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# A Review of the Analytical Methods Based on Chromatography for Analyzing Glyphosate in Foods

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## Abstract

Glyphosate is a pesticide widely used in agriculture, horticulture, and silviculture as well as around homes and gardens. It was introduced by Monsanto in the early 1970s, and it is a broad spectrum, nonselective, post-emergence herbicide that inhibits plants' shikimic acid pathway. Glyphosate is considered as "difficult herbicide" in terms of trace analysis. It has low molecular weight, low volatility, thermal lability, and good water solubility. These properties cause problems in its extraction, purification, and detection. The determination often requires additional processes that may allow quantification by chromatographic methods. Several analytical procedures have been developed based on solid-phase extraction, ion-exchange chromatography, or matrix solid phase dispersion. Most published methods involve liquid extraction followed by clean-up. This review would like to revise the literature on this issue discussing the relevant chromatographic methods reported in the literature in terms of analytical parameters for analyzing such compound in food chain.

**Keywords:** glyphosate, pesticide, herbicide, chromatography, GC, LC, MS, LOD/LOQ, food, recovery, human health

## 1. Introduction

Glyphosate (GLYP) (or, less commonly, but still used, glyphosphate), a broad-spectrum herbicide, is one of the most used pesticides in the world [1], nearly \$5 billion in sales and an annual global production about 825,800,000 kg [2]. Glyphosate is a nonselective herbicide; therefore, it is a molecule that eliminates all weeds without distinction.

Glyphosate [IUPAC N-(phosphonomethyl)glycine; CAS registry number 1071-83-6] is an aminophosphoric analogue of glycine and an important amino acid. It was discovered in the early 1950s by Henri Martin and was patented by Monsanto and sold as a Roundup® product for about 20 years; after 2001 (patent expiration date), free production of glyphosate was legally permitted [3, 4]. As of 2010, more than 750 glyphosate products have been on the market [5, 6]. The first important worldwide warning about the GLYP occurred in 2017: the Canadian Food Inspection Agency (CFIA) confirmed that 36.6% of the Canadian wheat samples had a high

presence of GLYP (3.9% above the legal limits, which in Canada is 5 ppm) [7]. In Canada, GLYP-based products are widely used for improving the wheat ripening and drying. Such occurrence has created a big supply problem in Europe where this practice is prohibited: for instance, Italy imported large amounts of wheat to make flour for pasta from Canada (and from the United States as well).

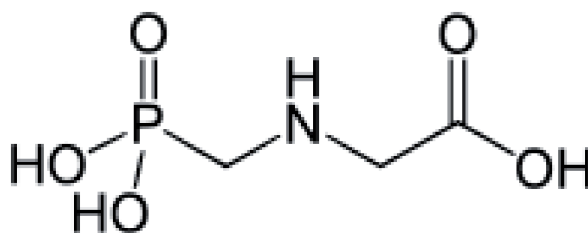
GLYP inhibits the 5-enolpyruvylshikimate-3-phosphate (EPSP) enzyme produced by plants, which is involved in the synthesis of three essential amino acids such as tyrosine, tryptophan, and phenylalanine. The mechanism of action is absorption through the foliage, and to a small extent through the roots, and transport to growth points. Since this enzyme is present only in the plant kingdom, glyphosate acts only on plant organisms.

GLYP is a leaf herbicide (it is absorbed by the leaves of the plant), systemic (once absorbed, it passes toward the growth points, causing the death of the plant), nonselective (in fact, it is active on all plants, if not genetically modified). Glyphosate-based products are activated by the addition of a surfactant, polyoxyethylene amine (POEA), which promotes penetration through the leaf surface of plants; other additives used are sulfuric acid and phosphoric acid. Its main metabolite is aminomethylphosphonic acid (AMPA). It should be noted that a fraction of AMPA could be due to degradation processes of the detergents/surfactants rather than from glyphosate. GLYP does not penetrate deeply into the soil (maximum 20 cm) and is easily degraded by bacteria. This means that the probability that it reaches the aquifers is very low and that its presence is certainly lower than that of other dangerous pollutants.

