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Urinary concentrations and determinants of glyphosate and glufosinate in pregnant Canadian participants in the MIREC study

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ABSTRACT

Background: Glyphosate is the most widely applied herbicide in agriculture. Glufosinate is a broad spectrum herbicide used to manage glyphosate-resistant weeds. Despite the widespread use of these herbicides, biomonitoring data – which inform risk assessment and management – are sparse.

Objectives: To identify determinants of urinary concentrations of these herbicides and their metabolites in pregnancy.

Methods: We measured urinary concentrations of glyphosate, glufosinate, and their primary metabolites aminomethylphosphonic acid (AMPA) and 3-methylphosphinicopropionic acid (3-MPPA) in a single spot urine specimen collected during the first trimester of pregnancy from the Maternal-Infant Research on Environmental Chemicals (MIREC) study. MIREC recruited about 2000 pregnant women from 10 Canadian cities between 2008 and 2011. We used UItra-Performance Liquid Chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) with sensitive limits of detection to quantify analyte concentrations. We examined urinary concentrations according to maternal sociodemographics, sample collection characteristics, reported pesticide use, and consumption of fruits, vegetables, legumes, and grain products. We used ANOVA models with specific gravity-standardized chemical concentrations as the dependent variable to determine associations with maternal and sample determinants.

Results: Among women with biobanked urine samples (n = 1829–1854), 74% and 72% had detectable concentrations of glyphosate and AMPA, respectively. In contrast, one and six percent of women had detectable concentrations of glufosinate and 3-MPPA, respectively. The specific gravity-standardized geometric mean (95% CI) concentrations of glyphosate and AMPA were 0.112 (0.099–0.127) μg/L and 0.159 (0.147–0.172) μg/L, respectively. We observed a dose-response relationship between consumption of whole grain bread and higher urinary glyphosate concentrations. Season of urine collection and self-reported pesticide use were not associated with increased concentrations of any analyte.

Conclusions: We detected glyphosate and AMPA in the majority of pregnant women from this predominantly urban Canadian cohort. Diet was a probable route of exposure.

Abbreviations: 3-MPPA, 3-hydroxy(methyl) phosphinoyl propionic acid; AMPA, aminomethylphosphonic acid; ATSDR, Agency Toxic Substances Disease Registry; LOD, limit of detection; LOQ, limit of quantification; MIREC, Maternal-Infant Research on Environmental Chemicals; PROTECT, Puerto Rico Testsite for Exploring Contamination Threats; TIDES, The Infant development and Environment study.

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1. Introduction

Glyphosate, a broad spectrum herbicide, is the most widely applied pesticide globally (US Department of Health and Human Services, 2020; Milesi et al., 2021; Myers et al., 2016; Vandenberg et al., 2017). Glyphosate is the active ingredient in many herbicide formulations and it is used in commercial, residential, and agricultural settings in Canada. In agricultural settings, glyphosate is registered for use both pre- and post-harvest (US Department of Health and Human Services. ATSDR, 2020). Following the development of glyphosate tolerant crops in 1996 (soybean, corn, alfalfa, cotton, sugar beets, and canola), use of glyphosate based herbicides increased rapidly (Benbrook, 2016) with suggestive, yet limited, evidence of corresponding increases in human exposure (Conrad et al., 2017; Mills et al., 2017). Consistent with other pesticides, the rise in glyphosate use was followed by the development of glyphosate resistant weeds. Glufosinate-ammonium, another broad spectrum herbicide, was introduced in 1993 and is used to treat glyphosate resistant weeds (Takano and Dayan, 2020). Biomonitoring data for both of these herbicides during vulnerable windows of development (i.e. fetal development) are lacking, yet are valuable for risk assessment and management (Connolly et al., 2020; Gillezeau et al., 2019; Kogevinas, 2021; Myers et al., 2016).

Glyphosate has been subject to intense research and in-depth scrutiny by national and international regulatory authorities and/or scientific organizations, as well as by non-governmental bodies and academic laboratories. Glyphosate disrupts the 5-enolpyruvylshikimate-3-phosphate synthase enzyme in the shikimate pathway. The shikimate pathway, essential for production of key amino acids in plants, is not found in human tissues (US Department of Health and Human Services, 2020; Casida, 2017) but may be present in some bacteria found in the human gastrointestinal tract (Mesnage and Antoniou, 2020) . Glyphosate was declared 'probably carcinogenic' with strong evidence of genotoxicity by the World Health Organization's (WHO) International Agency for Research on Cancer (IARC) (World Health Organization, 2017; Tarazona et al., 2017). Using a risk assessment based approach (in contrast to the hazard based approach of IARC), Health Canada, and international pesticide regulatory authorities worldwide concluded that glyphosate does not pose a carcinogenic risk to humans (US EPA, 2018; Health Canada, 2017). Non-cancer potential health effects of glyphosate exposure including adverse reproductive outcomes have been evaluated in epidemiological studies (De Araujo et al., 2016; Mink et al., 2011) but few large high-quality cohort studies with biomarkers of glyphosate and its metabolite have been conducted (Kogevinas, 2021; Myers et al., 2016). In a review of 13 non-occupational studies with glyphosate biomonitoring data, Connolly and colleagues (Connolly et al., 2020) identified 2 studies of pregnant women (Aris and Leblanc, 2011; Parvez et al., 2018), and 1 study during the postpartum time period (Mcguire et al., 2016). More recently, authors of The Infant Development and the Environment Study (TIDES) (n = 94) (Lesseur et al., 2021, 2022) and Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) (n = 208) (Silver et al., 2021) study have measured glyphosate during pregnancy and reported positive associations between anogenital distance (Lesseur et al., 2021), shortened gestational age (Lesseur et al., 2022), and preterm birth (Silver et al., 2021). Authors of the PROTECT study also recently reported positive associations between AMPA and biomarkers of oxidative stress measured between 16 and 20 weeks as well as 24–28 weeks (Eaton et al., 2022).

