2.36)	Reference
Inactive	125 (25.4)	1.50 (1.19, 1.88)	0.4430
Sedentary behaviour aged 12+ years			
High	418 (46.2)	0.93 (0.72, 1.20)	0.5315
Low	488 (53.8)	1.02 (0.86, 1.21)	Reference
Sedentary behaviour aged 6-11 years			
High	283 (57.7)	1.32 (0.94, 1.84)	0.1800
Low	208 (42.3)	1.69 (1.38, 2.07)	Reference

BMI, Body mass index; CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task Force; WHO, World Health Organization.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

In CMHS 2007-11, the odds of obesity by the CDC standard were 0.95 (95% CI: 0.55, 1.62) for those in the 4th quartile as compared to the 1st quartile (Table 7.14). The IOTF and WHO methods produced similar odds ratios and showed significant negative associations, while the CDC standard yielded weaker associations and did not reach significance. (Tables 7.15-7.16).

Overall the results varied more between the simple model and the age-stratified fully adjusted models than between the different BMI classification methods (Tables 7.14-7.17). Negative associations were stronger in the 12-17 year-old age group. There was little evidence of a trend where the comparison with the smallest difference in urine concentration, 2nd quartile to 1st quartile, often resulted in the highest odds ratio. The negative associations were generally stronger in girls, especially in the 6-11 year-old age group.

Table 7.14 Odds ratios (95% CI) for obesity by the CDC protocol in the 2nd, 3rd and 4th bisphenol A quartiles as compared to the 1st quartile.

	Odds Ratio (95% CI)	P-value
Total*		
Quartile 4	0.95 (0.55, 1.62)	0.8373
Quartile 3	0.57 (0.29, 1.09)	0.0890
Quartile 2	0.63 (0.40, 1.00)	0.0509
Quartile 1	1.00	Reference
6-11 years**		
Quartile 4	1.02 (0.40, 2.60)	0.9595
Quartile 3	0.55 (0.18, 1.68)	0.2967
Quartile 2	0.91 (0.39, 2.11)	0.8224
Quartile 1	1.00	Reference
12-17 years**		
Quartile 4	0.90 (0.41, 1.95)	0.7795
Quartile 3	0.53 (0.26, 1.10)	0.0867
Quartile 2	0.46 (0.20, 1.03)	0.0601
Quartile 1	1.00	Reference

CDC, Centers for Disease Control; CI, confidence interval.* Adjusted for age, sex and urine creatinine.
** Adjusted for additional variables including physical activity, sedentary behaviour, race and highest level of household education.

Table 7.15 Odds ratios (95% CI) for obesity by the IOTF protocol in the 2nd, 3rd and 4th bisphenol A quartiles as compared to the 1st quartile.

	Odds Ratio (95% CI)	P-value
Total		
Quartile 4	0.77 (0.51, 1.17)	0.2175
Quartile 3	0.59 (0.37, 0.95)	0.0284
Quartile 2	0.71 (0.49, 1.03)	0.0688
Quartile 1	1.00	Reference
6-11 years**		
Quartile 4	0.97 (0.42, 2.25)	0.9364
Quartile 3	0.66 (0.32, 1.40)	0.2813
Quartile 2	0.74 (0.50, 1.11)	0.1438
Quartile 1	1.00	Reference
12-17 years**		
Quartile 4	0.68 (0.39, 1.18)	0.1723
Quartile 3	0.55 (0.33, 0.92)	0.0220
Quartile 2	0.74 (0.50, 1.11)	0.1438
Quartile 1	1.00	Reference

CI, confidence interval; IOTF, International Obesity Task Force.

* Adjusted for age, sex and urine creatinine.

** Adjusted for additional variables including physical activity, sedentary behaviour, race and highest level of household education.

Table 7.16 Odds ratios (95% CI) for obesity by the WHO protocol in the 2nd, 3rd and 4th bisphenol A quartiles as compared to the 1st quartile.

	Odds Ratio (95% CI)	P-value
Total		
Quartile 4	1.13 (0.65, 1.96)	0.6741
Quartile 3	0.61 (0.32, 1.15)	0.1279
Quartile 2	0.62 (0.41, 0.96)	0.0305
Quartile 1	1.00	Reference
6-11 years**		
Quartile 4	0.93 (0.37, 2.36)	0.8828
Quartile 3	0.65 (0.24, 1.72)	0.3832
Quartile 2	0.88 (0.38, 2.03)	0.7638
Quartile 1	1.00	Reference
12-17 years**		
Quartile 4	1.12 (0.51, 2.44)	0.6192
Quartile 3	0.54 (0.26, 1.11)	0.0933
Quartile 2	0.45 (0.23, 0.89)	0.0214
Quartile 1	1.00	Reference

WHO, World Health Organization; CI, confidence interval.
* Adjusted for age, sex and urine creatinine.
** Adjusted for additional variables including physical activity, sedentary behaviour, race and highest level of household education.

Modeling log-transformed BPA against BMI, waist circumference or waist-to-hip ratio did not achieve significance, and there were no notable differences between age groups (Table 7.17). Modeling BPA quartiles produced even weaker associations (Table A.3). Log-transformed BPA was not significantly associated with any of the skinfold measures explored. (Table A.6).

The activity monitor subsample had generally lower odds ratios than the main sample, with more achieving significance (Table 7.18). The physical activity variable used (5 or more days with at least 60 minutes of moderate to vigorous physical activity) was stronger in the model than the physical activity variable used in the main sample, although the sedentary behaviour variable was not.

Table 7.17 Log-transformed bisphenol A modeled linearly against measures of adiposity.

	β coefficient*	P-value
Total		
Body mass index	-0.0533	0.6415
Waist circumference	-0.0734	0.8047
Waist-to-hip ratio	-0.0008	0.6648
6-11 years**		
Body mass index	0.0818	0.5631
Waist circumference	0.2320	0.5161
Waist-to-hip ratio	0.0017	0.2781
12-17 years**		
Body mass index	-0.0588393	0.5962
Waist circumference	-0.0117	0.6542
Waist-to-hip ratio	-0.0014	0.4894

* Adjusted for age, sex and urine creatinine.

** Adjusted for additional variables including physical activity, sedentary behaviour, race and highest level of household education.

Table 7.18 Activity monitor sample odds ratios (95% CI) for obesity in the 2nd, 3rd and 4th bisphenol A quartiles as compared to the 1st quartile.

	Odds Ratio (95% CI)*	P-value
CDC		
Quartile 4	0.60 (0.29, 1.21)	0.1527
Quartile 3	0.39 (0.16, 0.93)	0.0341
Quartile 2	0.54 (0.30, 0.99)	0.0473
Quartile 1	1.00	Reference
IOTF		
Quartile 4	0.57 (0.32, 1.03)	0.0630
Quartile 3	0.50 (0.27, 0.89)	0.0196
Quartile 2	0.64 (0.41, 0.99)	0.0455
Quartile 1	1.00	Reference
WHO		
Quartile 4	0.59 (0.29, 1.19)	0.1379
Quartile 3	0.45 (0.19, 1.05)	0.0637
Quartile 2	0.52 (0.30, 0.91)	0.0230
Quartile 1	1.00	Reference

CDC, Centers for Disease Control; CI, Confidence interval; IOTF, International Obesity Task Force; WHO, World Health Organization.

* Adjusted for sex, urine creatinine, age, physical activity, sedentary behaviour, race and highest level of household education.

7.2 Discussion

7.2.1 The Adult Sample

The geometric means of urinary BPA for subjects aged 18 years or over in CHMS 2007-11 were generally lower than those from other large studies. In NHANES, the 2005-06 cycle was the closest with 1.7 ng/ml (95% CI: 1.6, 1.9)[100], although the 2003-04 cycle found the much higher value of 2.4 ng/ml (95% CI: 2.1, 2.7)[100]. The mean urinary BPA concentration in NHANES 2003-08 was 2.0 ng/ml (95% CI: 1.9, 2.2)[100] and the creatinine adjusted value was 2.1 µg/g (95% CI: 2.0, 2.2)[100]. In a study of 715 older Italian adults, Galloway et al.[101] found a much higher geometric mean of 3.59 ng/ml (95% CI: 3.42, 3.77).

In the Songnan Community study, Wang et al.[98] found the low urinary BPA value of 0.81 ng/ml (95% CI: 0.47, 1.43), however this may be due to a difference in methods. Geometric means are commonly used when reporting urinary concentrations of BPA and similar environmental chemicals as they are more robust to outliers than arithmetic means. For example, Melzer et al.[97] calculated the geometric mean of NHANES 2005-06 to be 1.79 ng/ml (95% CI: 1.64, 1.96), and the arithmetic mean to be much higher at 3.30 ng/ml (95% CI: 2.88, 3.72). Wang et al.[98] did not specify what type of mean was used.

A very high geometric mean of 9.54 ng/ml (SD: 8.32) was found in a sample of middle aged Koreans[99], although a different method of detection was used. Yang et al.[99] used chromatography followed by fluorescence detection, while NHANES, CHMS, Songnan Community and InCHIANTI all used chromatography followed by mass spectrometry. A follow-up study[212] using a similar sample of Korean adults found geometric means of 6.88 ng/ml (SD: 3.72) and 5.01 ng/ml (SD: 3.16) for men and women, respectively. These values are much higher than those from NHANES or CHMS, but also had very high standard deviations. A similar

Korean study calculated exposure based on a model of BPA metabolites and found the much lower unspecified means of 2.82 ng/ml (standard error (SE): 0.73) for men, and 2.76 ng/ml (SE: 0.54) for women[77]. As the fluorescence method was validated against a chromatography/mass spectrometry method, it is difficult to assess how a difference in detection methods and general variability of urine measurements may have contributed to the high concentrations seen in the two previously mentioned studies.

Expressed as unadjusted geometric means, a higher urinary BPA concentration among men than women has been a common finding. Similar to CHMS 2007-09, significantly higher urinary BPA levels were observed in men than women in pooled NHANES 2003-08[100] and InCHIANTI[101]. Wang et al.[98] found that male sex was significantly positively associated with BPA quartile.

Similar to our results, BPA urine concentration has generally been observed to decrease with age in adults. This association was more pronounced and significant in CHMS 2007-09, but also evident in 2009-11. Significant negative associations with age have been observed in NHANES 2003-08[100], the Songnan Community study[98] and the InCHIANTI[101] study. Children have generally been found to have higher urinary concentrations than adults; however a negative association with age was also seen in the Songnan Community and InCHIANTI studies, which used older adults.

Education was not significantly related to urinary BPA level among adults in this analysis. A lack of association between urinary BPA concentration and education was also observed in NHANES 2003-08[100] and InCHIANTI[101]. However, urinary BPA was significantly negatively associated with income in NHANES 2003-08[100]. Those with higher incomes might be less likely to eat canned foods and other BPA containing products. Wang et al.[98] found a

significant *positive* association between education and BPA, but it became non-significant after adjustment for age, sex and urine creatinine.

Physical activity and sedentary behaviour were not significantly associated with urinary BPA in either cycle, and were not included in other studies. Similar to CHMS 2007-11, a lack of association between BPA concentration and BMI category was also observed in NHANES 2003-08[100], although BMI and waist circumference increased significantly across BPA quartiles in the study by Wang et al.[98]

Associations with race were not notable or consistent across cycles in CHMS 2007-11. Conversely, in NHANES 2003-04[10] non-Hispanic Blacks had higher urinary BPA than non-Hispanic Whites and significantly higher urinary BPA than Mexican Americans. In NHANES 2003-08, non-Hispanic blacks still had the highest concentrations but differences were non-significant.

Urinary BPA between racial groups in NHANES 2003-04[10] became more similar after adjustment for urine creatinine. This is likely due to higher urine creatinine concentrations in non-Hispanic blacks than non-Hispanic whites and Mexican Americans[204]. Racial variations in the metabolism of certain xenobiotics may exist[113], although is it also possible that racially associated lifestyle characteristics play an important role. The significant negative association with income in NHANES 2003-08[100] further suggests economic and social differences in BPA exposure. However, it is difficult to compare populations such as Canada and the U.S. with different racial compositions, and the analyses of NHANES[100] used more racial categories than this current study.

After adjustment for urine creatinine, urinary BPA was significantly higher in women than men in CHMS 2007-11. Men are known to have higher creatinine concentrations than women[204]. This was evident in NHANES 2003-04[10] and CHMS 2007-11 in that concentrations increased in women and decreased in men after adjustment. Adults have higher urine creatinine concentrations than children or elderly people[204], and this was evident in CHMS 2007-11 as negative associations with age were attenuated after creatinine adjustment. Urine creatinine is known to be associated with muscle mass and kidney function, which decrease with age and may partly explain these differences[204].

Lifestyle behaviours may play an important role in sex differences in BPA exposure. Cao et al.[213] matched BPA concentrations of food products in the Canadian Total Diet Survey conducted in Quebec with dietary intakes from the Canada Nutrition Survey to form estimates of BPA intake and found a higher intake among men. As food is the main form of BPA exposure[12], greater consumption of BPA containing food products may have contributed to the higher urinary BPA levels observed in men in NHANES 2003-08 and CHMS 2007-11.

It has been suggested that children have higher exposure than adults because their food consumption is proportionally greater in relation to their body weight[214], though it is unclear if this idea is relevant for older versus younger adults. The study by Cao et al.[213] also observed a negative association with age, suggesting that it may be more due to differences in lifestyle than physiology. However, this should be interpreted with caution as the data from the Canada Nutrition Survey were collected in the 1970s and may not be relevant to the current Canadian population.

Positive associations between BPA and androgen concentrations have also been found. In a Japanese study of 41 subjects, total and free testosterone were found to be significantly

associated with serum BPA concentration in both men and women[215]. Galloway et al.[101] also found a significant positive association between urinary BPA and testosterone, although only in men, and another study found a negative association with estradiol/testosterone ratio[216]. Serum BPA was found to be higher in women with elevated testosterone due to polycystic ovary syndrome than normal women[217]. The enzyme responsible for the glucuronidation of BPA, UGT2B1, is down regulated by testosterone in a dose dependent manner[218], and thus higher testosterone levels may slow BPA metabolism.

The reverse has also been suggested, where BPA affects testosterone metabolism by reducing the activity of aromatase, also called estrogen synthase, which converts testosterone to estradiol[101]. Aromatase activity[66,69] and estradiol[66] have been found to be reduced by BPA exposure, supporting this assertion. It has also been proposed that this association is bidirectional where increased testosterone slows BPA metabolism, and at high concentrations BPA can bind to the UDP enzyme and displace androgens, possibly increasing their circulating concentrations[218]. Thus differences in urinary BPA levels between the sexes are likely a combination of lifestyle and hormonal factors where BPA and testosterone may each have effects on the other's metabolism. It should be noted that these studies measured BPA in serum, which may be more sensitive to differences in metabolism than urine. Galloway et al.[101] stated that any metabolic changes are unlikely to be reflected in spot urine measurements at the population level.

Lakind et al.[106] calculated estimates of daily BPA intakes from NHANES 2003-2004 and CHMS 2007-2009 and found the highest intake in Canadian adults to be 24.9 ng/kg/day (95% CI: 22.2, 27.9) among 20-39 year olds. The current Canadian TDI is 25 µg/kg/day[37], although it was established in 1996 when much less research had been published about BPA and was based

on general toxicity parameters. The European Union TDI was recently lowered substantially from 50 µg/kg/day to 4 µg/kg/day[219] and may represent a more up to date reference point. Average daily intake among Canadians is ~1000 times lower than the Canadian TDI and ~100 lower than the European one, suggesting that BPA may have limited health effects in Canadian adults.

The logistic regression results for general obesity were non-significant, but did show the generally expected trend of increasing odds of obesity (BMI ≥30 m/kg^2) with increasing BPA quartile as compared to the 1st. Using a sample of 2,747 subjects from NHANES 2003-06, Carwile & Michels[104] found a slightly different pattern where the odds of obesity in the 2nd quartile of 1.96 (95% CI: 1.32, 2.94) were greater than in the 4th quartile of 1.85 (95% CI: 1.10, 3.09), as compared to the 1st. This trend remained in their fully adjusted model and was also evident when they modeled overweight (BMI ≥25 m/kg^2) as the dependent variable, which was also significantly associated with urinary BPA. Using data from NHANES 2003-08, Shankar et al.[110] found a significant association with an odds ratio of 1.69 (95% CI: 1.30, 2.20) for obesity in the 4th quartile as compared to the 1st. The authors also found a similar trend to that observed in this analysis, with increasing odds of obesity from the 2nd to 4th quartiles (Table 7.19).

Shankar et al.[110] and Carwile & Michels[104] both found significant positive associations of urinary BPA with abdominal obesity, while the CHMS results here did not support any association. No previous studies could be found that have modeled urinary BPA against waist-to-hip ratio, and the CHMS logistic regression results were non-significant. For both general obesity and elevated waist circumference, Carwile & Michels[104] found a stronger association in men (Supplementary Table 1[220]), however Shankar et al.[110] found a stronger one in women,

although by a smaller margin. This is an interesting difference as both studies used data from NHANES 2003-06, with the later also using the 2007-08 cycle.

In logistic regression, being female slightly increased the odds of obesity. Although very little to no association was found between urinary BPA and elevated waist circumference in the logistic regression analysis, female sex was significantly associated with elevated waist circumference. In general, women aged 20 to 79 years in Canada are significantly more likely to be abdominally obese than men[2]. Conversely, male sex was significantly associated with waist-to-hip ratio.

