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Tea consumption in pregnancy as a predictor of pesticide exposure and adverse birth outcomes: The MIREC Study

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ABSTRACT

Introduction: Pesticide residues in tea may contribute to exposure during pregnancy; however, the impact on maternal and infant health is not well understood. The aim of this study was to determine whether tea intake in the first trimester was associated with elevated concentrations of various pesticides in maternal blood or urine. Further, we examined the relationship between tea consumption and adverse birth outcomes.

Methods: Data from the Maternal–Infant Research on Environmental Chemicals (MIREC) Study, a pan-Canada pregnancy cohort, were used. All singleton, live births ($n = 1898$) with available biomarkers were included in the analyses. Descriptive statistics were used to characterize the population. The geometric means (GM) of organochlorine (OC) pesticide constituents or metabolites in maternal plasma (lipid adjusted) and organophosphate (OP) pesticide metabolites (adjusted for specific gravity) in maternal urine were calculated for participants who drank regular, green or herbal tea in the first trimester and for those who did not. Differences between groups were examined using chi-square or t-tests. Associations between frequency of drinking tea and adverse birth outcomes were examined using logistic regression (preterm birth and small-for-gestational-age) or generalized linear models (birthweight decile and head circumference).

Results: The GM of the OC pesticide constituent trans-nonachlor was 2.74 mg/g lipid, and for metabolites oxychlordane and p,p'-DDE this was 1.94 ng/g lipid and 55.8 ng/g lipid, respectively. OP pesticide metabolite concentrations adjusted for specific gravity, were dimethylphosphate (GM: 3.19 μg/L), dimethylthiophosphate (GM: 3.29 μg/L), dimethyldithiophosphate (GM: 0.48 μg/L), diethlphosphate (GM: 2.46), and diethylthiophosphate (GM: 0.67 μg/L). There was no significant difference in mean concentrations for these OC or OP pesticide constituents or metabolites between tea drinkers — of any type — and non-tea drinkers. Further, no association was found between tea intake and adverse birth outcomes.

Conclusions: Pesticide concentrations did not differ by tea intake. Further, tea intake in the first trimester was not associated with adverse birth outcomes. In this study population, there was no evidence for concern about tea intake being a source of the OP or OC pesticide metabolites measured or adversely affecting birth outcomes; however, tea intake was lower than national Canadian data for women of reproductive age.

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1. Introduction

Tea is a commonly consumed beverage worldwide. In 2009, Canada alone had an annual per capita consumption of 77 l (Statistics Canada, 2009). Tea producers often use pesticides, such as insecticides and fungicides to protect crops from devastating insect and disease infestation (Sood et al., 2004). There is evidence that some teas contain detectable pesticide residues, including organochlorines (OC) and organophosphates (OP) (Canadian Food Inspection Agency (2010–2011); Wang et al., 2014).; Current Canadian prenatal nutrition guidelines (Public Health Agency of

Canada, 2014) include caffeine content and medicinal properties that may lead to adverse health benefits but do not consider pesticide concentrations in the tea.

Since 2011, maximum residue limits (MRL) – regulated limits that are based on the maximum amount of pesticide residues expected to remain on dried tea, when a pesticide is used according to label directions, that is not considered a concern for human health – were established in Canada for six pesticides in dried tea leaves: lambda-cyhalothrin, ethiprole, fepropathrin, propiconazole, spiromesifen and etoxazole (Health Canada, 2015; Canadian Food Inspection Agency, 2011) A general MRL (GMRL) of 0.1 ppm for residues of pesticides is applied where a specific MRL is not established, and this is for compliance purposes, rather than based on a chemical-specific risk assessment. However, certain pesticides that may have been used in tea production, such as OC pesticides – including p,p′-dichlorodiphenyldichloroethylene (p,p′-DDE) and hexachlorobenzene (HCB) – and OP pesticides, have been shown to cross the blood-placenta barrier, and thus may put the fetus at risk (Aylward et al., 2014). For example, maternal exposure to HCB has been associated with an increased risk of preterm birth (PTB). Further, p,p′-DDE has demonstrated a positive relationship with prevalence of low birth weight, small-for-gestational age (SGA) and decreased head circumference, though these associations are inconsistent (Fenster et al., 2006; Wolff et al., 2007; Eggesbo et al., 2009; Ribas-Fito et al., 2002; Wojtyniak et al., 2010). OP pesticide exposure in pregnant women has also been be inversely associated with gestational age and birth weight (Rauch et al., 2012), though this association has not been consistently observed (Sathyanarayana et al., 2010). Further, potential associations have been made between prenatal exposure to dialkyl phosphate metabolites and poor neurobehavioural development in neonates (Zhang et al., 2014). Exposure to elevated levels of these potentially neurotoxic pesticides during the first trimester can be of concern, since the first trimester is a period of rapid fetal growth and development, particularly of the nervous system. Pesticide exposure may occur through various sources, for example consumption of foods that have been treated with pesticides, residing in an agricultural area where pesticides are used or personal use in gardening. Thus, it is important to assess the potential contribution of tea intake to pesticide exposure during pregnancy. To our knowledge, this is a novel examination of tea intake and pesticide exposure in a pregnancy cohort. Further, as there are other constituents and potential contaminants in tea, we aimed to determine if drinking tea was associated with adverse birth outcomes, including PTB and SGA, as has been reported in some studies (Okubo et al., 2015; Hoyt et al., 2014; Sengpiel et al., 2013).