The half-life of GLYP in the soil is between 2 and 197 days, a typical half-life of 47 days has been suggested. The soil and climate conditions on the persistence of glyphosate in the soil are very important. The average half-life of GLYP in water varies from few to 91 days. The AMPA metabolite of glyphosate has been found in Swedish forest soils for up to 2 years after a glyphosate application. In this case, the persistence of AMPA has been attributed to frozen soil for most of the year. The adsorption of glyphosate into the soil, and then its release from the soil, varies according to the type of soil. GLYP is generally less persistent in water than in land, with 12–60 days persistence observed in Canadian ponds, although persistence of more than a year has been recorded in American lake sediments.

GLYP (**Figure 1**) is a weak acid commonly used in the form of salt, distributed as a powder or as a water-soluble concentrate. At room temperature, it appears as a colorless crystalline solid, is completely soluble in water, and is highly insoluble in common organic solvents such as benzene and dichloromethane. GLYP is a nonvolatile and photo-resistant molecule, and its dissolution in water generates four chemical equilibria represented by the respective acid dissociation constants ( $K_a$ ). In logarithmic form,  $pK_a$  acquires the following values: 2.0, 2.6, 5.6, and 10.6. This aspect makes the molecule highly polar and amphoteric [8].

During the reactions involving the enzymes glyphosate oxidase and glyphosate N-acetyl transferase, glyphosate can form different metabolites: the main is



**Figure 1.**  
Glyphosate [*N*-(phosphonomethyl)glycine; CAS number 1071-83-6].

considered the amino-methylphosphonic acid (AMPA), whereas the others are glyoxylate, N-acetyl glyphosate, N-acetyl-AMPA, methylphosphonic acid, sarcosine, N-methyl-aminomethylphosphonic acid (MAMPA), hydroxymethylphosphonic acid, and phosphonoformic acid [9]. This behavior is important: these compounds should be considered when toxicity and environmental studies are performed for the risk assessment. Similarly, compounds used as adjuvants in commercial glyphosate formulations should be considered: for instance, polyoxyethylene amine (POEA), used as a surfactant in Roundup [10] or isopropylamine, ammonium and trimesium salts, or formulation impurities such as N-(phosphonomethyl)iminodiacetic acid and bis(phosphonomethyl)amine. This occurrence is really important because the adjuvants can modify the toxicity of pesticides based on glyphosate as active ingredient; so, the result is the need of a novel toxicological evaluation [11].

All these considerations play an important role in the GLYP toxicity. The toxicity of a substance is assessed according to its median lethal dose (lethal dose, 50% – LD<sub>50</sub>), that is, the dose that causes the death of 50% of the individuals taking the test substance: Class 1, high acute toxicity, LD<sub>50</sub> less than 50 mg per kg of live weight; Class 2, moderate toxicity, LD<sub>50</sub> between 50 and 500; Class 3, mild toxicity, LD<sub>50</sub> between 500 and 5000; and Class 4, harmless, LD<sub>50</sub> of over 5000 mg. The GLYP is in Class 3, while in Class 2, we find, for example, caffeine, aspirin, and boiling chloride, and in Class 1, the vitamin D<sub>3</sub>. In **Table 1**, acute toxicity assessment is reported.

Also, important is the concept of daily limit dose (expressed in milligrams per kilogram of body weight considered) definable as the maximum amount of herbicide that can be consumed daily without causing damage. Based on this concept, the glyphosate content of a food or a drink should be correctly evaluated using the milligrams of glyphosate per kilogram of body weight that can be taken per day as a unit of measurement. In this way, the European Food Safety Authority (EFSA) has set a daily limit dose of 0.5 mg kg<sup>-1</sup> of weight per day [12].

A tumor associated with glyphosate would be the non-Hodgkin lymphoma (NHL). In 2013, the German Federal Institute for Risk Assessment (BfR) found that “the available data are contradictory and far from convincing” in terms of the relationship between exposure to glyphosate formulations and the risk of various cancers, including the NHL [13–18]. A meta-analysis published in 2014 identified an increased risk of NHL in workers exposed to glyphosate formulations [19, 20].

