Exposure Estimation to Glyphosate through the Consumption of Lebanese Bread

A Thesis

presented to

the Faculty of Nursing and Health Sciences

at Notre Dame University-Louaize

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Alexandria Nivelle Mekanna

DECEMBER 2019

Notre Dame University - Louaize

Faculty of Nursing and Health Sciences

Department of Nursing and Health Sciences

We hereby approve the thesis of

Alexandria Nivelle Mekanna

Candidate for the degree of Master of Science in food safety and quality management

Dec 12th, 2019

Dr. Christelle Bou-Mitri — Supervisor, Chair

12/12/2019

Dr. Sara Moukarzel — Committee Member

Antoine G. Farhat — Dec. 13th, 2019

Prof. Antoine G. Farhat — Committee Member

Dedication

To my Mother, a true fighter and survivor.

Acknowledgment

I would like to express my special thanks of gratitude to Dr. Christelle Bou-Mitri as well as our Prof. Antoine Farhat, Dean of the Faculty of Nursing and Health Sciences who gave me the golden opportunity to do this wonderful project on the topic glyphosate in bread. My special thanks of gratitude as well to Dr. Sara Moukarzel.

Secondly I would also like to thank Dr. Jocelyne Bou Mosleh for her guidance and support as well as Mrs. Nada Maalouf and all lab assistants in addition to my colleague Sabine Dagher.

Contents

List of illustrations 8
Abstract 9
Chapter I 11
I.1. Background 11
I.2. Definition 11
I.3. Uses 12
I.4. Mechanism 12
I.5.History 13
I.6.Regulations 13
I.7.Risk to human and animal health 14
I.8.Exposure assessment 16
I.9.Exposure symptoms 19
I.10.Method of testing 20
I.11.Food Sources 20
I.12.Glyphosate in Lebanon 24
I.13.Bread production in Lebanon 24
Chapter II 26
II.1. Introduction 26
II. 2. Materials and Methods 28
II.2.1. Sample Collection 28
II.2.2. Sample preparation and derivatization 29
II.2.3. Glyphosate analysis 30
II.2.4. Ethical considerations 30
II.2.5. Assessment of the level of exposure to glyphosate 31
II.2.6. Data analysis 31
II. 3. Results and Discussion 31
II.3.1. Prevalence of glyphosate in pita bread 31
II.3.2 Exposure estimation 32

II.4.Discussion……………………………………………………………………………..35

II.4.1.Glyphosate prevalence in Lebanese bread………………………………………35

II.4.2.Glyphosate levels in Lebanese bread……………………………………………36

II.4.3.Glyphosate level in Lebanese bread per bread type and grain type………………….. .37

II.4.4.Exposure to glyphosate…………………………………………………………39

II.4.5.Limitations………….. ………………………………………………………40

Conclusion 42

References... 43

List of illustrations

List of figures

Figure 1: Chemical structure of glyphosate …………………………………………………12

List of tables

Table 1: Health effects of glyphosate on humans and animals, types of studies, participants, and references…………………………………………………………………...……………14

Table 2: Glyphosate residues detected and quantified in different foodstuffs from different countries based on previous literature including number of samples assessed, number of samples with residues above or equal to the limit of detection, average glyphosate content range, percentage of positive samples, method used, limit of detection, study limitation, and reference…………………………………………………………………………...………..21

Table 3: Number of samples detected positive for glyphosate in bread with their minimum, maximum, median, and mean values……………………………………………..…………33

Table 4: Risk characterization of daily exposure to glyphosate residues through intake of Lebanese Bread………………………………………………………………………………..……34

Abstract

Background and objective: Glyphosate, a nonselective herbicide widely-used in crop production, persists in food products leading to residues. Research findings on the health effects of glyphosate are highly controversial due to a perceived conflict of interest by the majority of research funders who are glyphosate manufacturers themselves. Glyphosate residues are detected in various tested food products globally, but data about exposure in Lebanon is lacking. Bread is a stable food in the Lebanese diet and the extend of its contamination with glyphosate is unknown. This study aimed at determining the concentration of glyphosate in the majority of bread products sold in Mount Lebanon and at estimating exposure to glyphosate through the consumption of bread.

Methods: Enzyme linked immunosorbent assay (ELISA) method was utilized to quantify glyphosate residues in 100% of Lebanese bread sold in Mount Lebanon (n=80) including white, brown, bran, whole wheat and unconventional (almond, quinoa, woodbees, healthy fiber, extra fiber, high protein, oat) types as all bakeries in Mount Lebanon were visited. Glyphosate levels where compared to maximum residue levels (MRL) of 30 mg/kg as listed by Codex in addition to MRLs of 30, 10, and 5 mg/kg for wheat and 15 mg/kg for bran as listed in the United States, Europe, and Canada, respectively. Exposure assessment of Lebanese population to glyphosate through bread consumption was also estimated.

Findings: The percentage of positive samples was 72.7, 69.2, 85.7, 78.9 and 100%, respectively for white, brown, bran, whole wheat, and unconventional bread with a median glyphosate level of 14.9, 18.7, 28.5, 25.7 and 52.9 ppb, respectively. White bread had significantly lower levels as compared to other bread types (P=0.004). Glyphosate levels in all products were below the MRL. The Estimated daily intake (EDI) of glyphosate through bread consumption in Lebanon was estimated at 0.0702 μg/kg BW/day which is only 0.000117% of the Acceptable Daily Intake (ADI) of 1 mg/kg/day as listed by Codex, and 0.00039% of the ADI of 0.5 mg/kg/day as listed by the German Federal Institute for Risk Assessment.

Conclusion: Despite more than two-thirds of samples containing glyphosate, levels fall below MRL, and Lebanese consumers do not seem to be exposed to unacceptable amounts of this herbicide through bread. Future studies need to investigate glyphosate contamination in other staple foods, and a more accurate assessment of exposure at the population level needs to be investigated.

Keywords: Glyphosate, Herbicide, Lebanese, Bread, Safety, Exposure.

Chapter I

I.1. Background

The agricultural use of chemicals, particularly to increase food production for human consumption, is common globally, leading to pesticide residues in foods (Valvanids, 2018; Alavanja, 2009). This can be problematic to humans since pesticide residues can be associated with adverse health effects (Roseboro, 2016). Several types of pesticides exist including herbicides such as glyphosate (Henderson *et al*., 2010).

I.2. Definition

Glyphosate is known as an herbicide applied to the leaves of plants. It is used to eliminate broadleaf plants and various types of weeds. It is present in several forms including solids or liquids derived from an acid and several salts which can regulate plant growth and ripen fruit (Henderson *et al.*, 2010). Its molecular formula is $C_3H_8NO_5P$ or $HOOCCH_2NHCH_2PO(OH)_2$ with a molecular weight of 169.073 g/mol. Glyphosate is an odorless white powder that decompose at 419°F or 215°C. Furthermore, glyphosate is a phosphonic acid resulting from the formal oxidative coupling of the methyl group of methylphosphonic acid with the amino group of glycine. It is a phosphonic acid and a glycine derivative (PubChem, 2018). The chemical structure of glyphosate is present in figure 1.

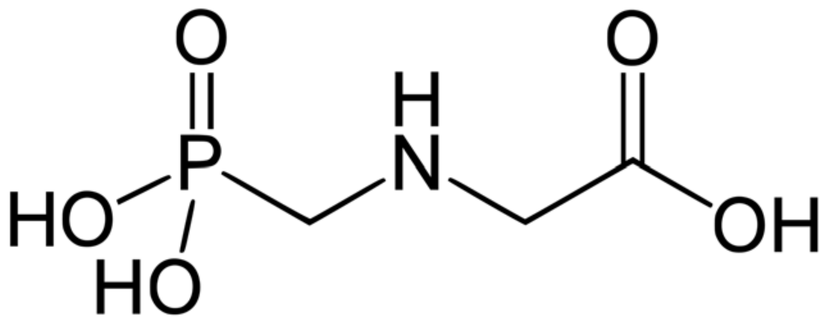

Figure 1: Chemical structure of glyphosate (PubChem, 2018).

I.3. Uses

Glyphosate is commonly used in agriculture and farming. It is mainly used as herbicide to control weeds in several kinds of plantings such as fruits, vegetables, greenhouses, and forest plantings. Its use extends to reach weed control in glyphosate resistant crop varieties such as wheat, corn, and canola (Schönbrunn *et al.,* 2001). Glyphosate use per type of crop was 0.70, 0.27, 0.27, 2.00, 0.24, and 4.00 kg for apple, barley, wheat, coffee, olives, and oranges respectively (Notarnicola *et al.,* 2017).