2. Methods

2.1. Study population

Data from the Canadian Maternal–Infant Research on Environmental Chemicals (MIREC) Study cohort were used for these analyses. The MIREC Study methods are outlined briefly here and in detail elsewhere (Arbuckle et al., 2014; Kramer et al., 2001). Two thousand women were recruited in the first trimester of pregnancy (< 14 weeks gestation) from obstetric and prenatal clinics in ten cities across Canada, from 2008 to 2011. Eligibility criteria included having the ability to consent and to communicate in English or French; being 18 years of age or older; planning to deliver at a local hospital; and participating in the cord blood collection component of the MIREC study. Women with a medical history of major chronic disease, threatened abortion, or illicit drug use were excluded. Potential participants were provided with

information on the objectives and design of the study and asked to sign the consent forms.

Questionnaires were administered during the 1st trimester visit to collect self-reported sociodemographic (i.e. maternal age, highest level of maternal education, annual household income, country of birth [Canada or other], and marital status) and behavioral (e.g. beverage consumption – including alcohol and coffee intake-and smoking) information as well as history of PTB. Pre-pregnancy body mass index (BMI) was calculated by dividing self-reported weight (kg) by measured height squared (m^2). Gestational hypertension was assessed through medical chart review. The study was reviewed and approved by the Health Canada Research Ethics Board and the ethics committees at the participating hospitals and research centers across Canada.

2.2. Tea intake

Intake of regular, green and herbal tea was evaluated in the first trimester, as well as intake of any type of tea. Women reported their frequency of consumption in terms of number of 6 ounce cups per day, week or month. In addition to a dichotomous variable (tea drinkers or non-tea drinkers), a variable was derived to describe frequency as number of cups per week (none to < 1, 1 to < 7, ≥ 7). Since the vast majority of participants in the "none to < 1 cup/week" category reported consuming tea "never" or "less than 2 times/month" – and consuming tea in these quantities was unlikely to represent a significant exposure to the contaminants being investigated – we collapsed these categories into "none to < 1 cup/week".

2.3. Neonatal factors

PTB was defined as a gestational age of less than 37 weeks, and included an indication of spontaneous PTB (Term, PTB or Spontaneous PTB). Gestational age was determined using ultrasound established dates where the discrepancy between early ultrasound date and date of last menstrual period exceeded 7 days. Birth weight and head circumference were abstracted from medical charts. Fetal growth was assessed using the Kramer sex-specific Canadian reference charts for birth weight for gestational age and infants were categorized as SGA where the birth weight was < 10th percentile for gestational age (Kramer et al., 2001).

2.4. Pesticide analysis

Maternal blood was collected in 10-ml sterile vacutainer tubes. Within 2 h of the blood draw, the samples were centrifuged and the plasma aliquoted into smaller cryovials to be stored at −20 °C until analysis. Maternal urine was collected in Nalgene® containers (Thermo-Fisher Scientific Inc., Rochester NY, USA), aliquoted and frozen at −20 °C.

Chemical analyses of maternal blood and urine were carried out by the Toxicology Laboratory, located in the Institut national de santé publique du Québec, https://www.inspq.qc.ca/ctq/, which is accredited by the Standards Council of Canada under ISO 17025 and CAN-P-43. The accuracy and precision of the analyses were evaluated on a regular basis through the laboratory's participation in external quality assessment programmes.

Fourteen OC pesticide metabolites α-chlordane, γ-chlordane, aldrin, cis-nonachlor, trans-nonachlor, oxychlordane, β-hexachloro-cylcohexane (β-HCH), γ-hexachlorocylcohexane (γ-HCH), p,p′-dichlorodiphenyltrichloroethane (DDT), p,p′-dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), mirex, toxaphene parlar 26 (Tox 26), toxaphene parlar 50 (Tox 50) were measured in 1st trimester maternal plasma. Plasma samples were enriched with internal standards and halogenated organic compounds were

retrieved by liquid–liquid extraction with a mixture of ammonium sulfate: ethanol:hexane (1:1:3). The extracts were concentrated, automatically purified on florisil column and then analyzed by gas chromatography coupled to a mass spectrometer (Agilent 6890N/ 5973 GC–MS). Measurements of ions generated after negative chemical ionization was performed in selective ion mode (SIM). The lower limits of detection ranged from 0.005 to 0.09 µg/L, depending on the analyte. Values were adjusted for total lipids using the following formula ([pesticide concentration]/[total lipids g/L]*1000).

