Urinary glyphosate concentration in pregnant women in relation to length of gestation

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Ethics approval and consent to participate
The TIDES study protocols were approved by the institutional review boards (IRB) of each study center (University of California, San Francisco, University of Rochester Medical Center, University of Minnesota, and University of Washington/Seattle Children's Hospital). Prior to study implementation and all subjects provided signed informed consent before starting any study activities. IRB approval was also obtained at the Icahn School of Medicine at Mount Sinai, which serves as the TIDES Coordinating Center since 2011.

Declaration of Competing Interest
The authors declare that they have no competing interests.

Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

Human exposure to glyphosate-based herbicides (GBH) is increasing rapidly worldwide. Most existing studies on health effects of glyphosate have focused on occupational settings and cancer outcomes and few have examined this common exposure in relation to the health of pregnant women and newborns in the general population. We investigated associations between prenatal glyphosate exposure and length of gestation in The Infant Development and the Environment Study (TIDES), a multi-center US pregnancy cohort. Glyphosate and its primary degradation product [aminomethylphosphonic acid (AMPA)] were measured in urine samples collected during the second trimester from 163 pregnant women: 69 preterm births (< 37 weeks) and 94 term births, the latter randomly selected as a subset of TIDES term births. We examined the relationship between exposure and length of gestation using multivariable logistic regression models (dichotomous outcome; term versus preterm) and with weighted time-to-event Cox proportional hazards models (gestational age in days). We conducted these analyses in the overall sample and secondarily, restricted to women with spontaneous deliveries (n=90). Glyphosate and AMPA were detected in most urine samples (>94%). A shortened gestational length was associated with maternal glyphosate (hazard ratio (HR): 1.31, 95% confidence interval (CI) 1.00–1.71) and AMPA (HR: 1.32, 95%CI: 1.00–1.73) only among spontaneous deliveries using adjusted Cox proportional hazards models. In binary analysis, glyphosate and AMPA were not associated with preterm birth risk (<37 weeks). Our results indicate widespread exposure to glyphosate in the general population which may impact reproductive health by shortening length of gestation. Given the increasing exposure to GBHs and the public health burden of preterm delivery, larger confirmatory studies are needed, especially in vulnerable populations such as pregnant women and newborns.

Keywords

1. Introduction

Glyphosate [N-(phosphonomethyl)glycine] is an organophosphorus compound and the active ingredient of glyphosate-based herbicides (GBHs) (such as Roundup®), the most commonly used herbicides, with over 8 million metric tons applied worldwide in the past decade (Benbrook, 2016; Guyton et al., 2015). The use of GBHs in agriculture has risen drastically since 1996 when genetically modified glyphosate-tolerant crops were introduced into the US market (Benbrook, 2016; Myers et al., 2016). Besides weed control during pre-plantation and growth, GBHs have also been used for pre-harvest desiccation of a wide variety of crops (Benbrook, 2016). Glyphosate can be metabolized by microorganisms into aminomethylphosphonic acid (AMPA), its primary metabolite. Glyphosate and AMPA residues have been widely detected in foods in the US (USDA, 2019), Canada (Kolakowski et al., 2020) and Europe (Zoller et al., 2018). Environmental contamination with these residues has also been reported in soil, dust, rain and surface water (Bai and Ogbourne,

2016). As a result, the general population may be exposed to GBHs through various routes including ingestion, inhalation, and dermal absorption. Recently, multiple studies have reported detectable glyphosate in human body fluids (mainly urine) from the general population (Gillezeau et al., 2019) and detection rates have been increasing over the last decades (Conrad et al., 2017; Mills et al., 2017). For example, an analysis of urine samples from California revealed an increase of mean glyphosate levels from 0.024 ng/mL in 1993–1996 to 0.314 ng/mL in 2014–2016, with the prevalence of samples with detectable glyphosate level rising from 12% to 70% in the same period (Mills et al., 2017).