	High toxicity	Moderate toxicity	Low toxicity	Very low toxicity
Acute oral <sup>a</sup>	≤50 mg kg <sup>-1</sup>	>50–500 mg kg <sup>-1</sup>	>500–5000 mg kg <sup>-1</sup>	>5000 mg kg <sup>-1</sup>
Inhalation <sup>b</sup>	≤0.05 mg L <sup>-1</sup>	>0.05–0.5 mg L <sup>-1</sup>	>0.5–2.0 mg L <sup>-1</sup>	>2.0 mg L <sup>-1</sup>
Dermal <sup>a</sup>	≤200 mg kg <sup>-1</sup>	>200–2000 mg kg <sup>-1</sup>	>2000–5000 mg kg <sup>-1</sup>	>5000 mg kg <sup>-1</sup>
Primary eye irritation	Corrosive or corneal involvement	Corneal involvement (8–21 days)	Corneal involvement (7 days)	Minimal effects clearing in 24 hours
Primary skin irritation	Corrosive	Severe irritation at 72 hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours

<sup>a</sup>As LD<sub>50</sub>.

<sup>b</sup>As lethal concentration, 50% (LC<sub>50</sub>).

**Table 1.**  
*Relationship between GLYP levels and toxicity.*



In March 2015, the International Agency for Research on Cancer (IARC) classified glyphosate “probably carcinogenic to humans” (Group 2a) based on epidemiological studies, animal studies, and in vitro studies: in particular, GLYP has been defined genotoxic through at least two mechanisms known to be associated with human carcinogens [21–23]. In contrast, EFSA concluded in November 2015 that “the substance is unlikely to be genotoxic (i.e., harmful to DNA), or pose a threat to humans.” Subsequently, EFSA itself states that while there may be formulations containing glyphosate that are carcinogenic, studies relating only to glyphosate as an active ingredient do not show this effect [24, 25]. The European Chemicals Agency (ECHA), on the basis of “the scientific evidence available at the moment,” classified GLYP, according to the CLP Regulation, as a chemical causing eye damage (H318) and being toxic to aquatic life with long-lasting effects (H411), but “the available scientific evidence did not meet the criteria in the CLP Regulation to classify glyphosate for specific target organ toxicity, or as a carcinogen, as a mutagen or for reproductive toxicity” [26]. The United States Environmental Protection Agency (US EPA) has classified glyphosate as a Group E chemical, meaning the agency has determined that there is “evidence of noncarcinogenicity to humans” [27, 28]. In any case, US EPA has established tolerances for GLYP residues in different commodities [29]. The difference of point of views depends on the fact that IARC and US EPA have analyzed different studies and applied different statistics. Further, EFSA analyses concern only the glyphosate molecule, whereas the studies considered by IARC also concern glyphosate-based products placed on the market [30].

This brief analysis shows that, in any case, international pesticide regulatory agencies and scientific organizations agree that there is no evidence that GLYP as an active substance is carcinogenic to humans, only IARC has classified glyphosate as “probably carcinogenic.”

Finally, it should be considered an interesting hypothesis by Samsel and Seneff [31]: they propose a relationship between celiac disease and imbalances in gut bacteria generated by the known GLYP effects on them.

The EFSA has renewed the authorization for GLYP, establishing the acute reference dose (ARfD) at  $0.5 \text{ mg kg}^{-1}$  of body weight, while the acceptable operator exposure level (AOEL) was set at  $0.1 \text{ mg kg}^{-1}$  body weight per day and the acceptable daily intakes (ADIs) for consumers are in line with the ARfD threshold,  $0.5 \text{ mg kg}^{-1}$  body weight per day.