Six dialkyl phosphate metabolites of OP pesticides (diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP), dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DMDTP)) were measured in maternal urine from the first trimester. The urine samples were enriched with internal standards and then derivatized at 70 °C (pentafluorobenzyl bromide) for 2 h. Benzylpentafluorinated derivatives were extracted with a mixture of hexane and dichloromethane and analyzed by gas chromatography coupled with mass spectrometry (GC–MS/ MS. The solvent was then evaporated to dryness, taken up in 2 mL of dichloromethane: hexane (20:80) and analyzed for dialkylphosphate metabolites on an Agilent 6890 Network gas chromatograph (GC) (Agilent Technologies; Mississauga, Ontario, Canada) coupled to a Waters Quattro Micro GC mass spectrometer in tandem (MS/MS) (Waters; Milford, MA). Agilent 7683, tandem mass detector Water Quattro Micro GC). The measurement of ions generated was performed in MRM mode with a source in negative chemical ionization mode (NCI). Concentrations were reported in micrograms per liter (µg/L). The limits of detection (LOD) reported were: 1.0 µg/L for DEP and DMP; 0.3 µg/L for DETP, DEDTP and DMDTP; and 0.6 µg/L for DMTP.

OP metabolites were adjusted for specific gravity using the following equation (Just et al., 2010):

$Pc=Pi \, [(SGm-1)/(SGi-1)]$

where Pc is the SG-adjusted metabolite concentration (ng per ml), Pi is the observed metabolite concentration, SGi is the specific gravity of the urine sample and SGm is the median SG for the cohort.

2.5. Statistical analysis

Analyte results below the limits of detection, defined as results indistinguishable from zero, were counted as half the detection limit. Descriptive statistics were used to characterize the population by mean gestational age, rate of PTB–including spontaneous PTB–and SGA. Differences between groups were examined using chi-square tests for categorical and t-test for continuous variables. The geometric means (GM) of OC pesticides in maternal plasma (lipid adjusted) and OP pesticides (adjusted for specific gravity) in maternal urine were calculated for participants who drank regular, green or herbal tea in the first trimester, and for those who did not, and differences were examined using t-tests.

Since the pesticides included in the MIREC may not be the only contaminants or constituents of concern related to tea intake, we used separate logistic regression analyses to examine associations between tea intake and PTB or SGA. Further, generalized linear models were used to determine the relationship between tea intake and birthweight deciles, as well as tea intake and head circumference. Potential confounders selected *a priori* included maternal factors, such as age, socioeconomic status, coffee intake, body mass index and smoking status.

To limit the bias caused by a high percentage of non-detects, only those chemicals with > 50% detectable concentrations were included in the analyses.

All singleton, live births were included in the analyses (n= 1898). For descriptive analyses of pesticides concentrations, all participants with a blood or urine pesticide concentration were included. Twenty pesticide samples were excluded from the analyses as they did not have specific gravity (n=3) or a total lipid concentration (n=17). The analyses were conducted using SAS version 9.3 software.

3. Results

3.1. Population characteristics

MIREC participants with singleton, live births are described in Table 1. The majority of the women were Canadian born (81%), 30 years of age or older (69%), non-smokers (94%), first pregnancy (71%) and with an annual household income of > $50,000 (82%). Prior PTB was reported for 7% of the participants and < 5% had gestational hypertension prior to or at delivery. Overall, mean gestational age was 38.9 weeks. Gestational age was significantly lower in women who were ≥ 35 years of age, in comparison with 30–34 years of age; in their first pregnancy; college educated in comparison with graduate degree; obese, in comparison with normal weight; alcohol consumption less than once per week in comparison to once or more per week; gestational hypertension prior to or at delivery; and those with prior PTB. The rate of PTB was 6.1%, and was higher among obese participants as compared to normal weight, those with gestational hypertension – prior to or at delivery – and prior PTB. Overall, 5.6% of participants delivered an SGA infant, and this rate was higher among multiparae, normal weight participants and those with gestational hypertension prior to or at delivery.

Only 21% of all women in the study reported consuming any type of tea in the first trimester, with less than 1% of tea-drinking women reported consuming all three types of tea and 15% reported drinking two types of tea (data not shown). Approximately 5% of women consumed seven or more cups of tea per week. A significantly higher proportion of any type of tea drinker, as well as green tea drinkers, were not Canadian born. Compared to women < 29 years of age, more women ≥ 35 reported drinking herbal tea in the first trimester.