Glyphosate exerts its herbicidal activity by inhibiting the 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS), a crucial enzyme for aromatic amino acid biosynthesis present in plants but not in mammals. Thus, glyphosate was initially considered relatively harmless to human health. However, evidence starts to emerge that links glyphosate and/or GBHs with adverse health outcomes in both animal models and humans (Landrigan and Belpoggi, 2018; Mesnage et al., 2015; Myers et al., 2016). For example, multiple studies in rodents have shown that GBH exposure has effects in the gut microbiome (Mao et al., 2018; Mesnage et al., 2019); since some bacteria and fungi use the shikimate pathway and are sensitive to its effects (Mesnage et al., 2021). Possible carcinogenic effects of glyphosate have been subject of intense public interest and scientific discussion (Benbrook, 2019), especially since it was classified as a probable human carcinogen (2A) by the International Agency for Research on Cancer (IARC, 2017).

Recent studies in animals have linked glyphosate and/or GBHs with endocrine-disrupting properties and reproductive toxicity outcomes (reviewed in (Ingaramo et al., 2020; Jarrell et al., 2020; Muñoz et al., 2020)). For example, in our previous study, rat pups exposed to low-dose GBHs (gestation and early-life) displayed longer anogenital distance (AGD), a marker of androgen exposure (Manservisi et al., 2019). Furthermore, more recently we reported an increase in anogenital distance in female infants born to mothers with high glyphosate and AMPA urinary levels (Lesseur et al., 2021). Few human studies have investigated the relationship between glyphosate exposure and reproductive outcomes like fertility and time to pregnancy. One study reported a continuous direct association between individual-level maternal urinary glyphosate (11–38 weeks of gestation) and shorter gestational length in 71 pregnant women in Indiana, U.S., however, the study only included two preterm births (<37 weeks of gestation) (Parvez et al., 2018). A recent study reported associations between urinary (26th weeks) glyphosate and AMPA with increased risk of preterm delivery in Puerto Rican women (Silver et al., 2021).

Preterm birth (PTB), defined as a live birth delivered prior to 37 weeks of gestation, is an important indicator of population health. PTB can occur spontaneously or be triggered by a medical indication of induction of labor or caesarian section delivery before 37 weeks (Walani, 2020). Complications from preterm birth are a frequent cause of infant mortality worldwide and in the United States (Ely and Driscoll, 2020; Liu et al., 2016). The prevalence of PTB in the US was 10% in 2018 and has been increasing (Martin et al., 2019). PTB imposes significant disease burden to the society as PTB-related complications may result in long-term neurodevelopmental disabilities (Wolke et al., 2019). PTB has been associated with multiple risk factors including maternal medical (e.g., chronic

disease) and reproductive history (e.g., previous c-section), sociodemographic characteristics (e.g., African American ethnicity), lifestyle factors (e.g. smoking), and current pregnancy characteristics (e.g. multiple gestation) (Frey and Klebanoff, 2016). Additionally, some environmental chemicals including lead, fine particulate matter, phthalates and some persistent pesticides (at high levels) have been associated with increased risk of PTB (Ferguson and Chin, 2017). Yet, our knowledge of the possible contribution of emergent contaminants like glyphosate to the risk of PTB is very limited.

Motivated by the recent evidence pointing to glyphosate (and GBHs) as a potential reproductive toxicant, we explored the relationships between individual-level maternal urinary concentrations of glyphosate and AMPA and length of gestation measured both as a continuous variable and dichotomized at 37 weeks to examine risk of PTB.