There are several exposure sources of humans to GLYP in the environment, for example, air, water, application to crops and target weeds, and food [32–34]. Solomon deeply reviewed the exposure data from the literature (PubMed and Google Scholar) and unpublished reports in different papers [35, 36]: in both papers, he reaches a similar conclusion: “In all cases, measured and estimated systemic exposures to glyphosate in humans and animals were less than the ADIs and the RfD. Based on this large dataset, these exposures represent a *de minimis* risk.” The conclusion reached by Gillezeau et al. [33] is instead intermediate by reviewing the same literature (PubMed and Google Scholar): they state that “additional studies are urgently needed to evaluate levels of glyphosate and related metabolites in the general population and in workers.” Further, they observe the great differences in the analyzed papers: they detected some bias such as the few studies on potential occupational GLYP exposure, or no study designed to address the hypothesis of seasonality in exposure, or the use of a few populations of farmers and relative collection of one-time spot urine. They rise serious doubts about the data generalizability, which they consider rather limited.

This paper would like to critically revise the literature on chromatographic methods developed for analyzing GLYP and AMPA in food matrices, specifically grains (e.g., rice, wheat, soybean, and maize), honey, olive and oil, vegetables,

fruit, beverages (e.g., drinking water, milk, tea, and coffee), cheese, and meat/fish products. In literature (source: Scopus database), there are 2666 papers using keywords “glyphosate” and “analysis” by the end of April 2020 and 361 using “chromatography” as third keyword.

## 2. Glyphosate determination in different food matrices

Starting from the Canadian study performed in 2017, the scientific attention on GLYP has become stronger, and several papers are annually published dealing the determination of such compound, along with its main metabolite AMPA, on different agricultural and food matrices. For avoiding dispersive information due to the big amount of studies aimed to this determination, the authors have focused their attention on the main innovative analytical methods based on chromatographic methods for determining both compounds in such matrices. It is also necessary to advise the reader that different matrices could be determined with same analytical protocols, at least showing different analytical parameters (multiresidue analyses), as well as in literature are present papers dealing important toxicological studies with no analytical information.

### 2.1 Approaching the determination

Before approaching the discussion on the different analytical methodologies developed for analyzing GLYP and AMPA in agricultural and food matrices, it should be necessary to resume some toxicological information on it along with some chemical characteristics to be taken into account for evaluating the analytical process.

First, a maximum residue level (MRL) is defined as the highest level of a pesticide residue legally tolerated in or on food or feed when pesticides are applied correctly [37]. For each product, an MRL of GLYP has been determined [38]. An example of this database is reported in **Table 2**.

A preliminary important information comes from the EU Reference Laboratories for Residues of Pesticides (EURL-SRM): for all the analytical steps, it is highly recommended the use of plastic vials because there is an interaction between the pesticide and the glass surface, especially when aprotic solvents are used. These interactions greatly affect the precision and accuracy, especially at low GLYP concentration. This statement is important because it influences its stability and degradation as well. Among the different solvents, water with 10% acetonitrile is considered a good storage solvent, whereas the compound is not stable in water and methanol. At room temperature, the degradation is very low within 14 days, whereas if extract is stored in the refrigerator, it is stable over 7 months [39].

Finally, the authors would like to remember some definitions regarding the parameter of an analytical method. Recovery is the term used in analytical and preparative chemistry to denote the fraction of the total quantity of a substance recoverable following a chemical procedure [40]. Accuracy is the difference between the mean of some measurements and the value considered as the true or correct value for the quantity measured, whereas precision is the measurement reproducibility, that is, the dispersion around a central value. In regard to the chromatographic separation, a signal-to-noise (S/N) ratio of 3 is acceptable for determining the limit of detection (LOD), that is, the lowest amount of analyte in a sample, which can be detected, whereas a ratio of 10 for the limit of quantification (LOQ), that is, the lowest amount of analyte in a sample, which can be quantitatively determined with precision and accuracy [41–44]. The S/N definition for chromatography is the ratio

Product	MRL
Tangerines, clementines, oranges, and grapes	0.5
Lemons, grapefruits, cedars, kumquats, apples, pears, peaches, apricots, cherries, plums, almonds, hazelnuts, strawberries, and table olives	0.1
Oil olives	1
Potatoes	0.5
Wild mushrooms	50
Other vegetables	0.1
Baked beans	2
Grain peas, lupines, and lentils	10
Other leguminous vegetables	0.1
Flax seeds, rapeseed, mustard, and cotton	10
Sunflower and soybeans	20
Other oil seeds	0.1
Wheat and rye	10
Barley, oats, and sorghum	20
Corn	1
Other cereals	0.1
Sugar beets (roots)	15
Forage from meadows and pastures, and alfalfa	0.1

**Table 2.**  
*Maximum residue levels for some food products.*

of the peak height relative to the middle of the noise range (S) to the difference between the maximum and minimum baseline signal values for the noise (N) [45].