I.4. Mechanism

Glyphosate is a broad spectrum systemic herbicide and crop desiccant absorbed by the foliage of the plant and moved throughout it. Once absorbed by the plant, glyphosate blocks the activity of an enzyme named 5-enolpyruvyl-3-shikimate phosphate synthase (EPSPS) in the plant. This enzyme is produced by plants to aid in the production of amino acids needed for protein synthesis responsible for its growth and development. As a result, plant growth is inhibited (Schönbrunn *et al.,* 2001).

I.5.History

The history of glyphosate goes back to the 1950s when it was first discovered by Henri Martin, a Swiss Chemist. At that stage of discovery, the product had no pharmaceutical purpose. Later, John uncovered glyphosate as a potent herbicide. As a result, glyphosate was marketed in the United Kingdom for wheat. With time, glyphosate's use was extended to reach other applications including pre-harvest in cereals and oilseed crops (Duke and Powles, 2008). In 1974, the product was first registered for use in the United States of America (U.S.A.). Nowadays, almost 750 products sold in the U.S.A. contain glyphosate (Valvanids, 2018).

I.6.Regulations

The United States Environmental Protection Agency (EPA) sets an acceptable daily intake level of 1.75 mg/kg/day. In contrast, The German Federal Institute for Risk Assessment sets the acceptable daily intake level at 0.3 to 0.5 mg/kg/day (Myers *et al*., 2016).

Belgium banned its use in 2017. Bermuda outlawed the private and commercial sale of all glyphosate-based herbicides. Same applies for Netherlands, Sri Lanka, and El Salvador. As for France, an outright ban on glyphosate was issued in November 2017 to take effect within the years. Middle Eastern countries including Saudi Arabia, Kuwait, Qatar, Oman and the United Arab Emirates have stopped glyphosate use (Organic Consumers Association, 2018).As for Lebanon, there are no specific regulations regarding glyphosate use. Thus, regulations from *Codex Alimentarius* can be used. "Glyphosate is the world's most used herbicide and a common weed killer in agriculture and home gardening. In March 2015 the International Agency for Research on Cancer (IARC) working group of experts classified Glyphosate in Group 2A (*probable human carcinogens*) with strong evidence for a genotoxic mechanism of carcinogenicity. The Joint WHO/FAO Expert Meeting on Pesticide Residues (JMPR), which is responsible for assessing the risk of pesticide residues in Food in Codex, originally evaluated Glyphosate in 2004. JMPR did not find evidence for carcinogenicity in humans and assigned an Acceptable Daily Intake. On that basis, the Codex Alimentarius Commission has established Maximum Residue Limits (MRL) for Glyphosate in a large number of food crops (2006-2014)." (*Codex Alimentarius*, 2006-2014). One of the food crops is cereal grains category and the MRL as per Codex is 30 mg/kg set in 2006 except for maize and rice. As for the acceptable daily intake (ADI), it is listed in Codex in JMPR related information. The ADI is 0-1 mg/kg. (*Codex Alimentarius,* 2006-2014).

I.7.Risk to human and animal health

The health effects of glyphosate on humans and animals are presented in the table that follows based on previous literature.

Table 1: Health effects of glyphosate on humans and animals, types of studies, participants, and references.

Effect	**Type of study**	**Participants**	**Reference**
Potent carcinogen	Review	Humans	IARC,2015
Induced cholangiocarcinoma (CCA) cell growth through non-genomic estrogen receptor/ERK1/2 signaling pathway	Review	Humans	Sritan *et al*. 2018
Induced breast cancer cells growth via estrogen receptors	Review	Humans	Thongprakaisang *et al*. 2013
Related to non-Hodgkin's lymphoma	Review	Humans	Centner *et al*. 2019
Bound to key nutrients in the soil such as iron, zinc, manganese and boron making them unavailable in the food products; Killed probiotics in the gut; Caused endocrine	Review	Humans	Murphy and Rowlands, 2013

disruptions; liver and kidney damage			
Destroyed the renal tissues of farmers when combined with nephrotoxic metals in hard water	Review	Humans	Jayasumana *et al*. 2014
Accumulated in environment resulting in shifts in microbial community composition in soils, plants and animal guts; Contributed to an increase in antibiotic resistance	Review	Animals	Bruggen *et al*. 2018
Harmful effect on endocrine function in male rats	Case-control	Male rats	Nardi *et al*. 2017
Lowered bee carotenoid contents	Experimental	Bees	Jumarie *et al*. 2017
Caused changes in the cellular structure of bees' glands	Experimental	Bees	Faita *et al*. 2018
Caused protein depletion, increases glycogen and triacylglycerol consumption in the liver, and changed the muscle glycogen and induced lipid deposition in the liver of adult catfish	Experimental	Adult catfish	Persch *et al*. 2018
Reduced the distance traveled and the mean speed, and reduced ocular distance with impairment in memory in adult Zebrafish	Experimental	Adult zebra fish	Bridi *et al*. 2017
Altered the ovarian follicular dynamics and gene expression, and the proliferative activity of the ovaries and uterus of lambs	Case-control	Lambs	Alarcon *et al*. 2019

I.8.Exposure assessment

Exposure assessment can be divided into human exposure and animal exposure.

Beginning with human exposure, farmers may be primarily exposed to glyphosate through occupational exposure. This happens if they get it on their skin, in their eyes, or inhale it upon applying to plants or even ingest it by accident. After application, glyphosate does not vaporize thus residues may remain increasing farmer's exposure. For instance, a study was done to test the presence of glyphosate in the urines of a farmer and his family who sprayed a glyphosate based herbicide on his land. Glyphosate residues were measured in urines a day before, during, and two days after spraying. Glyphosate reached a peak of 9.5 µg/L in the farmer after spraying, and 2 µg/L were found in him and in one of his children living at a distance from the field. The study suggested that oral or dermal absorptions could explain the differential pesticide excretions, even in family members at a distance from the fields (Mesnage *et al.,* 2012). A review was done to assess glyphosate exposure among workers in occupational settings as well as the general population. Scientific publications on glyphosate levels in humans were reviewed. In total, 19 studies were selected. Out of the 19 studies, 5 and 11 studies documented occupational exposure and general population exposure respectively, while 3 reported both. Results showed that urinary glyphosate levels were detected in 423 occupational exposures and 3298 exposures from the general population. Levels of glyphosate from occupational exposure were higher than urinary levels from the general population (Gillezeau *et al.,* 2019). People could be also exposed to glyphosate through the ingestion of food containing this herbicide. Studies have reported that although glyphosate is used on a large variety of crops, only a limited number of uses generate residues in the edible parts of the crops. The dietary exposure assessment revealed that even when considering the unrealistic worst case scenario where all the crops, fish, seafood and animal products would contain residues at the MRL or at 0.1 mg/kg if no current MRL exists, the consumer would only ingest 17% of the glyphosate ADI. No impact on human health is thus to be expected for the consumer of food treated with glyphosate according to good agricultural practices (European Commission, 2018). Another study was conducted in Germany on adults. Gas chromatography-mass spectrometry (GC-MS/MS) method was used to analyze the presence of glyphosate in 24 h- urine samples. These samples were retrieved from the German Environmental Specimen Bank using ones collected in 2001, 2003, 2005, 2007, 2009, 2011, 2012, 2013, 2014, and 2015. The samples originated from 20 to 29 years old adults living in Germany. A total of 399 urine samples were analyzed. Results showed that 127 or 31.8% of the samples had glyphosate concentrations at or above the limit of quantification (LOQ) of 0.1 µg/L. Following a time trend, results showed that glyphosate levels reached their peak in 2012 and 2013 at 57.5% and 56.4% respectively. In addition, the levels were at 10.01% in 2001 after which they have discontinuously increased. In 2014 and 2015, the levels decreased to 32.5% and 40.0% respectively. The study suggested that urinary glyphosate concentrations are higher in male gender. As for the decrease in levels obtained in 2014 and 2015, this is linked to the changes in glyphosate application in agricultural practices (Conrad *et al.,* 2017). A risk assessment was conducted to analyze the exposure to pesticides through the dietary intake of vegetables pertaining to the Mediterranean diet. The assessment was done in the Basque country in North of Spain. The number of samples analyzed was 221 by GC–MS/MS and liquid chromatography tandem mass spectrometry (LC–MS/MS). The sampling was established in regard to consumption frequency of each vegetable. 48% of the samples had no pesticide residues. However, 52% of the samples did. 6.8% of the samples had residue levels above the MRL. Risk calculation was done based on hazard quotient which reflected a range between 0.001-0.214 % risk to consumers through vegetable intake. This implies that exposure to pesticide residues from vegetables does not raise a health concern to consumers. Yet, residue levels in vegetables were high. (Lemos *et al.,* 2016). Moreover, a dietary risk assessment was also conducted by EPA for glyphosate. The total U.S.A. population had an exposure of 0.091 mg/kg/day glyphosate and children aged 1 to 2 years had the highest exposure of 0.23mg/kg/day. According to EPA, it did not raise a health concern (Reeves *et al.,* 2019).