In a sample of 3,390 Chinese adults aged 40 or over, Wang et al.[98] found weaker positive associations than the NHANES studies, but increasing odds of general obesity with increasing BPA quartile compared to the 1st quartile as reference (Table 7.19). The association was significant only for those in the fourth quartile for both simple and multivariate models and, similar to Carwile & Michels[104], modeling overweight as the dependent variable produced some significant associations. It should be noted that Wang et al. used lower standards of general (BMI $\geq$28 kg/m^2) and abdominal obesity (WC $\geq$90 cm for men 85 cm for women) designed for the Chinese population. The strength of association was similar for general and abdominal obesity in all three studies.

Shankar et al.[110] conducted a subgroup analysis and found that the positive association between urinary BPA and general obesity was slightly stronger in Mexican Americans than non-Hispanic whites and non-Hispanic blacks. Conversely, in NHANES 2003-04[10] Mexican Americans were found to have significantly lower urinary BPA levels than both non-Hispanic whites and non-Hispanic blacks. White race was generally a significant predictor for obesity in the CHMS models, although Canada and the U.S. have different racial compositions. No

significant differences in urinary BPA between racial groups were found in CHMS 2007-11. Furthermore, Canadians classified as "white" or "other" have been found to be significantly more likely to be obese than those classified as "Asian"[221]. Differences in urine creatinine between racial groups have been observed[204], but Shankar et al. did not use it as a covariate.

Table 7.19 Results of epidemiological studies on the association between urinary bisphenol A and general obesity in adults.

Analysis	Dataset and sample size	Quartile	Odds ratio (95% CI)
This study	CHMS (2007-2011) 4,713	Quartile 4	1.37 (0.87, 2.17)
		Quartile 3	1.01 (0.70, 1.73)
		Quartile 2	0.96 (0.60, 1.53)
		Quartile 1	1.00 (Reference)
Carwile & Michels 2011[104]	NHANES (2003-2006) 2,747	Quartile 4	1.76 (1.06, 2.94)
		Quartile 3	1.60 (1.05, 2.44)
		Quartile 2	1.85 (1.22, 2.79)
		Quartile 1	1.00 (Reference)
Shankar et al. 2012[110]	NHANES (2003-2008) 3,967	Quartile 4	1.69 (1.30, 2.20)
		Quartile 3	1.59 (1.25, 2.02)
		Quartile 2	1.40 (1.10, 1.76)
		Quartile 1	1.00 (Reference)
Wang et al. 2012[98]	Songnan Community Study 3,390	Quartile 4	1.50 (1.15, 1.97)
		Quartile 3	1.19 (0.90, 1.57)
		Quartile 2	1.14 (0.87, 1.50)
		Quartile 1	1.00 (Reference)

CHMS, Canadian Health Measures Survey; CI, Confidence interval; NHANES, National Health and Nutrition Examination Survey.

As stated earlier, Shankar et al.[110] and Carwile & Michels[104] used much of the same data but found stronger associations in the opposite sexes. This discrepancy may be a result of differing methods, or the addition of the 2007-08 cycle of NHANES in Shankar et al. However, the geometric mean for the 2007-08 cycle of 2.0 ng/ml (95% CI: 1.8, 2.1)[100] was not appreciably different from the previous two, suggesting that its addition would not have a dramatic effect. Urinary BPA was significantly higher for men in both the samples used (NHANES 2003-06 and 2003-08), and Silver at al.[100] showed that there were no significant demographic differences between the 2003-08 cycles of NHANES.

A notable difference in methods is that Carwile & Michels[104] used urine creatinine as a covariate, as did Wang et al.[98] and this current study, while Shankar et al.[113] did not. Urine creatinine was positively associated with all three measures of adiposity in regression analyses, but was generally non-significant. This suggests that its exclusion should not have a dramatic effect. Urine creatinine is generally higher in men, and positively associated with BMI[204]. In a small sample, Ye et al.[205] compared the Akaike information criteria of using no dilution correction, creatinine adjusted urinary BPA and urine creatinine as a covariate, and found the latter two methods to be superior. It has also been suggested that using creatinine adjusted concentrations instead of as a covariate in regressions may give misleading results as only the joint influence of BPA and creatinine will be seen[204], and creatinine is known to be associated with lean muscle mass[204]. Shankar et al.[110] also used many more covariates than Carwile & Michels[104] in their fully adjusted model including alcohol intake, physical activity, diabetes, hypertension and total serum cholesterol, and found that there were no appreciable differences in these variables between men and women.

Modeling continuous BMI against log-transformed urinary BPA produced a highly significant positive association in this current study (Table 7.6). After adjusting for age, sex, and study site, Galloway et al.[101] found a significant positive association between urinary BPA and body weight. A significant positive association was also found modeling BPA quartile against continuous BMI (Table 7.7). This has been a more common method and significant positive associations were found by Wang et al.[98], but not by Carwile & Michels[104] and Silver et al.[100]. Galloway et al.[101] found no linear association between BMI category as an ordinal variable and urinary BPA. From the lack of significance in their linear regression results, Carwile & Michels[104] suggest there may be a threshold effect, where BPA may only contribute to adiposity

past a certain level of exposure. Different models of dose-response have been discussed at length with regard to BPA exposure[222-225], as some hormones are known to exhibit similar responses at high and low levels, but a very different response at medium levels creating a 'U' shaped curve[226].

Table 7.20 Results of epidemiological studies on the association between urinary bisphenol A and abdominal obesity in adults.

Analysis	Dataset (sample size)	Quartile	Odds ratio (95% CI)
This study	CHMS 2007-11 (4,713)	Quartile 4	1.13 (0.74, 1.74)
		Quartile 3	0.84 (0.51, 1.38)
		Quartile 2	0.96 (0.62, 1.49)
		Quartile 1	1.00 (Reference)
Carwile & Michels 2011[104]	NHANES 2003-06 (2,747)	Quartile 4	1.58 (1.03, 2.42)
		Quartile 3	1.39 (1.02, 1.90)
		Quartile 2	1.62 (1.11, 2.36)
		Quartile 1	1.00 (Reference)
Shankar et al. 2012[110]	NHANES 2003-08 (3,967)	Quartile 4	1.59 (1.21, 2.09)
		Quartile 3	1.66 (1.28, 2.14)
		Quartile 2	1.63 (1.20, 2.22)
		Quartile 1	1.00 (Reference)
Wang et al. 2012[98]	Songnan Community (3,390)	Quartile 4	1.28 (1.03, 1.60)
		Quartile 3	1.28 (1.03, 1.59)
		Quartile 2	1.26 (1.02, 1.57)
		Quartile 1	1.00 (Reference)

CHMS, Canadian Health Measures Survey; CI, Confidence interval; NHANES, National Health and Nutrition Examination Survey.

Modeling waist circumference linearly also produced a significant positive association. Galloway et al.[101] also modeled log-transformed BPA against continuous waist circumference and found a significant positive association after adjusting for age, sex and study site. Similar to this analysis, Wang et al.[98] modeled BPA quartile as an indicator variable and found a significant positive association. Using similar methods, Carwile & Michels[104] did not report a p-value but found similar mean changes in waist circumference across BPA quartiles in their fully adjusted model, supporting their idea of a threshold effect. Modeling waist-to-hip ratio as the dependent

variable also produced a significant positive association with BPA whether continuous or as quartiles, although this measure has not been used in other studies.

The range of BPA concentrations found in CHMS and the other studies cited may account for some of the different strengths of association observed. NHANES, which yielded the strongest positive associations, had notably higher levels than CHMS 2007-11. Lakind et al.[106] estimated daily intake of BPA in NHANES 2007-08 and CMHS 2007-09 and found it to be ~70% higher in NHANES.

The Songnan Community Study had a lower mean concentration than either cycle of CHMS and higher odds ratios, but studied an older population that is less comparable to Canadian adults than NHANES. As Wang et al.[98] did not state what type of mean they used, it was likely arithmetic, which can give very different results than the geometric mean, making comparisons more difficult.

Galloway et al.[101] reported the highest geometric mean of the large studies and found no association between BMI category and urinary BPA in linear regression, but a highly significant one when weight was modeled continuously. Comparisons with the InCHIANTI study are difficult as it used an older study population with 63% of subjects aged 66-74 years. Galloway et al.[101] only used a simple model with sex, age and study site as covariates, while the other three studies cited used simple and adjusted models similar to this analysis. Similar to Shankar et al.[110], Galloway et al.[101] did not use urine creatinine as a covariate.

With respect to reverse causation, it is possible that individuals with an elevated BMI or waist circumference have higher urinary BPA through greater consumption of food. However, there would likely need to be a steady increase of consuming certain canned foods high in BPA

parallel to an increase in adiposity. As noted previously, in the Canadian studies on BPA containing food products, canned foods had notably higher levels than canned beverages, and canned meat/fish and soup had higher levels than vegetables[15,26,227].

A major contributor to obesity in the western world is the frequent consumption of energy dense foods[228], which generally refers to processed snack foods such as potato chips and fast food. Several canned foods can be accurately described as "energy dense", although many foods determined to have high BPA concentrations, such as canned tuna, vegetables and soups, are relatively low in calories.

Associations with other metabolic conditions have also been explored. Wang et al.[98] found a moderate positive association between BPA quartile and insulin resistance in linear regression. When stratified by weight status, the association became stronger in subjects with a BMI <24 kg/m^2, and weaker in overweight (BMI ≥24 kg/m^2) and obese (BMI ≥28 kg/m^2) subjects. Data from NHANES have also shown positive associations with diabetes[117,229], hypertension[119] and cardiovascular disease[117], and similar to Wang et al.[98], diabetes was more strongly associated with BPA in normal weight than overweight or obese subjects[117]. Using simple adjusted analysis, Wang et al.[98] also showed that fasting plasma glucose and total cholesterol increased significantly across BPA quartiles. Insulin resistance and related metabolic conditions have been observed in several rodent studies[62,230].

Gamma-glutamyltransferase (GGT) is an enzyme associated with liver abnormalities at elevated levels[231], which has been associated with urinary BPA in NHANES[117] and the Songnan Community Study[98]. Importantly, Lang et al.[117] found that GGT was associated with urinary BPA after excluding overweight and obese subjects, and those with cardiovascular disease or diabetes. Based on this observation, the authors concluded that reverse causation is unlikely.

Measurement variability is a notable issue in this field of research. Several studies have assessed the reliability of spot, morning void and simulated 24-hour void samples with varying results, and have found urinary BPA concentrations to vary throughout the day. For example, Ye et al.[205] collected samples over the course of a week and found that within-day variance for a given individual accounted for 70% of total variance, while between-person variance only accounted for 9%. In contrast, Mahalingaiah et al.[90] found a positive predictive value of 0.63 for one urine sample to correctly place a subject in the top tertile, and concluded that a single sample was representative of urinary levels in the long-term. The authors also found differences of up to 2 ng/ml, depending on the time of day, and within-day variability was found in NHANES 2003-04 as well[10].

As indicated earlier, the significantly lower geometric mean of NHANES 2005-06 than that of NHANES 2003-04[100] raises notable caveats when interpreting urinary data. It is unlikely that this change represented a genuine shift in the population, as it occurred over a short period of time.

Meeker et al.[232] assessed the measurement variability of a number of environmental chemicals in pregnant women, including triclosan, several parabens and other phenols, and found BPA to have the lowest ICC. Another study using pregnant women found an ICC of 0.10 for creatinine adjusted BPA concentrations[233]. Additionally, while it has been suggested that this variability is non-differential and will average out in large samples[234] such as NHANES, CHMS or the Songnan Community Study, the significant difference in urinary BPA between the NHANES 2003-04 and 2005-06 implies otherwise.

A notable driver of measurement variability is the speed at which a chemical is metabolized. In their commonly cited paper, Volkel et al.[87] demonstrated that BPA has a urinary

half-life of approximately 5.4 hours after administration of 5 mg. Smaller doses of 25 µg and 100 µg showed half-lives of 4 and 5 hours, respectively[87].

Stahlhut et al.[107] modeled log-transformed urinary BPA and fasting times from NHANES 2003-04, and found that fasting time did not predict the highest or lowest BPA levels. Additionally, BPA did not notably decline over fasting times of 0-4.5 hours, and the authors calculated population half-life of 43 hours from a fasting time of 0-24 hours. They concluded that BPA has a longer than expected half-life, or that there are significant sources of exposure other than food, or both. Conversely, fasting times may not be accurate enough to make this kind of modeling possible, and BPA did decline from 4.5-8.5 hours of fasting, with a population half-life of 4.1 hours, similar to previous results. As it is difficult to assess the validity of the study by Stahlhut et al.[107], the half-life estimates from Vogel et al.[87] are likely the best in the available literature.

Despite these caveats, CHMS 2007-09 and 2009-11 had very similar urinary BPA levels, adding confidence to the assertion that a large sample size will negate some of the measurement variability. The creatinine adjusted geometric means were significantly different between the cycles, although the method of urine collection changed from mid-stream in 2007-09 to first-catch in 2009-11[195]. It is unclear what effect this change may have had.

Comparisons to other environmental chemicals show that measurement variability is an inherent problem in biomonitoring. Analysis of BPA, and phthalate, a similarly studied group of chemicals, variability in the Nurse's Health Study show that most ICCs are under 0.50[167]. The assertion by Mahalingaiah et al.[90] that 0.63 is a sufficient positive predictive value for classifying subjects in the top tertile of urinary BPA adds confidence to our results in that a certain amount of variability is to be expected. Furthermore, Ye et al.[205] did not find that morning and 24-hour

voids provided dramatically less variability than spot samples, which were used in CHMS 2007-11.

Another prominent methodological issue is the detection of free BPA and not its conjugated form, BPA-glucuronide. Matthews et al.[78] compared the estrogenicity of BPA, BPA-glucuronide and estradiol on ERα and ERβ estrogen receptors *in vitro*, in mouse uteri and in MCF-7 breast cancer cells, which are sensitive to estrogen. While free BPA competed somewhat similarly with estradiol in all three preparations, 100 µM BPA-glucuronide did not displace more than 10% of the bound estradiol, the highest concentration examined. Similar results were observed in the MCF-7 cells where estradiol and BPA induced estrogen mediated gene expression but BPA-glucuronide did not at the highest concentration used (10 µM). This study is commonly cited in reference to this issue and no other studies could be found that make a similar direct comparison. Boucher et al.[235] found that BPA-glucuronide significantly increased differentiation in human and mouse adipocytes. Although BPA-glucuronide showed no estrogenic activity, co-treatment with the estrogen antagonist fulvestrant significantly reduced the adipocyte response and the authors concluded that BPA-glucuronide may increase adipocytes through a non-classical ER pathway or other mechanism. However, a direct comparison with unconjugated BPA was not made.

The methods used for detection of BPA in the CHMS 2007-09[194,195], NHANES[236,237] and Songnon Community[238] datasets involved treating urine samples with an enzyme (β-galactosidase) that cleaves the glucuronide group, releasing free BPA which is the only compound tested for and referred to as total BPA. This is useful as it theoretically accounts for all the BPA that originally entered an individual's body, but a lack of distinction between free BPA and BPA-glucuronide may create misleading results. This and several other

epidemiological analyses essentially assume that all BPA detected has estrogenicity, but research suggests that the majority of the BPA in urine and in active circulation is BPA-glucuronide.

Volkel et al.[95] analyzed 474 urine samples from 287 subjects, only finding free BPA in 10%. Other studies have generally been smaller, such as Ye et al.[239] who observed that free BPA made up 9.5% of total BPA in 30 urine samples from adult volunteers. Tsukiokai et al.[88] administered 0.050 mg of BPA to 25 subjects and found an average ratio of free to total BPA of 2.0% (95% CI: 0.34, 8.1%). Normal urine samples in another group of subjects in the same study were also analyzed and found to have an average ratio of free to total BPA of 12% (95% CI: 2.6, 29%).

Ginsberg & Rice[79] argue that β-glucuronidases are present throughout the body, which cleave the glucuronide group returning BPA to its free form as this is documented for several other xenobiotic substances[240]. This idea has not been explored with respect to BPA specifically, and the results of Volkel et al.[95] are cited in the European Food Safely Authority 2006 risk assessment of BPA[241].

As BPA is a lipophilic molecule, it has been suggested that it may become stored in adipose tissue, resulting in delayed elimination and possibly deconjugation. Fernandez et al.[242] tested adipose tissue samples from 20 women, finding BPA and several chlorinated derivatives in 11 of them and a mean of 9.00 µg/kg (SD: 9.22). The median for non-chlorinated BPA was 4.79 µg/kg (interquartile range 3.54, 7.12), which is relatively high compared to urinary concentrations. Unfortunately no other similar studies with human subjects could be found in the literature, although BPA was found to concentrate in brown adipose tissue when administered to ovariectomized rats[243].

Existing epidemiological data do suggest a causal positive association between urinary BPA and adiposity in adults, although the issues of measurement variability and detection of the free, hormonally active form of BPA make the conclusions of any study measuring total BPA contentious. Additionally, none of the three measures of adiposity achieved significance in logistic regression in this analysis. As stated earlier, this may be because the level of urinary BPA in Canada is not sufficient to promote general or central obesity, as it is lower than in NHANES[100] and InCHIANTI[101], which showed significant positive associations. Furthermore, positive linear associations were highly significant which may be more relevant to a discussion of adiposity, a continuous concept, rather than obesity specifically. Several other variables that were non-significant in logistic regression also became significant when modeled linearly such as physical activity, sedentary behaviour and smoking, thus part of this discrepancy may be due to a difference in the sensitivity of the methods used for these measures.