2. Materials and methods

2.1 Study population

The Infant Development and the Environment Study (TIDES) is a multicenter pregnancy cohort designed to investigate prenatal exposure to endocrine disrupting chemicals (EDCs) in relation to reproductive development. From 2010–2012, TIDES recruited women in their first trimester of pregnancy at four university-based medical centers: University of California, San Francisco, (UCSF), University of Rochester Medical Center (URMC), University of Minnesota (UMN) and the University of Washington (UW). Eligibility criteria included first trimester of pregnancy, > 18 years of age, able to read and write English, and planning to deliver at a study center hospital. Women with major medical complications were not eligible. Study participants completed questionnaires (including demographic factors, lifestyle, general health and reproductive history) and provided urine samples (in polypropylene cups) in each trimester of gestation. Urine specific-gravity (SpG) was measured within 30 minutes of collection with a hand-held refractometer (National Instrument Company, Inc., USA), then specimens were stored at $-80°C$. The outcomes for these analyses included gestational age at birth (continuous; defined as time (days) to delivery), spontaneous labor before delivery (binary), preterm birth (binary; <37 weeks or 259 days) and spontaneous preterm birth (binary; <37 weeks with spontaneous labor). Gestational age at birth was calculated based on first available ultrasound; if not available, the gestational age at birth estimate from the physician was used. Information on clinical presentation of spontaneous labor prior to delivery was abstracted from the medical records. Using the date of delivery as reference and the ICD codes O60.1, O60.2 and O42, we classified labor for each delivery as spontaneous or induced, and documented premature rupture of membranes (PROM, after 37 weeks) and preterm premature rupture of membranes (PPROM, before 37 weeks) when applicable. For 10% of the records, two independent reviewers abstracted the data, accuracy analyses showed high level of consistency between reviewers, as detailed elsewhere (Rosen et al., 2019). For this study, we identified all cases of preterm birth that were not extremely premature (gestational age 28–37 weeks) for whom 2nd trimester urine samples were available (n=69) (Supplementary Figure 1). Our control group was comprised of randomly selected mothers of term infants (37 weeks) for whom 2nd trimester urinary glyphosate and AMPA had previously been

measured for a separate analysis (n=94) (Lesseur et al., 2021). All TIDES protocols were approved by the Institutional Review Boards (IRB) of each participating center and all participants signed written informed consent prior to the start of the study.

2.2 Urinary glyphosate and AMPA measurements

Urinary glyphosate and AMPA measurements were performed at the Collaborative Center for Translational Mass Spectrometry (CCTMS) (TGen, Phoenix, AZ) following previously described methods (Jensen et al., 2016; Lesseur et al., 2021). Urinary levels of glyphosate and AMPA were quantified with ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using a Vanquish UHPLC coupled to a TSQ Altis triple quadrupole mass spectrometer (ThermoFisher, San Jose, CA). The calibration curves were performed in a blank urine matrix over a linear range of 0 to 5 ng/mL of glyphosate and AMPA concentrations (coefficient of determination $R2 > 0.99$). High creatinine samples (>160 mg/dL) were diluted 2-fold with water, samples with low (0 to 40 mg/dL) were injected at 100 μL and high and medium creatinine (40– 160 mg/dL) were injected at 50 μL. The limit of detection (LOD) was 0.014 ng/mL and the limit of quantitation (LOQ) was 0.041 ng/mL for glyphosate. The AMPA assay limits were: LOD 0.013 and LOQ: 0.04 ng/mL. Urine samples with glyphosate or AMPA levels below the LOD were imputed with the LOD/ 2 (Hornung and Reed, 1990). We used sample specific gravity (SpG) measurements to adjust glyphosate and AMPA measurements for urine concentration, using this formula: SpG_{adj}-Gly = Gly[1.014–1/(SpG-1)], where SpG_{adj}-Gly is the SpG adjusted glyphosate concentration (ng/mL), Gly is the observed glyphosate concentration and 1.014 is the mean SpG for all the samples (Boeniger et al., 1993).