3.2. Organochlorine pesticide constituent and metabolites

Lipid-adjusted maternal plasma concentrations of OC pesticide constituents and metabolites are presented in Table 2. Aldrin, α-chlordane, γ-chlordane, cis-nonachlor, γ-HCH, p,p′-Dichlorodiphenyltrichloroethane, HCB, Mirex, Tox 26 and Tox 50 had detectable concentrations in less than 50% of samples, thus differences between tea drinkers and non-tea drinkers could not be analysed. The GMs for the measurable pesticide constituent transnonachlor was 2.74 ng/g lipid; and for measurable pesticide metabolites the GM was 1.94 ng/g lipid for oxychlordane; and 55.8 ng/g lipid for p,p′-DDE. There was no difference between tea drinkers and non-tea drinkers for these three pesticide metabolites (Table 3). To determine the impact of substituting the LOD/2 for results below the limit of detection, we ran a sensitivity analysis that tested using the detection limit or zero in place of results below the limit of detection and the results were unchanged.

3.3. Organophosphate pesticide metabolites

OP pesticide metabolites, adjusted for specific gravity, with detectible concentrations were DMP (GM: 3.19 µg/L, > 79% detected), DMTP (GM: 3.29 µg/L, > 80% detected), DMDTP (GM: 0.48 µg/L, > 51% detected), DEP (GM: 2.46 µg/L, > 77% detected), and DETP (GM: 0.67 µg/L, > 85% detected (Table 2)). The percentage of samples with no detectable diethyldithiophosphate was

Table 1
Characteristics of the MIREC Cohort participants with Singleton, live births ($n=1898^*$), gestational age, rate of preterm births and SGA, MIREC, Canada, 2008–2011.

	N (%)	Mean gestational age (weeks)	Rate of PTB (%)	Rate of spontaneous PTB (% of PTB)	Rate of SGA (%)
Overall	1898	38.9	6.1	66.4	5.7
Maternal age (years)					
< 20	12 (0.6)	38.3	8.3	100	16.7
20–24	122 (6.4)	38.7	7.4	44.4	5.7
25–29	450 (23.7)	39.2	4.0	72.2	6.2
30–34	672 (35.4)	39.0[a]	5.7	84.2	5.1
≥ 35	642 (33.8)	38.7[b]	7.8	55.1	5.9
Maternal country of birth					
Canada	1550 (81.1)	38.9	5.9	64.8	5.4
Other	358 (18.9)	38.8	6.7	75.0	7.0
First pregnancy					
Yes	1356 (71.4)	38.8[a]	6.1	66.7	4.8[a]
No	542 (28.6)	39.2[b]	6.1	67.1	7.9[b]
Marital status					
Single	80 (4.2)	38.8	7.5	66.7	11.3
Married or common law	1808 (95.3)	38.9	6.0	66.7	5.5
Divorced or widowed	10 (0.5)	38.7	10.0	100	0
Maternal education					
Less than college	165 (8.7)	38.8	6.7	63.6	9.1
College educated	551 (29.0)	38.7[b]	7.4	67.5	4.4
Completed university	698 (36.8)	39.1	5.3	64.9	5.3
Graduate degree	484 (25.5)	39.0[a]	5.6	70.4	6.8
Household income ($)					
≤ 50,000	328 (18.1)	38.8	6.7	54.5	6.7
50,001–100,000	761 (42.0)	38.9	6.3	68.8	5.5
> 100,000	721 (39.8)	38.9	5.3	68.4	5.1
Maternal pre-pregnancy body mass index					
< 18.5	49 (2.8)	38.8	4.1	100	12.2
18.5–24.9	1069 (60.8)	39.1[a]	4.9[a]	75.0	6.4[a]
25–29.9	381 (21.7)	38.9	5.2	65.0	3.2[b]
≥ 30	258 (14.7)	38.3[b]	13.2[b]	51.5	5.4
Smoking in first trimester					
Daily	85 (4.5)	38.7	5.9	80.0	11.8
Occasionally	27 (1.4)	39.0	3.7	100	7.4
Not at all	1786 (94.1)	38.9	6.2	67.0	5.4

Table 1 (continued)

	N (%)	Mean gestational age (weeks)	Rate of PTB (%)	Rate of spontaneous PTB (% of PTB)	Rate of SGA (%)
Alcohol in first trimester of pregnancy					
Once/wk or more	62 (3.3)	39.2[b]	4.8	66.7	4.8
Less than once/wk	1836 (96.7)	38.9[a]	6.2	67.0	5.8
Gestational hypertension (prior to delivery)					
Yes	89 (4.9)	37.6[b]	24.7[b]	25.0	11.2[b]
No	1746 (95.1)	39.0[a]	5.2[a]	76.7	5.5[a]
Gestational hypertension (at delivery)					
Yes	81 (4.4)	38.0[b]	16.0[b]	30.8	9.9[b]
No	1756 (95.6)	39.0[a]	5.8[a]	71.3	5.7[a]
Prior PTB					
Yes	128 (6.7)	37.6[b]	17.2[b]	63.6	2.3
No	1770 (93.3)	39.0[a]	5.3[a]	67.7	6.0

Note: Missing data not shown, 7.4% of sample for maternal pre-pregnancy BMI.
*Excluded all multiple births and not born alive ($n=69$).
  [a] Reference.
  [b] Significant difference ($p < 0.05$).