In summary, this analysis of CMHS 2007-11 provided nationally representative estimates of urinary BPA and showed a positive association between BPA and adiposity in Canadian aged 18 years or older. The logistic regression results only showed a modest association, but log-transformed BPA was significantly associated with all three measures of adiposity. Even weaker positive associations were observed with the activity monitor sample in logistic regression. Previous epidemiological studies have shown compelling results, although methodological issues, most notably detection of free and not total BPA, exist. Furthermore, although the current Canadian TDI may be outdated and less relevant for a discussion of adiposity specifically, estimates of intake are far below it. Future research should measure free and not conjugated BPA, and continue to explore associations with elements of the metabolic syndrome to more properly frame the observed associations with adiposity.

7.2.2 The Child Sample

Urinary BPA concentrations in Canadians aged 6-17 years were found to be generally lower than those from similar studies. The geometric mean of children aged 6-18 years in NHANES 2003-10 was 2.6 ng/ml (95% CI: 2.4, 2.7)[244]. However, in NHANES 2003-04, the geometric means among 6-11 and 12-19 year-olds were much greater at 3.6 ng/ml (95% CI: 2.9, 4.3) and 3.7 ng/ml (95% CI: 3.3, 4.2), respectively[10]. In a much smaller sample of 35 children aged 6-10 years recruited from a community health centre in New York City, Teitelbaum et al.[245] found a similar level of urinary BPA with a geometric mean of 3.4 ng/ml. Becker et al.[246] also used a slightly younger sample of German children aged 3-14 years from the GerES IV study and found a relatively lower geometric mean of 2.66 ng/ml (95% CI: 2.44, 2.89). Wang et al.[103] found the much lower geometric mean of 0.45 ng/ml (95% CI: 0.37, 0.55) in a sample of Chinese primary and middle school students aged 8-14 years. Similar to adults, urinary BPA among children was found to be much higher in NHANES 2003-04[10] than 2005-06[109]. Lewis et al.[247] found a similar level as CHMS 2007-11 in a sample of 99 Mexican children aged 8-13 years with geometric means of 1.1 ng/ml and 1.2 ng/ml for boys and girls, respectively.

Urinary BPA among boys and girls aged 6-17 years was generally similar, which was also observed in Wang et al.[103] and Lewis et al.[247] The other cited studies did not stratify by sex. Men have been found to have higher urinary BPA than women in NHANES[100], and if this difference is lifestyle based, habits conducive to exposure may not be established yet over the ages of 6-17 years.

Unlike adults, there does not seem to be a trend of decreasing urinary BPA with age. In NHANES 2003-04[10] and 2005-06[109], there was a modest decrease with age, and Becker et al.[246] found a modest increase, though neither tested for significance. Conversely, Wang et al.[103] found

significantly greater urinary BPA in the 12-15 than the 8-11 year-old age group. This and several of the cited studies only used two age groups, which may not be enough to show true age related variation.

Significant negative associations between urinary BPA and age were seen in both CHMS cycles after creatinine adjustment. Creatinine is known to be associated with muscle mass[204], and has been found to be higher in 12-17 year olds than 6-11 year olds[204], so the strong association with age may be misleading. A similar pattern was observed in NHANES 2003-04[10]. Similar to adults, urinary BPA in CHMS 2007-09 was significantly greater than 2009-11 after creatinine adjustment. The urine collection method was changed from mid-stream in 2007-09 to first catch in 2009-2011 to optimize infectious disease testing[194,195], although it is unclear what effect this might have had on BPA levels.

BPA urine concentration was not found to vary significantly across BMI categories whether measured using the WHO, CDC or IOTF systems in unadjusted analyses. In contrast, Wang et al.[103] found significantly greater urinary BPA among overweight and obese subjects than normal weight ones using BMI cut-offs designed for Chinese children. Similarly Trasande et al.[112] found that BPA quartile was significantly positively associated with obesity in NHANES 2003-08 using the CDC BMI classification system. BPA did not significantly differ by race in this CHMS analysis, although being non-Hispanic black or non-Hispanic white was significantly associated with BPA quartile in children in NHANES 2003-08[112].

Similar to adults, mean urinary BPA among children in this study was notably less than from other large studies. NHANES 2003-04[10] had the highest levels among large scale studies, though it decreased to a similar level found in GerES[246] in 2005-06[109]. The dramatic decrease in urinary BPA seen in NHANES emphasizes concerns about measurement variability, although the

geometric mean was relatively stable between CHMS 2007-09 and 2009-11. Lakind et al. calculated daily intake of BPA in CHMS 2007-09 to be 24.4 ng/kg/day (95% CI: 21.8, 27.4) and 29.5 ng/kg/day (95% CI: 25.1, 34.8) for 6-11 and 12-17 year-olds, respectively[106]. These values are approximately 1,000 times below the current Canadian TDI of 25 µg/kg/day[37]. This TDI was based on a study in rats conducted in 1996 that used general toxicity parameters and did not consider metabolic or adiposity endpoints[37]. The European Union TDI of 50 µg/kg/day was changed in 2015 to 4 µg/kg/day[219] to factor in uncertainties about metabolic and other potential health effects. Thus, the current European TDI is more up-to-date with current research and may be a more relevant reference point regarding potential associations with adiposity. Geometric means of CHMS 2007-11 were still approximately 100 times below the European TDI, implying that it is unlikely that BPA is impacting the health, or adiposity, of Canadians aged 6-17 years.

In logistic regression analyses, the CDC and IOTF methods produced similar odds ratios, while the CDC system yielded weaker negative associations. Odds ratios between the three methods were more similar in the 6-11 year-old age group, although analysis of the 2004 Canadian Community Health Survey (CCHS) found a much higher prevalence of obesity by the WHO method than the other two in subjects aged 6-11 years[248].

For the 12-17 year-old age group, the WHO and CDC methods produced similar odds ratios, and the CCHS study[248] found that the WHO and CDC methods produced similar estimates in this age group. Odds ratios varied more by BMI classification system in the 12-17 year-old age group than in those aged 6-11 years. This is in contrast to the CCHS study[248] which found that the methods are more disparate over the ages of 6-11 years, and then converge slightly from there.

As it was designed to be a "gold standard" of child growth, the WHO method can be seen as a worst case scenario as it produces the highest estimates of child obesity[249]. Of the two methods based on reference populations, the CDC one may be superior in this case as it is based solely on the U.S. population[249], which is likely more comparable to the Canadian population than the mix of international samples used in the IOTF method[189]. An advantage of the IOTF method is it represents cut-offs intended to be equivalent to the adult cut-offs[189], and therefore may be more closely associated with obesity-related morbidity in adulthood such as insulin resistance and cardiovascular disease.

Similar to adults, the results of NHANES were generally more significant and had higher odds ratios than the CHMS. Two different analyses of NHANES 2003-08[112,113] with slightly different age ranges and covariates found similar results, achieving significance in every quartile comparison made. Bhandari et al.[113] found that children in the 4th quartile had 2.55 (95% CI: 1.65, 3.95) times the odds of obesity by the CDC standard as children in the 1st quartile in multivariate logistic regression. In addition to obesity, Trasande et al.[112] also found a borderline significant positive association with overweight using the CDC standard. When stratified by sex, the association in Bhandari et al.[113] lost significance in girls and was notably stronger in boys in Trasande et al.[112] Although the CHMS results were generally below the null, the models were stronger in boys than girls, especially in those aged 6-11 years. Similarly, a Korean study found no association between urinary BPA and obesity in a sample of young girls[250].

Eng et al.[111] analyzed data from NHANES 2003-10 and found significant positive associations with BMI ≥95th percentile and waist-to-height ratio in logistic regression. Contrary to Lang et al.[117] who found significant positives associations between BPA, cardiovascular

disease and diabetes in U.S. adults, Eng et al. found little association with several risk factors including triglycerides, HDL and insulin resistance[111].

Both NHANES studies stratified by race and observed that positive associations were much stronger in white than non-white subjects. Indeed, Trasande et al.[112] found that non-Hispanic white children aged 6-19 years in the 4th quartile of urinary BPA had 6.03 (95% CI: 2.88, 12.62) times the odds of obesity by the CDC standard as those in the 1st quartile. The other races had much lower odds ratios, with none achieving significance, and part of this dramatic difference may be due to the significantly higher urinary BPA the authors observed in non-Hispanic white children[112]. However, non-Hispanic black children also had significantly higher urinary BPA than the other races, but BPA was not significantly associated with obesity in regression modeling. These differences may also be due to non-Hispanic white children having a lower prevalence of obesity than children of other races in the U.S.[251] Furthermore, Bhandari et al.[113] demonstrated that positive associations between non-white boys and girls were similar, and that a stronger association in boys than girls was only evident in white subjects. Using data from NHANES 2003-10, Wells et al.[244] found a significant positive linear association between BPA quartile and waist-to-height ratio in subjects aged 6-18 years. In accordance with the other two NHANES studies[112,113], the association was stronger in boys than girls, but was similar between non-Hispanic whites and non-Hispanic blacks. Furthermore, unlike the logistic results in Trasande et al.[112], the association was significant in non-Hispanic blacks.

Table 7.21 Results of epidemiological studies on the association between urinary bisphenol A and adiposity in children.

Analysis	Dataset (sample size)	Quartile	Odds Ratios (95% CI)
This study CDC BMI Classification	CHMS 2007-11 (2,705)	Quartile 4	1.06 (0.62, 1.81)
		Quartile 3	1.15 (0.75, 1.77)
		Quartile 2	1.58 (1.00, 2.51)
		Quartile 1	1.00 (Reference)
Trasande et al. 2012[112]	NHANES 2003-08 (2,816)	Quartile 4	2.57 (1.72, 3.83)
		Quartile 3	2.08 (1.46, 2.96)
		Quartile 2	2.24 (1.54, 3.24)
		Quartile 1	1.00 (Reference)
Bhandari et al. 2013[113]	NHANES 2003-08 (2,200)	Quartile 4	2.55 (1.65, 3.95)
		Quartile 3	1.78 (1.13, 2.79)
		Quartile 2	2.35 (1.56, 3.53)
		Quartile 1	1.00 (Reference)

Abbreviations: BMI, Body mass index; CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, Confidence interval; NHANES, National Health and Nutrition Examination Survey

Bhandari et al.[113] suggested that these sharp differences might be due to lower estrogen levels in boys than girls and in non-Hispanic whites than other racial groups. Racial differences were not consistent with an analysis of adults in NHANES 2003-08[110] which found the lowest odds ratios in non-Hispanic whites and results for all races were generally similar.

Wang et al.[103] found a significant positive association between log-transformed BPA and BMI in a sample of Chinese school children aged 8-15 years, but also used log-transformed BMI. Specific amounts of normal, overweight and obese subjects were recruited to the study, which may have made it necessary to normalize the data through log-transformation. In contrast to Wells et al.[244] and Wang et al.[103], little association was found in the linear regression analysis of CHMS 2007-11. Another Chinese study of 1,326 schoolchildren found a significant positive association between urinary BPA and obesity and hip circumference, although only in girls aged 9-12 years and not boys or older girls[252]. The authors also found a positive association with

skinfold thickness, but it was non-significant and they did not specify which skinfold measurement was used. Despite the lack of association between urinary BPA and triceps and subscapular skinfolds in CHMS 2007-11 (Table A.6), these metrics are relevant to obesity in children[253-255] and should be explored in further studies and other datasets such as NHANES and/or future cycles of the CHMS.

The activity monitor subsample showed a generally stronger negative association. The activity monitor subsample is meant to provide direct, as opposed to reported, measurements of physical activity and sedentary behaviour. As expected, adherence to at least 60 minutes of MVPA at least 5 days a week was stronger in the logistic models than the reported measure of physical activity used in the main sample. However, average daily sedentary behaviour showed very little association, and less so than the reported measure. The physical activity variable was based on the standard endorsed by the WHO of 60 minutes of moderate to vigorous physical activity daily[210], while no standard exists for sedentary behaviour so the mean was used as the threshold. Previous analysis of the activity monitor subsample in CHMS 2007-09 found that only 6.7% of Canadians aged 6 to 19 years attained 60 minutes of MVPA even 6 days a week[210]. An important caveat is that subjects have different numbers of valid days of wear, creating an unequal opportunity to achieve this standard, although ~65% of subjects had at least 6 valid days. The standard was lowered to 60 minutes of MVPA 5 days of week to better suit regression modeling in this thesis. In linear regression, the probability of accumulating 60 minutes of MVPA 6 out of 7 days was highly significant in the models with BMI and waist circumference, much more so than the previously mentioned variable. Average daily sedentary activity showed a weaker positive association than reported sedentary activity in the main sample. The activity monitor sample did show stronger negative associations than seen in the main sample and

provided more robust measures of physical activity. Future research should establish standards of sedentary behaviour for Canadians aged 6-17 years so that the activity monitor data can be used more effectively.

The CHAMACOS study is a longitudinal study of children's growth and development started in California in 1999-00[115]. Urinary BPA was measured multiple times in 498 pregnant women and at 5 and 9 years of age in their resulting children. The BMI of the children was also measured at various time points. The authors modeled BMI z-score, waist circumference and percent body fat at 9 years of age against BPA tertile and log-transformed BPA during pregnancy and at 5 years-old and found no significant associations. Non-significant associations were generally negative for girls and positive for boys. Despite the lack of longitudinal association, urinary BPA at 9 years of age was cross-sectionally associated with all three measures of adiposity. The geometric means were 1.1 ng/ml during pregnancy and 2.5 ng/ml and 1.5 ng/ml at 5 and 9 years of age, respectively[115], lower than in NHANES and similar to CHMS 2007-11. The sample of pregnant women used in CHAMACOS was also 98% Hispanic and 62% at or below the poverty line, so unrepresentative of the general U.S. population. Interestingly, BPA concentrations in mothers during pregnancy were negatively associated with BMI, body fat % and obesity in their children at 9 years old[115]. A similar study found that log-transformed urinary BPA during the 1st and 3rd trimesters in 402 Spanish women was positively associated with BMI and significantly with waist circumference in their children at 4 years of age[256]. The geometric mean of urinary BPA of the mothers was 2.3 ng/ml (SD: 2.2), similar to NHANES and greater than CHMS 2007-11 and CHAMACOS.

Generally, the CHMS 2007-11 regression results show little evidence of a positive association between urinary BPA and adiposity in children. Unlike the adult sample, which

demonstrated a modest but non-significant positive association in logistic regression, odds ratios for children aged 6-17 years were generally below the null with some showing significant negative associations. Another major difference is that the adult linear regressions were highly significant while linear analysis of children showed little association either way. The significant positive associations seen in NHANES studies may be due to the higher level of urinary BPA in U.S. than Canadian children. In their comparison of NHANES 2007-08 and CHMS 2007-09, Lakind et al.[106] found daily intake to be ~40% higher in U.S. children. Conversely, Wang et al.[103] found a lower level in their sample of Chinese school children than this analysis and a significant positive linear association with BMI. Unlike NHANES and CHMS, this was not a nationally representative survey and recruited specific amounts of subjects in each BMI category. Furthermore, U.S. children are generally a much more comparable population to Canadian children. Despite odds ratios generally being below the null, they were higher in boys than girls. This is nonetheless similar to the significant results in NHANES, which found a stronger positive association in boys and no significant difference in urine concentrations. After adjustment for urine creatinine, girls had significantly greater urinary BPA in CHMS 2007-09, but not in 2009-11.

The opposite associations seen in adults and children are a notable result of this analysis. This is even more unexpected as urine concentrations were modestly higher in children than adults in CHMS 2007-11. It is possible that this is due to lower levels of endogenous hormones in children than adults. Testosterone and estrogen are known to increase through childhood and peak in the late teen years[257,258]. It has been suggested that testosterone increases circulating BPA by inhibiting the enzyme responsible for its glucurodation[217], and that this relationship may be bidirectional where BPA increases testosterone by decreasing its conversion to estradiol[101]. It has

also been suggested that the anti-androgenic activity of BPA blocks testosterone binding sites and increases circulating testosterone via feedback control mechanisms[101]. Thus, testosterone likely has a complicated relationship with BPA, which may result in very different metabolic profiles between groups with differing levels of testosterone (i.e., female versus male, children versus adults). BPA is known to be a weak estrogen by binding ERα and has been shown to increase plasma insulin *in vivo* in an estrogen-receptor-dependent manner via disruption of pancreatic β-cells[62,259,260]. Similar to estrogen[261], BPA has been shown to induce preadipocyte proliferation[262], and also inhibit the release of adiponectin, a hormone that increases insulin sensitivity[263]. In a rat study, BPA was found to be a partial agonist of ERα and increased peroxidase activity and levels of progesterone receptors as estradiol[53], although to a lesser extent. However, when estradiol and BPA were administered simultaneously, BPA acted as an antagonist and reduced the effect of estradiol on peroxidase activity and progesterone receptor levels[53].