2.3 Statistical analyses

The distributions of specific-gravity adjusted (SpG_{adj}) glyphosate and AMPA (ng/mL) concentrations were right skewed and were natural log-transformed for parametric statistical analyses. We compared the distributions of maternal and infant sociodemographic characteristics between term and preterm births in bivariate analyses (student's t-test or $\chi 2$ test). Next, we examined unadjusted differences between median exposure levels of glyphosate and AMPA in term and preterm births using Wilcoxon signed-rank tests. Since preterm deliveries were oversampled in this study, we used inverse probability sampling weights to give the cases and controls the same relative weight as in the original TIDES study (Richardson et al., 2007). To explore the relationship between exposure (glyphosate or AMPA) and time to delivery (gestational age at birth in days), we used weighted Cox proportional hazards models; hazard ratios larger than one indicated shorter time to delivery (increased hazard). Logistic regression models (not weighted) were used to evaluate possible associations between (SpG_{adj}) glyphosate or AMPA concentration and (dichotomous) PTB (< 37 or 37 weeks). We performed statistical analyses in the entire study population (N=163) or the subset of spontaneous births, using SpG-adjusted glyphosate and AMPA concentrations for those above the LOD and imputed values for those below the LOD (LOD/ 2). Sensitivity analyses were performed using imputed values for observations below the LOQ. Maternal age, race/ethnicity and education level were included as covariates in logistic and time-to-event models. Tests were two-sided and *p*-values 0.05 were

considered statistically significant. Analyses were conducted with R (version 4.0.0) and RStudio (version 1.2.5033) (Team, 2019).

3. Results

Clinical and sociodemographic characteristics of the study population are shown in Table 1. Like the overall TIDES cohort (Swan et al., 2015), our study population was predominantly white (71.2%) with an average maternal age of 31.1 years (range 18 – 44). On average the 2nd trimester maternal urine samples were collected at 19.8 weeks of gestation (range: 14.7 – 28.9). For term deliveries (n=94), median gestational age at birth was 39.6 weeks (range: 37 – 42) with 47.9% female and 52.1% male births. The median gestational age for preterm births (n=69) was 35.7 weeks (range 30.1 – 36.8) with similar sex distribution (47.8% females and 52.2% males). The majority of PTB cases (97%, n=67) were classified as moderate to late preterm (32–36 weeks) and 3% (n=2) as very preterm (28 – 31 weeks), according to the WHO classification of prematurity (Mactier et al., 2020; Moutquin, 2003). Of the 69 PTB cases, 37 women (53.6%) had spontaneous preterm labor leading to preterm delivery (sPTB), 28 deliveries (40.6%) were medically induced, and 4 (5.8%) could not be classified.

Glyphosate and AMPA were above the LOD in 96% and 94%, and above the LOQ in 87% and 80% of the samples, respectively. The median glyphosate concentration was 0.25 ng/mL (range 0.01 to 3.4); AMPA levels were lower with a median of 0.16 ng/mL (range: 0.01 – 6.1). Urinary concentrations of glyphosate and AMPA were correlated (Pearson's r =0.51, p=2×10^{-12}) (Supplementary Figure 2). Statistical summaries for maternal urinary glyphosate and AMPA levels before and after adjustment for SpG are shown in Supplementary Table 1. The median levels of glyphosate and AMPA stratified by time of delivery are shown in Table 2. In bivariate analyses, median exposure levels were not significantly different between preterm and term births.

In the overall study population (n=163), time-to-event Cox proportional hazards models with gestational age (in days) as a continuous outcome (Table 3) showed a non-significant increase in hazard for maternal urine levels of glyphosate (HR: 1.08, 95%CI: 0.91, 1.29) and AMPA (HR: 1.1, 95%CI: 0.93, 1.3). After limiting these analyses to spontaneous births, both glyphosate (HR: 1.31, 95%CI: 1.002, 1.71) and AMPA (HR: 1.32, 95%CI: 1.002, 1.73) were significantly associated with increased hazard ratio of delivery (shorter gestational age) after adjusting for maternal age, race/ethnicity and education level.

