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# Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada

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### ABSTRACT

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (GLUF) or insecticides such as the bacterial toxin bacillus thuringiensis (Bt). The aim of this study was to evaluate the correlation between maternal and fetal exposure, and to determine exposure levels of GLYP and its metabolite aminomethyl phosphoric acid (AMPA), GLUF and its metabolite 3-methylphosphinicopropionic acid (3-MPPA) and Cry1Ab protein (a Bt toxin) in Eastern Townships of Quebec, Canada. Blood of thirty pregnant women (PW) and thirty-nine nonpregnant women (NPW) were studied. Serum GLYP and GLUF were detected in NPW and not detected in PW. Serum 3-MPPA and CryAb1 toxin were detected in PW, their fetuses and NPW. This is the first study to reveal the presence of circulating PAGMF in women with and without pregnancy, paving the way for a new field in reproductive toxicology including nutrition and utero-placental toxicities.

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## 1. Introduction

An optimal exchange across the maternal-fetal unit (MFU) is necessary for a successful pregnancy. The placenta plays a major role in the embryo's nutrition and growth, in the regulation of the endocrine functions and in drug biotransformation [1–3]. Exchange involves not only physiological constituents, but also substances that represent a pathological risk for the fetus such as xenobiotics that include drugs, food additives, pesticides, and environmental pollutants [4]. The understanding of what xenobiotics do to the MFU and what the MFU does to the xenobiotics should provide the basis for the use of placenta as a tool to investigate and predict some aspects of developmental toxicity [4]. Moreover, pathological conditions in the placenta are important causes of intrauterine or perinatal death, congenital anomalies, intrauterine growth retardation, maternal death, and a great deal of morbidity for both, mother and child [5].

Genetically modified plants (GMP) were first approved for commercialization in Canada in 1996 then become distributed

worldwide. Global areas of these GMP increased from 1.7 million hectares in 1996 to 134 million hectares in 2009, a 80-fold increase [6]. This growth rate makes GMP the fastest adopted crop technology [6]. GMP are plants in which genetic material has been altered in a way that does not occur naturally. Genetic engineering allows gene transfer (transgenesis) from an organism into another in order to confer them new traits. Combining GMP with pesticides-associated GM foods (PAGMF) allows the protection of desirable crops and the elimination of unwanted plants by reducing the competition for nutrients or by providing insect resistance. There is a debate on the direct threat of genes used in the preparation of these new foods on human health, as they are not detectable in the body, but the real danger may come from PAGMF [6–10]. Among the innumerable PAGMF, two categories are largely used in our agriculture since their introduction in 1996: (1) residues derived from herbicide-tolerant GM crops such as glyphosate (GLYP) and its metabolite aminomethyl phosphoric acid (AMPA) [11], and gluphosinate ammonium (GLUF) and its metabolite 3-methylphosphinicopropionic acid (MPPA) [12]; and (2) residues derived from insect-resistant GM crops such as Cry1Ab protein [13,14].

Among herbicide-tolerant GM crops, the first to be grown commercially were soybeans which were modified to tolerate glyphosate [11]. Glyphosate [*N*-(phosphonomethyl) glycine] is a nonselective, post-emergence herbicide used for the control of a

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wide range of weeds [15]. It can be used on non-crop land as well as in a great variety of crops. GLYP is the active ingredient in the commercial herbicide Roundup®. Glyphosate is an acid, but usually used in a salt form, most commonly the isopropylamine salt. The target of glyphosate is 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), an enzyme in the shikimate pathway that is required for the synthesis of many aromatic plant metabolites, including some amino acids. The gene that confers tolerance of the herbicide is from the soil bacterium *Agrobacterium tumefaciens* and makes an EPSPS that is not affected by glyphosate. Few studies have examined the kinetics of absorption, distribution, metabolism and elimination (ADME) of glyphosate in humans [15,16]. Curwin et al. [17] reported detection of urinary GLYP concentrations among children, mothers and fathers living in farm and non farm households in Iowa. The ranges of detection were 0.062–5.0 ng/ml and 0.10–11 ng/ml for non farm and farm mothers, respectively. There was no significant difference between farm and non farm mothers and no positive association between the mothers' urinary glyphosate levels and glyphosate dust concentrations. These findings suggest that other sources of exposure such as diet may be involved.