Moving on to animal exposure, a study was conducted to assess glyphosate residues in animal feeds. Enzyme-linked immunosorbent assay (ELISA) technique was utilized for the analysis. 18 animal feeds from 8 manufacturers were tested. Results showed that average daily intakes by animals consuming feeds containing the median glyphosate concentration were estimated to result in exposures that are 0.68–2.5% of the ADI for humans in the U.S.A. and Europe, which were 1750 and 500 μg/kg respectively. The concentration of glyphosate in companion animal feeds was 7.83×10^1 to 2.14×10^3 μg/kg. This resulted in per exposure that is 4 to 12 times higher than that of humans on a per Kg basis (Zhao *et al.,* 2018). ELISA and GC-MS/MS were used to analyze glyphosate residues in urine and different organs of dairy cows in addition to urine of hares and rabbits. High correlation coefficients were identified between the two methods. German dairy cows samples were collected from conventional husbandry, cows kept in genetically modified free areas, organs from slaughtered cows from conventional husbandry in numbers of 343, 32, and 128 respectively. 32 samples were from gut wall, 41 from liver, 26 from kidney, 23 from lung and 6 from muscles. 242 urine samples were retrieved from Danish cows. German dairy cows had lower glyphosate excretion levels than Danish cows. Conventional husbandry cows had higher excretion levels than cows kept in genetically modified free area. Glyphosate was present in organs of slaughtered cows including intestine, liver, muscle, spleen and kidney. Higher levels were present in fattening rabbits as compared to hares (Kruger *et al.,* 2014).

I.9.Exposure symptoms

Exposure symptoms can be divided to those pertaining to humans and animals based on the gathered literature.

Beginning with humans, a review was done for 80 intentional ingestion cases, 79 of which were suicide attempts. Researchers identified typical symptoms of erosion of the gastrointestinal tract, dysphagia or difficulty swallowing, and gastrointestinal hemorrhage. Seven cases resulted in death. A review explained the EPA's no observed adverse effect level (NOAEL) and reference dose values for glyphosate. Given the absence of an accurate toxicological endpoint attributable to a single dose, there is no acute reference dose for glyphosate. The identified NOAEL was 100 mg/kg/day. After dividing the NOAEL by 10 to convert data from animals to humans, the resulting value was also divided by 10 to account for susceptible populations. The resulting chronic reference dose for glyphosate was 1.00 mg/kg/day (National Pesticide Information Center, 2015).

Moving on to animals, the acute oral lethal dose (LD_{50}) in rats was shown to be greater than 4320 mg/kg. In addition, the acute oral LD_{50} was greater than 10,000 mg/kg in mice and 3530 mg/kg in goats. Furthermore, the acute dermal LD_{50} in rabbits was greater than 2 g/kg. Concerning data on signs of toxicity, animals exposed to formulated glyphosate herbicides have displayed anorexia, lethargy, hyper salivation, vomiting, and diarrhea. Symptoms persisted for 2 to 24 hours following exposure. Chronic toxicity data showed a no observed effect level (NOEL) greater than or equal to 500 mg/kg/day in beagle dogs. As for rats, the low observed effect level (LOEL) was 940 and 1183 mg/kg/day for males and females, respectively (National Pesticide Information Center, 2015).

I.10.Method of testing

Several methods could be used to identify and quantify glyphosate in food including strip kit, ELISA, high performance liquid chromatography (HPLC) with ultraviolet visible spectroscopy (UV-Vis) or fluorescence and LC-MS/MS analysis (Valle *et al*., 2019). Each of these techniques has advantages and disadvantages. LC-MS/MS analysis is a method recognized by regulators worldwide for glyphosate testing in food (Murphy and Rowlands, 2013). HPLC is the most commonly used; however, it requires derivatization to improve the sensitivity and selectivity by including detectors for UV-Vis and fluorescence (Melo *et al.,* 2018). Thus, a substitute method is ELISA. Several studies compared ELISA with HPLC and LC-MS/MS pertaining to accuracy and precision for the detection and quantification of glyphosate. Results showed that ELISA application is cost-effective and reliable (Rubio *et al.,* 2003). Therefore, ELISA was considered a cheap, sensitive, easy and simple technique to be used for the identification and quantification of this molecule. Moreover, ELISA presented good repeatability and high precision for detection (Qiang *et al.,* 2016).

I.11.Food Sources

Several studies in the literature were reported on the detection and quantification of glyphosate in different food products throughout the world. Tested food products included those pertaining to the basic food groups (milk, meat, fruits, vegetables, breads and cereals) in addition to some organic foods, creative foods, drinks, and desserts. These studies are reported in the table 2.

Table 2: Glyphosate residues detected and quantified in different foodstuffs from different countries based on previous literature including number of samples assessed, number of samples with residues above or equal to the limit of detection, average glyphosate content range, percentage of positive samples, method used, limit of detection, study limitation, and reference.

Foodstuff	Number of samples assessed	Number of samples with residues ≥ limit of detection	Average Glyphosate Content Range	Percentage of positive samples (%)	Method used	Limit of detection	Study limitation	Reference
Grains, rice, flour, bread, cereal based food for infants	1168	283	0.47 mg/kg	24	-	0.1 mg/kg	No mention of method used	Stephenson and Harris, 2016
Soda, soy milk, tofu, fruit drink, sports drink, organic milk, corn starch, nonorganic milk, beef, cucumber, chicken juice, fish corn, popsicle, beer, coffee, honey, tea	23	23	0.83 ppb	100	ELISA	0.075 ppb	-	Joshuva and Liu, 2018
Flour, cornflakes, oatmeal, bagels, yogurt, bread, hash browns, potatoes, cream of wheat, eggs, non-dairy creamers, dairy based coffee creamers	24	10	134.64 ppb	42	ELISA	-	No mention of LOD	The alliance for natural health USA, 2016
Soy based infant formula	105	-	0.55 mg/kg	-	Liquid chromatography with fluorescence detection	0.02 mg/kg	No mention of number of positive samples	Rodriguez *et al.* 2018

Soybean, corn, eggs, milk, fruits, vegetables, grains, infant foods, nuts, cereals	6049	2178	-	36	-	-	Review paper for grouped data; no mention of LOD, method, and glyphosate levels	Reeves *et al.* 2019
Baby food, beer, cereals, grains, honey, pancake and corn syrup, soy milk, soy sauce, tofu, wine	312	84	550.3 ppb	27	ELISA	17.625ppb	-	Glaze *et al.* 2017
Cereals, pulses, pasta, wine, fruit juice, honey	243	-	-	-	Liquid chromatography triple quadrupole mass spectrometry	Between 0.0005 and 0.0025 mg/kg	No access to number of positive samples and glyphosate levels.	Zoller *et al.* 2018
Honey	74	22	79.5 ppb	30	ELISA and validation with liquid chromatography and tandem mass spectrometry	15 ppb	-	Berg *et al.* 2018
Honey	140	18	0.07 mg/kg	13	Ultra performance liquid chromatography - tandem mass spectrometer	0.05 mg/kg	-	Raimets *et al.* 2020

Soybean, barley, wheat, corn	-	-	-	36	Liquid Chromatography - tandem mass spectrometry	-	Review paper based on group data; no mention of sample number and glyphosate level	Vicini *et al.* 2019
Food samples tested in Canada between years 2015 and 2016	3188	956	-	30	-	-	Review paper based on group data; no mention of glyphosate level and method used	Ledoux *et al.* 2020
Organic honeys, beef muscle pools, sea bass muscle pools	30	0	0	0	Ion chromatography-high resolution spectrometry	-	No mention of LOD	Chiesa *et al.* 2019

I.12.Glyphosate in Lebanon

Data is available on the amount of glyphosate in Lebanese chickpeas products exported to Quebec. Based on Canadian Food Inspection Agency (CFIA) tests, on 14 July 2015 the chickpea dip or hummus showed a glyphosate concentration of 0.149 µg/g, and was above the acceptable limit, whereas it was acceptable when tested on 24 March, 2016. The number of tested samples was not mentioned in the CFIA report (CFIA tests 2016). A study conducted in Lebanon evaluated the efficacy of glyphosate against *Phelipanche aegyptiaca* in potato. *P. aegyptiaca* is an obligate holoparasite which can cause damage to potatoes. Based on the two years field and greenhouse studies that were done in Lebanon, glyphosate application was found to be beneficial for potatoes by reducing *P. aegyptiaca* in potatoes (Haidar and Shdeed, 2015).