We also examined PTB as a binary outcome (gestational age < 37 vs   37 weeks) using multivariable logistic regression analyses (Table 4). In analyses including all births, point estimates (odds ratios, ORs) were above 1 for glyphosate (OR: 1.19, 95% confidence interval (CI): 0.86, 1.64) and AMPA (OR: 1.19, 95% CI: 0.91–1.58). Similar results were obtained in analyses limited to spontaneous births; glyphosate (OR: 1.54, 95% CI: 0.97–2.57) and AMPA (OR: 1.06, 95% CI: 0.71–1.58). However, none of the results from the logistic regression analyses reached statistical significance. Additionally, we performed sensitivity analyses using imputed exposure values for samples with glyphosate or AMPA

concentrations below the LOQ; these showed similar results in both time-to event and logistic regression models (Supplementary Table 2).

4. Discussion

In the current study, we detected glyphosate and its breakdown product AMPA in almost all the examined maternal urine samples (>94%) from a multi-center US birth cohort, suggesting ubiquitous exposure to these chemicals. We also observed associations between maternal urinary glyphosate and AMPA levels in the 2nd trimester and shorter gestational length in time to event analyses amongst women with spontaneous deliveries.

Despite intense research and public interest on the carcinogenic effects of glyphosate (and GBHs), epidemiological evidence of effects on human reproductive health remains limited. Previously, a single-site study in Central Indiana reported an inverse association between urinary glyphosate levels and gestational length (r = –0.28, *p*=0.02), but that study was smaller (n=79) with only two preterm deliveries (Parvez et al., 2018). Notably, a recent nested case-control study in the PROTECT cohort in Puerto Rico reported associations between glyphosate and AMPA urine levels in 3rd trimester maternal urine and increased risk of preterm birth; with larger effect sizes when examining spontaneous deliveries (Silver et al., 2021). Our results are consistent with these studies and were derived in a multi-center pregnancy cohort covering multiple geographic areas in the US (New York, Minnesota, California, and Washington) and distinct demographics (in terms of race/ethnicity, SES, urban/suburban) that roughly reflect the US general population. Moreover, similar to the Puerto Rico study (Silver et al., 2021), we also observed stronger associations in analyses stratified to spontaneous deliveries. However, in our study we only detected significant associations with shorter gestational length in spontaneous deliveries only. It is worth pointing out that in a previous report from the Ontario Farm Family Health Study, a cohort study in an agriculture setting, exposure to glyphosate (assessed from questionnaires) was marginally associated with increased risk of spontaneous abortion (19 weeks; OR: 1.4, 95%CI: 1.0–2.1), especially late abortions (12 – 19 weeks; OR: 1.7, 95% CI: 1.0 – 2.9) (Arbuckle et al., 2001). In the same population, glyphosate exposure inferred from paternal farming activity was associated with a non-significant elevated risk of PTB (OR:2.4, 95%CI: 0.8–7.9) (Savitz et al., 1997). Collectively, these results add weight to the evidence that GBHs are human reproductive toxicants.

Preterm birth is a complex and heterogeneous phenotype that has been associated with a myriad of risk factors that could trigger premature labor individually or in concert. These include reproductive and obstetric characteristics (*e.g.,* short cervical length, previous PTB, multiple gestation, infections), as well as maternal demographic and lifestyle factors (*e.g.* age, low socioeconomic status, stress, and smoking) (Frey and Klebanoff, 2016). Although some environmental chemicals have been linked to PTB risk (Ferguson and Chin, 2017), their mechanistic role in the pathogenesis of PTB is not well understood, particularly for emerging exposures such as glyphosate. Systemic oxidative stress and intrauterine inflammation have been proposed as biological mechanisms that may play a role in PTB (Aponte and Agarwal, 2013; McElrath et al., 2008; Peiris et al., 2017). Elevated oxidative stress as well as pro-inflammatory biomarkers in pregnant women have been associated with

PTB and shortened gestation length (Ferguson et al., 2015; Ferguson et al., 2014; Longini et al., 2007; Peter Stein et al., 2008). Prior work in the TIDES cohort demonstrated that third trimester urinary concentrations of 8-iso-prostaglandin F2α, which may be indicative of both oxidative stress and inflammation (van et al., 2019), were associated with PTB risk in the TIDES cohort (n=740)(Rosen et al., 2019).