Glufosinate (or glufosinate) [ammonium dl-homoalanin-4-(methyl) phosphinate] is a broad-spectrum, contact herbicide. Its major metabolite is 3-methylphosphinopropionic acid (MPPA), with which it has similar biological and toxicological effects [18]. GLUF is used to control a wide range of weeds after the crop emerges or for total vegetation control on land not used for cultivation. Glufosinate herbicides are also used to desiccate (dry out) crops before harvest. It is a phosphorus-containing amino acid. It inhibits the activity of an enzyme, glutamine synthetase, which is necessary for the production of the amino acid glutamine and for ammonia detoxification [12]. The application of GLUF leads to reduced glutamine and increased ammonia levels in the plant's tissues. This causes photosynthesis to stop and the plant dies within a few days. GLUF also inhibits the same enzyme in animals [19]. The gene used to make plants resistant to glufosinate comes from the bacterium *Streptomyces hygroscopicus* and encodes an enzyme called phosphinothricine acetyl transferase (PAT). This enzyme detoxifies GLUF. Crop varieties carrying this trait include varieties of oilseed rape, maize, soybeans, sugar beet, fodder beet, cotton and rice. As for GLYP, its kinetics of absorption, distribution, metabolism and elimination (ADME) is not well studied in humans, except few poisoned-case studies [16,20,21]. Hirose et al. reported the case of a 65-year-old male who ingested BASTA, which contains 20% (w/v) of GLUF ammonium, about 300 ml, more than the estimated human toxic dose [20]. The authors studied the serial change of serum GLUF concentration every 3–6 h and assessed the urinary excretion of GLUF every 24 h. The absorbed amount of GLUF was estimated from the cumulative urinary excretion. The changes in serum GLUF concentration exhibited  $T_{1/2\alpha}$  of 1.84 and  $T_{1/2\beta}$  of 9.59 h. The apparent distribution volume at b-phase and the total body clearance were 1.44 l/kg and 86.6 ml/min, respectively. Renal clearance was estimated to be 77.9 ml/min.

The Cry1Ab toxin is an insecticidal protein produced by the naturally occurring soil bacterium *Bacillus thuringiensis* [22,23]. The gene (truncated *cry1Ab* gene) encoding this insecticidal protein was genetically transformed into maize genome to produce a transgenic insect-resistant plant (Bt-maize; MON810) and, thereby, provide specific protection against Lepidoptera infestation [13,14]. For more than 10 years, GM crops have been commercialized and approved as an animal feed in several countries worldwide. The Cry toxins (protoxins) produced by GM crops are solubilized and activated to Cry toxins by gut proteases of susceptible insect larvae. Activated toxin binds to specific receptors localized in the midgut epithelial cells [24,25], invading the cell membrane and forming cation-selective ion channels that lead to the disruption

of the epithelial barrier and larval death by osmotic cell lysis [26–28].

Since the basis of better health is prevention, one would hope that we can develop procedures to avoid environmentally induced disease in susceptible population such as pregnant women and their fetuses. The fetus is considered to be highly susceptible to the adverse effects of xenobiotics. This is because environmental agents could disrupt the biological events that are required to ensure normal growth and development [29,30]. PAGMF are among the xenobiotics that have recently emerged and extensively entered the human food chain [9], paving the way for a new field of multidisciplinary research, combining human reproduction, toxicology and nutrition, but not as yet explored. Generated data will help regulatory agencies responsible for the protection of human health to make better decisions. Thus, the aim of this study was to investigate whether pregnant women are exposed to PAGMF and whether these toxicants cross the placenta to reach the fetus.

## 2. Materials and methods

### 2.1. Chemicals and reagents

For the analytical support (Section 2.3), GLYP, AMPA, GLUF, APPA and *N*-methyl-*N*-(tert-butyl)dimethylsilyl) trifluoroacetamide (MTBSTFA) + 1% tert-butyltrimethylchlorosilane (TBDMCS) were purchased from Sigma (St. Louis, MO, USA). 3-MPPA was purchased from Wako Chemicals USA (Richmond, VA, USA) and Sep-Pak Plus PS-2 cartridges, from Waters Corporation (Milford, MA, USA). All other chemicals and reagents were of analytical grade (Sigma, MO, USA). The serum samples for validation were collected from volunteers.