Pregnant women may be exposed to glyphosate via ingestion of residues on food or in drinking water, dermal contact when handling the pesticide or from contact with treated surfaces, or inhalation of spray drift (Milesi et al., 2021). The established urinary biomarkers of exposure for glyphosate are the parent glyphosate and its primary metabolite aminomethylphosphonic acid (AMPA) (Zoller et al., 2020). AMPA has been measured during pregnancy in few biomonitoring studies (Lesseur et al., 2021; Mcguire et al., 2016; Silver et al., 2021). Glyphosate has a relatively short excretion half-life in humans of three to 14 h (Connolly et al., 2019; Zoller et al., 2020); however, authors of a study examining glyphosate kinetics following a controlled oral dose in humans observed a two-phase excretion pattern with half-lives between 6 and 9 h in the rapid phase and between 18 and 33 h in the slower phase (Faniband et al., 2021). This two-phase pattern is consistent with animal excretion (Williams et al., 2000). As reviewed by Williams and colleagues (Williams et al., 2000) and summarized by the US Agency for Toxic Substance and Disease Registry (ATSDR) (US Department of Health and Human Services. ATSDR, 2020), evidence from oral dosing studies in rats suggests that glyphosate is rapidly absorbed from the gastrointestinal tract but minimally metabolized; approximately one-third of ingested glyphosate was absorbed and excreted in urine with the remaining amount excreted unchanged in feces Human data on the toxicokinetics of glyphosate are scarce (Connolly et al., 2020). In contrast with the experimental evidence, authors of two recent studies in humans report that excreted urinary concentrations of unchanged glyphosate concentrations were 1–6% of the total dose (Faniband et al., 2021; Zoller et al., 2020). Using an estimated excretion rate of 1%, Campbell et al. reported that the maximum urinary glyphosate concentration in Australian farmers was 1.7 times higher than the acceptable daily intake (Campbell et al., 2022). *In vitro* studies of human skin samples as well as studies in rhesus monkeys suggest dermal penetration is low; human and animal data on inhalation are lacking (US Department of Health and Human Services, 2020; Williams et al., 2000).

The majority of urinary AMPA detected in human biomonitoring studies likely stems from direct, exogenous exposure to this metabolite via foods or water rather than via endogenous metabolism of glyphosate (Connolly et al., 2020; Zouaoui et al., 2013). In addition to microbial degratation of glyphosate, AMPA in drinking water may stem from the use and release of phosphonates into the environment (Grandcoin et al., 2017). Zoller et al. (2020) estimated the elimination half-life of AMPA in humans to be 8 h. Authors of one identified study examining glyphosate kinetics in humans (n = 3) reported that urinary AMPA concentrations were 0.01–0.04% of the total administered dose of glyphosate (Faniband et al., 2021). This finding is consistent with experimental literature demonstrating that less than one percent of administered glyphosate is metabolized to AMPA in Sprague-Dawley rats (Brewster et al., 1991; Panzacchi et al., 2018). In an oral dosing study in rats, 20% of administered AMPA was absorbed and excreted unchanged in urine; 74% of the dose was excreted in feces (Williams et al., 2000); corresponding human data are lacking (Connolly et al., 2020; Zoller et al., 2020).

Although glufosinate is commonly used in agriculture to treat glyphosate-resistant weeds, little is known about population level glufosinate exposures (Takano and Dayan, 2020). Glufosinate targets glutamine synthetase (Casida, 2017; Takano and Dayan, 2020) and has an estimated elimination half-life of 9 h (Hirose et al., 1999). The toxicity of glufosinate and its impact on glutamine synthetase in multiple organ system has been well studied in animal models (EFSA, 2005; US EPA, 2019; World Health Organization, 1999). Although glufosinate is intended to be applied to crops only once and usually early in the growing season (Takano and Dayan, 2020), residues on food have been detected (Health Canada, 2020; US EPA, 2019) suggesting that diet is a potential route of exposure. Based on evidence from pharmokinetic studies, glufosinate is poorly absorbed in the gut with the majority of administered glufosinate (74–88% of the parent compound) excreted unchanged in feces (US EPA, 2019). Glufosinate applied to plants may be metabolized by soil microbes and lead to potential for exogenous exposure to glufosinate metabolites (Takano and Dayan, 2020). 3-hydroxy(methyl) phosphinoyl propionic acid (3-MPPA) is one of the primary metabolites of glufosinate (EFSA, 2005). Data are insufficient to determine if 3-MPPA urinary concentrations are driven by endogenous metabolism of glufosinate or exogenous, environmental sources of 3-MPPA. With the exception of one small study that measured serum concentrations of glufosinate and 3-MPPA in both pregnant and non-pregnant women (Aris and Leblanc, 2011), data on these two analytes in urine are limited to reports of poisoning (Hirose et al., 1999; Lee

and Kim, 2019; Takahashi et al., 2000), occupational exposures (Koureas et al., 2014), and laboratory methods development (Bienvenu et al., 2021).

We conducted the present study to measure urinary glyphosate, AMPA, glufosinate, and 3-MPPA concentrations in predominantly urban, pregnant Canadian women, and to quantify associations between these analytes and sociodemographics, urine sampling characteristics, reported pesticide use, as well as the reported frequency of consuming fruits, vegetables, legumes, and grain products.

2. Materials and methods

2.1. Study population

The Maternal-Infant Research on Environmental Chemicals (MIREC) study is a national-level pregnancy cohort of 2001 women from 10 Canadian cities (Vancouver, Edmonton, Winnipeg, Sudbury, Ottawa, Kingston, Toronto, Hamilton, Montreal, and Halifax) (Arbuckle et al., 2013). Briefly, women were recruited between 2008 and 2011 in their first trimester and followed through delivery. Women were eligible for participation in MIREC if they had no serious medical complications, were at least 18 years old, less than 14 weeks gestation at the time of recruitment and could communicate in either English or French (Arbuckle et al., 2013). The present analysis used stored frozen first trimester urine samples from women who consented to use of their data and biological specimens in future research. Of the 2001 women recruited, 43 did not consent to the biobank, 18 withdrew from the study, and 60 urine samples were not collected leaving 1880 available for the present analysis.

The MIREC study was reviewed by the Health Canada Research Ethics Board as well as by ethics committees at all recruitment sites. Participants provided informed consent prior to participation.

2.2. Data collection

Women completed detailed questionnaires throughout pregnancy to provide information on their sociodemographic characteristics, health history, and lifestyle. At the first trimester visit (range: 6–13 weeks), women provided a spot urine sample. Research staff noted the date and time of urine collection, and time since last void. In the first trimester, women were asked specific questions about whether they or anyone in their house used pesticides since the beginning of their pregnancy. In the second trimester, women completed a food frequency questionnaire and reported whether they had eaten the food in the prior month, their frequency of consumption (e.g., number of servings per day, week, or month), as well as whether their average serving size was greater or smaller than average. We included vegetables, fruits, grains, and legumes in our analysis because these crops may be treated with herbicides. We created categories of food consumption for 21 foods in the food frequency questionnaire (FFQ) as follows: never consumed, low consumption ($\leq$ median consumption based on the distribution of servings per day within the sample), and high consumption (>median). Women reported whether their estimated serving size was greater or smaller than average. We applied a 33% adjustment to responses that were greater (+33%) or smaller (−33%) than average. For example, if a women reported her typical serving size of pasta was greater than the average serving of one cup, we increased her consumption by 33% (Morisset et al., 2016). In the post-partum questionnaire, women were asked to indicate their principal source of drinking water at home.