Of note, we observed stronger associations between glyphosate and gestational length among women who underwent spontaneous labor deliveries, which may shed additional light on possible mechanisms. Whereas spontaneous preterm delivery (preterm labor, pre-labor premature rupture of membrane, placental abruption, and cervical insufficiency) is thought to be driven by intrauterine inflammation and oxidative stress, medically indicated premature deliveries (e.g. preeclampsia) are typically characterized by placental dysfunction and relative absence of inflammation (McElrath et al., 2008). Thus, the stronger association between glyphosate and shorter gestational length in spontaneous deliveries may provide some insight into potential mechanisms (possibly increased oxidative stress and pro-inflammation) linking glyphosate and prematurity.

Glyphosate and/or GBHs have been shown to induce oxidative stress and inflammation in multiple model systems including human cells (Chaufan et al., 2014; Martínez et al., 2020; Wozniak et al., 2018) and rodents (Zhang et al., 2019). For example, production of reactive oxygen species in peripheral blood mononuclear cells was enhanced by glyphosate and more substantially by Roundup®, while AMPA had no impact (Wozniak et al., 2018). In a study of mouse oocytes, glyphosate induced reactive oxygen species (ROS) and changes in the mRNA expression of related antioxidant enzyme genes (Zhang et al., 2019). Similarly, a recent study in rats shows oxidative stress related changes in the blood metabolome of rats exposed to glyphosate (Mesnage et al., 2021). In addition, glyphosate, AMPA and GBHs have also been linked to immune cell responses (Barbasz et al., 2020; Peillex and Pelletier, 2020). Whether these effects may contribute to shortened gestational length and PTB in humans requires further study.

In the current study, urinary glyphosate and AMPA levels were positively correlated (Pearson's r =0.51, p=2×10^{-12}), consistent with previous reports (Conrad et al., 2017; Soukup et al., 2020; Stajnko et al., 2020). Similarly, the high detection rate with median level of 0.24 ng/mL for glyphosate in the current study (in which samples were collected from 2010–2012) are consistent with previous reports (Gillezeau et al., 2019; Mills et al., 2017). We analyzed glyphosate and AMPA separately. While both showed similar associations (direction of effect) with gestational length, associations were stronger for glyphosate. In the environment, microorganisms transform glyphosate into AMPA, the most commonly detected breakdown product. Some studies have attempted to combine these two analytes as a surrogate measure of total glyphosate residue exposure (Mills et al., 2020). However, AMPA can also come from degradation of phosphonates compounds commonly used domestically, such as detergents, and is frequently detected in surface water (Grandcoin et al., 2017). Additionally, the toxicity or biological effects of these two compounds may not be comparable (Blot et al., 2019; Wozniak et al., 2018).

Notably, despite a high prevalence of detectable glyphosate in urine, the concentrations are low. From these urinary levels, we can extrapolate the external dose for glyphosate exposure using a model established by Niemann et al (Niemann et al., 2015) and recently revised by Connolly *et al* (Connolly et al., 2020). Assuming daily urine excretion of 2 liters and mean body weight of 60 kg for an adult person, and 1% urinary excretion rate (Zoller et al., 2020), the mean urinary glyphosate levels of 0.24 ng/mL and maximum level of 3.4 ng/mL observed in our study would translate to daily external doses of 0.0008 mg/kg and 0.0011 mg/kg, corresponding to 0.16% and 2.3% of the European ADI (0.5 mg/kg (EFSA 2014)), respectively. Similarly, the maximum value observed in this study corresponds to 0.06 % of the 1.75 mg/kg US ADI value (EPA, 1993). However, whether this regulatory reference dose is truly safe to humans remains a key question. Recent evidence from animal studies suggests that glyphosate as an endocrine disruptor (Ingaramo et al., 2020), thus re-evaluation of safety determination is warranted to protect human health, especially in vulnerable populations.