### 2.2. Study subjects and blood sampling

At the Centre Hospitalier Universitaire de Sherbrooke (CHUS), we formed two groups of subjects: (1) a group of healthy pregnant women ( $n=30$ ), recruited at delivery; and (2) a group of healthy fertile nonpregnant women ( $n=39$ ), recruited during their tubal ligation of sterilization. As shown in Table 1 of clinical characteristics of subjects, eligible groups were matched for age and body mass index (BMI). Participants were not known for cigarette or illicit drug use or for medical condition (i.e. diabetes, hypertension or metabolic disease). Pregnant women had vaginal delivery and did not have any adverse perinatal outcomes. All neonates were of appropriate size for gestational age ( $3423 \pm 375$  g).

Blood sampling was done before delivery for pregnant women or at tubal ligation for nonpregnant women and was most commonly obtained from the median cubital vein, on the anterior forearm. Umbilical cord blood sampling was done after birth using the syringe method. Since labor time can take several hours, the time between taking the last meal and blood sampling is often a matter of hours. Blood samples were collected in BD Vacutainer 10 ml glass serum tubes (Franklin Lakes, NJ, USA). To obtain serum, whole blood was centrifuged at 2000 rpm for 15 min within 1 h of collection. For maternal samples, about 10 ml of blood was collected, resulting in 5–6.5 ml of serum. For cord blood samples, about 10 ml of blood was also collected by syringe, giving 3–4.5 ml of serum. Serum was stored at  $-20^\circ\text{C}$  until assayed for PAGMF levels.

Subjects were pregnant and non-pregnant women living in Sherbrooke, an urban area of Eastern Townships of Quebec, Canada. No subject had worked or lived with a spouse working in contact with pesticides. The diet taken is typical of a middle

**Table 1**  
Characteristics of subjects.

	Pregnant women ( $n=30$ )	Nonpregnant women ( $n=39$ )	<i>P</i> value <sup>a</sup>
Age (year, mean $\pm$ SD)	32.4 $\pm$ 4.2	33.9 $\pm$ 4.0	NS
BMI (kg/m <sup>2</sup> , mean $\pm$ SD)	24.9 $\pm$ 3.1	24.8 $\pm$ 3.4	NS
Gestational age (week, mean $\pm$ SD)	38.3 $\pm$ 2.5	N/A	N/A
Birth weight (g, mean $\pm$ SD)	3364 $\pm$ 335	N/A	N/A

BMI, body mass index; N/A, not applicable; data are expressed as mean  $\pm$  SD; NS, not significant.

<sup>a</sup> *P* values were determined by Mann–Whitney test.

class population of Western industrialized countries. A food market-basket, representative for the general Sherbrooke population, contains various meats, margarine, canola oil, rice, corn, grain, peanuts, potatoes, fruits and vegetables, eggs, poultry, meat and fish. Beverages include milk, juice, tea, coffee, bottled water, soft drinks and beer. Most of these foods come mainly from the province of Quebec, then the rest of Canada and the United States of America. Our study did not quantify the exact levels of PAGMF in a market-basket study. However, given the widespread use of GM foods in the local daily diet (soybeans, corn, potatoes, ...), it is conceivable that the majority of the population is exposed through their daily diet [31,32].

The study was approved by the CHUS Ethics Human Research Committee on Clinical Research. All participants gave written consent.

### 2.3. Herbicide and metabolite determination

Levels of GLYP, AMPA, GLUF and 3-MPPA were measured using gas chromatography–mass spectrometry (GC–MS).

#### 2.3.1. Calibration curve

According to a method described by Motojyuku et al. [16], GLYP, AMPA, GLUF and 3-MPPA (1 mg/ml) were prepared in 10% methanol, which is used for all standards dilutions. These solutions were further diluted to concentrations of 100 and 10 µg/ml and stored for a maximum of 3 months at 4 °C. A 1 µg/ml solution from previous components was made prior herbicide extraction. These solutions were used as calibrators. A stock solution of DL-2-amino-3-phosphonopropionic acid (APPA) (1 mg/ml) was prepared and used as an internal standard (IS). The IS stock solution was further diluted to a concentration of 100 µg/ml. Blank serum samples (0.2 ml) were spiked with 5 µl of IS (100 µg/ml), 5 µl of each calibrator solution (100 µg/ml), or 10, 5 µl of 10 µg/ml solution, or 10, 5 µl of 1 µg/ml solution, resulting in calibration samples containing 0.5 µg of IS (2.5 µg/ml), with 0.5 µg (2.5 µg/ml), 0.1 µg (0.5 µg/ml), 0.05 µg (0.25 µg/ml), 0.01 µg (0.05 µg/ml) 0.005 µg (0.025 µg/ml) of each compound (i.e. GLYP, AMPA, GLUF and 3-MPPA). Concerning extraction development, spiked serum with 5 µg/ml of each compound was used as control sample.