2.3. Laboratory analysis

The methodology used for measuring each herbicide and metabolite has been previously described (Bienvenu et al., 2021). Briefly, urine samples were enriched with labeled internal standards. After derivatization, the analytes were recovered by liquid-liquid extraction with methyl tertbutyl ether (MTBE) using the flash freeze technique. The extracts were evaporated to dryness and taken up in a solution of ammonium acetate in acetonitrile. The derivatized analytes were then analyzed by Ultra-Performance Liquid Chromatography (Waters Acquity) coupled to tandem mass spectrometry (Waters Xevo TQ-XS) (UPLC-MS/MS) in MRM mode using an electrospray ionization source in the positive mode. The inter-day precision for each analyte was tested in three different quality control levels (low 0.45, medium 2.5 and high 12 μg/L) and were as follows: glyphosate: 4.8–6.8%, AMPA: 7.9–8.3%, glufosinate: 6.1–7.7%, 3-MPPA: 4.9–7.9%. The limits of detection (LOD) and limits of quantification (LOQ) were calculated as 3- and 10- times, respectively, of the standard deviation of 10 replicates in an analysis of a sample with concentrations between 7- and 10- times the estimated LOD. This process was repeated on three identical instruments. The final LODs and LOQs were the highest values validated on these instruments and were as follows: glyphosate (0.08 and 0.26 μg/L), AMPA (0.09 and 0.29 μg/L), glufosinate (0.08 and 0.28 μg/L) and 3-MPPA (0.08 and 0.28 μg/L). The overall quality and accuracy of the analytical method for glyphosate and AMPA was monitored by participation with the following interlaboratory programs: German External Quality Assessment Scheme (G-EQUAS; Erlangen, Germany), the Organic Substances in urine Quality Assessment Scheme (OSEQAS; Centre de Toxicologie du Québec (CTQ)/INSPQ, Québec, Canada) and the Human Biomonitoring for Europe (HBM4EU, Wageningen, Netherlands). Machine readings data were available for all measurements below the LOD. Machine readings values of zero were substituted with one-half of the smallest positive value in statistical models.

2.4. Statistical analysis

We calculated the descriptive statistics of all chemicals using machine readings data for values below the LOD and substituted values for machine readings values of zero. For chemicals with a detection rate greater than 50% we reported the geometric mean and calculated Spearman's correlation coefficients. We calculated specific gravity (SG)-standardized concentrations using the following formula adapted from Just et al. (2010) and Duty et al. (2005): $P_c = P_i\ [(SG_m - 1)/(SG_i - 1)]$ where P_c is the *SG*-adjusted metabolite concentration (μg/L), P_i is the observed metabolite concentration, and SG_i is the specific gravity of the urine sample and SG_m is the median SG for the cohort.

We investigated whether chemical concentrations differed according to: sociodemographic and sample collection characteristics, reported pesticide use and diet. We calculated the geometric mean concentrations within the strata of each characteristic and tested for differences between the group specific means using ANOVA models. The specific gravity standardized $\log_{10}$-transformed analyte concentrations were the dependent variable and the maternal characteristics were the independent variables. Separate models were developed for each individual characteristic and analyte. We conducted model diagnostics by checking normality and homoscedasticity of the residuals. We calculated the statistical significance of the overall group effect. When the overall group effect was statistically significant (p-value <0.05), we calculated pairwise comparisons. The pairwise p-values were corrected for multiple comparisons using the Bonferroni correction.

In the analyses of dietary data, we adjusted for variables that were associated with glyphosate and AMPA. Unlike the descriptive analysis of the sociodemographic and sampling characteristics, there are clear independent variables (foods) and covariates (variables associated with glyphosate or AMPA in the descriptive analysis) in the FFQ analyses. There is, therefore, rationale for investigating whether these associations are confounded by the sociodemographic and sampling characteristics. Moreover, unlike many of the sociodemographic and sampling characteristics, herbicide concentrations in foods are amenable to intervention. 3 RESULTS

3. Results

The majority of participants in this analysis were over 30 years of age, born in Canada, non-smokers, and were in the normal range for pre-pregnancy body mass index (Supp Table 1). Glyphosate and AMPA were above the LOD in 74% and 72% and above the LOQ in 49% and 43% of participant urine samples, respectively. Glufosinate and 3-MPPA were detected in one and six percent of participant urine samples respectively (Table 1). Due to these low detection rates, we did not undertake further statistical analysis of glufosinate and 3-MPPA. Using machine readings data, specific gravity-standardized geometric mean glyphosate and AMPA concentrations were 0.112 μg/L (95% CI: 0.099, 0.127) and 0.159 μg/L (95% CI: 0.147, 0.172). The Spearman correlation coefficient between glyphosate and AMPA was 0.61 ($p < 0.001$).

Women born in Canada had 0.041 μg/L higher urinary concentrations of glyphosate than women born elsewhere (p-value = 0.02); however, we observed no differences in glyphosate concentrations between women who were White and those of another race. In contrast, women who were White had 0.015 μg/L lower concentrations of AMPA in urine than those women of another race (p-value = 0.03). We observed no differences in AMPA concentrations according to country of birth. Glyphosate and AMPA concentrations were both positively associated with pre-pregnancy BMI and education (Table 2). No associations were observed between analyte concentrations and maternal age, household income, parity, smoking or infant sex (Table 2).

Urinary glyphosate and AMPA concentrations were higher in samples collected later in the day (18:00–24:00) than those collected prior to 9:00. We observed no association between time since last void or season of collection and concentrations of either analyte. We also observed no association between source of drinking water or reported pesticide use by MIREC participants or anyone in their home and concentrations of either analyte (Table 3).

In adjusted models, consumption of raw spinach, soy or rice beverages, whole grain bread, or pasta was associated with higher glyphosate concentrations (overall p-values <0.05). The pairwise comparison of high vs no consumption was statistically significant ($p < 0.05$) for raw spinach, whole grain bread, and pasta but geometric mean concentrations in the low consumption group were higher than those in the high consumption group for pasta (Fig. 1, Suppl Table 2). Geometric mean glyphosate concentrations according to consumption of whole grain bread was suggestive of a dose-response association. The geometric mean and lower 95% CI concentrations of glyphosate were both < LOD for women who ate no whole grain bread. In the low and high consumptions groups, glyphosate geometric means (95% CI) were 0.105 (95% CI: 0.081, 0.137) and 0.182 (95% CI: 0.138, 0.240) μg/L respectively. Consumption of fruit juices and drinks, other than orange juice with calcium, was inversely associated with glyphosate concentrations (overall and pairwise p-values <0.05). No other foods were associated with glyphosate concentrations.

Geometric mean AMPA concentrations were higher among women who consumed more than the median number of servings per day of fruit juice, whole grain bread, and pasta (overall p-values <0.05) but the pasta and fruit juice associations were non-monotonic and none of these associations was suggestive of a statistically significant dose-response relationship. No other foods were associated with AMPA concentrations (Fig. 2, Suppl Table 2).