In discussing the disparately stronger association between BPA and obesity in non-Hispanic white boys than other race/sex groups, Bhandari et al.[113] suggested that the potential effects of BPA may depend on the level of endogenous estrogens. The authors also suggested that BPA may act as an estrogen agonist in a lower estrogen environment and an antagonist in a higher estrogen environment. This relates to the concept of non-monotonic or U-shaped dose-response curves, where very different effects are seen at low versus high doses and are common in toxicology[226]. Hugo et al. found that inhibition of adiponectin release by BPA in abdominal adipose explants showed a U-shaped curve, where similar decreases were observed at 0.1 nM and 10 nM of BPA, while a much greater decrease was seen at 1 nM[263]. Estradiol showed a similar U-shaped curve of adiponectin release, however, only BPA had a U-shaped when the

experiment was conducted in breast explant tissue[264]. The effect on BPA on aromatase has also varied, where decreases have been seen in normal Leydig[66] and granulosa cells[68], although increases have been observed in a tumorigenic Leydig cell line[68], which likely has different levels of hormones. BPA was found to modestly increase aromatase activity in placental cancer cells at concentrations of 50 µM and 100 µM, but decrease it by ~50% at 25 µM. Furthermore, 18 hours after administration aromatase activity was decreased below the baseline at all three concentrations[265]. The experiment was repeated in embryonic kidney cells where increases in aromatase activity were observed 1-6 hours after administration but decreases at all three concentrations were observed at 18 hours[265].

Several studies[266,267] have suggested that the health effects of BPA and other endocrine disruptors operate on a non-monotonic dose-response curve. In logistic regression for the child sample, a traditional dose-response profile of increasing odds of obesity with increasing BPA quartile as compared to the 1st was not seen, where the strongest negative associations were often produced by the 3rd and 2nd quartile comparisons. Evidence of a traditional dose-response relationship was sparse in the two NHANES studies on BPA and obesity in children[112,113], where the 2nd quartile comparison (i.e., the smallest difference in urinary BPA) often produced the strongest associations.

Thus, it is apparent that BPA might follow a non-monotonic dose-response curve where opposite effects can be seen at different concentrations. Furthermore, the effects of BPA might depend on the levels of endogenous hormones present, such as estradiol and testosterone, and its effects on enzymes important to hormone regulation like aromatase may differ based on cell type and the frequency of exposure.

In addition to differences in endogenous hormones between the sexes, the levels in the CMHS 2007-11 child sample are likely highly variable given the age range of 6-17 years old. Subjects at the lower end of the range who have not started puberty would have relatively low levels of estrogen[268] and testosterone[269], and subjects at the higher end would have hormone concentrations similar to many adults. Furthermore, the 12-17 year-old age groups also contains subjects who are at different stages of puberty[268] and are likely to have varying levels of endogenous hormones.

Thus, the stark differences in logistic regression for adults and children might be due to differing levels of endogenous hormones such as estradiol and testosterone, which are a possible mechanism for BPA to contribute to obesity and other metabolic conditions such as type 2 diabetes and insulin resistance. Furthermore, the 12-17 year-old age group includes subjects at varying stages of puberty and may have a wide range of hormone concentrations making a single measure of association possibly misleading. Dose-response relationships may also change with varying levels of hormones, further complicating comparison of child and adult samples. Future research should focus on elucidating dose-response relationships in BPA with specific regard to hormones such as estradiol and testosterone. Future research should also focus on samples where levels of these hormones are likely to be more stable to increase confidence in presenting single measures of association.

A major consideration in this field of research is the measurement variability of urine samples. There have been no highly detailed metabolic studies such as Volkel et al.[87] for children and adolescents but Frederickson et al.[270] did collect two first morning voids and one 24-hour void in a study of Danish schoolchildren. The two first morning voids had similar median concentrations while the 24-hour void was notably lower. However, when expressed as total

BPA, the morning voids were still similar but the 24-hour void was notably higher. This shows the generally expected relationship where the 24-hour void contains a greater amount but a lower concentration as it is divided by a higher volume of urine than the morning void. If one is to accept the 24-hour void as the most accurate measurement of long-term urinary BPA, this study demonstrates the weaker utility of first morning voids relative to 24-hour voids as they overestimated the median concentration. NHANES and CHMS used spot urine samples, which, as demonstrated by studies in adults[271,272], may be even more weakly representative of the long-term than morning voids.

Teitelbaum et al.[245] collected multiple urine samples from 35 children aged 6 to 10 years over the course of 6 months and found ICCs of 0.22 and 0.35 for raw and creatinine-corrected BPA measurements, respectively. This is similar to the ICC observed in adults by Nepomnaschy et al.[273] of 0.43 (95% CI: 0.31, 0.56) for creatinine-adjusted values based on three samples taken over the much smaller time frame of 28 days. The Spearman correlation coefficients for samples taken four weeks apart in Teitelbaum et al., the approximate time frame of the other study, were 0.41 and 0.56 for the creatinine-corrected and raw measurements, respectively. This is again somewhat similar to the Spearman coefficient found by Nepomnaschy et al. of 0.30 (95% CI: 0.04, 0.51) also for a period of four weeks. Another study used children less than 6 years of age and may be less relevant to this analysis but did find an ICC of 0.51 from four samples taken over two days[274]. The authors also found that the fraction of subjects that a single sample could place in the correct tertile was 0.68 (95% CI: 0.52, 0.84)[274]. From the available data for children and adolescents, it is reasonable to conclude that they illicit similar measurement variability to adults. Despite the ICCs generally being below 0.50, they were higher for creatinine adjusted values, showing the effect of correcting for dilution. Furthermore, using the Akaike criteria Ye et

al.[205] showed that including urine creatinine in regression models results in a lower degree of variance.

In summary, this analysis of CHMS 2007-11 provided nationally representative estimates of urinary BPA in Canadians aged 6-17 years, which were found to be lower than in U.S. children and ~1000 times below the current Canadian TDI. Furthermore, a lack of association between BPA urine concentration and obesity by the CDC, IOTF and WHO standards in logistic regression was demonstrated, with some significant negative associations observed. No notable associations were observed when BPA was modeled linearly against BMI, waist circumference and waist-to-hip ratio. The activity monitor subsample showed stronger and more significant negative associations than the main sample, and the directly measured physical activity variables were stronger in the models. This analysis shows that it is unlikely that BPA exposure is contributing to obesity in Canadians aged 6-17 years.

8. DI-(2-ETHYLHEXYL) PHTHALTE

8.1 Results

8.1.1 Adult Sample

Table 8.1 shows the geometric means of urinary MEHP, MEHHP and MEOHP in adults in CMHS 2007-11. MEHP and MEHHP were significantly higher in men than women, while MEOHP showed no notable difference. MEHHP and MEOHP showed significant negative associations with age, while MEHP showed a modest negative association. All three metabolites were significantly different between the 2007-09 and 2009-11 cycles.

Table 8.2 shows the geometric means of urinary MEHP, MEHHP and MEOHP in adults in CHMS 2007-11 adjusted for urine creatinine. MEHHP and MEOHP were significantly higher in women than men. All three metabolites were significantly different between the 2007-09 and 2009-11 cycles.

Table 8.1 Geometric mean (95% CI) of urinary MEHP, MEHHP and MEOHP in Canadians aged 18 years or older in CHMS 2007-2011 stratified by sex and age.

Characteristic	N (%)*	Geometric mean (ng/ml)	P-value
MEHP			
Total	2,037	2.70 (2.45, 2.96)	<0.0001**
Sex			
Men	1,026 (50.4)	3.08 (2.69, 3.52)	Reference
Women	1,011 (49.6)	2.36 (2.12, 2.62)	0.0003
Age (years)			
18-29	814 (40.0)	2.79 (2.48, 3.15)	Reference
30-39	529 (26.0)	2.69 (2.24, 3.23)	0.7316
40-49	694 (34.1)	2.59 (2.22, 3.02)	0.3800
MEHHP			
Total	2,056	15.67 (14.32, 17.14)	<0.0001**
Sex			
Men	1,038 (50.5)	17.13 (15.0, 19.57)	Reference
Women	1,018 (49.5)	14.30 (12.88, 15.88)	0.0212
Age (years)			
18-29	811 (39.4)	17.01 (15.23, 19.0)	Reference
30-39	541 (26.3)	14.10 (11.77, 16.9)	0.0481
40-49	704 (34.3)	15.46 (13.54, 17.65)	0.2194
MEOHP			
Total	2,056	9.22 (8.46, 10.05)	<0.0001**
Sex			
Men	1,038 (50.5)	9.72 (8.50, 11.11)	Reference
Women	1,018 (49.5)	8.74 (7.92, 9.65)	0.1846
Age (years)			
18-29	811 (39.4)	10.25 (9.21, 11.41)	Reference
30-39	541 (26.3)	8.26 (6.96, 9.79)	0.0247
40-49	704 (34.3)	8.89 (7.68, 10.29)	0.0901

CHMS, Canadian Health Measures Survey; CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 8.2 Geometric means (95% CI) of urinary MEHP, MEHHP and MEOHP adjusted for urine creatinine in Canadians aged 18 years or older in CHMS 2007-2011 stratified by sex and age.

Characteristics	N (%)*	Geometric mean (ng/ml)	P-value
MEHP			
Total	2,037	2.54 (2.33, 2.76)	<0.0001**
Sex			
Men	1,026 (50.4)	2.45 (2.22, 2.72)	Reference
Women	1,011 (49.6)	2.62 (2.33, 2.95)	0.3454
Age (years)			
18-29	814 (40.0)	2.37 (2.10, 2.67)	Reference
30-39	529 (26.0)	2.62 (2.17, 3.18)	0.3789
40-49	694 (34.1)	2.68 (2.30, 3.12)	0.1914
MEHHP			
Total	2,056	15.11 (13.99, 16.32)	<0.0001**
Sex			
Men	1,038 (50.5)	13.96 (12.71, 15.33)	Reference
Women	1,018 (49.5)	16.38 (14.72, 18.24)	0.0130
Age (years)			
18-29	811 (39.4)	14.60 (13.22, 16.12)	Reference
30-39	541 (26.3)	14.16 (11.88, 16.87)	0.7276
40-49	704 (34.3)	16.53 (14.63, 18.67)	0.1036
MEOHP			
Total	2,056	8.75 (8.12, 9.43)	<0.0001**
Sex			
Men	1,038 (50.5)	7.82 (7.12, 8.58)	Reference
Women	1,018 (49.5)	9.82 (8.84, 10.89)	0.0004
Age (years)			
18-29	811 (39.4)	8.70 (7.86, 9.63)	Reference
30-39	541 (26.3)	8.19 (7.00, 9.60)	0.4821
40-49	704 (34.3)	9.27 (8.13, 10.57)	0.4284

CHMS, Canadian Health Measures Survey; CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 8.3 Odds ratios (95% CI) for BMI ≥ 30kg/m^2 in 2nd, 3rd and 4th MEHP, MEHHP or MEOHP quartiles as compared to the 1st quartile.

	Model 1*		Model 2**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
MEHP				
Quartile 4	0.70 (0.32, 1.56)	0.3885	0.70 (0.30, 1.63)	0.4094
Quartile 3	0.64 (0.37, 1.13)	0.1243	0.65 (0.36, 1.16)	0.1444
Quartile 2	1.14 (0.55, 2.36)	0.7340	1.24 (0.59, 2.63)	0.5689
Quartile 1	1.00	Reference	1.00	Reference
MEHHP				
Quartile 4	1.38 (0.68, 2.80)	0.3748	1.36 (0.65, 2.86)	0.4153
Quartile 3	1.99 (1.07, 1.91)	0.0289	2.13 (1.09, 4.17)	0.0268
Quartile 2	1.66 (0.83, 3.30)	0.1508	1.78 (0.85, 3.74)	0.1265
Quartile 1	1.00	Reference	1.00	Reference
MEOHP				
Quartile 4	1.53 (0.71, 3.30)	0.2837	1.49 (0.64, 3.45)	0.3556
Quartile 3	1.59 (0.86, 2.97)	0.1423	1.59 (0.83, 3.05)	0.1642
Quartile 2	2.19 (1.17, 4.10)	0.0147	2.19 (1.08, 4.44)	0.0300
Quartile 1	1.00	Reference	1.00	Reference

BMI, Body mass index; CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

* Adjusted for urine creatinine, age and sex.

** Adjusted for race, education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

MEHHP and MEOHP were significantly associated with obesity in logistic regression (Table 8.3). Non-significant positive associations were seen between MEHHP and MEOHP and elevated waist circumference and waist-to-hip ratio (Tables 8.4-8.5). MEHHP and MEOHP were also significantly associated with obesity and waist circumference in linear regression whether log-transformed or by quartile (Tables 8.6-8.7). Modest positive associations were seen between MEHP and waist circumference and waist-to-hip ratio. The metabolite ratios MEHP/MEHHP and MEHP/MEOHP were significantly negatively associated with BMI and waist circumference, and MEHP/MEHHP was also significantly negatively associated with waist-to-hip ratio (Table 8.8).

Table 8.4 Odds ratios (95% CI) for elevated waist circumference in 2nd, 3rd and 4th MEHP, MEHHP or MEOHP quartiles as compared to the 1st quartile.

	Model 1*		Model 2**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
MEHP				
Quartile 4	1.15 (0.62, 2.11)	0.6661	1.12 (0.62, 2.02)	0.7182
Quartile 3	0.94 (0.52, 1.71)	0.8484	0.96 (0.54, 1.71)	0.8983
Quartile 2	1.13 (0.62, 2.07)	0.6881	1.20 (0.63, 2.30)	0.5842
Quartile 1	1.00	Reference	1.00	Reference
MEHHP				
Quartile 4	1.50 (0.89, 2.53)	0.1255	1.46 (0.83, 2.56)	0.1898
Quartile 3	1.48 (0.90, 2.45)	0.1249	1.61 (0.90, 2.89)	0.1074
Quartile 2	1.41 (0.83, 2.40)	0.2103	1.43 (0.76, 2.72)	0.2683
Quartile 1	1.00	Reference	1.00	Reference
MEOHP				
Quartile 4	1.61 (0.85, 3.05)	0.1404	1.57 (0.78, 3.16)	0.2044
Quartile 3	1.24 (0.75, 2.06)	0.3979	1.24 (0.73, 2.13)	0.4263
Quartile 2	1.51 (0.94, 2.43)	0.0920	1.46 (0.82, 2.60)	0.1986
Quartile 1	1.00	Reference	1.00	Reference

CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate. * Adjusted for urine creatinine, age and sex.

** Adjusted for race, education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table 8.5 Odds ratios (95% CI) for elevated waist-to-hip ratio in 2nd, 3rd and 4th MEHP, MEHHP or MEOHP quartiles as compared to the 1st quartile.

	Model 1*		Model 2**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
MEHP				
Quartile 4	0.97 (0.53, 1.77)	0.9128	0.94 (0.49, 1.81)	0.8535
Quartile 3	1.28 (0.74, 2.22)	0.3831	1.23 (0.68, 2.23)	0.4888
Quartile 2	1.44 (0.78, 2.67)	0.2424	1.47 (0.77, 2.80)	0.2455
Quartile 1	1.00	Reference	1.00	Reference
MEHHP				
Quartile 4	1.59 (0.84, 3.01)	0.1514	1.56 (0.82, 2.98)	0.1756
Quartile 3	1.78 (0.99, 3.18)	0.0524	1.79 (0.99, 3.25)	0.0557
Quartile 2	1.38 (0.86, 2.21)	0.1848	1.53 (0.94, 2.48)	0.0871
Quartile 1	1.00	Reference	1.00	Reference
MEOHP				
Quartile 4	1.55 (0.77, 3.12)	0.2186	1.52 (0.77, 2.99)	0.2245
Quartile 3	1.62 (0.84, 3.13)	0.1482	1.68 (0.86, 3.30)	0.1291
Quartile 2	1.49 (0.90, 2.48)	0.1228	1.60 (0.94, 2.74)	0.0859
Quartile 1	1.00	Reference	1.00	Reference

CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.* Adjusted for urine creatinine, age and sex.

** Adjusted for race, education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table 8.6 Log-transformed MEHP, MEHHP or MEOHP modeled linearly against measures of adiposity.

	Model 1*		Model 2**	
	β coefficient	P-value	β coefficient	P-value
MEHP				
Body mass index	0.1139	0.5005	0.1875	0.2583
Waist circumference	0.1301	0.7353	0.3304	0.3882
Waist-to-hip ratio	0.0020	0.3837	0.0022	0.3591
MEHHP				
Body mass index	0.4841	0.0055	0.5321	0.0029
Waist circumference	1.2601	0.0020	1.3772	0.0010
Waist-to-hip ratio	0.0044	0.0021	0.0039	0.0086
MEOHP				
Body mass index	0.3758	0.0455	0.4055	0.0344
Waist circumference	0.9857	0.0251	1.0490	0.0207
Waist-to-hip ratio	0.0041	0.0969	0.0044	0.0796

CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.
* Adjusted for urine creatinine, age and sex.
** Adjusted for race education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table 8.7 MEHP, MEHHP or MEOHP quartile modeled linearly against measures of adiposity.

	MEHP		MEOHP		MEHHP	
	β coefficient*	P-value	β coefficient*	P-value	β coefficient*	P-value
Body mass index	0.0671	0.6886	0.3567	0.0433	0.4733	0.0041
Waist circumference	0.0595	0.8757	0.9717	0.0195	1.2562	0.0009
Waist-to-hip ratio	0.0014	0.5480	0.0039	0.1716	0.0044	0.0727

CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.
* Adjusted for age sex, urine creatinine, race, education, smoking, physical activity and sedentary behaviour

Table 8.8 DEHP metabolite ratios modeled linearly against measures of adiposity.