#### 2.3.2. Extraction procedure

The calibration curves and serum samples were extracted by employing a solid phase extraction (SPE) technique, modified from manufacturer's recommendations and from Motojyuku et al. [16]. Spiked serum (0.2 ml), prepared as described above, and acetonitrile (0.2 ml) were added to centrifuge tubes. The tubes were then vortexed (15 s) and centrifuged (5 min, 1600 × g). The samples were purified by SPE using 100 mg Sep-Pak Plus PS-2 cartridges, which were conditioned by washing with 4 ml of acetonitrile followed by 4 ml of distilled water. The samples were loaded onto the SPE cartridges, dried (3 min, 5 psi) and eluted with 2 ml of acetonitrile. The solvent was evaporated to dryness under nitrogen. The samples were reconstituted in 50 µl each of MTBSTFA with 1% TBDMCS and acetonitrile. The mixture was vortexed for 30 s every 10 min, 6 times. Samples of solution containing the derivatives were used directly for GC–MS (Agilent Technologies 6890N GC and 5973 Invert MS).

#### 2.3.3. GC–MS analysis

Chromatographic conditions for these analyses were as followed: a 30 m × 0.25 mm Zebron ZB-5MS fused-silica capillary column with a film thickness of 0.25 µm from Phenomenex (Torrance, CA, USA) was used. Helium was used as a carrier gas at 1.1 ml/min. A 2 µl extract was injected in a split mode at an injection temperature of 250 °C. The oven temperature was programmed to increase from an initial temperature of 100 °C (held for 3 min) to 300 °C (held for 5 min) at 5 °C/min. The temperatures of the quadrupole, ion source and mass-selective detector interface were respectively 150, 230 and 280 °C. The MS was operated in the selected-ion monitoring (SIM) mode. The following ions were monitored (with quantitative ions in parentheses): GLYP (454, 352; AMPA (396), 367; GLUF (466); 3-MPPA (323); IS (568), 466.

The limit of detection (LOD) is defined as a signal of three times the noise. For 0.2 ml serum samples, LOD was 15, 10, 10 and 5 ng/ml for GLYP, GLUF, AMPA and 3-MPPA, respectively.

#### 2.4. Cry1Ab protein determination

Cry1Ab protein levels were determined in blood using a commercially available double antibody sandwich (DAS) enzyme-linked immunosorbent assay (Agdia, Elkhart, IN, USA), following manufacturer's instructions. A standard curve was prepared by successive dilutions (0.1–10 ng/ml) of purified Cry1Ab protein (Fitzgerald Industries International, North Acton, MA, USA) in PBST buffer. The mean absorbance (650 nm) was calculated and used to determine samples concentration. Positive and negative controls were prepared with the kit Cry1Ab positive control solution, diluted 1/2 in serum.

#### 2.5. Statistical analysis

PAGMP exposure was expressed as number, range and mean ± SD for each group. Characteristics of cases and controls and PAGMP exposure were compared using the Mann–Whitney *U*-test for continuous data and by Fisher's exact test for categorical data. Wilcoxon matched pairs test compared two dependent groups.

**Table 2**

Concentrations of GLYP, AMPA, GLUF, 3-MPPA and Cry1Ab protein in maternal and fetal cord serum.

	Maternal (n = 30)	Fetal cord (n = 30)	P value <sup>a</sup>
<i>GLYP</i>			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD			
<i>AMPA</i>			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD (ng/ml)			
<i>GLUF</i>			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD (ng/ml)			
<i>3-MPPA</i>			
Number of detection	30/30 (100%)	30/30 (100%)	<i>P</i> < 0.001
Range of detection (ng/ml)	21.9–417	8.76–193	
Mean ± SD (ng/ml)	120 ± 87.0	57.2 ± 45.6	
<i>Cry1Ab</i>			
Number of detection	28/30 (93%)	24/30 (80%)	<i>P</i> = 0.002
Range of detection (ng/ml)	nd–1.50	nd–0.14	
Mean ± SD (ng/ml)	0.19 ± 0.30	0.04 ± 0.04	

GLYP, glyphosate; AMPA, aminomethyl phosphoric acid; GLUF, glufosinate ammonium; 3-MPPA, 3-methylphosphinopropionic acid; Cry1Ab, protein from *Bacillus thuringiensis*; nd, not detectable; nc, not calculable because not detectable. Data are expressed as number (*n*, %) of detection, range and mean ± SD (ng/ml).