4. Discussion

Most pregnant women in this large, primarily urban cohort had detectable concentrations of glyphosate and its metabolite AMPA in their first trimester urinesamples. Few women had detectable concentrations of glufosinate or its metabolite 3-MPPA. These findings represent the time period of sample collection (2008–2011) but, given changing patterns of herbicide use, may not reflect contemporary exposure patterns. Our results support the hypothesis that consumption of grains and certain fruits and vegetables contributes to glyphosate and AMPA exposure; however, our results must be interpreted with caution because glyphosate geometric mean concentrations were consistently below the LOQ (0.26 μg/L). The absence of associations with season of collection and pesticide use suggest that spray drift and direct exposure to these analytes were not major routes of exposure for MIREC participants, most of whom reside in urban regions.

At the time of participant recruitment and sample collection, glyphosate was registered for use in Canadian agricultural and residential settings (Health Canada, 2022). Glufosinate was also registered for use in agricultural settings at the time of urine collection (Health Canada, 2020) but domestic sales were consistently lower than glyphosate throughout the study recruitment period (2008–2011) (Health Canada, 2008, 2009, 2011).Thus, although our observed concentrations may not be reflective of contemporary Canadian exposure patterns, they are comparable to or lower than other recent North American pregnancy cohort studies with biomonitoring data and overlapping years of participant recruitment (Aris and Leblanc, 2011; Ferguson et al., 2019; Kongtip et al., 2017; Lesseur et al., 2021; Parvez et al., 2018; M. K. Silver et al., 2021) (Suppl Table 3). Exposure patterns and resulting biomonitoring concentrations have likely changed since the mid-2000s due to the rise in use of glyphosate as a harvest aid (Benbrook, 2016). Median glyphosate urine concentrations in the TIDES study of 94 mother-infant pairs from four medical centers in the US (0.22 μg/L) (Lesseur et al., 2021) are comparable to those observed in MIREC (0.25 μg/L), whereas AMPA concentrations are slightly lower (TIDES: 0.14 μg/L; MIREC 0.21 μg/L). Geometric mean glyphosate (0.44 μg/L) and AMPA (0.25 μg/L) urine concentrations at 18 weeks gestation among PROTECT study (Puerto Rico) participants are both higher than observed in MIREC (Silver et al., 2021). Median glyphosate concentrations (3.25 μg/L) in the central Indiana study of 71 pregnant women (Parvez et al., 2018) are notably higher than those observed in the TIDES, PROTECT, or MIREC studies. Differences in the observed concentrations among the TIDES, PROTECT, Indiana and MIREC studies

Table 1
Descriptive statistics of herbicides and metabolites in first trimester urine samples, MIREC study, 2008–2011 (μg/L).

Analyte	N		LOD	%<LOD	25th %ile	Median	75th %ile	95th %ile	Max	Geometric Mean[a] (95% CI)
Not SG-standardized										
Glyphosate	1829	0.08		26	< LOD	0.225	0.523	1.711	7.813	0.098 (0.086, 0.112)
AMPA	1848	0.09		28	< LOD	0.119	0.470	1.275	5.725	0.140 (0.127, 0.153)
Glufosinate	1855	0.08		99	< LOD	< LOD	< LOD	< LOD	6.053	NA
3-MPPA	1854	0.08		94	< LOD	< LOD	< LOD	0.113	4.043	NA
SG-standardized										
Glyphosate	1826	0.08		26	0.120	0.249	0.466	1.092	3.907	0.112 (0.099, 0.127)
AMPA	1845	0.09		28	0.111	0.213	0.384	0.872	6.139	0.159 (0.147, 0.172)
Glufosinate	1852	0.08		99	< LOD	< LOD	< LOD	< LOD	3.421	NA
3-MPPA	1851	0.08		94	< LOD	< LOD	< LOD	0.091	6.570	NA

SG: specific gravity: LOD: limit of detection; AMPA aminomethylphosphonic acid; 3-MPPA 3-hydroxy(methyl) phosphinoyl propionic acid; NA = not applicable.
[a] Geometric means not calculated for glufosinate or 3-MPPA due to low detection rates.

Table 2
Specific gravity-standardized urinary concentrations of glyphosate and AMPA according to sociodemographic characteristics.

Variable	Glyphosate				AMPA			
	N	GM (95% CI) (μg/L)	Overall p-value	Pairwise[a] p-value	N	GM (95% CI) (μg/L)	Overall p-value	Pairwise p-value
Sociodemographic								
Maternal Age (y)								
<25	119	0.115 (<LOD, 0.187)	0.58		120	0.159 (0.118, 0.215)	0.60	
25-29	425	0.112 (0.086, 0.145)			429	0.168 (0.144, 0.196)		
30-34	659	0.117 (0.095, 0.143)			670	0.170 (0.150, 0.193)		
≥35	623	0.107 (0.086, 0.132)			626	0.143 (0.123, 0.167)		
Country of birth								
Canada	1482	0.121 (0.106, 0.138)	**0.02**		1496	0.164 (0.150, 0.178)	0.33	
Other	344	< LOD (<LOD, 0.110)			349	0.142 (0.116, 0.173)		
Race								
Non-white	1535	0.114 (0.100, 0.131)	0.47		1553	0.155 (0.142, 0.169)	**0.03**	
White	291	0.099 (0.070, 0.140)			292	0.184 (0.153, 0.222)		
Pre-pregnancy BMI (kg/m^2)								
< 25	1080	0.108 (0.092, 0.127)	**0.03**	–	1081	0.149 (0.134, 0.166)	**<0.01**	
25 - < 30	368	0.121 (0.093, 0.158)		0.99	377	0.157 (0.131, 0.189)		0.66
≥30	250	0.128 (0.090, 0.183)		**0.03**	253	0.212 (0.175, 0.256)		**<0.01**
Household income (CAD$)								
≤50,000	316	0.110 (0.081, 0.150)	0.08		311	0.181 (0.151, 0.216)	0.41	
50,001–100,000	722	0.111 (0.090, 0.136)			738	0.162 (0.143, 0.183)		
>100,000	709	0.107 (0.088, 0.130)			716	0.146 (0.128, 0.168)		
Education								
≤ High School	254	0.148 (0.107, 0.204)	**<0.01**	–	252	0.206 (0.174, 0.245)	**0.02**	–
≤ College Diploma	422	0.091 (<LOD, 0.120)		**<0.01**	431	0.162 (0.137, 0.191)		0.22
≥ University	1148	0.113 (0.097, 0.132)		**0.04**	1161	0.150 (0.135, 0.166)		**0.01**
Parity								
0	815	0.106 (0.088, 0.128)	0.40		820	0.159 (0.142, 0.179)	0.83	
1	733	0.116 (0.095, 0.141)			746	0.154 (0.135, 0.176)		
2+	278	0.118 (0.086, 0.162)			279	0.174 (0.145, 0.208)		
Smoking[b]								
Never	1104	0.111 (0.095, 0.131)	0.07		1121	0.159 (0.095, 0.131)	0.99	
Former	505	0.127 (0.101, 0.159)			507	0.150 (0.101, 0.159)		
Smoker	215	0.085 (<LOD, 0.123)			215	0.183(0.144, 0.231)		
Infant Sex								
Male	960	0.110 (0.093, 0.131)	0.47		972	0.159 (0.142, 0.178)	0.27	
Female	859	0.113 (0.095, 0.135)			867	0.153 (0.136, 0.172)		