	MEHP/MEHHP		MEHP/MEOHP	
	β coefficient*	P-value	β coefficient*	P-value
Body mass index	-6.2115	<0.0001	-2.3950	0.0290
Waist circumference	-17.6589	<0.0001	-7.6362	0.0040
Waist-to-hip ratio	-0.0736	0.0051	-0.0275	0.0655

CI, confidence interval, DEHP, Di(-2-ethylhexyl) phthalate; MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

* Adjusted for age sex, urine creatinine, race, education, smoking, physical activity and sedentary behaviour

8.1.2 Child Sample

Table 8.9 shows the geometric means of urinary MEHP, MEHHP and MEOHP in subjects aged 6-17 years in CHMS 2007-11. MEOHP was significantly negatively associated with age, while MEHP and MEHHP showed modest negative associations. All three metabolites showed no significant difference by sex and were significantly different between the 2007-09 and 2009-11 cycles of CHMS.

Table 8.9 Geometric mean (95% CI) of urinary MEHP, MEHHP and MEOHP concentration in Canadians aged 6-17 years in CHMS 2007-11 stratified by sex and age.

	N (%)*	Geometric mean (ng/ml)	P-value
MEHP			
All	2,702	2.84 (2.58, 3.12)	0.0009**
Sex			
Boys	1,409 (52.2)	2.96 (2.67, 3.28)	Reference
Girls	1,293 (47.8)	2.72 (2.43, 3.04)	0.1139
Age (years)			
6-11	1,242 (46.0)	2.98 (2.72, 3.26)	Reference
12-17	1,460 (54.0)	2.73 (2.41, 3.09)	0.1073
MEHHP			
All	2,719	23.68 (21.81, 25.72)	<0.0001**
Sex			
Boys	1,420 (52.2)	24.50 (22.27, 26.95)	Reference
Girls	1,299 (47.8)	22.82 (20.53, 25.36)	0.2262
Age (years)			
6-11	1,249 (45.9)	26.92 (24.71, 29.33)	Reference
12-17	1,470 (54.1)	21.24 (18.83, 23.95)	0.0004
MEOHP			
All	2,719	14.96 (13.78, 16.24)	<0.0001**
Sex			
Boys	1,420 (52.2)	15.25 (13.92, 16.71)	Reference
Girls	1,299 (47.8)	14.65 (13.16, 16.31)	0.4804
Age (years)			
6-11	1,249 (45.9)	17.18 (15.71, 18.78)	Reference
12-17	1,470 (54.1)	13.31 (11.86, 14.93)	<0.0001

BMI, Body mass index; CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

Table 8.10 shows the geometric means of urinary MEHP, MEHHP and MEOHP adjusted for urine creatinine in subjects aged 6-17 years in CHMS 2007-11. All three metabolites showed significant negative associations with age and were significantly different between the 2007-09 and 2009-11 cycles of CHMS.

Table 8.10 Geometric mean (95% CI) of urinary MEHP, MEHHP and MEOHP concentration adjusted for urine creatinine in Canadians aged 6-17 years in CHMS 2007-11 stratified by sex and age.

Characteristics	N (%)*	Geometric mean (µg/g)	P-value
MEHP			
Total	2,702	2.90 (2.67, 3.15)	<0.0001**
Sex			
Boys	1,409 (52.2)	2.90 (2.63, 3.19)	Reference
Girls	1,293 (47.8)	2.91 (2.63, 3.22)	0.9359
Age (years)			
6-11	1,242 (46.0)	3.90 (3.58, 4.26)	Reference
12-17	1,460 (54.0)	2.26 (2.04, 2.50)	<0.0001
MEHHP			
Total	2,719	24.62 (23.18, 26.15)	<0.0001**
Sex			
Boys	1,420 (52.2)	24.43 (22.66, 26.33)	Reference
Girls	1,299 (47.8)	24.83 (22.98, 26.84)	0.7316
Age (years)			
6-11	1,249 (45.9)	35.88 (33.52, 38.40)	Reference
12-17	1,470 (54.1)	17.88 (16.35, 19.56)	<0.0001
MEOHP			
Total	2,719	15.31 (14.40, 16.26)	<0.0001**
Sex			
Boys	1,420 (52.2)	14.95 (13.93, 16.05)	Reference
Girls	1,299 (47.8)	15.70 (14.48, 17.03)	0.3009
Age (years)			
6-11	1,249 (45.9)	22.55 (20.99, 24.23)	Reference
12-17	1,470 (54.1)	11.01 (10.10, 12.00)	<0.0001

BMI, Body mass index; CI, confidence interval, MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.

* N (%) is weighted to the population using standardized weights.

** P-value for the difference in geometric mean between the 2007-09 and 2009-11 cycles.

In logistic regression, the odds ratios for MEHP and obesity were mostly below the null, with significant negative associations seen in the 6-11 year-old age group (Table 8.11). In the 12-17 year-old age group, the 3rd to 1st quartile comparison resulted in a modest positive association and odds ratios were generally higher than in the 6-11 year-old age group.

Table 8.11 Odds ratios (95% CI) for obesity in 2nd, 3rd and 4th MEHP quartiles as compared to the 1st quartile

CDC	Total*	6-11 years**	12-17 years**
Quartile 4	0.75 (0.49, 1.14)	0.53 (0.22, 1.30)	0.61 (0.27, 1.37)
Quartile 3	1.03 (0.61, 1.77)	0.72 (0.31, 1.66)	1.35 (0.53, 3.43)
Quartile 2	0.56 (0.33, 0.96)	0.47 (0.23, 0.96)	0.61 (0.24, 1.55)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

IOTF	Total*	6-11 years**	12-17 years**
Quartile 4	0.71 (0.55, 0.91)	0.47 (0.25, 0.85)	0.78 (0.48, 1.28)
Quartile 3	0.83 (0.64, 1.08)	0.47 (0.27, 0.81)	1.23 (0.72, 2.13)
Quartile 2	0.60 (0.38, 0.95)	0.71 (0.42, 1.23)	0.54 (0.29, 0.99)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

WHO	Total*	6-11 years**	12-17 years**
Quartile 4	0.72 (0.48, 1.10)	0.51 (0.22, 1.18)	0.57 (0.25, 1.29)
Quartile 3	1.10 (0.64, 1.92)	0.73 (0.32, 1.65)	1.49 (0.54, 4.14)
Quartile 2	0.57 (0.33, 0.97)	0.46 (0.24, 0.89)	0.63 (0.25, 1.61)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

CDC, Centers for Disease Control; CI, confidence interval; IOTF, International Obesity Task Force; MEHP, Mono-(2-ethylhexyl) phthalate; WHO, World Health Organization.
* Model 1 adjusted for age, sex and urine creatinine.
** Model 2 adjusted for highest level of household education, race, physical activity and sedentary behaviour in addition to model 1 covariates.

The odds ratios for MEHHP and obesity were mostly below the null, especially in the 6-11 year-old age group (Table 8.12). A significant negative association was seen with the IOTF standard of obesity in the 6-11 year-old age group. The 4th to 1st quartile comparison in the 12-17 year-old age group produced modest positive associations.

Table 8.12 Odds ratios (95% CI) for obesity protocol in 2nd, 3rd and 4th MEHHP quartiles as compared to the 1st quartile

CDC	Total*	6-11 years**	12-17 years**
Quartile 4	1.37 (0.62, 3.02)	0.70 (0.17, 2.87)	1.61 (0.61, 4.24)
Quartile 3	0.73 (0.39, 1.35)	0.77 (0.26, 2.29)	0.43 (0.15, 1.23)
Quartile 2	0.79 (0.49, 1.28)	0.55 (0.21, 1.44)	0.79 (0.45, 1.37)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
IOTF	Total*	6-11 years**	12-17 years**
Quartile 4	0.83 (0.50, 1.38)	0.37 (0.18, 0.76)	1.30 (0.72, 2.37)
Quartile 3	0.79 (0.51, 1.22)	0.55 (0.28, 1.05)	0.96 (0.50, 1.82)
Quartile 2	0.75 (0.53, 1.06)	0.39 (0.23, 0.68)	0.99 (0.61, 1.62)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
WHO	Total*	6-11 years**	12-17 years**
Quartile 4	1.38 (0.61, 3.09)	0.64 (0.17, 2.44)	1.72 (0.62, 4.79)
Quartile 3	0.74 (0.39, 1.40)	0.78 (0.28, 2.14)	0.42 (0.14, 1.33)
Quartile 2	0.84 (0.52, 1.37)	0.56 (0.22, 1.45)	0.85 (0.49, 1.50)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

CDC, Centers for Disease Control; CI, confidence interval; IOTF, International Obesity Task Force; MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; WHO, World Health Organization.
* Model 1 adjusted for age, sex and urine creatinine.
** Model 2 adjusted for highest level of household education, race, physical activity and sedentary behaviour in addition to model 1 covariates.

Odds ratios for MEOHP were mostly below the null and significant negative associations were seen in the 6-11 year-old age group (Table 8.13). The 4th to 1st quartile comparison produced modest positive associations in the 12-17 year-old age group. Overall, odds ratios varied notably between age groups where the odds of obesity were generally higher for the 12-17 than the 6-11 year-old age group. The IOTF standard produced the most significant negative associations, especially in the 6-11 year-old age group. Results varied notably by method of classifying obesity for MEHHP and MEOHP. Some odds ratios were well below the null by the CDC and WHO protocols in the 12-17 year-old age group, while the same quartile comparisons with the IOTF method resulted in much higher odds ratios close to the null.

Table 8.13 Odds ratios (95% CI) for obesity in 2nd, 3rd and 4th MEOHP quartiles as compared to the 1st quartile

CDC	Total*	6-11 years**	12-17 years**
Quartile 4	1.12 (0.50, 2.52)	0.47 (0.14, 1.60)	1.69 (0.69, 4.17)
Quartile 3	0.89 (0.45, 1.74)	0.96 (0.32, 2.88)	0.63 (0.20, 2.02)
Quartile 2	0.56 (0.35, 0.89)	0.37 (0.15, 0.91)	0.63 (0.36, 1.12)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
IOTF	Total*	6-11 years**	12-17 years**
Quartile 4	0.82 (0.48, 1.40)	0.30 (0.14, 0.60)	1.57 (0.87, 2.83)
Quartile 3	0.85 (0.53, 1.36)	0.69 (0.36, 1.32)	1.01 (0.45, 2.24)
Quartile 2	0.69 (0.53, 0.88)	0.33 (0.19, 0.57)	1.01 (0.63, 1.64)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
WHO	Total*	6-11 years**	12-17 years**
Quartile 4	1.16 (0.51, 2.64)	0.47 (0.14, 1.56)	1.79 (0.70, 4.55)
Quartile 3	0.92 (0.45, 1.89)	1.01 (0.34, 3.03)	0.63 (0.18, 2.20)
Quartile 2	0.62 (0.39, 0.99)	0.42 (0.16, 1.07)	0.70 (0.40, 1.21)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

CDC, Centers for Disease Control; CI, confidence interval; IOTF, International Obesity Task Force; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate; WHO, World Health Organization.
* Model 1 adjusted for age, sex and urine creatinine.
** Model 2 adjusted for highest level of household education, race, physical activity and sedentary behaviour in addition to model 1 covariates.

Log-transformed MEHP was significantly negatively associated with BMI and waist circumference in linear regression, but lost significance when stratified by age. Log-transformed MEHHP and MEOHP showed modest positive associations in the 12-17 year-old age group and modest negative associations in the 6-11 year-old age group. Modeling linearly by quartile produced similar but attenuated associations.

Table 8.14 Log-transformed MEHP modeled linearly against measures of adiposity

	β coefficient*	P-value
Total		
Body mass index	-0.2724	0.0149
Waist circumference	-0.6422	0.0116
Waist-to-hip ratio	-0.0016	0.2263
6-11 years		
Body mass index	-0.2769	0.0999
Waist circumference	-0.8949	0.0543
Waist-to-hip ratio	-0.0031	0.2785
12-17 years		
Body mass index	-0.2621	0.1253
Waist circumference	-0.2775	0.4698
Waist-to-hip ratio	0.0010	0.5187

MEHP, Mono-(2-ethylhexyl) phthalate.
* Adjusted for age, sex and urine creatinine.

Table 8.15 Log-transformed MEHHP modeled linearly against measures of adiposity

	β coefficient*	P-value
Total		
Body mass index	-0.0645	0.7072
Waist circumference	0.1160	0.7643
Waist-to-hip ratio	0.0020	0.2024
6-11 years		
Body mass index	-0.2253	0.1512
Waist circumference	-0.6752	0.0850
Waist-to-hip ratio	-0.0008	0.7330
12-17 years		
Body mass index	0.0118	0.9662
Waist circumference	0.6789	0.2469
Waist-to-hip ratio	0.0047	0.0558

MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate.
* Adjusted for age, sex and urine creatinine.

Table 8.16 Log-transformed MEOHP modeled linearly against measures of adiposity

	β coefficient*	P-value
Total		
Body mass index	-0.1107	0.5366
Waist circumference	0.0171	0.9653
Waist-to-hip ratio	0.8912	0.1944
6-11 years		
Body mass index	-0.3364	0.0489
Waist circumference	-0.8970	0.0416
Waist-to-hip ratio	-0.0010	0.7016
12-17 years		
Body mass index	-0.0029	0.9919
Waist circumference	0.6722	0.2594
Waist-to-hip ratio	0.0049	0.0441

MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.
* Adjusted for age, sex and urine creatinine.

Table 8.17 DEHP metabolite quartile modeled linearly against measures of adiposity

Measure	β coefficient*	P-value
MEHP		
Body mass index	-0.1316	0.1276
Waist circumference	-0.3948	0.0561
Waist-to-hip ratio	-0.0013	0.3793
MEHHP		
Body mass index	0.0091	0.9729
Waist circumference	0.1584	0.6300
Waist-to-hip ratio	0.0021	0.1761
MEOHP		
Body mass index	-0.2943	0.3648
Waist circumference	-0.2557	0.7538
Waist-to-hip ratio	0.0013	0.7460

DEHP, Di-(2-ethylhexyl) phthalate; MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.
* Adjusted for age, sex and urine creatinine.

The MEHP/MEHHP and MEHP/MEOHP metabolite ratios were significantly negatively associated with all three measures of adiposity.

Table 8.18 DEHP metabolite ratios modeled linearly against measures of adiposity

	MEHP/MEHHP		MEHP/MEOHP	
	β coefficient*	P-value	β coefficient*	P-value
Body mass index	-4.1315	0.0160	-2.2673	0.0216
Waist circumference	-13.9406	0.0019	-7.7386	0.0022
Waist-to-hip ratio	-0.0697	0.0006	-0.0400	0.0005

DEHP, Di-(2-ethylhexyl) phthalate. MEHHP, Mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; MEOHP, Mono-(2-ethyl-5-oxohexyl) phthalate.
*Adjusted for age sex and urine creatinine.

8.2 Discussion

8.2.1 Adult Sample

The levels of urinary MEHP in the Canadian adults are similar to that found in the U.S. In NHANES 1988-94 and 1999-00, values of 3.5 ng/ml (interquartile range: 1.4, 7.0)[171] and 3.21 ng/ml (95% CI: 2.95, 3.49)[122] were observed, although the 1988-94 sample was not representative. The geometric means for MEHHP and MEOHP for subjects 6 years or older in NHANES 2001-06 were 21.2 ng/ml (interquartile range: 10.2, 37.8)[275] and 13.9 ng/ml (interquartile range: 6.71, 24.6)[275], respectively.

Several German studies have found much higher levels, but reported using medians, which are often different than geometric means. For example Wittassek et al.[276] used samples taken over the course of 15 years from 634 German university students aged 20-29 years and found medians levels of 7.6 ng/ml, 21.0 ng/ml and 16.7 ng/ml for MEHP, MEHHP and MEOHP, respectively. Even higher medians of 10.3 ng/ml, 46.8 ng/ml and 36.5 ng/ml were found in a sample of 85 Germans aged 7 to 64 years[163]. Interestingly, Wittassek et al.[276] show a steady decline in concentration of MEHP, MEHHP and MEOHP from 1988-03.

DEHP metabolites showed steadily decreasing levels with age in adults in NHANES[277,278], and MEHHP and MEOHP were significantly greater in the 18-29 than the 30-39 year-old age group in CHMS 2007-11. Levels of phthalates have been found to be higher in children than adults in NHANES and the authors suggest that it may be due to children having higher food intake and air inhalation in proportion to their body weight[279]. This may also apply to younger versus older adults, although the oldest subjects in the CHMS sample were 49 years old. Differences in metabolism may also play an important role in age related variation.

Similar to the CHMS results, levels of DEHP metabolites have generally been found to be higher in men. All three metabolites were higher in men in the German studies conducted by Wittassek et al.[174] and Fromme et al.[166], and urinary MEHP was marginally higher in men than women in NHANES 1999-00[279]. As the main source of exposure to DEHP metabolites is thought to be through food[280], it is possible that men consume more food products containing DEHP and differences in metabolism between the sexes may also play an important role. After adjustment for urine creatinine, women had significantly greater MEHHP and MEOHP concentrations. This pattern is expected as women generally have lower urine creatinine than men[204] and is seen in other studies[276,279]. As younger adults generally have higher creatinine than older adults[204], previous negative associations with age were attenuated and lost significance after adjustment.

Yaghjyan et al.[193] used data from NHANES 1999-2004 in ordered logistic regression and found that urinary MEHP was associated with a significant increase in BMI in women aged 18 years or older with an odds ratio of 1.12 (95% CI: 1.03, 1.23). In contrast, MEHHP and MEOHP showed little to no association with any of the outcomes tested including total cholesterol, triglycerides, HDL and LDL. The strongest positive associations they found were between the ratio of MEHP/MEHHP and BMI, 1.21 (95% CI: 1.09, 1.34), and waist circumference, 1.20 (95% CI: 1.10, 1.31)[193].