<sup>a</sup> *P* values were determined by Wilcoxon matched pairs test.

Other statistical analyses were performed using Spearman correlations. Analyses were realized with the software SPSS version 17.0. A value of *P* < 0.05 was considered as significant for every statistical analysis.

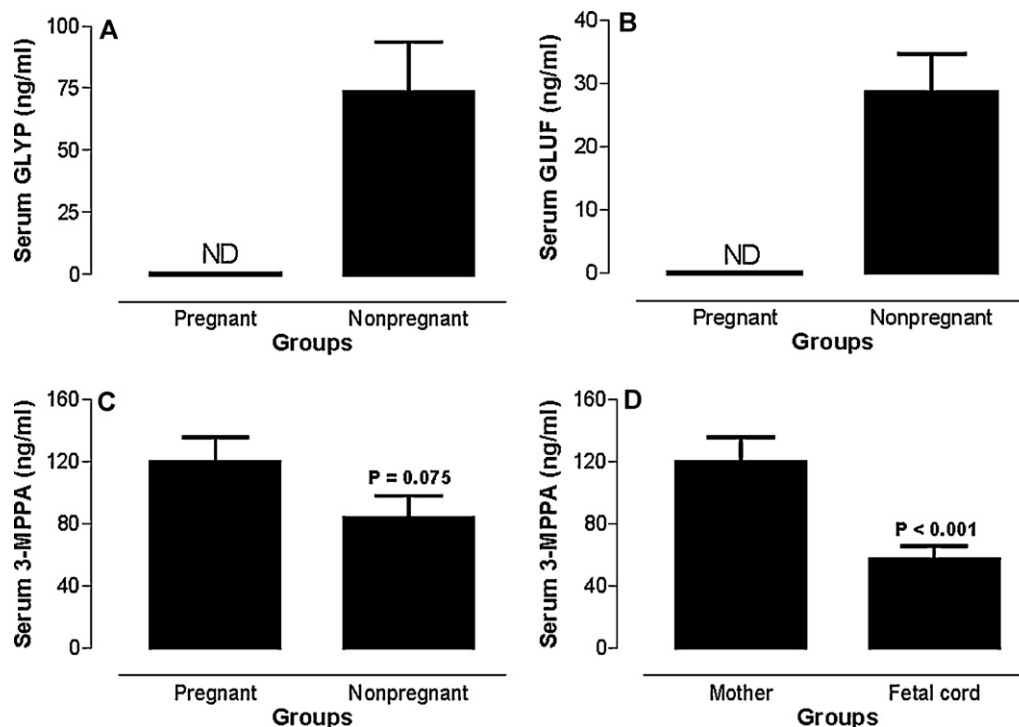
## 3. Results

As shown in Table 1, pregnant women and nonpregnant women were similar in terms of age and body mass index. Pregnant women had normal deliveries and birth-weight infants (Table 1).

GLYP and GLUF were non-detectable (nd) in maternal and fetal serum, but detected in nonpregnant women (Table 2, Fig. 1). GLYP was [2/39 (5%), range (nd–93.6 ng/ml) and mean ± SD (73.6 ± 28.2 ng/ml)] and GLUF was [7/39 (18%), range (nd–53.6 ng/ml) and mean ± SD (28.7 ± 15.7 ng/ml)]. AMPA was not detected in maternal, fetal and nonpregnant women samples. The metabolite 3-MPPA was detected in maternal serum [30/30 (100%), range (21.9–417 ng/ml) and mean ± SD (120 ± 87.0 ng/ml)], in fetal cord serum [30/30 (100%), range (8.76–193 ng/ml) and mean ± SD (57.2 ± 45.6 ng/ml)] and in nonpregnant women serum [26/39 (67%), range (nd–337 ng/ml) and mean ± SD (84.1 ± 70.3 ng/ml)]. A significant difference in 3-MPPA levels was evident between maternal and fetal serum (*P* < 0.001, Table 2, Fig. 1), but not between maternal and nonpregnant women serum (*P* = 0.075, Table 3, Fig. 1).