I.13.Bread production in Lebanon

Wheat is planted in the Bekaa valley and the Ministry of Economy and Trade buys the seasonal crops from the farmers and distributes them to several mills as needed according to their yearly production. On the other hand, wheat is also imported and stored in silos at Beirut port from several sources including Australia, Turkey, and Ukraine amongst others. Several types of flours are also imported to be used in the production of Lebanese pita bread worldwide. Imports are then bought by different mills in Lebanon. Then, smaller bakeries receive the main bread ingredients which are flour and wheat as per their own specifications (Decision number 15 issued by the Ministry of Economy and Trade, 2017). Lebanese standards institution (LIBNOR) standards for Lebanese bread are available in which the bread on this standard is classified into different categories. White Lebanese Bread is the bread which is characterized by a semi-circular loaf of bread formed from two layers of two-dimensional joints, and separated from the inside. It does not exceed the thickness of 3 millimeters. Brown Lebanese bread is the bread which is characterized by a semi-circular form formed from two layers of two-dimensional joints. Its thickness is no more than 4 millimeters. Wheat flour category number 135 is used or 225, or wheat flour with bran combined, thus giving the brown color. Fiber should not be less than 1.5%. Bran Lebanese bread is the bread which is characterized by a semi-circular form formed from two layers of two-dimensional joints. Its thickness is no more than 5 millimeters. Wheat flour is used with bran. Fiber should not be less than 2%. Whole wheat Lebanese bread is the bread that is characterized by the use of flour and whole wheat flour. These are made up of the germ, starchy endosperm, and bran. The Lebanese bread should have a list of general conditions including that the materials used in the Lebanese bread industry should be in conformity with their Lebanese standards, to be free from bitter taste or any stranger taste or smell, to be free from lumps such as flour or salt lumps that indicate that dough ingredients are not mixed well, to be free from dust, sand and insects or all kinds of strange material, to be free from colorants, and to be free from cancerous substances. Wheat flour enriched with vitamins and minerals can be used. Bread should be free from mineral oils. Moisture percentage should not exceed 25%, 26%, and 26% for white, brown, and bran respectively. Materials that may be added to the bread include sugar, milk, soybean flour, and calcium and / or sodium propionate, to resist mold, but not exceeding a total of 0.3%. Heavy metals in bread should not exceed 0.2, 10, and 0.2 milligram per kilogram for lead, copper, and cadmium respectively. Zearalenone, Deoxynivalenol, Ochratoxin A, and Aphlatoxin total should not exceed 50, 500, 3, and 3 mg per kg respectively. As for radionuclides it should be as specified in *Codex alimentarius* standard number 193 (Lebanese Standards, 2010).

Chapter II

II.1. Introduction

Agricultural chemicals use is widespread and increasing throughout the years (Alavanja, 2009). Several types of pesticides exist including herbicides such as glyphosate. Glyphosate is used to eliminate broadleaf plants and various types of weeds, regulate plant growth or ripen fruit (Valvanidis, 2018). Glyphosate averts the plants from producing proteins essential for plant growth through inhibiting the shikimic acid pathway (Valvanids, 2018). It is present in several forms including solids or liquids derived from an acid and several salts (Henderson *et al.,* 2010). Their use is mainly growing in crop production leading to residues in foods consumed by humans (Valvanids, 2018).

Evidences showed that glyphosate residues were detected in different food products throughout the world (Stephenson and Harris, 2016, The alliance for natural health USA, 2016, Glaze *et al.,* 2017, Joshuva and Liu, 2018, Berg *et al.,* 2018, Rodriguez *et al.,* 2018, Zoller *et al.,* 2018, Reeves *et al.,* 2019, Vicini *et al.,* 2019, Chiesa *et al.,* 2019, Raimets *et al.,* 2020, Ledoux *et al.,* 2020). Tested food products included those pertaining to the basic food groups (milk, meat , fruits, and vegetables, cereals and cereals products) (Stephenson and Harris, 2016, The alliance for natural health USA, 2016, Glaze *et al.,* 2017, Joshuva and Liu, 2018, Zoller *et al.,* 2018, Reeves *et al.,* 2019, Vicini *et al.,* 2019, Chiesa *et al.,* 2019, Ledoux *et al.,* 2020) in addition to some organic foods (Joshuva and Liu, 2018, Chiesa *et al.,* 2019), creative foods (Glaze *et al.,* 2017, Rodriguez *et al.,* 2018, Joshuva and Liu, 2018) drinks (Glaze *et al.,* 2017, Zoller *et al.,* 2018, Joshuva and Liu, 2018), and desserts (The alliance for natural health USA, 2016, Glaze *et al.,* 2017, Joshuva and Liu, 2018, Zoller *et al.,* 2018, Berg *et al.,* 2018, Chiesa *et al.,* 2019, Raimets *et al.,* 2020). In the United Kingdom, glyphosate was detected in nearly two in three bread loaves. In the US most of the breakfast cereals were contaminated (Solo Syndication, 2018). As per Xu et al. 2019, glyphosate concentration in wheat grain was 11 mg/kg wheat based. This concentration was concentrated in the bran fraction but greatly less in the white flour. In processing, removal of outer layers of grains was shown effective to decrease herbicide residues. Cooking on the other hand does not affect glyphosate levels. Concentration of glyphosate in grain based food products were reported in the literature and ranged between 0.001 and 11.1 mg/kg in wheat, between 0.4 and 8.8 mg/kg in soybean and much less in barley, oat and rye (Rubio *et al*., 2014; Granby *et al*., 2018; Gelinas *et al.,* 2018). All the reported levels were below MRLs set by FAO/WHO at 30 mg/kg (Xu *et al.,* 2019). Studies also showed that glyphosate residues were higher in whole wheat grains as compared to the more refined ones (Granby *et al*., 2003). Glyphosate was even detected in Australian water in Melbourne at levels of 77, 79, and 4% respectively for wetlands, urban streams, and rural streams (Okada *et al.,* 2020).

Controversial results were reported in the literature about the risks of glyphosate ingestion on human health. However, most of the preliminary studies on glyphosate's health effects were considered biased due to the influence of the glyphosate manufacturers on the results. In brief, glyphosate induced cholangiocarcinoma (CCA) cell growth through non-genomic estrogen receptor/ERK1/2 signaling pathway in humans (Sritan *et al*., 2018). It was related to non-Hodgkin's lymphoma (Centner *et al*., 2019), and bound to key nutrients in the soil making them unavailable to humans (Murphy and Rowlands, 2013). It destroyed the renal tissues of farmers when combined with nephrotoxic metals in hard water (Jayasumana *et al*., 2014).

The Environmental Protection Agency (EPA), The European Food Standards Agency (EFSA), and the United Nations Food and Agriculture Organization (FAO) considered glyphosate products not likely to be carcinogenic to humans (Thongprakaisang *et al*., 2013, Sritan *et al.,* 2018, Centner *et al.,* 2019, Zhang *et al.,* 2019). The International Agency for Research on Cancer (IARC) has listed glyphosate as a probable carcinogen in humans since the year 2015 (Thongprakaisang *et al.,* 2013, Sritan *et al.,* 2018, Centner *et al.,* 2019, Zhang *et al.,* 2019).

Most of the countries are actively involved in the regulation of glyphosate use (Organic Consumers Association, 2018). EPA sets an acceptable daily intake (ADI) level of 1.75 mg/kg/day. In contrast, the German Federal Institute for Risk Assessment is more stringent and sets the ADI level at 0.3 to 0.5 mg/kg/day (Myers *et al.,* 2016). Belgium banned its use in 2017 (Organic Consumers Association, 2018). Bermuda outlawed the private and commercial sale of all glyphosate-based herbicides (Organic Consumers Association, 2018). Same applies for Netherlands, Sri Lanka, and El Salvador (Organic Consumers Association, 2018). As for France, an outright ban on glyphosate was issued in November 2017 to take effect within the years (Organic Consumers Association, 2018). Middle Eastern countries including Saudi Arabia, Kuwait, Qatar, Oman and the United Arab Emirates have stopped glyphosate use (Organic Consumers Association, 2018). In Lebanon, standards from *Codex Alimentarius* are mainly adopted. The maximum residue level (MRL) as per Codex is 30 mg/kg set in 2006 for cereal grains except for maize and rice (*Codex Alimentarius*, 2006). The adopted ADI is 1 mg/kg/ BW (*Codex Alimentarius*, 2014). Data concerning this herbicide in Lebanon are still lacking. Considering that Lebanese consume bread in or with almost each meal, the objectives of this study were to quantify the amount of glyphosate in Lebanese pita bread and assess the exposure to glyphosate.