The odds ratio for the sum of all DEHP metabolites and BMI was 0.93 (95% CI: 0.84, 1.03)[193], suggesting that the source of the association is MEHP and that the inclusion of the oxidative metabolites in addition may reduce the apparent association. The majority of odds ratios for all DEHP metabolites were close to the null for BMI, waist circumference, LDL and triglycerides.

Hatch et al.[281] conducted a similar study using NHANES 1999-02 found that MEHP quartile was not linearly associated with BMI in men, and may be negatively associated in women. Furthermore, MEHHP was significantly associated with BMI in men aged 20 to 59 years and women aged 6-19 years. Stahlhut et al.[277] used data from NHANES 1999-00, modeling log-transformed urinary phthalate concentrations linearly against waist circumference and limiting their analysis to men. No correlation was found with MEHP, but of all the phthalates tested, MEHHP and MEOHP showed the strongest positive associations with waist circumference.

Similar to Hatch et al.[192], Svenssen et al.[282] found that urinary MEHP was moderately negatively correlated with BMI and waist circumference using Spearman coefficients in a sample of 182 Mexican women. Furthermore, MEHHP and MEOHP were associated with waist circumference and waist-to-hip ratio, and significantly so for MEHHP. The sum of DEHP metabolites was also associated with waist circumference and significantly with waist-to-hip ratio. In their crude logistic regression model, the authors only investigated diabetes as an outcome and found that MEHHP, MEOHP and the sum of DEHP metabolites but not MEHP were significantly associated with it[282]. All associations lost significance in the full adjusted model, although the sum of DEHP metabolites was borderline with an odds ratio of 1.64. Stahlhut et al.[277] used the homeostatic model assessment, a measure of insulin resistance[283], as an outcome but found no associations with DEHP metabolites.

It has been suggested that MEHP is the most biologically active of the three DEHP metabolites used in this analysis[156]. Hauser et al.[156] stated that because MEHHP and MEOHP are more easily excreted in urine than MEHP, conversion to the oxidative metabolites could be protective if MEHP is the most active. This is roughly represented in Yaghjyan et al.[193] as MEHP

showed greater positive association than the other metabolites and the MEHP/MEHHP ratio was significantly positively associated with BMI and waist circumference. Our results contrast sharply with this notion as MEHHP and MEOHP showed much stronger positive associations than MEHP to measures of adiposity in logistic and linear regression. Furthermore, modeling MEHP/MEHHP and MEHP/MEOHP produced significant negative associations with BMI and waist circumference in CHMS 2007-11. A notable difference is that the NHANES sample only included women[193].

Hauser et al.[156] found that MEHP was more strongly associated with DNA damage in sperm when MEHHP and MEOHP were included in the model, although their previous analyses found no association. MEHHP and MEOHP also showed strong negative associations with DNA damage in sperm[156]. Many laboratory studies only evaluate the effects of DEHP or MEHP compared to other phthalate diesters and not potential differences between DEHP metabolites. Stroheker et al.[284] found that DEHP and MEHP were not androgen receptor antagonists in rats, but that two unidentified metabolites, which could be MEHHP and MEOHP, did show anti-androgenic activity. MEHP was found to be negatively associated with testosterone in a sample of 295 adult men[285]. Interpretation of results may be further complicated by differences in metabolism as some studies have found different relative amounts of MEHP, MEHHP and MEOHP[122,138]. This represents a possible route for reverse causation, as a faster or slower metabolism of DEHP or MEHP caused by a different exposure or health condition could create variation in the metabolite ratios and produce spurious associations[157]. Natural variation in metabolism among populations such as by sex or age is also possible and further complicates interpretation. The half-lives of MEHHP and MEOHP are much longer than MEHP[164] and the timing of urine collection may cause differences in their relative concentrations[286]. General

variability is an issue in human biomonitoring and analysis of repeated samples in 10 men found MEHP to have weaker sensitivity and specificity than other phthalate monoesters such as MBzP, and MEP[287]. Another study found that MEHP and MEHHP had higher variability than several other phthalates, although the ICCs were generally below 0.5[288]. The unadjusted and creatinine corrected geometric means of all three metabolites were significantly different between the 2007-09 and 2009-11 cycles. This may represent high variability as it is unlikely that exposure would change significantly over a few years.

The results of Yaghjyan et al.[193] who used data from NHANES 1999-04 contrast with ours in that MEHP and MEHP/MEHHP showed positive associations and no notable associations were observed for MEHHP and MEOHP. However, the results of Stahlhut et al.[277] and Hatch et al.[281] are in greater agreement with ours in that MEHHP and MEOHP were more strongly associated with BMI and waist circumference than MEHP. The study by Yaghjyan et al.[193] only included women, but Hatch et al.[192] found a significant negative association between urinary MEHP and obesity in women aged 19-80 years and little to no association in men. Hatch et al.[192] used data from NHANES 1999-02 so this may be due to differences in methods as Hatch et al. modeled phthalate linearly by quartile while Yaghjyan et al.[193] used ordered logistic regression. Svensson et al.[282] also found that MEHP had a modest negative Spearman correlation with BMI and MEHHP a modest positive association, also in a female only sample.

Further research should focus on understanding the relative biological activity and health effects of MEHP, MEHHP and MEOHP. Further studies of metabolism to identify age and sex related differences would help contextualize any differences in association between the metabolites. Large samples can be an important tool in reducing measurement variability in urine samples and future cycles of NHANES and the CMHS can be combined with previous ones to

increase reliability. Investigating possible associations between DEHP metabolites and outcomes related to obesity such as diabetes and insulin resistance would help add to existing work on obesity.

This analysis of CMHS 2007-11 provided nationally representative estimates of urinary MEHP, MEHHP and MEOHP in Canadians aged 18-49 years. Both MEHHP and MEOHP showed significant positive associations with BMI ≥30 kg/m^2, while MEHP did not. MEHHP and MEOHP also showed some significant positive associations when modeled linearly, whether log-transformed or by quartile. The DEHP metabolite ratios of MEHP/MEHHP and MEHP/MEOHP produced highly significant negative associations when modeled linearly. These strong negative associations and the lack of an association between MEHP and measures of adiposity have been found in analyses of NHANES, although it has been suggested that MEHP is the most biologically active.

8.2.2 Child Sample

Generally the levels of DEHP metabolites found in Canadians aged 6-17 years are lower than in similar studies from other countries. In a sample of 599 German children aged 3-14 years, Becker at al.[289] found geometric means of 6.4 ng/ml (95% CI: 6.0, 6.8), 47.9 ng/ml (95% CI: 45.1, 50.8) and 37.0 ng/ml (95% CI: 34.9, 39.1) for MEHP, MEHHP and MEOHP, respectively. In NHANES 1999-2000[122], geometric means of MEHP for subjects aged 6-11 and 12-19 years were also higher at 5.12 ng/ml (95% CI: 4.25, 6.16) and 3.75 ng/ml (95% CI: 3.30, 4.27), respectively. A study of 521 black and Hispanic children aged 6-8 years from New York City did not calculate geometric means but found medians of MEHP, MEHHP and MEOHP of 6.3 ng/ml, 50.4 ng/ml and 75.7 ng/ml for boys and 6.5 ng/ml, 72.0 ng/ml, and 44.8 ng/ml for girls, respectively[290].

A smaller sample of 99 Mexican children found geometric means of 7.0 ng/ml, 20.9 ng/ml and 12.1 ng/ml for boys and 6.7 ng/ml, 23.4 ng/ml and 13.8 ng/ml for girls of MEHP, MEHHP and MEOHP, respectively[247]. A study of 259 Chinese children aged 8-15 years found a lower MEHHP geometric mean than CMHS 2007-11 of 16.1 ng/ml (SE: 1.0), although levels of MEHP were much higher at 21.3 ng/ml (SE:1.0) and MEOHP modestly higher at 22.9 ng/ml (SE:1.1)[291]. The authors also found a significant positive association between MEHP and age, while a modest decrease with age was seen in CHMS 2007-11.

Becker et al.[289] did not test for significance but found that MEHP levels increased steadily with age from 3 to 14 years-old. The authors found no notable differences by sex, similar to CHMS 2007-11 and NHANES 1999-02[279]. Urinary MEHP was higher in children aged

6-11 years than those aged 12-19 years in NHANES 1999-02[279]. Overall DEHP exposure was found to be higher in 6-11 year-olds than 12-19 year-olds in NHANES 2003-06[292].

Adjustment for urine creatinine had the expected effect of strengthening the negative associations with age, as it is generally lower in the 6-11 year-old age group than the 12-17 year-old age group[204]. Generally Canadians aged 6-17 years had lower levels of DEHP metabolites than that in similar populations such as the U.S. and Germany. Interestingly, children had similar urinary MEHP as adults in CHMS 2007-11, but much greater concentrations of MEHHP and MEOHP and a similar pattern is seen among girls and women in NHANES 1999-02[281]. This may be representative of age related differences in metabolism, although could also be due to differences in sources of exposure between adults and children. Higher consumption of food in relation to body weight has also been suggested[293].

Overall, this analysis found mostly negative associations between the urine concentrations of DEHP metabolites and measures of adiposity in Canadians aged 6-17 years. Similarly to CHMS 2007-11, in NHANES 1999-00 Hatch et al.[192] found negative associations between urinary MEHP and BMI in girls and a significant negative association in girls aged 12-19 years in linear regression by quartile. MEHHP quartile was positively associated with BMI in boys aged 12-19 years and girls aged 6-19 years. No notable associations were seen in CHMS 2007-11 when modeling MEHHP linearly against BMI whether log-transformed or by quartile. Modest positive associations were seen when modeling MEHHP in logistic regression in the 12-17 year-old age group, but other odds ratios were below the null. Trasande et al.[292] used data from NHANES 2003-06, but only included an overall sum of urinary DEHP metabolites in their regression modeling and found no notable associations with overweight, obesity or BMI z-score. In a sample of 387 black and Hispanic children from New York City, Teitalbaum et al.[290] found

no association between log-transformed DEHP metabolite concentrations and BMI z-score in linear regression. Log-transformed MEHP and MEHHP were modestly associated with waist circumference, and all three metabolites were negatively associated with height. In CHMS 2007-11, the DEHP metabolite ratios showed stronger positive linear associations than each of the metabolites by themselves, suggesting that MEHP may be more negatively associated with adiposity in children than MEHHP or MEOHP, despite their odds ratios being roughly similar. No previous studies that modeled DEHP metabolite ratios in children could be found in the literature.

A notable result of this analysis is that MEHHP and MEOHP were significantly positively associated with adiposity in adults, but negatively associated in children, especially in the 6-11 year-old age group. Furthermore, children were found to have higher concentrations of these chemicals than adults, while having a similar level of MEHP. MEHP steadily decreased with age in NHANES[122], although it was initially much higher than in CHMS 2007-11[281].

One of the possible mechanisms of DEHP exposure could contribute to adiposity is the inhibition of aromatase by activation of peroxisome proliferator-activated receptors (PPARs)[140,294,295]. Aromatase is an enzyme responsible for the conversion of testosterone to estradiol[140], and its suppression would theoretically increase circulating testosterone. A study of 425 adult men found that MEHP was negatively associated with testosterone, estradiol and an index of free androgens, and MEHHP and MEOHP exhibited similar weaker negative associations[296]. Furthermore, all three metabolites were similarly associated with estradiol/testosterone ratio[296]. PPARs also play important roles in adipocyte differentiation and lipid metabolism[294].

Other studies have found that DEHP exposure may have an anti-androgenic effect and decrease testosterone[162]. Most laboratory studies use DEHP as their exposure and very few have been conducted on these metabolites specifically. Desdoits-Lethimonier[141] concluded that MEHHP is probably responsible for some of the observed anti-androgenic activity from DEHP exposure[141]. Stroheker et al.[284] found that two unidentified metabolites of DEHP, which can reasonably be assumed to be MEHHP and MEOHP had anti-androgenic activity *in vitro*, while DEHP and MEHP did not. However a similar study found that MEHP, DEHP and two unidentified metabolites did not reduce testosterone production in cultured fetal rat testis[161]. Interestingly, MEHP was significantly negatively correlated with testosterone in studies of adult men[285] and 3 month old boys exposed via breast millk[297].

Data on the specific effects of MEHHP and MEOHP are scarce and studies of DEHP and MEHP have not been consistent. Assuming a hormonal mechanism is responsible for the positive associations between DEHP metabolites and many of the sex-based and metabolic outcomes explored in the literature, hormonal interactions may be very different in adults and children. As has been suggested with BPA[113], the overall effects of DEHP exposure may depend on levels of endogenous hormones, which would be very different between adults and children but also show notable variation at the ages of 6-17 years. This is further complicated by the fact that hormonal interactions in general often do not follow typical dose-response curves and may operate on a U-shaped (or inverted U-shaped) curve where opposite effects may be observed at larger and smaller doses[226].

The urinary levels of all three DEHP metabolites in this study showed significant differences between the 2007-09 and 2009-11 cycles. Measuring DEHP metabolites in urine can exhibit a high degree of variability as shown by Teitelbaum et al.[298] who found a joint ICC of

0.23 for the sum of MEHP, MEHHP and MEOHP in a sample of New York City children. Using data from NHANES 2001-10, Zota et al.[299] showed a high degree of variation in 11 different phthalates including DEHP metabolites over the 5 cycles. Given that the study covered a 10-year time span, it is possible that actual changes in the exposure of the U.S. population occurred, although large changes between consecutive cycles suggest general variability.

Other outcomes have also been explored in regard to DEHP exposure and children's health. Studies have found significant positive associations between DEHP in house dust and asthma[300] and wheezing[301], and well as DEHP metabolites and attention deficit hyperactivity disorder[302]. Furthermore, several other phthalates such as DBP[302], diethyl phthalate[301], and di-n-octyl phthalate[301] were tested and found not to have these associations. Other studies have found positive associations between MEHP and DEHP concentrations in maternal cord blood and low birth weight[303], and negative associations with gestational age[304], although that may not be relevant to a discussion of Canadians aged 6-17 years. A study of NHANES 2003-06 found that DEHP, and MEHP, MEHHP and MEOHP were significantly associated with systolic blood pressure in subjects aged 6-19 years[305]. Urinary DEHP was also significantly associated with insulin resistance in children in NHANES 2003-06[306].

The CHMS 2007-11 results provide little evidence of a positive association between adiposity and urinary DEHP metabolites in Canadians aged 6-17 years. Hatch et al.[281] found modest positive association between MEHHP and BMI and a significant negative association for MEHP in girls, but Trasande et al.[292] and Teitelbaum et al.[307] found little evidence of an association either way. Future work should focus on increasing sample sizes and standardizing methods for greater comparability, as well further elucidating the relative effects of MEHP, MEHHP and MEOHP.

This analysis of CMHS 2007-11 provided nationally representative estimates of urinary MEHP, MEHHP and MEOHP in Canadians aged 6-17 years. There was little indication of a positive association between any of the metabolites and adiposity, and all three DEHP metabolites showed some significant negative associations. Furthermore, ratios of the metabolites produced stronger negative associations, suggesting differences in biological activity between MEHP and the oxidative metabolites MEHHP and MEOHP. These results contrast with the adult sample where MEHHP and MEOHP showed significant positive associations with adiposity. If DEHP metabolites have an effect on adiposity through a hormonal (e.g., anti-androgenic) mechanism, it may depend on levels of endogenous hormones and have opposite effects at different concentrations.

9. GENERAL STRENGTHS AND LIMITATIONS

This analysis has several important strengths and limitations to consider. A strength of the CHMS is the use of direct measurements for measures of adiposity such as BMI, waist circumference and waist-to-hip ratio. Self-reporting of these measures may be subject to bias as subjects may under-report their BMI or waist circumference due to stigma about obesity. Other measures such as physical activity and sedentary behaviour may also be subject to social desirability bias due to stigma of a sedentary lifestyle.

The survey as a whole and the urine draw sample had response rates of ~50% in both cycles[194,195]. The activity monitor sample had response rates of ~40% and ~50% in 2007-09 and 2009-11, respectively[194,195]. The phthalate sample was included in the environmental urine sample in 2009-11 but in 2007-09 it was its own subsample with an overall response rate of ~50%[194,195]. These low response rates represent a potential form of bias. As stated above, participants may feel stigma about anthropomorphic variables such as BMI and waist circumference that may deter them from participating and induce a differential bias. Individuals may also not participate due to lack of interest which would likely represent a non-differential bias. Nevertheless, Statistics Canada made corrections in the sample weights to account for these non-responses biases.

A general issue with large surveys is that the people who participate in them tend to be wealthier and more educated than the general population. As having a low level of education and being low-income is associated with obesity, this sample may not accurately represent the obese population of Canada. However, the financial compensation offered for participation in the

CHMS as well as the sample adjusted weights employed by Statistics Canada should have offset these participation tendencies.

Outliers in with BPA or DEHP metabolites concentrations may have a significant impact on analysis and produce misleading results. A sensitivity analysis was conducted by generating scatter plots, eliminating apparent outliers and repeating analyses to observe any changes in results or significance. In each of the samples there were several data points that fell far away from the majority of data yet their removal did not appreciably change results. A more aggressive approach was then taken where data points with high values that arguably were not outliers were eliminated, with ~5% of the sample being removed. Predictably, this had a more noticeable effect where associations were weakened and some lost significance. Overall, very few data points were apparent outliers and their removal had minimal impact.