98%. No significant differences in concentrations of these OP pesticide metabolites were found between tea and non-tea drinkers (Table 3). Sensitivity analysis that tested using the detection limit or zero in place of results below the limit of detection did not change the results.

3.4. Tea and adverse birth outcomes

No significant association was found between consuming tea and the risk of preterm birth, spontaneous preterm birth or SGA (Table 4). Small sample sizes precluded the examination of risks by type of tea (black, green or herbal). Similarly, no associations were found between tea intake and birth weight or head circumference (data not shown).

4. Discussion

This study found no evidence of an association between drinking tea (regular, green or herbal) in the first trimester of pregnancy and elevated mean concentrations of certain OC or OP pesticides. Further, a relationship was not established between tea intake in the first trimester and several birth outcomes (i.e. PTB, SGA, birth weight or head circumference).

Among a 2008 nationally-representative sample of women in the general Canadian population, approximately 30% of 1–30 year olds and 35% of 31–50 year olds reported tea consumption, and those who consumed tea reported an average daily intake of approximately 2 cups (Garriguet, 2008). Only 21% of the pregnant women in this study reported consuming any type of tea in the first trimester, with 5% reporting a frequency of ≥ 7 cups per week. The largest proportions of tea drinkers were not born in Canada. Recent figures from 2013 estimate that Canadians drink an average of 8.3 cups of tea per week (Tea Association of Canada, 2014). The demographics of the study population and possible changes in tea consumption with pregnancy may explain the lower rates of tea intake in our study.

Table 2

Detection and Concentration of lipid-adjusted organochlorine pesticide constituents or metabolites in maternal plasma and specific gravity-adjusted organophosphate pesticide metabolites in maternal urine in MIREC participants with singleton live births, Canada, 2008–2011.

	N	% < LOD[a]	Geometric mean, ng/g lipid	Percentiles, ng/g lipid				
				25th	50th	75th	95th	99th
Type of organochlorine pesticide constituents or metabolites								
Aldrin	1854	88	< 0.025[b]	–	–	–	–	–
alpha-Chlordane	1842	100	< 0.005[b]	–	–	–	–	–
gamma-Chlordane	1851	100	< 0.005[b]	–	–	–	–	–
cis-Nonachlor	1852	89	< 0.0025[b]	–	–	–	–	–
trans-Nonachlor	1852	16	2.74	1.92	2.89	4.22	7.34	13.0
Oxychlordane	1851	8	1.94	1.43	2.09	2.90	4.60	6.88
gamma –Hexachlorocylcohexane	1852	100	< 0.005[b]	–	–	–	–	–
p,p'-Dichlorodiphenyltrichloroethane	1853	96	< 0.025[b]	–	–	–	–	–
p,p'-Dichlorodiphenyldichloroethylene	1853	10	56.0	33.8	48.3	77.3	263.2	176.5
Hexachlorobenzene	1852	70	< 0.04[b]	–	–	–	–	–
Mirex	1852	92	< 0.005[b]	–	–	–	–	–
Toxaphene parlar 26	1852	97	< 0.0025[b]	–	–	–	–	–
Toxaphene parlar 50	1852	87	< 0.0025[b]	–	–	–	–	–
			Geometric mean, µg/L	Percentiles, µg/L				
Type of organophosphate pesticide metabolite								
Dimethylphosphate	1850	21	3.19	1.63	3.25	6.27	16.9	32.5
Dimethylthiophosphate	1848	20	3.29	1.21	3.43	8.67	32.2	68.3
Dimethyldithiophosphate	1851	49	0.48	0.20	0.39	0.98	5.03	14.1
Diethylphosphate	1851	23	2.46	1.37	2.38	4.23	9.97	21.3
Diethylthiophosphate	1849	15	0.67	0.35	0.65	1.18	3.52	8.13
Diethyldithiophosphate	1851	98	< 0.15[b]					

[a] LOD: < 0.01 µg/L for Aldrin, alpha-chlordane, trans-Nonachlor, gamma-Hexachlorocyclohexane, mirex; < 0.05 µg/L p,p'-Dichlorodiphenyltrichloroethane; < 0.09 µg/L p,p'-Dichlorodiphenyldichloroethylene; < 0.04 µg/L hexachorobenzene; < 0.005 µg/L for gamma-chlordane, cis-Nonachlor, oxychlordane, Toxaphene parlar 26, Toxaphene parlar 50; < 1 Dimethylphosphate, Diethylphosphate; < 0.6 µg/L Dimethylthiophosphate, Diethylthiophosphate; < 0.3 µg/L Dimethyldithiophosphate, Diethyldithiophosphate.