Serum insecticide Cry1Ab toxin was detected in: (1) pregnant women [28/30 (93%), range (nd–1.5 ng/ml) and mean ± SD (0.19 ± 0.30 ng/ml)]; (2) nonpregnant women [27/39 (69%), range (nd–2.28 ng/ml) and mean ± SD (0.13 ± 0.37 ng/ml)]; and (3) fetal cord [24/30 (80%), range (nd–0.14 ng/ml) and mean ± SD (0.04 ± 0.04 ng/ml)]. A significant difference in Cry1Ab levels was evident between pregnant and nonpregnant women's serum (*P* = 0.006, Table 3, Fig. 2) and between maternal and fetal serum (*P* = 0.002, Table 2, Fig. 2).

We also investigated a possible correlation between the different contaminants in the same woman. In pregnant women, GLYP, its metabolite AMPA and GLUF were undetectable in maternal blood and therefore impossible to establish a correlation between them. In nonpregnant women, GLYP was detected in 5% of the subjects, its metabolite AMPA was not detected and GLUF was detected in 18%, thus no significant correlation emerged from these contam-



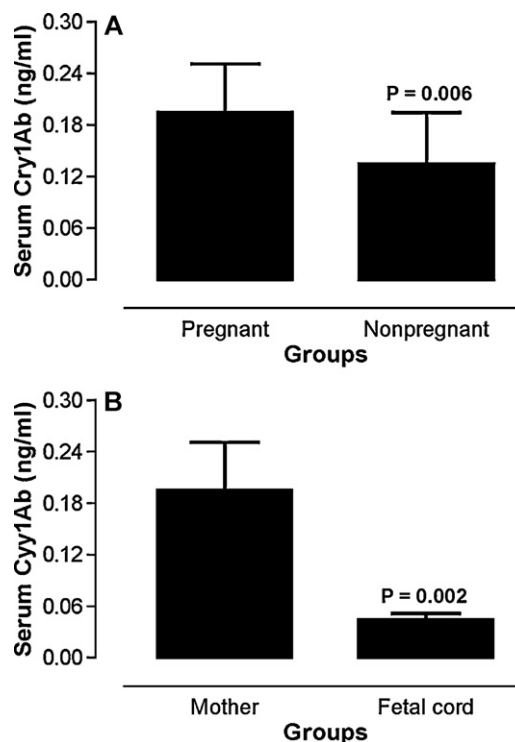
**Fig. 1.** Circulating concentrations of Glyphosate (GLYP: A), Glufosinate (GLUF: B) and 3-methylphosphinicpropionic acid (3-MPPA: C and D) in pregnant and nonpregnant women (A–C) and in maternal and fetal cord blood (D). Blood sampling was performed from thirty pregnant women and thirty-nine nonpregnant women. Chemicals were assessed using GC–MS. *P* values were determined by Mann–Whitney test in the comparison of pregnant women to nonpregnant women (A–C). *P* values were determined by Wilcoxon matched pairs test in the comparison of maternal to fetal samples (D). A *P* value of 0.05 was considered as significant.

**Table 3**  
 Concentrations of GLYP, AMPA, GLUF, 3-MPPA and Cry1Ab protein in serum of pregnant and nonpregnant women.

	Pregnant women (n = 30)	Nonpregnant women (n = 39)	<i>P</i> value <sup>a</sup>
<i>GLYP</i>			
Number of detection	nd	2/39 (5%)	nc
Range of detection (ng/ml)		nd–93.6	
Mean ± SD		73.6 ± 28.2	
<i>AMPA</i>			
Number of detection	nd	nd	nc
Range of detection (ng/ml)			
Mean ± SD (ng/ml)			
<i>GLUF</i>			
Number of detection	nd	7/39 (18%)	nc
Range of detection (ng/ml)		nd–53.6	
Mean ± SD (ng/ml)		28.7 ± 15.7	
<i>3-MPPA</i>			
Number of detection	30/30 (100%)	26/39 (67%)	<i>P</i> = 0.075
Range of detection (ng/ml)	21.9–417	nd–337	
Mean ± SD (ng/ml)	120 ± 87.0	84.1 ± 70.3	
<i>Cry1Ab</i>			
Number of detection	28/30 (93%)	27/39 (69%)	<i>P</i> = 0.006
Range of detection (ng/ml)	nd–1.50	nd–2.28	
Mean ± SD (ng/ml)	0.19 ± 0.30	0.13 ± 0.37	

GLYP, glyphosate; AMPA, aminomethyl phosphoric acid; GLUF, glufosinate ammonium; 3-MPPA, 3-methylphosphinicpropionic acid; Cry1Ab, protein from bacillus thuringiensis; nd, not detectable; nc, not calculable because not detectable. Data are expressed as number (*n*, %) of detection, range and mean ± SD (ng/ml).