Y years, BMI body mass index, GM geometric mean.
[a] Pairwise p-values are comparisons between categories and referent value and are corrected for multiple comparisons using the Bonferroni correction. For example, the glyphosate p-value for the comparison of BMI≥30 to <25 is 0.03. Pairwise comparisons were only calculated when the overall p-value was <0.05.
[b] Smokers are individuals who reported currently smoking during the first trimester visit or had quit when they knew they were pregnant.

may be explained by variability in glyphosate use patterns and exposure potential; the use of different labs and related laboratory conditions may also contribute to some of the observed variability across studies (Suppl Table 3). The specific gravity standardized geometric mean and median glyphosate concentrations in MIREC were lower than the Health Canada derived biomonitoring equivalent of 5.4 μg/L (Tech et al., 2021). All subgroup geometric means were below this threshold. The biomonitoring equivalent is a screening tool used to identify biomonitoring results that require further evaluation and to set priorities for future research or exposure reduction (Aylward et al., 2013; Government of Canada, 2016). The value for glyphosate was developed similar to other biomonitoring equivalents for urine (Aylward et al., 2011; Government of Canada, 2016). It was based on the Health Canada acceptable daily intake reference value (Government of Casida, 2017), takes into account the different bioavailability of glyphosate following oral doses in humans compared to rats and that absorbed glyphosate is rapidly excreted via urine in humans (NTP, 1992; ATSDR, 2020).

Due to the lack of data, sources of glufosinate exposure in the general population, including pregnant women, are largely unknown. Based on published regulatory assessments (Health Canada, 2020; US EPA, 2019), we speculate that women may be primarily exposed via diet but we did not have the capacity to assess potential dietary sources given the low detection rates. Only 6% of urine samples in the MIREC study had detectable concentrations of 3-MPPA and 1% had detectable concentrations of glufosinate. These low detection frequencies likely reflect the modest use of glufosinate during the time of sample collection (2008–2011). Contemporary detection rates may be higher due to glyphosate resistance and subsequent changing patterns of herbicide use (Takano and Dayan, 2020). In the only other identified study of these analytes in pregnancy, authors measured delivery and umbilical cord serum concentrations of glyphosate, glufosinate, AMPA, and 3-MPPA in 39 pregnant women who resided in the Eastern Townships of Quebec (Aris and Leblanc, 2011). Glyphosate, AMPA, and glufosinate were not detected in any of the serum delivery or cord samples; however, 3-MPPA was detected in all maternal and cord samples. Due to the use of different matrices (serum vs urine), it is not possible to directly compare detection limits or rates between this study and MIREC.

We observed similar patterns between sociodemographic characteristics and both glyphosate and AMPA concentrations. The absolute differences in concentrations among sociodemographic strata were marginal and of questionable biological significance. For example, the difference between geometric mean glyphosate concentrations among women with an obese pre-pregnancy BMI and those with an underweight or normal BMI was 0.02 μg/L which is 18% of the population geometric mean (0.112 μg/L) and roughly equivalent to the width (0.028) of the corresponding confidence interval (0.099, 0.127). Interestingly, race was associated with AMPA concentrations whereas country of birth was associated with glyphosate concentrations. Although we do not have the capacity to disentangle the underlying explanations for these differences, our results indicate the need to consider women's sociodemographic profiles from multiple perspectives. No differences in glyphosate or AMPA concentrations were noted for sociodemographic characteristics in the TIDES study (Lesseur et al., 2021). We found that urine specimens collected later in the day had

Table 3
Specific gravity-standardized urinary concentrations of glyphosate and AMPA according to sample collection characteristics, drinking water source and pesticide exposure.

Variable	Glyphosate				AMPA			
	N	GM (95% CI) (μg/L)	Overall p-value	Pairwise p-value	N	GM (95% CI) (μg/L)	Overall p-value	Pairwise p-value
Sample Collection								
Time of urine collection								
6:00–9:00	27	<LOD (<LOD, 0.287)	**<0.01**	–	28	0.134 (0.090, 0.198)	**<0.01**	–
9:00–12:00	787	0.082 (<LOD, 0.101)		1.0	799	0.107 (0.093, 0.123)		1.0
12:00–15:00	621	0.114 (0.092, 0.142)		1.0	623	0.186 (0.165, 0.211)		0.11
15:00–18:00	355	0.198 (0.159, 0.247)		0.71	358	0.280 (0.243, 0.323)		**<0.01**
18:00–24:00	33	0.306 (0.178, 0.526)		0.22	34	0.333 (0.264, 0.419)		**<0.01**
Time since last void (minutes)								
≤75	473	0.119 (0.093, 0.151)	0.53		479	0.144 (0.122, 0.171)	0.92	
76–120	576	0.098 (<LOD, 0.123)			573	0.157 (0.135, 0.182)		
121–170	253	0.127 (0.093, 0.175)			261	0.182 (0.153, 0.215)		
>170	430	0.118 (0.091, 0.152)			439	0.171 (0.146, 0.200)		
Season of collection								
Spring	434	0.118 (0.093, 0.151)	0.57		439	0.158 (0.134, 0.187)	0.18	
Summer	436	0.099 (<LOD, 0.130)			438	0.142 (0.118, 0.170)		
Fall	518	0.098 (<LOD, 0.126)			525	0.151 (0.129, 0.176)		
Winter	438	0.139 (0.110, 0.176)			443	0.192 (0.169, 0.219)		
Source of Drinking Water								
Municipal	705	0.114 (0.093, 0.140)	0.28		711	0.145 (0.126,0.166)	0.48	
Private well	57	0.123 (<LOD, 0.252)			55	0.174 (0.105,0.287)		
Bottled	142	0.081 (<LOD, 0.128)			147	0.159 (0.117, 0.215)		
Other	26	< LOD (<LOD, 0.118)			26	0.107 (<LOD, 0.156)		
Don't know	96	< LOD (<LOD, 0.192)			97	0.163 (<LOD, 0.33)		
Pesticide Use								
Have you used pesticides for lawn/garden weeds during pregnancy								
No	1760	0.113 (0.100, 0.129)	0.57		1760	0.159 (0.147, 0.173)	0.53	
Yes	56	0.082 (<LOD, 0.166)			56	0.156 (0.099, 0.245)		
Have you used chemicals to control weeds in lawn or garden								
No	1791	0.112 (0.099, 0.127)	0.93		No	0.159 (0.146, 0.172)	0.35	
yes	32	<LOD (<LOD, 0.203)			yes	0.184 (0.100, 0.337)		
Has anyone in your home used chemicals to control weeds in lawn or garden								
No	1731	0.111 (0.098, 0.126)	0.32		yes	0.161 (0.148, 0.175)	0.82	
Yes	87	0.119 (<LOD, 0.211)				0.120 (0.084 0.173)		