[b] Geometric mean and percentiles not calculated because detection frequency is < 50%

The percentage of samples with no detectable lipid-adjusted OC pesticides studied in MIREC participants were similar to females 20 to 39 years of age in the 2007–2009 Canadian Health Measures Survey (CHMS) (Health Canada (2007–2009)) for trans-nonachlor (MIREC: 16% vs. CHMS: 19%) and oxychlordane (MIREC: 8% vs. CHMS: 8%), but higher for p,p′-DDE (MIREC: 10% vs. CHMS: 0.7%). The lipid-adjusted GMs for oxychlordane and trans-nonachlor for women aged 20–39 years in the 2007–2009 CHMS were 2.31 µg/kg lipid and 3.07 µg/kg lipid, respectively, which is similar to the findings for MIREC participants (Table 5). However, p,p′-DDE – an indicator of long-term historical exposure to DDT – differed with a GM of 102.15 µg/kg lipid, which is almost double that of our

MIREC participants. Lower concentrations of certain pesticides in pregnant women is consistent with other studies, for example, using data from the 2003–2004 NHANES, it was demonstrated that mean concentrations of lipid-adjusted p,p′-DDE were lower in pregnant versus non-pregnant women (140.39 ng/g lipid and 151.04 ng/g lipid, respectively), which may be due to transfer to the fetus and excretion in breast milk (Woodruff et al., 2011). Comparing levels of OP pesticides for MIREC participants with those of females 20–39 years of age from the CHMS (2007–2009), MIREC participants had a lower prevalence of DMDTP samples below the limit of detection (MIREC: 20% vs. CHMS: 66%) but a higher prevalence of DEDTP samples below the limit of detection

Table 3

Geometric mean concentrations of organochlorine pesticide constituents or metabolites in maternal plasma and organophosphate pesticide metabolites in maternal urine by tea intake in the first trimester in MIREC participants, Canada, 2009–2012.

	Regular tea		Green tea		Herbal tea		Any Tea	
N(%)	Yes 280 (14.8) Geometric mean ng/g lipid (95% CI)	No 1618 (85.3)	Yes 113 (6.0) Geometric mean ng/g lipid (95% CI)	No 1785 (94.1)	Yes 68 (3.6) Geometric mean ng/g lipid (95% CI)	No 1830 (96.4)	Yes 398 (21.0) Geometric mean ng/g lipid (95% CI)	No 1500 (79.0)
Type of organochlorine pesticide constituents or metabolites								
trans-Nonachlor	2.77 (2.55, 3.01)	2.73 (2.64, 2.82)	2.96 (2.58, 3.38)	2.72 (2.64, 2.81)	3.09 (2.57, 3.72)	2.72 (2.64, 2.81)	2.78 (2.59, 2.98)	2.72 (2.63, 2.81)
Oxychlordane	1.92 (1.77, 2.07)	1.94 (1.88, 2.00)	1.98 (1.75, 2.23)	1.93 (1.88, 1.99)	2.10 (1.74, 2.54)	1.93 (1.87, 1.98)	1.92 (1.80, 2.05)	1.94 (1.88, 2.00)
p,p'-DDE	57.7 (52.5, 63.3)	55.7 (53.5, 58.1)	59.6 (51.5, 68.9)	55.8 (53.7, 58.0)	62.6 (52.5, 74.7)	55.8 (53.7, 58.0)	57.8 (53.5, 62.5)	57.8 (53.2, 58.0)
Type of organophosphate pesticide metabolites								
Dimethylphosphate	3.15 (2.82, 3.52)	3.20 (3.04, 3.36)	3.73 (3.07, 3.31)	3.16 (3.01, 3.31)	3.44 (2.68, 4.42)	3.18 (3.04, 3.33)	3.25 (2.95, 3.57)	3.17 (3.02, 3.34)
DimethylTP	3.01 (2.55, 3.56)	3.35 (3.13, 3.59)	3.99 (3.07, 5.19)	3.26 (3.05, 3.48)	3.99 (2.88, 5.53)	3.28 (3.07, 3.49)	3.20 (2.78, 3.67)	3.33 (3.10, 3.57)
DimethylDTP	0.44 (0.38, 0.51)	0.49 (0.46, 0.52)	0.55 (0.43, 0.71)	0.48 (0.45, 0.51)	0.52 (0.39, 0.69)	0.48 (0.46, 0.51)	0.45 (0.40, 0.51)	0.49 (0.46, 0.52)
Diethylphosphate	2.33 (2.10, 2.58)	2.49 (2.39, 2.60)	2.43 (2.04, 2.88)	2.47 (2.37, 2.57)	2.44 (2.02, 2.94)	2.46 (2.37, 2.57)	2.33 (2.13, 2.54)	2.50 (2.39, 2.61)
Diethylthiophosphate	0.66 (0.59, 0.74)	0.67 (0.64, 0.70)	0.73 (0.61, 0.88)	0.66 (0.64, 0.69)	0.62 (0.49, 0.79)	0.67 (0.64, 0.70)	0.65 (0.59, 0.72)	0.67 (0.64, 0.71)