<sup>a</sup> *P* values were determined by Mann–Whitney test.



**Fig. 2.** Circulating concentrations of Cry1Ab toxin in pregnant and nonpregnant women (A), and maternal and fetal cord (B). Blood sampling was performed from thirty pregnant women and thirty-nine nonpregnant women. Levels of Cry1Ab toxin were assessed using an ELISA method. *P* values were determined by Mann–Whitney test in the comparison of pregnant women to nonpregnant women (A). *P* values were determined by Wilcoxon matched pairs test in the comparison of maternal to fetal samples (B). A *P* value of 0.05 was considered as significant.



inants in the same subjects. Moreover, there was no correlation between 3-MPPA and Cry1Ab in the same women, both pregnant and not pregnant.

#### 4. Discussion

Our results show that GLYP was not detected in maternal and fetal blood, but present in the blood of some nonpregnant women (5%), whereas its metabolite AMPA was not detected in all analyzed samples. This may be explained by the absence of exposure, the efficiency of elimination or the limitation of the method of detection. Previous studies report that glyphosate and AMPA share similar toxicological profiles. Glyphosate toxicity has been shown to be involved in the induction of developmental retardation of fetal skeleton [33] and significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood [34]. Also, glyphosate was harmful to human placental cells [35,36] and embryonic cells [36]. It is interesting to note that all of these animal and *in vitro* studies used very high concentrations of GLYP compared to the human levels found in our studies. In this regard, our results represent actual concentrations detected in humans and therefore they constitute a referential basis for future investigations in this field.

GLUF was detected in 18% of nonpregnant women's blood and not detected in maternal and fetal blood. As for GLYP, the non detection of GLUF may be explained by the absence of exposure, the efficiency of elimination or the limitation of the method of detection. Regarding the non-detection of certain chemicals in pregnant women compared with non pregnant women, it is assumed that the hemodilution caused by pregnancy may explain, at least in part, such non-detection. On the other hand, 3-MPPA (the metabolite of GLUF) was detected in 100% of maternal and umbilical cord blood samples, and in 67% of the nonpregnant women's blood samples. This highlights that this metabolite is more detectable than its precursor and seems to easily cross the placenta to reach the fetus. Garcia et al. [37] investigated the potential teratogenic effects of GLUF in humans found and increased risk of congenital malformations with exposure to GLUF. GLUF has also been shown in mouse embryos to cause growth retardation, increased death or hypoplasia [18]. As for GLYP, it is interesting to note that the GLUF concentrations used in these tests are very high (10 µg/ml) compared to the levels we found in this study (53.6 ng/ml). Hence, our data which provide the actual and precise concentrations of these toxicants, will help in the design of more relevant studies in the future.

On the other hand, Cry1Ab toxin was detected in 93% and 80% of maternal and fetal blood samples, respectively and in 69% of tested blood samples from nonpregnant women. There are no other studies for comparison with our results. However, trace amounts of the Cry1Ab toxin were detected in the gastrointestinal contents of livestock fed on GM corn [38–40], raising concerns about this toxin in insect-resistant GM crops; (1) that these toxins may not be effectively eliminated in humans and (2) there may be a high risk of exposure through consumption of contaminated meat.

#### 5. Conclusions

To our knowledge, this is the first study to highlight the presence of pesticides-associated genetically modified foods in maternal, fetal and nonpregnant women's blood. 3-MPPA and Cry1Ab toxin are clearly detectable and appear to cross the placenta to the fetus. Given the potential toxicity of these environmental pollutants and the fragility of the fetus, more studies are needed, particularly those using the placental transfer approach [41]. Thus, our present results will provide baseline data for future studies

exploring a new area of research relating to nutrition, toxicology and reproduction in women. Today, obstetric-gynecological disorders that are associated with environmental chemicals are not known. This may involve perinatal complications (i.e. abortion, prematurity, intrauterine growth restriction and preeclampsia) and reproductive disorders (i.e. infertility, endometriosis and gynecological cancer). Thus, knowing the actual PAGMF concentrations in humans constitutes a cornerstone in the advancement of research in this area.

#### Conflict of interest statement

The authors declare that they have no competing interests.

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