II. 2. Materials and Methods

II.2.1. Sample Collection

A total of 80 samples of bread including white (n=22), brown (n=13), bran (n=14), whole wheat (n=19) and unconventional (n=12) (almond, quinoa, woodbees, healthy fiber, extra fiber, high protein, oat) were purchased from bakeries throughout Mount Lebanon. 27.5 % of the samples were from white bread, 16.3% from brown, 17.5% from bran, 23.8% from whole wheat, and 15% from unconventional. Woodbees bread brand is known to be high in protein, high in fibers, low in sodium with no sugar and preservatives added. All registered 28 bakeries, producing pita bread, in Mount Lebanon, were visited in September 2019. All samples were analyzed in duplicate within 24 h.

II.2.2. Sample preparation and derivatization

The glyphosate residues in bread were carried out using the ELISA test kit PN 500086 (Abraxis, USA). Sample preparation, derivatization and analysis were conducted according to the manufacturer's instructions. A 0.5 g of bread sample was weighed into an appropriately labeled 20 mL glass vial. Boiling deionized water (10 mL) was added to each sample at a 1:20 dilution. The mixture was then rotated for 10 min at 150 rpm using an orbital shaker Stuart SSL1 (United Kingdom) then removed to be settled for 2 min. A 2 mL aliquot of the supernatant were then transferred to an appropriately labeled micro centrifuge tube and centrifuged on Heraeus Fresco 21 centrifuge (United States of America) for 5 min at 8000 g. Finally, 800 μL of glyphosate sample diluent were added to a glass vial, and mixed with 200 μL of the supernatant in the vial. The derivatization reagent was diluted by adding 3.5 mL of the derivatization reagent diluent to the derivatization reagent vial mixed thoroughly on a mini vortexer. Standard solutions of glyphosate were provided in each test kit with concentrations 0, 0.25, 0.5, 0.75, 1 and 4 ppb. An aliquot of 250 μL of each standard, control, and sample were added to appropriately labeled 4 mL glass vials. Glyphosate assay buffer (1 mL) was added to each vial and vortexed for 2 s. A100 μL of the diluted derivatization reagent was added to each standard, control and sample successively. Each tube was vortexed for 15-30 s then incubated at room temperature for 10 min. For the glyphosate analysis, 50 µL of the derivatized standard solutions, control, and samples were added to each well followed by the addition of 50 µL anti-glyphosate antibody solution and covered then mixed for 30 s. After incubation at room temperature for 30 min, 50 µL of the enzyme conjugate was added and covered and mixed for 30 s then incubated at room temperature for 60 min. The plates were then washed three times with 250 µL of washing buffer. A 150 µL of substrate/color solution was added and the strips were incubated for 30 min at room temperature and away from direct sunlight. Following the addition of 100 µL of stop solution to each well, the absorbance was measured at 450 nm by a micro plate reader (Thermo Lab systems Opsus MR, USA).

II.2.3. Glyphosate analysis

Absorbance levels for each sample analyzed were retrieved and analysis was performed on ELISA Skanit software version 4.1. For the evaluation of the results obtained, the ELISA results were multiplied by a factor of 100 to account for the necessary dilution. The limit of detection (LOD) was 7.5 ppb. Samples showing a concentration lower than 7.5 ppb were reported as negative. Samples showing a higher concentration than 7.5 ppb were reported as positive. Glyphosate levels in tested samples were compared to MRL set by codex at 30 mg/kg (Codex, 2006-2014) in addition to MRLs set by other countries including US, Europe, and Canada which set MRLs for wheat at 30, 10 and 5 mg/kg with an exception of 15 mg/kg for bran in Canada (Code of Federal Regulations, European Commission, Health Canada, 2019).

II.2.4. Ethical considerations

Institutional review board (IRB) approval was granted to this study. Bread samples were coded to protect supplier confidentiality. Glyphosate disposal was done based on material safety data sheet provided by the supplier (Abraxis, USA).

II.2.5. Assessment of the level of exposure to glyphosate

The daily intake was estimated using the equation suggested by Codex, 2014. Estimated Daily Intake (EDI) (µg/kg BW/day) = Σ [(Daily bread intake (kg/person/day) × Median residue concentration (µg/kg)] ÷ BW (kg). The values obtained from this formula were then compared to the acceptable daily intake to glyphosate (ADI) as set by Codex at 1 mg/kg BW (Codex, 2006-2014). The results were also compared to ADI of 0.3 to 0.5 mg/kg/day set by the German Federal Institute for Risk Assessment. The following equation was used for comparison: % ADI = 100 × Intake (µg/day) ÷ [(ADI (µg/kg BW/day) × BW (kg)] (Codex, 2014). Reference daily bread intake was 0.136 kg bread/day (Al Medawar, 2015) and body weight was 60 kg (Codex, 2014).

II.2.6. Data analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences Software (SPSS) version 21 (IBM, USA) and $p<0.05$ was considered statistically significant. The data was analyzed for normality using Kolmogorov-Smirnov test, and the data was not normally distributed for glyphosate concentration. Therefore, the median and interquartile range (IQR) were reported and used for exposure assessment estimation. The glyphosate residues level medians were based on positive samples. Negative samples were assigned a value of one half of the LOD when calculating the median glyphosate residue concentration. Percentages of positive samples by bread type were calculated.

II. 3. Results and Discussion

II.3.1. Prevalence of glyphosate in Lebanese bread

The occurrence of samples detected positive for glyphosate in white, brown, bran, whole grain and unconventional type of bread with their maximum, minimum, median, mean, percentage, and comparison to MRL was reported in Table 3. Unconventional bread category showed the highest percentage of positive samples (100%) among all bread types tested. The lowest percentage of positive samples was found in brown bread category at 69.2%. The difference in percentage positive samples among different bread types and bread brands was not significant (p=0.291, 0.079, respectively). The glyphosate median residue levels were significantly highest in the unconventional breads (52.9 ppb) as compared to the bran (28.5 ppb), whole wheat (25.7 ppb), brown (18.7 ppb) and white bread (14.9 ppb) with p= 0.004. However, the glyphosate levels were statistically similar among the different assessed bread brands (p=0.203). All tested samples (n=80) prevailed glyphosate concentration below the MRLs.

II.3.2 Exposure estimation

The EDI of glyphosate residue from the consumption of bread is represented in Table 4 along with the calculated percent ADI. Hence, the EDI of glyphosate through bread consumption in Lebanon was found to be 0.0702 μg/kg BW/day which makes up 0.000117% of the ADI of 1 mg/kg/day as listed by Codex, and 0.00039% of the ADI of 0.3 to 0.5 mg/kg/day as listed by the German Federal Institute for Risk Assessment.

Table 3: Number of samples detected positive for glyphosate in bread with their minimum, maximum, median, and mean values.

Bread Type	Positive[a]/n	% Positive	Min/Max of the Positive samples (ppb)	Median (ppb)	Interquartile Range (IQR)	Mean ±SD (ppb)	%> MRL
White	16/22	72.7	7.5/56.5	14.9	25.8	21.0±14.5	0
Brown	9/13	69.2	7.5/60.5	18.7	27.1	22.0±17.2	0
Bran	12/14	85.7	7.5/146.8	28.5	29.9	32.0±35.4	0
Whole	15/19	78.9	7.5/160.1	25.7	45.1	45.5±48.9	0
Unconventional	12/12	100	12.7/183.5	52.9	65.4	70.8±54.3	0
Total	64/80	80.0	7.5/183.5	31.0	33.4	39.5±39.9	0

[a]Positive samples: samples in which glyphosate residue level exceeded the limit of detection (LOD). LOD glyphosate: 7.5 ppb.

p= 0.004 for differences in glyphosate concentration by bread type (kruskal Wallis test)

p= 0.291 for differences in positive results by bread type (chi square test)

p= 0.079 for difference in positive results by bread brand (chi square test)

p= 0.203 for differences in glyphosate concentration by bread brand (kruskal Wallis test)

Table 4: Risk characterization of daily exposure to glyphosate residues through intake of Lebanese bread.

Reference	Herbicide	Intake (kg/bread/day)	EDI	%ADI (Codex)	%ADI (German)
Al Medawar, 2015	Glyphosate	0.136	0.0702	0.000117	0.00039%

EDI=Estimated daily intake (ug/kg BW/day). EDI (μg/kg BW/day)=Σ[(Daily bread intake (kg/person/day)×Mean residue concentration (μg/kg)] ÷ BW (kg). % ADI=100×Intake (μg/day)/[(ADI (μg/kg BW/day)×BW (kg)]. ADI=Acceptable daily intake (Codex): $ADI_{glyphosate}$ =0-1 mg/kg BW. ADI=Acceptable daily intake (German Federal Institute for Risk Assessment): $ADI_{glyphosate}$ =0.3-0.5 mg/kg BW.