GM geometric mean.
[1]Pairwise p-values are comparisons between categories and referent value and are corrected for multiple comparisons using the Bonferroni correction.

higher urinary glyphosate and AMPA concentrations. This pattern has been observed in other MIREC studies examining determinants of phthalates (Arbuckle et al., 2014), free triclosan (Arbuckle et al., 2015) and metabolites of organophosphate insecticides (Sokoloff et al., 2016). Given the relatively short half-life of glyphosate and AMPA, it is possible that evening concentrations reflect exposures, such as ingestion of food containing pesticide residues, that occurred throughout the day. Time of urine collection was associated with AMPA, but not glyphosate, concentrations in the TIDES study; the authors did not specify what time period tended to have the highest concentrations (Lesseur et al., 2021). Considering the influence of this variable on urinary concentrations, we recommend that studies examining the health effects of glyphosate use a standard time of urine collection or - in the absence of standardized timing - include this variable in multivariable statistics.

The increasingly common practice of applying glyphosate close to the time of harvest may contribute to herbicide residues on food (Myers et al., 2016). In 2015–2016, the Canadian Food Inspection Agency's (CFIA) tested for glyphosate residues in 3188 food samples and detected glyphosate (sum of glyphosate and AMPA residues) in 29.7% of all samples; glyphosate concentrations exceeded the Canadian maximum residue limit (MRL) in 1.3% of samples. No samples of fruits, vegetables, soy products, or infant foods exceeded the Canadian MRL; however, 36.6% of grain products had detectable concentrations of glyphosate residues and 3.9% were above the MRL (Canadian Food Inspection Agency, 2017). Authors of a separate analysis of 7955 food product samples in the Canadian retail market detected glyphosate in 42% of all samples. The highest levels of glyphosate were observed in beans (dried, flour), chickpeas (flour), and wheat products (wheat bran); the lowest concentrations were found in fresh fruits (Kolakowski et al., 2020). Both of these reports summed glyphosate and AMPA concentrations because the glyphosate MRL is based on the sum of glyphosate and AMPA (Government of Health Canada, 2020; Kolakowski et al., 2020). Our observation that pasta and whole grain bread consumption was positively associated with glyphosate urinary concentrations is consistent with these reports assuming that these reported food residues are representative of what was consumed at the time of urine collection. Using the previously described method for dealing with nondetects, the observed difference between those who consumed more than the median servings of whole grain bread and those who never ate it was 0.119 μg/L which is more than four times the width of the 95% CI of the population geometric (0.028). A similar pattern was observed for AMPA, although the magnitude of difference between those who ate more than the median and those who never ate whole grain bread was smaller (0.058 μg/L).

Our findings raise questions about dietary sources of AMPA. When examined separately, AMPA was detected less frequently than glyphosate (Government of Health Canada, 2020; Kolakowski et al., 2020). The Food and Agricultural Organization of the United Nations reported that mean glyphosate residue concentrations in food were consistently higher than AMPA in supervised food residues trials on lentils, beans, and tree nuts (World Health Organization, 2019). These data may not be applicable to fruit juice due to differing application rates and glyphosate metabolism patterns Individuals may be directly exposed to AMPA via diet following microbial degradation of glyphosate on plants and in soil (US Department of Health and Human Services, 2020; Gomes et al., 2014). Environmental AMPA concentrations can also originate from the

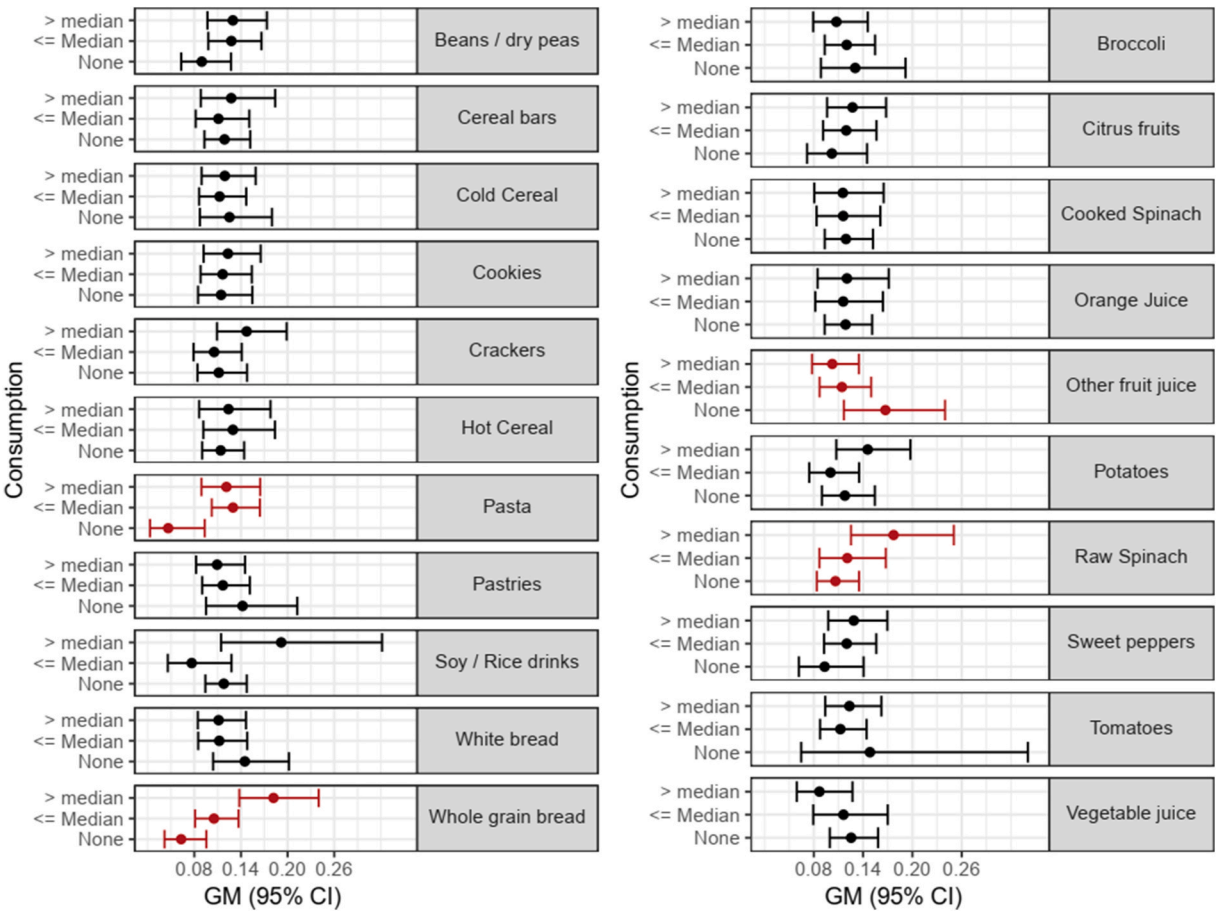

Fig. 1. Adjusted geometric mean (95% CI) standardized urinary concentrations of glyphosate according to dietary consumption. Food groups with a red 95% CI error bar and geometric mean point have statistically significant pairwise comparisons ($p < 0.05$) between consumption above the median and none. The glyphosate LOD (0.08 μg/L) and LOQ (0.26 μg/L) are indicated on the axis; concentrations < LOD are machine readings data. All concentrations below the LOQ should be interpreted with caution. Models are adjusted for country of birth, time of urine collection, pre-pregnancy BMI and education. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