A notable difference between the cycles was that the method of urine collection for measuring urine creatinine was changed from midstream in CHMS 2007-09[194] to first catch in CHMS 2009-11[195]. This change was implemented in order to include an infectious disease module, and it is unclear what effect it may have had on this analysis. Another notable difference was that adjustments were made to the 2007-09 phthalate data[194] to correct inaccuracies and to enable comparability between cycles.

As stated earlier, analyte concentrations in urine biomonitoring have been observed to vary significantly over the course of a day, and between days in a given subject. ICCs for BPA have generally been low, where a value of 0.09 was found for spot samples taken over the course of a week[89]. A similar study collected daily urine samples for 8 days and found higher ICCs for MEHP, MEHHP and MEOHP of 0.29, 0.24 and 0.23, respectively[166]. Fisher et al.[308] collected six urine samples from a group of pregnant women from before 20 weeks gestation to 2-3

months after delivery. The positive predictive values for a single sample to classify a subject above or below a reference CHMS geometric mean were 0.61, 0.86, 0.74 and 0.76 for BPA, MEHP, MEHHP and MEOHP, respectively[308]. These analyses highlight the issue of exposure misclassification, which is especially relevant in logistic regression where subjects are divided into quartiles. If all subjects exhibit the same degree of variation in the analyte content of their urine, then the potential bias is referred to as non-differential which is classically believed to shift measures of association like odds ratios closer to the null[309]. If, for example, certain subjects metabolize BPA faster than others, then they might show a higher level of variation and would create a differential bias. The effect of differential misclassification is difficult to predict and could result in the under- or overestimation of a true value.

As several different measures of adiposity were used in this analysis, there exists the possibility that significance was achieved by chance through multiple testing. This is not seen as a major concern as all outcomes are interrelated and interpreted in the context of one another. For example, Lang et al.[117] investigated associations between urinary BPA concentrations and many disparate outcomes such as arthritis, asthma, cancer, diabetes, stroke and thyroid disease. This is a study design where associations are interpreted much more independently and likely has a greater risk of achieving significance through multiple testing.

A major limitation of this analysis is that it is cross-sectional, meaning that causality cannot be inferred and observed positive associations may be due to reverse-causation. Longitudinal or intervention research is needed to establish causality and show a temporal association between exposure to BPA or phthalates and adiposity. However, large sample sizes are an asset in human biomonitoring to reduce variability and, due to cost, longitudinal studies generally have lower sample sizes.

Hays et al.[310] used data from NHANES 2009-12, which unlike previous cycles collected data on urinary flow, to investigate the possibility of reverse causation. The authors found that urinary output rates do not increase linearly per kilogram body weight and suggested that urine is naturally more concentrated in individuals with a higher BMI. This could result in reverse causation where a higher BMI reduces urinary flow rate and increases BPA urine concentration. The authors state that using an excretion rate rather than a concentration corrects for this issue and showed that the excretion rate of BPA actually has a significant negative association with BMI category, while an unadjusted concentration shows a significant positive association[310]. The authors also state that methods to correct for dilution such as urine creatinine do not fully take into account the systematic variations in urinary flow rate seen in their analysis, and urine creatinine is also known to vary systematically, for example, by age, sex and BMI[204]. Fernandez et al.[311] found a mean BPA concentration in human adipose tissue of 3.16 ng/g (SD: 4.11 ng/g), and it has been suggested that the accumulation of BPA in adipose tissue could be a route of reverse causation[110]. However, Fernandez et al.[311] found no association between BPA concentration in adipose tissue and BMI.

Another important limitation in this study is the possibility of confounding where excessive intake of BPA-containing and energy-dense foods, such as soft drinks, causes both increased adiposity and increased BPA concentrations in urine. However, two Canadians studies showed that the BPA concentration in soft drinks is ~1000 times less than in canned foods, 270 ng/l[312] vs. 35 ng/g[15], and are not necessarily major contributors to obesity. This may be offset by individuals consuming much greater amounts of soft drinks as Lakind & Naiman[109] found that soda consumption, but not canned tuna, was significantly associated with a higher urinary BPA concentration in NHANES 2005-06. Another study found that soft drink consumption was

significantly associated with BPA urine concentration in children 9 years of age, but not in their mothers during pregnancy[115]. It is possible that this idea is less relevant for adults than children and adolescents as soft drink consumption decreases with age[313].

Another notable issue is the possibility of urine samples being contaminated with extraneous BPA. House dust is known to contain traces of BPA, and one study detected a median of 553 µg/kg in samples from 12 different homes[95]. Laboratory equipment such as the containers used to collect and store urine are sometimes made with BPA containing materials and are also a potential source of contamination[234]. In a review of BPA exposure and associations with obesity, only three of the nine studies on urine concentration were deemed to have provided sufficient information on whether BPA-free materials had been used[314]. The CHMS used laboratory blanks of deionized water as a measure of contamination and their BPA concentration was subtracted from each analytical sequence[201,315]. In CHMS 2007-09 the blanks had a mean BPA concentration of 0.41 ng/ml and a range of 0.08 to 1.27 ng/ml, approximately one third of the concentration of 1.13 ng/ml (95% CI: 1.07, 1.20) found in this analysis in the 2009-11 sample. Urinary BPA consists mostly of its conjugated form, which is only formed *in vivo* and can add confidence that contamination was not a significant factor[316], although the CHMS did not measure BPA and BPA-glucuronide separately. A similar concept can be applied to measuring the metabolites of DEHP, where the oxidative metabolites MEHHP and MEOHP are also only formed *in vivo* but, a large presence of the monoester MEHP may indicate contamination[316].

10. CONCLUSION

This analysis of CMHS 2007-11 provided national representative estimates of urinary BPA in Canadians aged 6 years or older, and urinary MEHP, MEHHP and MEOHP in Canadians aged 6-49 years. In both adults and children, BPA levels were lower than in similar studies such as NHANES[100]. Unadjusted geometric means showed greater urine concentrations among men, although they became significantly greater in women after adjustment for urine creatinine. A significant negative association with age was seen in children after creatinine adjustment. In logistic regression, BPA showed modest positive associations with BMI ≥30 kg/m^2 and elevated waist-to-hip ratio, and little association with waist circumference. Log-transformed BPA was significantly associated with all three measures of adiposity in linear regression. Conversely, the results from the child sample showed little indication of a positive association with adiposity while several significant negative associations were seen. These disparate results may be due to differing effects of BPA based on levels of endogenous hormones such as estrogen and testosterone[113], which would be lower in children than adults and also vary considerably over the age range of our child sample of 6-17 years[317]. This is further complicated by the fact that hormones often do not follow typical dose-response patterns[226], and may elicit opposite effects at different concentrations. An expected dose-response would be that the more disparate quartile comparisons (i.e., 4^{th} to 1^{st} quartile) would produce the strongest associations and the 2^{nd} to 1^{st} quartile comparison would produce the weakest. This pattern was only sparsely seen in this and previous analyses.

A notable issue in BPA research is that the methods of detection commonly employed in large studies do not distinguish between BPA and its major metabolite BPA-glucuronide. Smaller studies have measured them separately and found most urinary BPA to be BPA-

glucuronide[88,95]. Furthermore, while direct comparisons are sparse, BPA-glucuronide appears to have very little to no hormonal activity[78] – in other words, it appears that free BPA may an obesogen, whereas BPA-glucuronide is not. Further research should focus on confirming this and modeling associations separately between free BPA and BPA-glucuronide.

Analysis of urinary DEHP metabolites in adults showed concentrations similar to other studies. Levels were generally higher in men, but became higher in women after adjustment for urine creatinine. Negative associations with age were attenuated after creatinine adjustment. In logistic and linear regression analysis, MEHHP and MEOHP showed significant positive associations with adiposity, while MEHP showed little association either way. It has been suggested that MEHP may be the most biologically active of the three and that conversion to MEHHP and MEOHP may be protective[156], as it reduces the bioavailability of MEHP. Modeling the metabolite ratios MEHP/MEHHP and MEHP/MEOHP produced significant negative associations in linear regression and do not support this notion. However, several epidemiological studies[192,277] are in agreement with this analysis in finding stronger positive associations with MEHHP and MEOHP than MEHP.

Urinary DEHP metabolites in children were found to be lower than in similar studies such as NHANES[281] and a German study[318]. Interestingly, urinary MEHP concentrations were similar in adults and children, but MEHHP and MEOHP levels were notably greater in children. This may be due to a combination of different metabolisms and sources of exposure between adults and children, although detailed studies of metabolism in children are needed to confirm this.

In the child sample, all three metabolites showed significant negative associations with adiposity, and most odds ratios were below the null. With respect to MEHHP and MEOHP, this

bears similarity to the BPA analysis where opposite associations were observed in adults and children. Laboratory studies of DEHP metabolites are scarce, although there is indication of anti-androgenic activity[284]. Varying levels of endogenous hormones and the non-monotonic dose-response relationships of may help explain this discrepancy. Future research should include detailed studies of metabolism in children similar to Koch et al.[164] as well as establishing the relative biological activity of MEHP, MEHHP and MEOHP.

A notable issue in human monitoring in general is measurement variability. Urinary BPA was similar between CHMS 2007-09 and 2009-11, but all three DEHP metabolites showed significantly different geometric means between the cycles. The cycles were conducted in a short enough time span that it seems unlikely that significant changes in exposure in a large population could take place, although analysis of NHANES 2001-10[299] shows that levels of phthalates have steadily declined, in some cases significantly.

The large sample sizes used in this analysis also likely help alleviate some of the variability. Children are believed to present special challenges as they are still developing and may be more sensitive to hormone like effects. Children may also have greater exposure in proportion to their body weight than adults. As several significant negative associations were observed, this analysis found that it is unlikely that BPA and DEHP are contributing to adiposity in Canadians aged 6-17 years. Observed positive associations in adults between BPA, MEOHP and MEHHP and adiposity require further study to elucidate.

11. DIRECTIONS FOR FUTURE RESEARCH

It is important that future research establish the relatively estrogenicity of BPA and BPA-glucuronide. Several reports have shown that BPA-glucuronide makes up the majority of BPA in urine[88,95,239], while most of the large studies such as NHANES and CHMS only measure total BPA. Matthews et al.[78] show that BPA-glucuronide has little to no estrogenic activity *in vitro*, but further studies that make similarly direct comparisons between the two are needed to confirm this. Studies of BPA metabolism in children similar to Volkel et al.[87] would be helpful to elucidate possible differences in metabolism between children and adults. Another important direction for future research is establishing a TDI more specific to metabolic outcomes and not developmental or reproductive toxicity. This would allow researchers to more effectively interpret epidemiological data, and make more specific conclusions about the potential health effects of BPA. Future epidemiological research should focus on standardizing methods, as some studies[101,110] did not use urine creatinine or another method to correct for dilution. It is also important that future studies use only geometric means, and not arithmetic means or medians.

An important future direction for DEHP research is establishing the relative biological activity of MEHP, MEHHP and MEOHP, which would help to interpret differing associations between them. Epidemiological studies on DEHP and adiposity or related conditions should use the metabolite ratios MEHP/MEHHP and MEHP/MEOHP as the primary independent variable in regression models to corroborate previous results.

Generally variability is an inherent problem in urine biomonitoring, and future studies should continue to combine available cycles of large surveys such as CHMS and NHANES to reduce variability and add confidence to assertions.

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13. APPENDICES

13.1 Appendix A: Additional Bisphenol A analysis

13.1.1 Adult Sample

Table A.1 Log-transformed urinary bisphenol A modeled linearly against measures of adiposity in the activity monitor sample.

Dependent variable	β coefficient*	P-value
Body mass index	0.3441	0.0035
Waist circumference	0.9861	0.0035
Waist-to-hip ratio	0.0052	0.0445

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

Table A.2 Urinary bisphenol A quartile modeled linearly against measures of adiposity in the activity monitor sample.

Dependent variable	β coefficient*	P-value
Body mass index	0.2911	0.0313
Waist circumference	0.7125	0.1039
Waist-to-hip ratio	0.0035	0.3034

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

13.1.2 Child Sample

Table A.3 Urinary bisphenol A quartile modeled linearly against measures of adiposity.

Dependent variable	β coefficient*	P value
Body mass index	-0.0662	0.5767
Waist circumference	-0.0484	0.8760
Waist-to-hip ratio	0.0001	0.9619

* Adjusted for age, sex and urine creatinine

Table A.4 Log-transformed urinary bisphenol A modeled linearly against measures of adiposity in the activity monitor sample.

Dependent variable	β coefficient*	P-value
Body mass index	-0.3570	0.0168
Waist circumference	-0.9350	0.0189
Waist-to-hip ratio	-0.0036	0.0880

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

Table A.5 Urinary bisphenol A quartile modeled linearly against measures of adiposity in the activity monitor sample.

Dependent variable	β coefficient*	P-value
Body mass index	-0.3470	0.0244
Waist circumference	-0.8355	0.0417
Waist-to-hip ratio	-0.0024	0.2476

* Adjusted for urine creatinine, age, sex, race, education, smoking, physical activity and sedentary behaviour.

Table A.6 Log-transformed urinary bisphenol A concentration modeled linearly against skinfold measurements.

Dependent variable	β coefficient*	P-value
Triceps	-0.0095	0.9551
Biceps	-0.0712	0.4964
Subscapular	-0.0464	0.8288
Iliac crest	0.0013	0.9963
Medial calf	0.0081	0.9753
Sum of skinfolds	-0.1176	0.9055

* Adjusted for age, sex and urine creatinine

13.2 Appendix B: Additional analysis of phthalates Monobenzyl Phthalate, Monethyl Phthalate and Mono-n-butyl Phthalate

13.2.1 Adult Sample

Table A.7 Geometric means (95% CI) of urinary MBzP, MEP and MBP in Canadians aged 18 or older in CHMS 2007-11 stratified by sex and age.

Characteristic	N (%)*	Geometric mean (ng/ml)	P-value
MBzP			
Total	2,056	8.49 (7.53, 9.58)	0.0208**
Sex			
Men	1,038 (50.5)	8.99 (7.78, 10.38)	Reference
Women	1,018 (49.5)	8.02 (6.81, 9.44)	0.2226
Age (years)			
18-29	811 (39.4)	10.64 (8.90, 12.71)	Reference
30-39	541 (26.3)	7.07 (5.57, 8.98)	0.0027
40-49	704 (34.3)	7.55 (6.57, 8.68)	0.0012
MEP			
Total	2,056	55.26 (47.68, 64.05)	0.0419**
Sex			
Men	1,038 (50.5)	58.44 (48.03, 71.12)	Reference
Women	1,018 (49.5)	52.20 (44.19, 61.65)	0.2782
Age			
18-29	811 (39.4)	61.77 (49.43, 77.20)	Reference
30-39	541 (26.3)	50.34 (40.64, 62.36)	0.0837
40-49	704 (34.3)	52.23 (43.62, 62.52)	0.2199
MBP			
Total	2,054	20.18 (18.42, 22.10)	0.2854**
Sex			
Men	1,039 (50.6)	20.79 (18.59, 23.26)	Reference
Women	1,015 (49.4)	19.56 (17.59, 21.76)	0.3107
Age			
18-29	811 (39.4)	23.06 (20.72, 25.66)	Reference

30-39	538 (26.2)	18.93 (15.97,	0.0245
40-49	705 (34.3)	18.17 (15.76, 20.94)	0.0018

CHMS, Canadian Health Measures Survey; CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.
* N (%) is weighted to the population using standardized weights.
** P-value for the different in geometric mean between the 2007-09 and 2009-11 cycles.

Table A.8 Geometric means (95% CI) of urinary MBzP, MEP and MBP adjusted for urine creatinine in Canadians aged 18 or older in CHMS 2007-11 stratified by sex and age.

Characteristic	N (%)*	Geometric mean (µg/g)	P-value
MBzP			
Total	2,056	8.05 (7.17, 9.04)	<0.0001**
Sex			
Men	1,038 (50.5)	7.22 (6.41, 8.14)	
Women	1,018 (49.5)	9.00 (7.74, 10.45)	0.0014
Age			
18-29	811 (39.4)	9.01 (7.56, 10.73)	Reference
30-39	541 (26.3)	7.00 (5.57, 8.81)	0.0521
40-49	704 (34.3)	7.87 (6.92, 8.95)	0.1964
MEP			
Total	2,056	52.42 (46.62, 58.95)	<0.0001**
Sex			
Men	1,038 (50.5)	46.98 (40.39, 54.64)	Reference
Women	1,018 (49.5)	58.63 (50.34, 68.28)	0.0199
Age			
18-29	811 (39.4)	52.42 (43.07, 63.81)	Reference
30-39	541 (26.3)	49.92 (40.81, 61.05)	0.6914
40-49	704 (34.3)	54.43 (46.26, 64.05)	0.7746
Total	2,054	19.08 (18.0, 20.22)	<0.0001**
Sex			
Men	1,039 (50.6)	16.70 (15.68, 17.80)	Reference
Women	1,015 (49.4)	21.86 (20.06, 23.81)	<0.0001
Age			
18-29	811 (39.4)	19.54 (18.30, 20.86)	Reference
30-39	538 (26.2)	18.60, (16.16, 21.40)	0.4702
40-49	705 (34.3)	18.92 (17.12, 20.91)	0.5664

CHMS, Canadian Health Measures Survey; CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.

* N (%) is weighted to the population using standardized weights.
** P-value for the different in geometric mean between the 2007-09 and 2009-11 cycles.