II.4. Discussion

II.4.1. Glyphosate prevalence in Lebanese bread

The results in this study showed that glyphosate residue was present in almost all the breads. Similarly, in the United Kingdoms (U.K.), 32 of 196 (16%) white bread samples tested positive for glyphosate, and 73 of 167 (44%) wheat bread with bran samples were also positive (Stephenson and Harris, 2016). Also, in the U.K., 63% of bread loaves in supermarkets contain pesticide residues with highest amounts for glyphosate (Carrington, 2014). In Philadelphia, US metropolitan area, the assessment of several samples showed that 94.65% (106 of 112) of the beer samples were positive and 87.5 % of the cereals (14 out of 16) (Glaze *et al.,* 2017). As for Canadian data, 29.7% of samples collected from fruits, vegetables, grains, and infant foods had glyphosate residues (Reeves *et al.,* 2019). Moving on to European data, out of 5329 samples of fruits, vegetables, nuts, cereals, infant foods and some animal products tested for glyphosate residue, 3.1% of the samples showed residues (Reeves *et al.,* 2019). Moreover, in the U.S.A., glyphosate herbicide residues were also found in bread samples separated into groups of conventional gluten free (3 samples), organic (5 samples), conventional white (7 samples), conventional whole wheat (6 samples) (Honeycutt, 2018). The difference between countries and between Europe and U.S.A. was present because glyphosate use in U.S.A. is higher as compared to other countries. Hence, two-thirds of the total volume of glyphosate applied in the U.S. from 1974 to 2014 has been sprayed in just the last 10 years. The corresponding share globally is 72 % (Benbrook, 2016).

The high prevalence of glyphosate in Lebanese bread could be due to the lack of regulations on the use of this herbicide in the Lebanese agriculture unlike other countries where regulations on glyphosate are highly implemented (Organic Consumers Association, 2018). These include Bermuda, for Netherlands, Sri Lanka, and El Salvador, which outlawed the private and commercial sale of all glyphosate-based herbicides (Organic Consumers Association, 2018). An outright ban on glyphosate was issued in November 2017 in France to take effect within two years (Organic Consumers Association, 2018). Middle Eastern countries including Saudi Arabia, Kuwait, Qatar, Oman and the United Arab Emirates have stopped glyphosate use (Organic Consumers Association, 2018). Germany set to ban this herbicide from the end of 2023 (Made from minds, 2019). Health Canada still approves the use of glyphosate and had decided to keep its decision unchanged in 2017 which approved glyphosate use for 15 years (Health Canada, 2018). As for U.S.A., glyphosate is not banned. However, some states issued a statewide ban on glyphosate such as California, Colorado, Minnesota, New Mexico, Oregon, some areas in New York, New Jersey, Nevada and others (Texas Organic Research Center, 2018).

We propose the Lebanese Ministry of Agriculture takes appropriate action towards the implementation of laws and standards to control the use of glyphosate herbicide in the Lebanese agriculture sector. This in turn would result in lower glyphosate contamination in Lebanese food products specifically bread.

II.4.2. Glyphosate levels in Lebanese bread

The findings showed that the glyphosate residue levels were all below MRL and the median ranged between 14.9 and 52.9 ppb for white and unconventional bread, respectively.

Similarly, the reported glyphosate concentrations showed values in bread ranging between 1 and 4.58 ppb in other grains averages were all below the MRL set by FAO/WHO at 30 mg/kg (30,000 ppb) with values less than 450, 80, 40, 1070-1130, and 1-12.4 ppb for barley, oat, rye, wheat and other cereal products, respectively (Xu *et al.,* 2019).

In contrast, Canadian data showed 1.3% above the maximum residue level of glyphosate for samples collected from fruits, vegetables, grains, and infant foods in 2016 where total number of samples was 3188. Moreover, in Europe, 0.09% of 5329 samples of fruits, vegetables, nuts, cereals, infant foods and some animal products tested for glyphosate residue had levels above the maximum residue level (Reeves *et al.,* 2019).

The difference between the results reported in our study and Canadian and European data could be due to the high number of samples collected in both countries (n=5329) as compared to ones collected in this study (n=80). However, the difference is not related to the MRL adopted in this study since we adopted MRLs set by different countries. Hence, glyphosate levels where compared to maximum residue levels (MRL) of 30 mg/kg as listed by Codex in addition to MRLs of 30, 10, and 5 mg/kg for wheat and 15 mg/kg for bran as listed in the United States, Europe, and Canada, respectively. Still, even if Canadian and European data showed samples above MRL, the number of samples above MRL was low. This may be due to the high MRL set at 30 mg/kg (30,000 ppb).

II.4.3. Glyphosate levels in Lebanese bread per bread type and grain type

The glyphosate median residue levels were significantly highest in the unconventional breads (52.9 ppb) as compared to the other breads (p= 0.004). However, the glyphosate levels were statistically similar among the different assessed bread brands (p=0.203).

Similarly, glyphosate level in white bread was 0.1-0.2 mg/kg and in wheat bread with bran 0.1-1.3 mg/kg in the U.K. which prevailed higher percentages for bran as compared to white bread (Stephenson and Harris, 2016). In addition, glyphosate herbicide residues in American bread samples were assessed in 2017, and the results showed that averages were 6.47 ppb in gluten free (n=3), 12.24 ppb in organic (n=5), 14.13 ppb in conventional white bread (n=7) and 140.98 ppb in conventional whole wheat bread (n=6) (Honeycutt, 2018). This conforms to the results reported in this study of higher glyphosate in whole wheat bread as compared to white one. Moreover, glyphosate concentrations were reported in a review paper which stated that concentration in wheat grain is 11 mg/kg wheat. This concentration is concentrated in the bran fraction but greatly less in the white flour (Xu *et al.,* 2019).

The significant difference in glyphosate concentration among different bread types is due to the difference in processing methods used for the different bread types. Hence, glyphosate is sprayed on wheat as a drying agent and the hulls on whole wheat would retain those residues. However, in white bread, the hulls are removed resulting in lower glyphosate residues. Glyphosate level was significantly higher in unconventional bread because these types are processed from whole wheat flour as well. The insignificant difference among different bread brands may be due to the lack of presence of all the bread types in Lebanese bakeries. For instance, upon sample collection, some bakeries would have all bread types available when others did not.

It is suggested that Lebanese focus more on consuming white bread to reduce the consumption of glyphosate from whole wheat bread regardless of the bread brand. This suggestion contradicts the traditional nutritional recommendation of the replacement of dietary white bread by whole wheat bread. Thus here the risk to benefit can be analyzed to come up with a final conclusion on this matter. Hence the question that may be asked is whether nutritional benefits of whole wheat bread are more important than the protection of the consumer from the consumption of a potent carcinogen from whole wheat bread.

II.4.4. Exposure to glyphosate

The findings showed that the estimated daily intake (EDI) of glyphosate through bread consumption in Lebanon was 0.0702 µg/kg BW/day which makes up 0.000117% of the ADI of 1 mg/kg/day as listed by Codex, and 0.00039% of the ADI of 0.5 mg/kg/day as listed by the German Federal Institute for Risk Assessment. This implies glyphosate intake level below the acceptable intake level.

The reported exposure in Lebanon is much lower than that in Europe, where it is estimated that consumers ingested 17% of the glyphosate ADI (European Commission, 2018). In addition, in Spain a risk assessment was conducted to analyze the exposure to pesticides through the dietary intake of vegetables pertaining to the Mediterranean diet. The assessment was done in the Basque country in North of Spain. Risk calculation was done based on hazard quotient which reflected a range between 0.001-0.214% risk to consumers through vegetable intake (Lemos *et al.,* 2016). The Spanish risk calculation reflected percentage risk higher than the one reported in this study, but still both reflected an intake lower than the ADI.

The exposure estimation to glyphosate through Lebanese bread consumption was expected to be below the ADI due to previous findings reported. However, it would be important to highlight the fact that even if exposure is below the ADI, glyphosate may have the tendency to accumulate in the human body and cause long term health implications (Murphy and Rowlands, 2013). The health implications can include liver and kidney damage. Some studies suggested that it is readily excreted from the body, whereas others confirmed its ability to accumulate in the human body.