Our study has multiple strengths. Our non-occupational multicenter study is geographically and demographically diverse, thus increasing the potential generalizability of the study. We used robust and sensitive analytical methods to measure urinary glyphosate and AMPA. We examined the relation between exposure as both a continuous and a binary outcome the parent study, TIDES, was designed to examine relationships between several environmental exposures *in utero* in relation to reproductive outcomes and the study population is well characterized so that potential bias can be evaluated and minimized. We acknowledge that our study is not without limitations. First, the sample size is limited, and the number of preterm births was small. Second, glyphosate and AMPA were measured at a single time point (2nd trimester) which may not represent long-term exposure, particularly since glyphosate has a short half-life (Connolly et al., 2019; Zoller et al., 2020) and rapidly decreases after dietary changes (Fagan et al., 2020). Thus, future studies may benefit from assessing exposure at multiple time points. However, continuous exposure could occur in the general population because diet is the most likely source of glyphosate. Importantly, a recent study in Puerto Rican women examined exposure at 18 weeks and 26 weeks of pregnancy, reported weak correlations between the two measurements for both glyphosate and AMPA (Spearman $\rho = 0.36$ $p < 0.001$ and $\rho = 0.19$, $p = 0.03$, respectively) (Silver et al., 2021). Lastly, in this study we did not measure additives present in GBH formulations, thus we cannot rule out that glyphosate and AMPA are proxies of these adjuvants (Defarge et al., 2018; Mesnage and Antoniou, 2017). This is an important issue that needs to be addressed in future investigations.

In conclusion, our findings from a multi-center US birth cohort confirm widespread exposure to glyphosate in the general population, although external exposure levels are likely to be below regulatory limits. However, even at these levels, we observed inverse relationships between urinary glyphosate and AMPA levels with gestational length, associations that were stronger among spontaneous deliveries. Given the prevalent and rising exposures to glyphosate and GBHs, confirmatory studies are needed to explore reproductive effects of glyphosate and GBHs to re-assess their safety on human health and to explore possible programming consequences to lifelong health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations:

AMPA	aminomethylphosphonic acid
GBHs	glyphosate-based herbicides
LOD	Limit of detection
LOQ	Limit of quantification
SD	standard deviation
SE	standard error
SpG	specific gravity
TIDES	The Infant Development and the Environment Study
UPLC-MS/MS	ultra-high-performance liquid chromatography-tandem mass spectrometry
UCSF	University of California, San Francisco
URMC	University of Rochester Medical Center
UMN	University of Minnesota
UW	University of Washington.

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Table 1.

Characteristics of the study population.