Table A.9 Odds ratios (95% CI) for BMI ≥ 30kg/m^2 in 2nd, 3rd and 4th MBzP, MEP or MBP quartiles as compared to the 1st quartile.

	Model 1*		Model 2**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
MBzP				
Quartile 4	1.97 (1.16, 3.34)	0.0117	1.75 (0.99, 3.10)	0.0550
Quartile 3	1.50 (0.77, 2.90)	0.2324	1.43 (0.69, 2.94)	0.3345
Quartile 2	1.03 (0.61, 1.76)	0.9062	0.97 (0.54, 1.73)	0.9130
Quartile 1	1.00	Reference	1.00	Reference
MEP				
Quartile 4	1.27 (0.59, 2.70)	0.5432	1.41 (0.67, 2.98)	0.3676
Quartile 3	1.12 (0.60, 2.09)	0.7322	1.21 (0.62, 2.36)	0.5747
Quartile 2	1.14 (0.56, 2.32)	0.7246	1.21 (0.56, 2.62)	0.6236
Quartile 1	1.00	Reference	1.00	Reference
MBP				
Quartile 4	0.84 (0.41, 1.72)	0.6294	0.85 (0.38, 1.90)	0.6953
Quartile 3	1.25 (0.49, 3.21)	0.6378	1.23 (0.48, 3.15)	0.6645
Quartile 2	1.65 (0.82, 3.30)	0.1591	1.59 (0.83, 3.05)	0.1614
Quartile 1	1.00	Reference	1.00	Reference

CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.
* Adjusted for urine creatinine, age and sex.
** Adjusted for race education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table A.10 Odds ratios (95% CI) for elevated waist circumference in 2nd, 3rd and 4th MBzP, MEP or MBP quartiles as compared to the 1st quartile.

	Model 1*		Model 2**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
MBzP				
Quartile 4	2.14 (1.07, 4.28)	0.0317	2.02 (1.01, 4.03)	0.0477
Quartile 3	2.19 (1.15, 4.18)	0.0170	2.11 (1.01, 4.41)	0.0477
Quartile 2	1.48 (0.89, 1.46)	0.1269	1.40 (0.81, 2.42)	0.2311
Quartile 1	1.00	Reference	1.00	Reference
MEP				
Quartile 4	1.21 (0.60, 2.45)	0.5916	1.31 (0.63, 2.69)	0.4697
Quartile 3	0.84 (0.47, 1.51)	0.5664	0.89 (0.49, 1.62)	0.6986
Quartile 2	0.96 (0.54, 1.73)	0.8965	1.05 (0.59, 1.86)	0.8671
Quartile 1	1.00	Reference	1.00	Reference
MBP				
Quartile 4	1.29 (0.64, 2.58)	0.4770	1.39 (0.66, 2.94)	0.3937
Quartile 3	1.20 (0.54, 2.64)	0.6573	1.29 (0.58, 2.88)	0.5320
Quartile 2	1.30 (0.76, 2.23)	0.3423	1.25 (0.76, 2.04)	0.3839
Quartile 1	1.00	Reference	1.00	Reference

CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.

* Adjusted for urine creatinine, age and sex.

** Adjusted for race education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table A.11 Odds ratios (95% CI) for elevated waist-to-hip ratio in 2nd, 3rd and 4th MBzP, MEP or MBP quartiles as compared to the 1st quartile.

	Model 1*		Model 2**	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
MBzP				
Quartile 4	1.29 (0.66, 2.53)	0.4611	1.41 (0.72, 2.77)	0.3194
Quartile 3	1.41 (0.97, 2.05)	0.0735	1.46 (0.94, 2.26)	0.0909
Quartile 2	0.87 (0.48, 1.55)	0.6305	0.81 (0.44, 1.47)	0.4796
Quartile 1	1.00	Reference	1.00	Reference
MEP				
Quartile 4	1.41 (0.71, 2.79)	0.3274	1.33 (0.66, 2.66)	0.4264
Quartile 3	1.11 (0.56 2.18)	0.7726	1.11 (0.54, 2.26)	0.7801
Quartile 2	1.45 (0.78, 2.70)	0.2410	1.47 (0.78, 2.75)	0.2338
Quartile 1	1.00	Reference	1.00	Reference
MBP				
Quartile 4	0.77 (0.37, 1.63)	0.5010	0.72 (0.33, 1.60)	0.4218
Quartile 3	0.80 (0.41, 1.58)	0.5212	0.87 (0.42, 1.83)	0.7149
Quartile 2	1.25 (0.85, 1.83)	0.2614	1.20 (0.83, 1.71)	0.3343
Quartile 1	1.00	Reference	1.00	Reference

CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.

* Adjusted for urine creatinine, age and sex.

** Adjusted for race education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table A.12 Log-transformed MBzP modeled linearly against measures of adiposity.

	Model 1*		Model 2**	
	β coefficient	P-value	β coefficient	P-value
MBzP				
Body mass index	0.6173	<0.0001	0.5238	0.0034
Waist circumference	1.773	<0.0001	1.3791	0.0023
Waist-to-hip ratio	0.0075	0.0241	0.0068	0.0402
MEP				
Body mass index	0.2167	0.2209	0.2883	0.1075
Waist circumference	0.3506	0.4039	0.5387	0.2036
Waist-to-hip ratio	0.0014	0.4685	0.0015	0.4474
MBP				
Body mass index	0.0261	0.9239	0.0.1213	0.6639
Waist circumference	-0.1821	0.7912	0.0995	0.8858
Waist-to-hip ratio	-0.0004	0.9059	0.0000	0.9815

MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.
* Adjusted for urine creatinine, age and sex.
** Adjusted for race education, smoking, physical activity and sedentary behaviour in addition to model 1 covariates.

Table A.13 MBzP, MEP and MBP quartile modeled linearly against measures of adiposity

	MBzP		MEP		MBP	
	β coefficient*	P-value	β coefficient*	P-value	β coefficient*	P-value
Body mass index	0.7109	0.0002	0.2412	0.5152	0.2550	0.4593
Waist circumference	1.9548	0.0004	0.6910	0.4475	0.4031	0.6089
Waist-to-hip ratio	0.0082	0.0126	0.0009	0.8352	0.0039	0.2530

MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.
* Adjusted for age sex, urine creatinine, race, education, smoking, physical activity and sedentary behaviour

12.2.2 Child Sample

Table A.14 Geometric means (95% CI) of urinary MBzP in Canadians aged 6-17 years in CHMS 2007-11 stratified by sex and age.

Characteristics	N (%)*	Geometric mean (ng/ml)	P-value
MBzP			
Total	2,719	17.46 (15.88, 19.20)	0.0164**
Sex			
Boys	1,420 (52.2)	18.16 (16.08, 20.52)	Reference
Girls	1,299 (47.8)	16.73 (14.88, 18.81)	0.2722
Age			
6-11	1,249 (45.9)	19.83 (17.57, 22.38)	Reference
12-17	1,470 (54.1)	15.67 (14.08, 17.45)	0.0003
MEP			
Total	2,719	38.78 (35.11, 42.85)	0.4106
Sex			
Boys	1,420 (52.2)	35.72 (31.20, 40.90)	Reference
Girls	1,299 (47.8)	42.43 (38.14, 47.20)	0.0218
Age			
6-11	1,249 (45.9)	27.64 (24.19, 31.57)	Reference
12-17	1,470 (54.1)	51.72 (46.05, 50.08)	<0.0001
MBP			
Total	2,718	32.99 (30.92, 35.19)	0.8477
Sex			
Boys	1,419 (52.2)	33.28 (29.85, 37.10)	Reference
Girls	1,299 (47.8)	32.67 (30.25, 35.29)	0.7914
Age			
6-11	1,248 (45.9)	34.89 (31.57, 38.55)	Reference
12-17	1,470 (54.1)	31.45 (28.78, 34.37)	0.1324

CHMS, Canadian Health Measures Survey; CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.

* N (%) is weighted to the population using standardized weights.

** P-value for the different in geometric mean between the 2007-09 and 2009-11 cycles.

Table A.15 Geometric means (95% CI) of urinary MBzP adjusted for urine creatinine in Canadians aged 6-17 years in CHMS 2007-11 stratified by sex and age.

Characteristics	N (%)*	Geometric mean (µg/g)	P-value
MBzP			
Total	2,719	17.86 (16.31 (19.56)	<0.0001
Sex			
Boys	1,420 (52.2)	17.78 (16.03, 19.72)	Reference
Girls	1,299 (47.8)	17.94 (15.91, 20.23)	0.8948
Age			
6-11	1,249 (45.9)	26.03 (23.30, 29.09)	Reference
12-17	1,470 (54.1)	12.97 (11.74, 14.32)	<0.0001
MEP			
Total	2,719	39.64 (36.17, 43.44)	0.0010
Sex			
Boys	1,420 (52.2)	34.95 (31.41, 38.9)	Reference
Girls	1,299 (47.8)	45.48 (40.89, 50.57)	<0.0001
Age			
6-11	1,249 (45.9)	36.32 (31.73, 41.58)	Reference
12-17	1,470 (54.1)	42.69 (38.87, 46.88)	0.0222
MBP			
Total	2,718	33.75 (31.80, 35.82)	0.0004
Sex			
Boys	1,419 (52.2)	32.64 (29.48, 36.15)	Reference
Girls	1,299 (47.8)	35.00 (32.75, 37.40)	0.2715
Age			
6-11	1,248 (45.9)	45.85 (42.28, 49.73)	Reference
12-17	1,470 (54.1)	26.02 (24.11, 28.07)	<0.0001

CHMS, Canadian Health Measures Survey; CI, confidence interval; MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.

* N (%) is weighted to the population using standardized weights.

** P-value for the different in geometric mean between the 2007-09 and 2009-11 cycles.

Table A.16 Odds ratios (95% CI) for obesity by protocol in 2nd, 3rd and 4th MBzP quartiles as compared to the 1st quartile.

CDC	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	1.26 (0.75, 2.12)	0.91 (0.32, 2.54)	2.02 (0.84, 4.86)
Quartile 3	1.17 (0.67, 2.03)	0.86 (0.28, 2.65)	1.55 (0.52, 4.62)
Quartile 2	0.81 (0.52, 1.26)	0.84 (0.34, 2.05)	0.75 (0.32, 1.77)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

IOTF	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	0.86 (0.55, 1.34)	0.58 (0.26, 1.27)	1.12 (0.65, 1.93)
Quartile 3	0.86 (0.55, 1.35)	0.54 (0.24, 1.19)	1.16 (0.58, 2.33)
Quartile 2	0.91 (0.61, 1.35)	0.64 (0.32, 1.30)	1.09 (0.67, 1.75)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

WHO	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	1.20 (0.70, 2.07)	0.92 (0.33, 2.51)	1.72 (0.74, 4.00)
Quartile 3	1.16 (0.68, 1.99)	0.91 (0.31, 2.69)	1.45 (0.50, 4.18)
Quartile 2	0.83 (0.54, 1.29)	0.95 (0.38, 2.37)	0.70 (0.30, 1.62)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task; MBzP, Monobenzyl phthalate; WHO, World Health Organization.

* Model 1 adjusted for age, sex and urine creatinine.

** Model 2 adjusted for physical activity, sedentary behaviour, race and highest level of household education in addition to model 1 covariates.

Table A.17 Odds ratios (95% CI) for obesity by protocol in 2nd, 3rd and 4th MEP quartiles as compared to the 1st quartile.

CDC	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	1.02 (0.57, 1.81)	0.69 (0.19, 2.48)	1.05 (0.38, 2.94)
Quartile 3	1.77 (1.04, 3.02)	2.21 (1.00, 4.91)	1.07 (0.24 4.70)
Quartile 2	1.21 (0.74, 2.00)	1.33 (0.64, 2.76)	1.19 (0.34, 4.16)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

IOTF	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	0.89 (0.50, 1.59)	0.79 (0.34, 1.84)	0.87 (0.37, 2.02)
Quartile 3	1.34 (0.90, 1.99)	2.35 (1.16, 4.75)	0.80 (0.38, 1.67)
Quartile 2	1.09 (0.76, 1.55)	1.20 (0.65, 2.21)	0.99 (0.53, 1.85)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

WHO	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	0.98 (0.55, 1.76)	0.74 (0.23, 2.34)	0.97 (0.35, 2.73)
Quartile 3	1.86 (1.09, 3.19)	2.37 (1.05, 5.35)	1.16 (0.26, 5.20)
Quartile 2	1.20 (0.73, 1.95)	1.28 (0.61, 2.67)	1.18 (0.33, 4.15)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task Force; MEP, Monoethyl phthalate. WHO, World Health Organization.

* Model 1 adjusted for age, sex and urine creatinine.

** Model 2 adjusted for physical activity, sedentary behaviour, race and highest level of household education in addition to model 1 covariates.

Table A.18 Odds ratios (95% CI) for obesity by protocol in 2nd, 3rd and 4th MBP quartiles as compared to the 1st quartile.

CDC	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	0.66 (0.25, 1.71)	0.17 (0.05, 0.61)	1.61 (0.37, 7.01)
Quartile 3	0.95 (0.44, 2.05)	0.36 (0.12, 1.12)	1.35 (0.45, 4.06)
Quartile 2	0.89 (0.51, 1.57)	0.58 (0.24, 1.39)	0.96 (0.49, 1.90)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

IOTF	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	0.47 (0.25, 0.86)	0.15 (0.08, 0.32)	0.91 (0.35, 2.39)
Quartile 3	0.64 (0.36, 1.14)	0.37 (0.15, 0.90)	0.77 (0.43, 1.38)
Quartile 2	0.72 (0.48, 1.08)	0.54 (0.27, 1.05)	0.79 (0.42, 1.51)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

WHO	Total	6-11 years	12-17 years
	Odds ratio (95% CI)*	Odds ratio (95% CI)**	Odds ratio (95% CI)**
Quartile 4	0.61 (0.23, 1.58)	0.17 (0.05, 0.58)	1.38 (0.31, 6.17)
Quartile 3	0.92 (0.43, 1.95)	0.38 (0.13, 1.15)	1.22 (0.46, 3.26)
Quartile 2	0.89 (0.50, 1.56)	0.65 (0.28, 1.51)	0.84 (0.45, 1.57)
Quartile 1	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)

CDC, Centers for Disease Control; CHMS, Canadian Health Measures Survey; CI, confidence interval; IOTF, International Obesity Task Force; MBP, Mono-n-butyl phthalate; WHO, World Health Organization.

* Model 1 adjusted for age, sex and urine creatinine.

** Model 2 adjusted for physical activity, sedentary behaviour, race and highest level of household education in addition to model 1 covariates.

Table A.19 Log-transformed MBzP modeled linearly against measures of adiposity.

Measure	β coefficient*	P-value
Body mass index	0.0320	0.7970
Waist circumference	0.4122	0.2209
Waist-to-hip ratio	0.0057	0.0024
	6-11 years	
Body mass index	0.0984	0.5284
Waist circumference	0.2519	0.5259
Waist-to-hip ratio	0.0050	0.0310
	12-17 years	
Body mass index	-0.0711	0.7298
Waist circumference	0.4336	0.3945
Waist-to-hip ratio	0.0068	0.0036

MBzP, Monobenzyl phthalate.
* Model 1 adjusted for age, sex and urine creatinine.
** Model 2 adjusted for physical activity, sedentary behaviour, race and highest level of household education in addition to model 1 covariates.

Table A.20 Log-transformed MEP modeled linearly against measures of adiposity.

Measure	β coefficient*	P-value
Body mass index	0.1116	0.3079
Waist circumference	0.4074	0.1151
Waist-to-hip ratio	0.0018	0.3792
	6-11 years	
Body mass index	0.1145	0.4812
Waist circumference	0.3990	0.2784
Waist-to-hip ratio	0.0033	0.1668
	12-17 years	
Body mass index	0.0775	0.6471
Waist circumference	0.5365	0.2047
Waist-to-hip ratio	0.0024	0.4208

MEP, Monoethyl phthalate.

* Model 1 adjusted for age, sex and urine creatinine.
** Model 2 adjusted for physical activity, sedentary behaviour, race and highest level of household education in addition to model 1 covariates.

Table A.21 Log-transformed MBP modeled linearly against measures of adiposity.

	β coefficient*	P-value
Body mass index	-0.3477	0.0703
Waist circumference	-0.8153	0.0727
Waist-to-hip ratio	0.0009	0.6996
	6-11 years	
Body mass index	-0.4125	0.0120
Waist circumference	-1.1401	0.0010
Waist-to-hip ratio	0.0012	0.6235
	12-17 years	
Body mass index	-0.2252	0.5742
Waist circumference	-0.2111	0.8096
Waist-to-hip ratio	0.0029	0.3231

MBP, Mono-n-butyl phthalate.
* Model 1 adjusted for age, sex and urine creatinine.
** Model 2 adjusted for physical activity, sedentary behaviour, race and highest level of household education in addition to model 1 covariates.

Table A.22 MBzP, MEP and MBP quartile modeled linearly against measures of adiposity.

	MBzP		MEP		MBP	
	β coefficient*	P-value	β coefficient*	P-value	β coefficient*	P-value
Body mass index	0.1410	0.3546	0.1943	0.0943	-0.2526	0.1634
Waist circumference	0.6521	0.0799	0.6770	0.0220	-0.5247	0.2113
Waist-to-hip ratio	0.0059	0.0019	0.0036	0.1392	0.0017	0.2668

MBP, Mono-n-butyl phthalate; MBzP, Monobenzyl phthalate; MEP, Monoethyl phthalate.
* Adjusted for age sex and urine creatinine.