The exposure estimation presented was below the ADI, and this is not due to the ADI adopted in this study since several ADIs where used. Hence, ADIs adopted where both pertaining to Codex and the German Federal Institute for Risk Assessment. Both of them are set at different levels of 1 and 0.5 mg/kg/day respectively. Furthermore, the fact that Lebanese may be exposed to glyphosate from other food products should not be forgotten. Hence, as previously stated glyphosate is widespread in various food products. This will lead to even higher consumption when considering a daily intake of different food products by the consumer. Also, Lebanese are exposed to potent carcinogens on a daily basis from environmental exposure through carbon monoxide from fumes and traffic and other lifestyle behaviors such as smoking. All of this combined will put them at higher risk of developing long term complications especially cancer. It would also be important to highlight the fact that exposure estimation was overestimated for Lebanese females and underestimated for Lebanese males since the body weight adopted for calculation was 60 kg. This weight may be lower for Lebanese females and higher for Lebanese males practically.

It is suggested that local marketplaces aid the consumer in purchasing organic food products to reduce the consumption of glyphosate residues. Even if glyphosate levels reported in this study were low, however glyphosate is widespread in various food products. Thus, residues may accumulate in the human body from different food products resulting in higher levels. By consuming organic foods, exposure to glyphosate would decrease. In addition, local authorities are encouraged to support local manufacturers to be able to increase organic production in the private sector. Also, selling prices of organic food products are to be reconsidered to increase their sales.

II.4.5. Limitations

The limitations of this study include that it was only carried out in Mount Lebanon instead of all Lebanon. Hence, several Lebanese areas were missed. In addition, glyphosate analysis was only carried out on bread. Glyphosate is known to be widespread in many other food products. An additional study conducted on other food products as well will lead to a higher exposure to glyphosate which may even become higher than the acceptable daily intake. Moreover, the sample size adopted in this study was low which resulted in low glyphosate levels in bread even if high percentage of positive samples was shown. A higher sample size can result in major differences in glyphosate levels.

Conclusion

The overall results showed that almost all Lebanese pita bread contain glyphosate residues. Glyphosate levels were lower in white bread as compared to the other types including brown, bran whole wheat and the unconventional which is due to processing steps where hulls are removed. However, the levels fall below MRL and exposure assessment showed that Lebanese are not consuming unacceptable amounts of this herbicide through bread. Keeping in mind that glyphosate may still accumulate in the human body, the exposure assessment would not be the only factor for final judgment on Lebanese consumer safety. Based on the findings reported in this study, we recommend local authorities including the involved ministries to take action towards consumer protection. This can be done through appropriate testing of glyphosate in different food products consumed by Lebanese, and implementing local laws and standards concerning this herbicide.

References

Alarcon, R.; INgaramo, P.; Rivera, O.; DIoguardi, G.; Repetti, M.; Demonte,L.;Milesi,M.;Varayoud,J.;Toro,M.; and Luque,E.(2019). Neonatal exposure to a glyphosate-based herbicide alters the histofunctional differentiation of the ovaries and uterus in lambs. *Molecular and cellular endocrinology*, (482), 45-56.

Alavanja, M. (2009). Pesticides use and Exposure Extensive Worldwide. *Environmental health,* 24(4), 303-309.

Al Medawar, M.; Nasredine, L.; Olabi,A.;Hamade,H.;Awad,E.;Toufeili,I.Arnaout,S.; and Ismael,H.(2015). Sodium intake reduction efforts in Lebanon, *Cardiovascular diagnosis and therapy,* 5(3), 178-185.

Benbrook, C. (2016). Trends in glyphosate herbicide use in the United States and globally, *Environmental sciences Europe*, 28(1), 3.

Berg, C.; Kng, P.; Delenstarr, G.; Kumar, R.; Rubio, F.; and Glaze, T. (2018). Glyphosate residue concentrations in honey attributed through geospatial analysis to proximity of large-scale agriculture and transfer off-site by bees. *PLoS ONE*, 13(7), 1-18.

Bridi,D.;Altenhofen,S.;Gonzalez,J.;Reolon,G.; and Bonan,C.(2017). Glyphosate and Roundup® alter morphology and behavior in zebra fish. *Toxicology*, (392), 32-39.

Bruggen,V.;He,M.;Shin,K.;Mai,V.;Jeong,K.C.;Finckh,M.; and Morris,J.(2018).Environmental and health effects of the herbicide glyphosate. *Science of the total environment*, (616-617), 255-268.

Carrington, D. (2014). Over 60% of breads sold in the UK contain pesticide residues, tests show. *The Guardian*, (1), 1.

Centner, T.; Russell, L.; and Mays, M. (2019). Viewing evidence of harm accompanying uses of glyphosate-based herbicides under US legal requirements. *Science of the total environment,* (648), 609-617.

CFIA tests. (2016). Glyphosate in chickpea. https://equiterre.org/sites/fichiers/food_matters_appendix_i.pdf

Chiesa, L.; Nobile, M.; Panseri, S. and Arioli, F. (2019). Detection of glyphosate and its metabolites in food of animal origin based on ion-chromatography-high resolution mass spectrometry (IC-HRMS). *Food additives and contaminants*, (36), 592-600.

Code of Federal Regulations (2019). https://gov.ecfr.io/cgi-bin/ECFR?page=browse

Codex Alimentarius (2006-2014). Codex pesticide residues in Food online database.

Codex Alimentarius (2014). Guideline for the simple evaluation of dietary exposure to food additives. *CAC/GL 3-1989* revision 2014.

Conrad,A.;Kermani,C.;Hopp,H.;Ruther,M.;Pieper,S.; and Gehring,M.K. (2017). Glyphosate in German adults – Time trend (2001 to 2015) of human exposure to a widely used herbicide. *International journal of hygiene and environmental health*, (220), 8-16.

Duke, S.; Powles, S. (2008). Mini-review Glyphosate: a once-in-a-century herbicide. *Pest Management Science,* (64), 319-325.

European commission (2018). Evaluation of the impact of glyphosate residues in food on human health. *EFSA journal,* (16), 1-5.

European commission (2019). http://www.ec.europa.eu

Faita, M.; Oliveira, E.; Junior, V.; Orth.A.; and Nodari, R. (2018). Changes in hypo pharyngeal glands of nurse bees (Apis mellifera) induced by pollen-containing sub lethal doses of the herbicide Roundup®. *Chemosphere*, (211), 566-572.

Gillezeau,C.; Gerwen,M.; Shaffer,R.; Rana,I.; Zhang,L.;Sheppard,L.; and Taioli,E.(2019). The evidence of human exposure to glyphosate: a review, *Environmental health,* (18), 2.

Glaze, T.; Rubio, F.; and Enguehard, S. (2017). Analysis of Glyphosate in Food and Beverage Samples by ELISA and Lateral Flow Immunoassay. *Abraxis,* (1), 1.

Haidar, M.; and Shdeed, E. (2015). Phelipanche aegyptiaca Management with Glyphosate in Potato. *American journal of plant sciences,* (6), 2540-2549.

Henderson, A. M.; Gervais, J. A.; Luukinen, B.; Buhl, K.; Stone, D.; Cross, A.; and Jenkins, J. (2010). Glyphosate General Fact Sheet. *National Pesticide Information Center, Oregon State University Extension Services,* (1), 1.

Health Canada (2019). http://www.hc-sc.gc.ca/

Health Canada (2018). Health Canada doubles down on glyphosate cancer fight, (1),1.

Honeycutt, Z. (2018). Got Monsanto's glyphosate in your lunch? *Moms across America,* (1), 1-2.

Jayasumana, C.; Gunatilake, S.; and Senanayake, P. (2014). Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits Behind the Epidemic of Chronic Kidney Disease of Unknown Etiology in Sri Lanka? *International journal of environmental research and public health,* 11(2), 2125-2147.

Joshuva, J.; and He, L. (2018). Glyphosate monitoring in water, foods, and urine reveals an association between urinary glyphosate and tea drinking: A pilot study*, international journal of environmental health engineering,* (7), 2.

Jumaire, C.; Aras, P.; and Boily, M. (2017). Mixtures of herbicides and metals affect the redox system of honey bees. *Chemosphere,* (168), 163-170.

Kabrite,S.;Bou Mitri,C.;Fares,J.;Hassan,H.; and Bou Mosleh,J.(2019). Identification and dietary exposure assessment of tetracycline and penicillin residues in fluid milk, yogurt, and labneh: A cross-sectional study in Lebanon, *Veterinary World*, 12(4), 527-534.

Krüger,M.;Schledorn,P.;Schrödl,W.; Hoppe.H.W.;Lutz,W.; and Shehata,A.A.(2014). Detection of Glyphosate Residues in Animals and Humans. *Environmental and analytical toxicology,* 4(2), 1-5.

Lebanese standards, Lebanese bread, *NL 240*, third edition, 2010.

Ledoux, M.; Hettiarachchy, N.; Yu, X.; Howard, L. and Sun-Ok, L. (2020). Penetration of glyphosate into the food supply chain and the incidental impact on the honey supply and bees. *Food Control,* (109), 106859.