2.2 Cereal grains

Grain is the largest and well-studied matrix in this field. Many papers deal the glyphosate determination in cereals and legumes due to the worldwide use of such herbicide in the relative cereal crops. We must remember that, as said at the beginning, the first warning came precisely by analyzing several Canadian wheat samples and finding almost 37% of them with high presence of the pesticide. So, after this occurrence, scientific and health attention has been very high and focused on cereals in general, for example, maize corn, millet, barley, oats, rice, wheat wild rice, amaranth, and quinoa.

The literature analysis for the GLYP determination in such matrix is very large; for this reason, the authors focused their attention on the main publications starting from the last deep review, that is, by Tadeo et al. [46]. The same method will be applied to the revision of the analytical methods for GLYP determination in vegetables and fruit matrices.

A routine control method based on extraction with water by ultrasonication was developed by Granby et al. [47] for analyzing several Danish mill products. It was one of the first studies based on green chemistry, that is, the authors used no organic solvents or chemicals except diluted solutions of NaHCO<sub>3</sub> (as eluent) and, in some cases, H<sub>2</sub>SO<sub>4</sub>. The samples (rye or wheat in grain and flour) were subjected to online clean-up and separation by in-series system of ion chromatography

(IC) and high performance liquid chromatography (HPLC) with detection by electrospray ionization mass spectrometry in the negative-ion mode. The method, investigated in the range of 0.03–0.33 mg kg<sup>-1</sup>, shows a GLYP recovery of 85%, a repeatability between 1 and 14%, a reproducibility from 4 to 16%, and a LOD of 0.02 mg kg<sup>-1</sup> (LOQ was not reported).

A very interesting paper was published by Tseng et al.: they used the gas chromatography coupled with a pulsed flame photometric detector (PFPD) for a simultaneous determination of GLYP and glufosinate (DL-homoalanin-4-yl-(methyl)phosphinic acid, GLUF) along with their main metabolites including AMPA [48] after a single-step derivatization with trimethyl orthoacetate (TMOA). In particular, the authors studied the influence of the heating temperature (70–90°C) and time (90–120 min) on the AMPA and 3-(methylphosphinico) propionic acid (3-MPPA, a GLUF metabolite) derivatization. They optimized the method on soybean sprouts and rice samples and determined the different analytical parameters (recoveries 72–81, 71–86, 101–119, and 83–90%; LOD of 0.02, 0.03, 0.02, and 0.01 µg g<sup>-1</sup>; and LOQ of 0.06, 0.10, 0.06, and 0.04 µg g<sup>-1</sup> for glyphosate, AMPA, GLUF, and 3-MPPA, respectively; RSD < 10%). On the other hand, Li et al. used fluorenylmethyl chloroformate (FMOC-Cl) as derivatizing agent followed by HPLC-MS/MS for analyzing GLYP and AMPA residues in different matrices such as rice, wheat, vegetables, fruits and tea, pig and chicken muscles, aquatic products, chestnut, and honey [49]. Further, they also used an isotope-labeled 1,2-<sup>13</sup>C<sup>15</sup>N GLYP for increasing the accuracy and the precision of the measurements at low GLYP concentration. In this way, they obtained recoveries between 80.0 and 104% and RSDs from 6.7 and 18.2% with a LOQ of 0.05 mg kg<sup>-1</sup> for both compounds and a correlation of 0.998 in the linear range of 0.20–10 µg L<sup>-1</sup>.