use of phosphonates (Grandcoin et al., 2017). Authors of a controlled study with a known concentration of glyphosate and AMPA in a falafel dish reported that, depending upon different assumptions regarding the amount of glyphosate metabolized to AMPA, between 1 and 23% of ingested AMPA was excreted in urine as AMPA (Zoller et al., 2020). Another experimental study administered glyphosate to three volunteers and reported that urinary AMPA concentrations were between 0.01 and 0.04% of the total glyphosate dose (Faniband et al., 2021). Together, these two studies suggest that glyphosate metabolism is not the primary source of AMPA concentrations in MIREC participants. The moderate degree of correlation between glyphosate and AMPA in our study suggests that glyphosate and AMPA may have a shared source of exposure such as dietary residues. The similar patterns between consumption of several foods (e.g. soy or rice beverages, whole grain bread, fruit juices) and both glyphosate and AMPA concentrations provide further support for this hypothesis. For example, glyphosate was associated with soy or rice beverage consumption (overall p-value = 0.0294); a similar direction of association was observed between AMPA and soy or rice beverage consumption, but the association was not statistically significant (p = 0.0854). Several recent studies have examined associations between diet and urinary glyphosate and AMPA concentrations (Lemke et al., 2021; Soukup et al., 2020; Stajnko et al., 2020) but none have evaluated these associations in pregnant women.

Type of diet and agricultural practices may influence extent of exposure from food and drink and their respective residue levels. A recent intervention study (n = 16 participants) reported that adoption of an organic diet for six days resulted in 70% decreases in both glyphosate and AMPA urinary concentrations (Fagan et al., 2020). Three observational studies examined type of diet and glyphosate urine concentrations with one reporting higher glyphosate urine concentrations in those who ate conventional diets compared to predominantly organic diets (Krüger et al., 2014); however, the other two found no statistically significant difference between organic food intake and urinary concentrations in lactating (Mcguire et al., 2016) and pregnant women (Parvez et al., 2018). The MIREC study did not have any questions on conventional vs organic diets so we were unable to distinguish analyte concentrations according to type of diet. Herbicide application frequency, intensity, and timing can also influence food residue levels (Myers et al., 2016). Pre-harvest application of glyphosate, done to promote crop drying and faster, more consistent maturation, and easier harvesting, results in higher residue levels (Bøhn et al., 2014; Bøhn and Millstone, 2019; Myers et al., 2016). In contrast, pre-plant applications are less likely to lead to detectable residue levels (Bøhn and Millstone, 2019; Duke et al., 2003). In addition, genetically modified foods may have higher residue levels than conventional foods possibly due to repeated spraying of the plants with glyphosate-based herbicides throughout the growing season (Bøhn et al., 2014; Bøhn and Millstone, 2019; Duke et al., 2003).

Both AMPA and glyphosate have been detected in surface water and groundwater in Canada (Grandcoin et al., 2017; Struger et al., 2015; Van Stempvoort et al., 2016). A study of southern Ontario surface water samples reported that three-quarters of 52 measured samples had detectable concentrations of AMPA and glyphosate but none of the

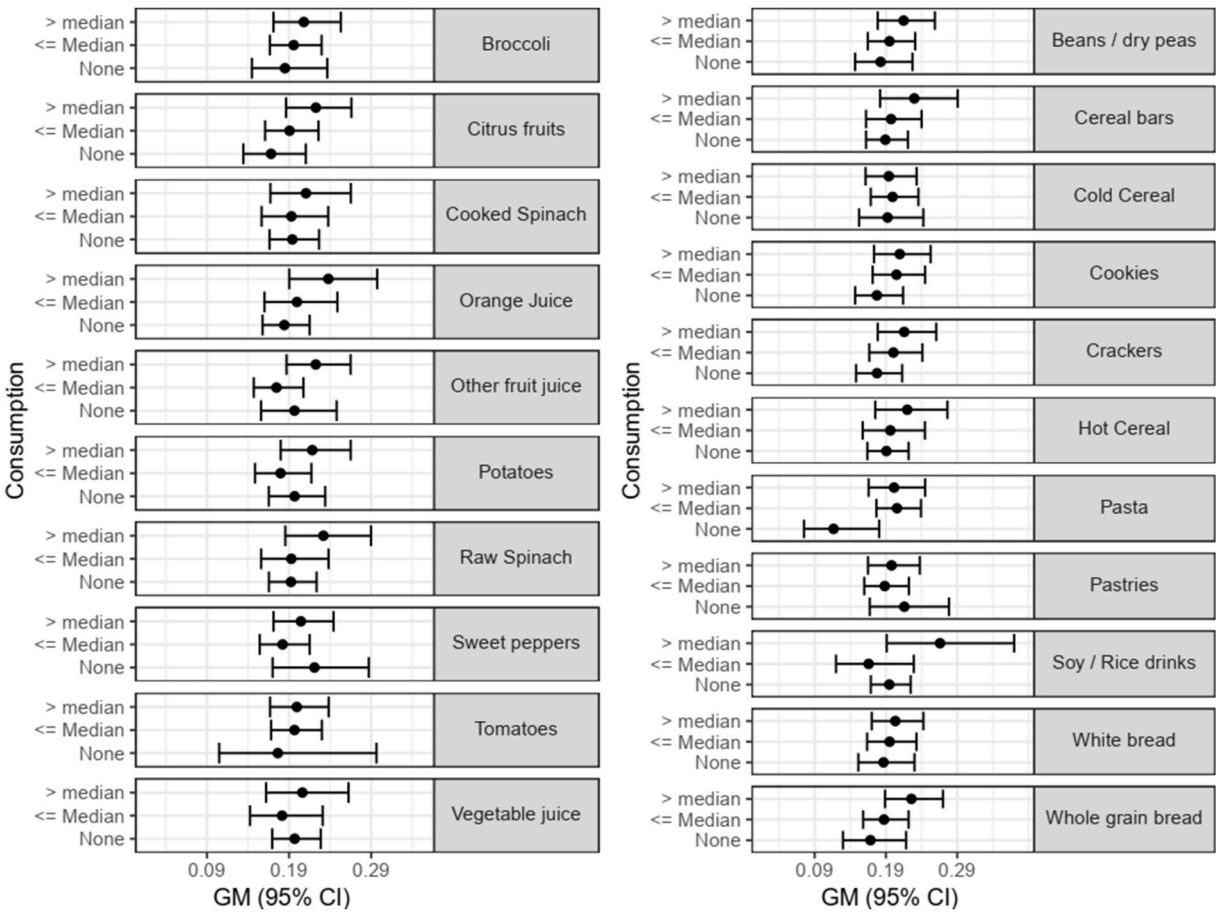

Fig. 2. Adjusted geometric mean (95% CI) standardized urinary concentrations of AMPA according to dietary consumption. The AMPA LOD (0.09 μg/L) and LOQ (0.29 μg/L) are indicated on the axis; concentrations < LOD are machine readings data. All concentrations < LOQ should be interpreted with caution. Models are adjusted for race, time of urine collection, BMI and education.