Lemos, J.; Sampedro, M.; Arino, A.; Ortiz, A.; and Barrio, R. (2016). Risk assessment of exposure to pesticides through dietary intake of vegetables typical of the Mediterranean diet in the Basque Country. *Journal of food composition and analysis*, (49), 35-41.

Leu, A. (2007). Monsanto's toxic herbicide glyphosate: a review of its health and environmental effects. *Organic consumers association,* (18), 1-4.

Made from Minds (2019). Germany set to ban glyphosate from end of 2023, (1),1.

Martinez, A.; and Al-Ahmad, A. (2019). Effects of glyphosate and aminomethylphosphonic acid on an isogeneic model of the human blood-brain barrier. *Toxicology letters,* (304), 39-49.

Melo, KG.; De Nucci, G.; Trape A.Z.; Jacobucci, S.R.F.; Garlippo, C.R.; and Rosa P.C.P. (2018). Brief review analytical methods for the determination of glyphosate. *Med crave online journal of toxicology,* 4(1), 39-42.

Mesnage,R.; Moesch,C.; Le grand,R.; Lauthier,G.;Spiroux, J.; Gress,S.; and Seralini,E.(2012). Glyphosate exposure in a farmer's family. *Journal of environmental protection*, (3), 1001-1003.

Ministry of Economy and Trade. *Decision number 15*. (2017).

Murphy, D.; and Rowlands, H. (2013). Glyphosate: unsafe on any plant. *Food democracy now and the detox project,* (1), 1-29.

Myers,J.; Antoniou,M.; Blumberg,B.; Carroll,L.; Colborn,T.; Everett,L.; Hansen,M.; Landrigan,P.; Lanphear,B.; Mesnage,R.; Vandenberg,L.;Saal,F.; Welshons,W.; and Benbrook,C. (2016). Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environmental Health*, (15), 19.

Nardi, J.; Moras, P.; Koeppe, C.; DDallegrave, E.; BainylM; Grazziotini, L.; and Grando, R. (2017). Prepubertal sub chronic exposure to soy milk and glyphosate leads to endocrine disruption. *Food and chemical toxicology*, (100), 247-252.

Notamicola, B.; Tassielli, G.; Renzulli, P.; Castellani, V.; and Sala, S. (2017). Environmental impacts of food consumption in Europe. *Journal of cleaner production*, (140), 753-765.

Organic Consumer's Association. (2018). Germany +13 other countries say no to glyphosate: What about the U.S.? *Organic consumers association,* (1), 1.

Okada, E.; Allinson, M.; Barral, M.; Clarke, B. and Allinson, G. (2020). Glyphosate and aminomethylphosphonic acid (AMPA) are commonly found in urban streams and wetlands of Melbourne, Australia. *Water research,* (168), 115139.

Persch,T.;Silva,P.;Santos,S.;Freitas,B.; and Oliveira,G.(2018). Changes in intermediate metabolism and oxidative balance parameters in sexually matured three-barbeled catfishes exposed to herbicides from rice crops (Roundup®, Primoleo® and Facet®*).Environmental toxicology and pharmacology,* (58), 170-179.

PubChem. (2004). Glyphosate. *National Center for Biotechnology Information*, compound summary for CID 3496.

Ramadan, M.; and Perry, M. (2018). Human Evidence for Parental Glyphosate Exposure on Developmental Malformations and Neurodevelopmental Effects: Systematic Literature Review via the Navigation Guide Methodology. *Health sciences research commons,* (351), 66.

Raimets,R.;Bontsutsnaja,A.;Bartkevics,V.;Pugajeva,I.;Kaart,T.;Pussepp,L.;Pihlik,P.;Keres,I.;Vinalass,H.;Mand,M.; and Karise,R. (2020). Pesticide residues in beehive matrices are dependent on collection time and matrix type but independent of proportion of foraged oilseed rape and agricultural land in foraging territory. *Chemosphere,* (238), 124555.

Reeves, W.; McGuire, M.; Stokes, M.; and Vicini, J. (2019). Assessing the Safety of Pesticides in Food: How Current Regulations Protect Human Health. *Advances in Nutrition*, 10(1), 80-88.

Rodrigues, N.; and Souza, A. (2018). Occurrence of glyphosate and AMPA residues in soy-based infant formula sold in Brazil. *Food additives and contaminants*, (35), 724-731.

Roseboro, K. (2016). Grim reaping: Many food crops sprayed with weed killer before harvest. *The organic and non-GMO report.* (1), 1-4.

Rubio, F.; Veldhuis, L.; Clegg, B.S.; Fleeker, J.R.; and Hall, J.C. (2003). Comparison of a Direct ELISA and an HPLC Method for Glyphosate Determinations in Water. *Journal of agriculture and food chemistry,* 51 (3), 691–696

Salameh, P.; Waked, M.; Baldi, I.; Brochard, P.; and Abi Saleh, B. (2006). Respiratory diseases and pesticide exposure: a case-control study in Lebanon. *Journal of epidemiology and community health,* 60(3), 256–261.

Schönbrunn,E.; Eschenburg,S.; Wendy A.; Shuttleworth.;Schloss J.V.; Amrhein,N.; Jeremy N. S. Evans.; and Kabsch W.(2001). Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail. *Proceedings of the national academy of sciences of the United States of America,* 98 (4), 1376-1380.

Solo Syndication (2018). Traces of glyphosate discovered in bread and cereal. *Poulter Sean*. (1), 1-2.

Sritana, N.; Suriyo, T.; Kanitwithayanun, J.; Songvasin BH.; Thiantanawat, A.; and Satayavivad, J. (2018). Glyphosate induces growth of estrogen receptor alpha positive cholangiocarcinoma cells via non-genomic estrogen receptor/ERK1/2 signaling pathway. *Food and chemical toxicology*, (118), 595-607.

Stephenson, C.L.; Harris, C.A. (2016). An assessment of dietary exposure to glyphosate using refined deterministic and probabilistic methods. *Food and Chemical Toxicology*, (95), 28-41.

Texas Organic Research Center. (2018). GLYPHOSATE - WHERE IS IT RESTRICTED OR BANNED IN THE UNITED STATES? (1),1.

The alliance for natural health, USA. (2016). Glyphosate levels in breakfast food: What is safe? (1), 1-5.

Thongprakaisang,S.;Thiantanawat,A.;Rangkadilok.N.;Suriyo,T.; and Satayavivad,J.(2013). Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food and chemical toxicology,* (59), 129-136.

Valle, A.L.; Mello, F.C.C.; Balvedi, R.P.; Rodrigues, L.P.; and Goulart, L.R. (2019). Glyphosate detection: methods, needs and challenges. *Environmental chemistry letters*, (17), 291-317.

Valvanidis, A. (2018). Glyphosate, the Most Widely Used Herbicide. Health and safety issues. Why scientists differ in their evaluation of its adverse health effects. *Chem-tox-ecotox scientific reviews,* (1), 1-40.

Vicini, J.; Reeves, W.; SW, J.; Karberg, K. (2019). Glyphosate in livestock: feed residues and animal health. *Journal of animal science*, 97 (11), 1-41.

Wozniak,E.;Sicinska,P.;Michalowicz,J.;Wozniak,K.;Reszka,E.; Huras,B.;Zakrzewski,J.; and Bukowska,B.(2018). The mechanism of DNA damage induced by Roundup 360 PLUS, glyphosate and AMPA in human peripheral blood mononuclear cells - genotoxic risk assessment, *Food and chemical toxicology,* (120), 510-522.

Xu,J.;Smith,S.;Smith,G.;Wang,W.; and Li,Y.(2019). Glyphosate contamination in grains and foods: an overview. *Food control*, (106), 106710.

Youssef, L.; Youness, G.; Kouzayha, A.; and Jaber, F. (2015). Occurrence and levels of pesticides in South Lebanon Water. *Chemical speciation and bioavailability*, (27), 62-70.

Zhang, L.; Rana, I.; Shaffer, R.; Taioli, E.; and Sheppard, L. (2019). Exposure to Glyphosate-Based Herbicides and Risk for Non-Hodgkin Lymphoma: A Meta-Analysis and Supporting Evidence. *Mutation research*, (781), 1-62.

Zhao,J.; Pacenka,S.; Wu,J.; Richards,B.; Steenhuis,T.;Simpson,K.; and Hay,A.(2018). Detection of glyphosate residues in companion animal feeds. *Environmental pollution,* (243), 1113-1118.

Zoller,O.;Rhyn,P.;Rupp.H.;Zarn.J.; and Geiser,C.(2018). Glyphosate residues in Swiss market foods: monitoring and risk evaluation. *Food additives and contaminants,* 11 (2), 83-91.