In 2007, Granby's group published a paper on the (six) laboratory intercomparison for determining GLYP, chlormequat, and mepiquat (these two are plant growth regulators, also used for the growth reduction of the lowest straw part) residues in cereals [50]. GLYP was analyzed by treating the samples twice with MilliQ water by ultrasonication followed by centrifugation, filtration, clean-up on polystyrene-based reverse phase column, and separation by IC-HPLC-MS/MS, whereas the other two compounds were extracted by Ultra-Turrax and cleaned-up by SPE-C<sub>18</sub>. The results showed very different LOQs and recoveries reached by the six laboratories (0.01 and 0.3 mg kg<sup>-1</sup> and 29 and 109% for GLY) with a good within-laboratory precision and a poor between-laboratory precision [51]. For glyphosate, the authors stated the presence of a systematic component between laboratories to be the reason of such large data variability.

Simple sample preparation and fast chromatographic analysis are the main features of the paper by Martins-Júnior et al. [52]. They analyzed GLYP and AMPA in soybean samples by means of liquid-liquid partition with dichloromethane and protein precipitation followed by HPLC-MS/MS determination (in positive and negative electrospray ionization, ESI, mode). This paper highlights the choice of the liquid-liquid partition and protein precipitation. Particularly, the paper evidences the importance of the second step, that is, the protein precipitation for eliminating the matrix interference: different solvents, that is, acetone, acetonitrile, and methanol, were tested, and methanol was found the best for reducing it (but it does not eliminate it). The authors took advantage of the great performance of the tandem mass spectrometry (MS/MS) and reached LODs of 0.09 and 0.1 mg kg<sup>-1</sup> and LOQs of 0.30 and 0.34 mg kg<sup>-1</sup> for GLYP and AMPA, respectively, with recoveries between 79.6 and 109.1% and RSD below 12.2%. Further, the authors suggested to apply this analytical protocol to other crop matrices, where GLYP is largely used, for instance, corn and cotton.



As just noted above and especially using the LC-MS/MS as GLYP detection, the matrix effect is not negligible. In literature, different possibilities have been studied for reducing this artifact: for instance, sample dilution [53], injection of smaller volumes [54], the optimization of sample preparation and/or chromatographic parameters [55], or the use of expensive internal standard (IS). Ding et al. [56] developed a combination of C18 and SAX cartridge for reducing the matrix effect. After to have optimized the analytical conditions, the authors used hydrophilic interaction chromatography (HILIC)/WAX mixed-mode stationary phases for glyphosate retention and LC-MS/MS in negative ion mode for the detection. They used this methodology for analyzing soybean, corn, spicy cabbage, apple, and carrot samples. GLYP is investigated in a linear range between 0.02 and 10 mg kg<sup>-1</sup>, with a  $R^2 > 0.999$ , and the intra- and inter-day errors are 2.7 and 1.8%, respectively, whereas the precision as RSD is below 7%. Using the developed analytical procedure, the authors reached good LOD and LOQ, 0.02 and 0.005 mg kg<sup>-1</sup>, respectively, and recoveries ranging between 83.1 and 100.8% according to the different matrices analyzed, specifically 89–96% for soybean, 84–101% for corn, 86–94% for carrot, 85–93% for spicy cabbage, and 83–100% for apple. Quite interesting in this paper are both the possibilities to quantify such herbicides in different plant-derived or processed foods (this is not so common in the literature) and to use solution calibration curves instead of matrix-match calibration curve for the analysis.

Botero-Coy et al. explored for first the possibility to analyze GLYP in rice, maize, and soybean without derivatization step but just direct LC-MS/MS with a triple quadrupole instrument after water extraction and SPE using Oasis HLB cartridge [57]. The method has allowed to reach high correlation coefficients ( $<0.99$ ) in the range of 1–250 µg L<sup>-1</sup>, recoveries between 77 and 100% with RSDs below 17%, and good LODs and LOQs (0.007–0.12 mg kg<sup>-1</sup> and 0.1 and 2 mg kg<sup>-1</sup>, respectively) for all matrices.