NOTE: Only including pesticides with at least 50% > LOD; p,p'-DDE=Dichlorodiphenyldichloroethylene; DimethylTP=Dimethylthiophosphate; DimethylDTP=Dimethyldithiophosphate

Table 4
Dose-response analyses of tea consumption in first trimester and the risk of pre-term birth or SGA.

	No PTB	PTB	Crude OR	Adjusted[†] OR
	(n=1635)	(n=116)	(95% CI)	(95% CI)
All tea (cups*/week)				
None to < 1	1286	92	1	1
≥ 1	349	24	1.04 (0.65, 1.66)	0.99 (0.61, 1.61)
	No spontaneous PTB	Spontaneous PTB	Crude OR	Adjusted[†] OR
	(n=1633)	(n=77)	(95% CI)	(95% CI)
All tea (cups*/week)				
None to < 1	1284	62	1	1
≥ 1	349	15	1.12 (0.63, 2.00)	1.08 (0.59, 1.98)
	No SGA	SGA	Crude OR	Adjusted[†] OR
	(n=1634)	(n=109)	(95% CI)	(95% CI)
All tea (cups*/week)				
None to < 1	1285	91	1	1
≥ 1	349	18	1.37 (0.82, 2.30)	1.43 (0.83, 2.46)

NOTE: The reference group for each outcome does not include the other two outcomes examined in this table (e.g. no PTB does not include SGA or spontaneous PTB).

* Defined in questionnaire as 6 ounces.

† Model adjusted for maternal age, country of birth, maternal pre-pregnancy body mass index, household income, highest level of education, coffee intake and smoking in the first trimester.

Table 5
Comparison of adjusted geometric mean concentrations of certain organochlorine and organophosphate pesticides from MIREC (2008–2011) and CHMS (2007–2009) females 20–39 years of age.

Type of pesticide	MIREC	CHMS
	µg/kg lipid	µg/kg lipid
Oxychlordane	1.94	2.31
Trans-Nonachlor	2.74	3.07
p, p'-DDE	56.0	102
	µg/L	µg/g creatinine
Dimethylphosphate	3.19	3.82
Dimethylthiophosphate	3.29	2.46
Diethylphosphate	2.46	2.99

NOTE: CHMS=Canadian Health Measures Survey; p,p'-DDE=Dichlorodiphenyl-dichloroethylene

(MIREC: 98% vs. CHMS: 64%) (Health Canada (2007–2009)). GMs for MIREC participants for the OP pesticides were similar to women in this sub-group of the Canadian population for DMP and DEP, but slightly higher for DMTP, with a GM of 3.29 µg/L in MIREC and 2.46 µg/L for the Canadian population.

Our findings did not support the hypothesis that tea drinkers would have significantly higher exposure to OC or OP pesticides, despite studies that have demonstrated detectable concentrations of pesticide residues in teas sold in Canada (Canadian Food Inspection Agency (2009–2010); Canadian Food Inspection Agency, 2011). For example, a 2010–2011 Canadian Food Inspection Agency (CFIA) study tested 267 tea samples and found 25% had at least one pesticide violation of the GMRL, or an established MRL (Canadian Food Inspection Agency, 2011). Of the samples in violation, 41% were green teas and 36% were black teas. Certain detectable pesticides measured in MIREC — such as trans-nonachlor and oxychlordane — were not found to be in violation of the General MRL, however, not all MIREC

measured pesticides were analysed in the dried tea samples. Concentrations of the 19 detected pesticides, including 1 OC and 2 OPs, ranged from 0.025 to 2.42 ppm. Only fepropathrin and lambda-cyhalothrin have a specified MRL for tea (dried leaves) of 2 ppm, as the levels for these ranged from 0.189 to 0.705 ppm and 0.045 to 0.499 ppm, respectively. Since OC pesticides are persistent organic pollutants (POPs) that have a long biological half-life, these pesticides may continue to exist in soil (Environment Canada, 2005). Considering the varied countries of origin for tea, there is the potential for exposure to pesticides that are not permitted for use in Canada. Further, pesticide residues transfer from dried to brewed tea and-though transfer rates vary depending on the solubility of the pesticide, steeping time and water temperature-there has been speculation that this may pose a risk to consumers (Wang et al., 2014). However, our study did not demonstrate higher mean blood concentrations for certain pesticides among tea drinkers. The POP concentrations in our population may be lower as pregnant women can excrete some stored POPs during pregnancy and lactation (Woodruff et al., 2011), thus an investigation of pesticide exposure and tea intake in the general population is needed to confirm the null associations reported here.