glyphosate concentrations exceeded the Canadian Drinking Water quality guideline of 280 μg/L (Struger et al., 2015). No drinking water guidelines exist for AMPA. Van Stempvoort et al. (2016) detected glyphosate and AMPA in 10.5% and 5.0% of groundwater samples from an agricultural catchment region with all concentrations below the Canadian guideline. Groundwater in agricultural regions may be particularly subject to AMPA contamination (Grandcoin et al., 2017). As previously noted, the presence of AMPA in water may originate from the use and release of phosphonates into the environment (Grandcoin et al., 2017); however, an analysis of wastewater effluent suggests that AMPA water concentrations were largely linked to glyphosate applications (Struger et al., 2015). The one identified pregnancy cohort study that measured glyphosate in drinking water concentrations did not detect glyphosate in any drinking water samples from residents of Central Indiana (Parvez et al., 2018). In the MIREC study, the majority of participants live in urban regions and receive their water from municipal sources. In the absence of drinking water samples analyzed for glyphosate, we were unable to determine whether drinking water is a source of AMPA or glyphosate exposure and how exposure in drinking water compares to that from food residues.

This study is characterized by several notable strengths including the large sample size, extensive sociodemographic characteristics, multi-site recruitment, and a sensitive laboratory method using conservative determinations of the LOD and LOQ. We analyzed glyphosate and AMPA in 92% of enrolled MIREC participants and, therefore, are subject to minimal, if any, bias due to attrition. Other pregnancy cohorts often had smaller sample sizes or recruited from a small geographic region (Lesseur et al., 2021; Parvez et al., 2018; Silver et al., 2021). Although MIREC is a large pan-Canadian study, participants are primarily women who resided in urban areas at the time of recruitment, were of moderate to high socioeconomic status and born in Canada (Arbuckle et al., 2013). Our results, therefore, are not generalizable to populations from marginalized or rural communities. Interpretation of our results is also limited by the availability of one spot urine sample in early pregnancy. Repeated glyphosate concentrations in the PROTECT study were variable throughout pregnancy with an intraclass correlation coefficient of 0.24. AMPA concentrations were less variable with an intraclass correlation coefficient of 0.63 (Silver et al., 2021). The potential variability throughout pregnancy does not impair our descriptive statistics or our analysis of associations with sociodemographic and health history characteristics because we did not rely on any inferences regarding stability of glyphosate or AMPA concentrations throughout pregnancy. Although we did adjust the dietary analyses according to sociodemographic and sampling characteristics, it is possible that some of the observed associations between dietary consumption and analyte concentrations may be confounded by co-occurring patterns. For example, the inverse association between fruit juices and glyphosate concentrations is likely explained by co-consumption of another food group or beverage among women who did not drink any fruit juice. Furthermore, we relied on self-reported pesticide use and dietary data both of which may have contributed to exposure misclassification. The FFQ was completed at the second trimester visit (and referred to the previous month's diet) and subsequent to the first trimester urine collection. We conducted our analysis based on the assumption that the consumption patterns reported in the FFQ were reflective of women's consumption patterns in the first trimester but nondifferential misclassification is

possible due to this assumption and the uncertainty associated with recall. We minimized this potential misclassification by categorizing women into groups of consumption (none, (≤median, and > median). Last, we note that subgroup geometric means were often below the LOQ and that results below the LOQ may be more imprecise and should be interpreted with caution. Nevertheless, there is sufficient epidemiological and laboratory rationale for using rather than censoring these results. Using the data below the LOQ allows us to preserve the rank order of the data rather than imposing an arbitrary distribution. Furthermore, all observations are subject to measurement error and background noise; presenting data above a threshold with the implication of no measurement error creates a false dichotomy and leads to substantial loss of data (Whitcomb and Schisterman, 2008). We recognize that making decisions about individual diagnostics is not advised when the signal to noise ratio is low (as in the case of values < LOQ) but in population based analyses such as MIREC these data points contain information of value (Browne and Whitcomb, 2010). Furthermore, the INSPQ laboratory calculated the LOQ by running the analysis on three different identical instruments and selecting the highest value obtained out of these three LOQs. This more conservative approach to defining the LOQ ensures that the variations that may occur at low concentrations are taken into consideration when providing a result. Nevertheless, we recognize the potential lower imprecision in values below the LOQ. For this reason, we have focused our interpretation on dose-response patterns and precision rather than specific geometric mean concentrations. The associations of whole grain bread and glyphosate was the only observed monotonic relationship where the 95% CIs of the GMs for women who ate more than the median servings of these foods did not overlap with the 95% CI of the GMs for women who never ate these foods.

5. Conclusions

This pan-Canadian study provides biomonitoring data on these herbicides and primary metabolites in the largest sample of pregnant women to date. Most women had detectable concentrations of glyphosate and AMPA but glufosinate and 3-MPPA were rarely detected. We conclude that diet is a potential source of exposure and that direct pesticide use and spray drift are unlikely routes of exposure to glyphosate or AMPA in this largely urban Canadian population. We suggest interpreting results below the LOQ with caution. Future research in the MIREC study will examine associations between these herbicides and maternal-child health.

Author Contribution statement

Jillian Ashley-Martin: Conceptualization, Writing – Original Draft, Writing – Review and Editing, Visualization Rong Huang: Formal analysis, Writing – Review and Editing Susan MacPherson: Formal analysis, Writing – Review and editing Orly Brion: Formal analysis, Writing – Review and Editing James Owen: Formal analysis, Writing – Review and Editing Eric Gaudreau: Laboratory methodology and analysis, Writing – Review and Editing Jean-Francois Bienvenu: Laboratory methodology and analysis, Writing – Review and Editing Mandy Fisher: Writing – Review and Editing Michael M. Borghese: Writing – Review and Editing Maryse F. Bouchard: Writing – Review and Editing Bruce Lanphear: Writing – Review and Editing Warren G. Foster: Writing – Review and Editing Tye E. Arbuckle: Conceptualization, Writing – Review and Editing, Funding Acquisition.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Warren G. Foster reports a relationship with Hollingsworth LLC that includes: paid expert testimony.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114842.

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