	Total n = 163	Term n = 94	Preterm n = 69	p-value[a]
Maternal age (yrs.)	31.1 (5.9)	31.2 (5.8)	30.9 (6.0)	0.79
Maternal BMI (kg/m^2)	27.2 (6.9)	26.9 (7.0)	27.7 (6.8)	0.50
Birthweight (kg)	3.1 (0.7)	3.5 (0.5)	2.6 (0.6)	<.001
Gestational age at urine collection (weeks)	20.6 (3.2)	20.8 (3.3)	20.2 (3.0)	0.24
Time of urine collection (hours)	11.9 (2.7)	11.9 (2.8)	11.9 (2.6)	0.94
Spontaneous labor				1.00
No	66 (40.5%)	38 (40.4%)	28 (40.6%)	
Yes	90 (55.2%)	53 (56.4%)	37 (53.6%)	
Missing	7 (4.3%)	3 (3.2%)	4 (5.8%)	
Infant sex				1
Female	78 (47.9%)	45 (47.9%)	33 (47.8%)	
Male	85 (52.1%)	49 (52.1%)	36 (52.2%)	
Infant race				0.67
Other	46 (28.2%)	25 (26.6%)	21 (30.4%)	
White	116 (71.2%)	69 (73.4%)	47 (68.1%)	
Missing	1 (0.6%)	0 (0%)	1 (1.4%)	
Study center				1.00
UCSF	35 (21.5%)	20 (21.3%)	15 (21.7%)	
UMN	44 (27%)	25 (26.6%)	19 (27.5%)	
UR	55 (33.7%)	32 (34%)	23 (33.3%)	
UW	29 (17.8%)	17 (18.1%)	12 (17.4%)	
Urine collection season				
Winter	32 (19.6%)	21 (22.3%)	11 (15.9%)	0.32
Spring	53 (32.5%)	30 (31.9%)	23 (33.3%)	
Summer	46 (28.2%)	22 (23.4%)	24 (34.8%)	
Autumn	32 (19.6%)	21 (22.3%)	11 (15.9%)	
Maternal smoking				0.45
No	140 (85.9%)	80 (85.1%)	60 (87%)	
Yes	14 (8.6%)	10 (10.6%)	4 (5.8%)	
Missing	9 (5.5%)	4 (4.3%)	5 (7.2%)	
Maternal alcohol use				0.70
No	149 (91.4%)	88 (93.6%)	61 (88.4%)	
Yes	5 (3.1%)	2 (2.1%)	3 (4.3%)	
Missing	9 (5.5%)	4 (4.3%)	5 (7.2%)	
Maternal Education				
Less than college degree	114 (69.9%)	67 (71.3%)	47 (68.1%)	0.90
College degree or more	48 (29.4%)	27 (28.7%)	21 (30.4%)	
Missing	1 (0.6%)	0 (0%)	1 (1.4%)	

[a]*p*-values from student's t-test or χ2 test comparing each variable between the term and preterm groups.

Abbreviations: SD, standard deviation; UCSF, University of California San Francisco; UMN, University of Minnesota; URMC, University of Rochester Medical Center; UW, University of Washington.

Table 2.

Median [25th-75th percentiles] levels of SpG adjusted glyphosate and AMPA (ng/mL) by timing of delivery.

	All births			
	Overall n = 163	**Term n = 94**	**Preterm n = 69**	**p**
Glyphosate	0.25 [0.13 ,0.53]	0.23 [0.12 ,0.53]	0.27 [0.14 ,0.49]	0.36
AMPA	0.16 [0.08 ,0.36]	0.14 [0.08 ,0.29]	0.18 [0.1 ,0.37]	0.15
	Spontaneous births			
	Overall n = 90	**Term n = 53**	**Preterm n = 37**	**p**
Glyphosate	0.25 [0.15 ,0.46]	0.22 [0.12,0.39]	0.27 [0.19 ,0.59]	0.1
AMPA	0.16 [0.09 ,0.35]	0.13 [0.09 ,0.28]	0.21 [0.1 ,0.38]	0.27

P-values from Wilcoxon signed rank test.

Table 3.

Cox proportional hazards models of maternal glyphosate or AMPA and gestational length in days

	All births (n=163)			Spontaneous births (n=90)		
	HR	95% CI	*p*	HR	95% CI	*p*
Glyphosate	1.08	(0.91, 1.29)	0.37	1.31	(1.002, 1.71)	0.05
AMPA	1.1	(0.93, 1.30)	0.25	1.32	(1.003, 1.73)	0.05

HR: hazard ratio; CI, Confidence Interval. Models adjusted for maternal age, race/ethnicity and education level.

Table 4.

Logistic regression models of preterm vs. term birth and maternal glyphosate or AMPA

	All births (n=163)			Spontaneous births (n=90)		
	OR	95% CI	*p*	OR	95% CI	*p*
Glyphosate	1.19	(0.86, 1.64)	0.29	1.55	(0.97, 2.57)	0.08
AMPA	1.19	(0.91, 1.58)	0.21	1.06	(0.71, 1.58)	0.76

OR: odds ratio; CI, Confidence Interval. Models adjusted for maternal age, race/ethnicity and education level.