Our study also did not demonstrate a relationship between tea intake in the first trimester and various fetal growth outcomes, which is somewhat inconsistent with findings from other prenatal cohorts. For example, a 2002–2009 birth cohort of over 56,000 Norwegian mothers and children, which aimed to examine caffeine intake, did not demonstrate a relationship between black tea intake — estimated in the first four or five months of pregnancy — and risk of preterm delivery, but did show a positive association with early spontaneous preterm delivery (between 22 and 34 weeks) (OR 1.61, 95% CI: 1.10–2.35) (Sengpiel et al., 2013). Further, in the US National Birth Defects Prevention Study, which examined 7943 pregnant women and their offspring between 1997 and 2007, those with a tea intake of ≥ 3 cups/day in the first trimester, in comparison to < 1 cup/month, were more likely to have a small for gestational age offspring (OR 2.00, 95% CI: 1.46–2.74) (Chen et al., 2014). Our finding of a lack of an association between tea intake in the first trimester and adverse birth outcomes may be due to our inability to examine heavy tea drinkers – as defined in other studies-separately due to the small sample size. The contribution of tea to overall caffeine intake may also be a potential determinant of increased risk of adverse birth outcomes and a recent meta-analysis of 60 studies did report an association between incremental increases in caffeine consumption (per 100 g) and increased risk of low birth weight (7% [95%CI: 1–12%]) and SGA (10% [95% CI: 6–14%]), though the models used were highly heterogeneous suggesting the need for caution in interpreting these findings (Greenwood et al., 2014).

The MIREC cohort has multiple strengths, including the large, national-level prenatal population that provides biomonitoring data on the potential distribution of pesticide exposures in pregnant women. Further, analyses for MIREC and the CHMS were conducted at the same laboratory, which minimizes potential inter-laboratory variation. As reported by Helsel (1990), substituting values less than the LOD with zero produce estimates of mean and median which were biased low, while substituting with the reporting limit results in estimates above the true value. While not ideal, when the percentage of non-detects is low, researchers frequently use substitutions such as the LOD/2 for results below the LOD. Sensitivity analysis that tested using the detection limit or zero in place of results below the limit of detection did not change our conclusions. Since MIREC is not population-based, participants differed from the Canadian population giving birth in 2009, for example, our population tended to be older, more educated and less likely to be a current smoker. Thus, our results may not be generalizable to the Canadian population (Arbuckle et al.,

2013).Our study was limited by a lack of measured pesticide concentrations at the second or third trimester, thus a longitudinal analysis over the entire pregnancy was not possible. Later pregnancy exposure can be relevant to neurobehavioural outcomes due to the continued growth and development of the brain throughout pregnancy. However, an examination of cord blood from MIREC participants demonstrated more than 90% non-detects for concentrations of trans-nonachlor, oxychlordane and p,p′-DDE.

Tea intake was self-reported in our study, which may have introduced reporting bias, such as social desirability bias. This could lead to an under-estimation of actual tea intake in our population. Our sample included mostly low to moderate intakes of tea per week, which limited the power to detect associations with adverse birth outcomes and did not allow for the study of heavy tea intake. Data on pre-conception tea intake was not collected, thus we could not assess the effect of behavior change post-conception and whether the concentration of persistent pesticides reflected long-term exposure rather than actual exposure from tea intake reported at the first trimester. Sensitivity analysis, examining women with no tea consumption at all as the reference group, did not change the results of the analyses. Further, other than general categories (e.g. black, green) we did not collect specific data on the properties of the tea consumed, such as country of origin. A study examining the pesticide content of tea, by type and country of origin, in relation to blood or urine concentrations may provide further insight.

This study did not find any evidence that low to moderate tea intake in the first trimester of pregnancy was associated with elevated concentrations of certain POPs or OP pesticides or adverse birth outcomes. Our findings do not substantiate concerns regarding exposure to the pesticides we studied through tea intake in pregnancy. Future research should investigate heavy tea intake and the potential implications of OC or OP pesticide exposure through tea intake during the entire pregnancy on fetal growth and development.

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