A Chinese-French scientific paper in 2018 dealt the determination of GLYP and GLUF in 136 food samples, of which 34% of samples with high (banana, apple, orange, potato, carrot, and juice) and low (biscuits or bread) water contents and 66% of animal origin samples (milk-based foods included, e.g., milk, cheese, and butter) [58]. After a solvent extraction (acidified water, methanol, and dichloromethane), the authors performed a derivatization by means of FMOC and a solid phase extraction (SPE) C<sub>18</sub> for purifying and concentrating the extract and a HPLC-MS/MS analysis for determining the two compounds. Using these conditions, recoveries between 82 and 112%, LODs and LOQs of 1.7 and 5 µg kg<sup>-1</sup>, respectively, and RSDs below 20% for both compounds were achieved.

An ion chromatography-tandem mass spectrometry-based method was developed by Adams et al. for analyzing 14 polar pesticides including GLYP in cereal and grape samples [59]. The extraction is based on quick polar pesticide (QuPPE). Although the method is interesting, not all the analytical parameters are reported except the recoveries for cereals (specifically, oat flour) ranging between 85 and 104%.

A simple method based on acidified methanol solution extraction followed by centrifugation and filtration and LC-MS/MS analysis was developed by Santilio et al. for analyzing GLYP in rice and maize [60]. The authors highlighted the importance of using GLYP isotope labeled in the matrix effect reduction. LODs of 2 µg kg<sup>-1</sup> for rice and 4 µg kg<sup>-1</sup> for maize and a LOQ of 10 µg kg<sup>-1</sup> for both matrices were reached in a linearity range of 0.01–1.5 mg kg<sup>-1</sup> ( $R^2$  0.9982) with recoveries ranging between 74 and 98% and RSD  $< 20\%$ . Finally, it should be reported that the authors' principal aim was to develop a method to be routinely used for analyzing rice and maize, taking into account the relative MRLs established, and to extend it to other matrices.

Finally, Herrera López et al. set up a multiresidue analysis for determining 14 highly polar pesticides (parents and metabolites) in 352 samples including oat and soya beans, lettuce, grapes, and oranges [61]. After a solvent extraction step, a LC system coupled with a hybrid quadrupole/linear ion trap mass spectrometer system (with ESI source) (LC-ESI-QTRAP-MS) was used for reaching high analytical performances: linearity range between 0.01 and 10 mg kg<sup>-1</sup> with  $r^2 > 0.99$ , recoveries between 70 and 120% with RSD < 22% (specifically, GLYP between 83 and 118% with RSD < 22%), LOQs between 20 and 500 µg kg<sup>-1</sup> (particularly, for GLYP 500 and 20 µg kg<sup>-1</sup>) for all the investigated matrices. The clean-up procedure was not involved because no appropriate sorbent was found to increase the protocol, and the derivatization step was not necessary, whereas these authors also stated that the use of an isotopically labeled internal standard helps in the matrix effect correction.

### 2.3 Meat, fish, and cheese

The scientific attention on GLYP contamination in this food class is on the rise recently. Only few papers are available on such matrices. In fact, if the GLYP behavior in the aquatic environment is studied since many years [62–64], poor information is presented on its presence in foods.

Starting from the paper by Botero-Coy et al. [57], Chiesa et al. developed a method based on IC coupled to high resolution mass spectrometry (IC-HRMS) for determining GLYP, GLUF, and AMPA in different foods of animal origin without a derivatization step [65]. The authors focused their attention on the matrix, particularly on the lipid composition, which is the major interfering group because co-extracted with the analytes. The main contribution of this study was to identify the best extraction solvent: among different assays, the best solution is 30% of methanol and 70% of acidified water (1% formic acid). Thirty samples among fish (bass), bovine muscle, and organic honey were analyzed. The detector, an orbitrap quipped with heated electrospray ionization (HESI) source, allowed to reach very low LOQs (4.26–5.38 ng g<sup>-1</sup>, 6.25–6.47 ng g<sup>-1</sup>, and 4.30–9.26 ng g<sup>-1</sup> for fish, bovine, and honey, respectively), good recoveries (96.9, 76.1, and 97.0%, respectively), RSDs <13.1%, and good correlation coefficients ( $R^2 > 0.992$ ).

Actually, in literature, there are other few papers showing the determination of GLYP and AMPA in muscle meat (bovine, cow, pig, and chicken), but the LODs are higher (50 ng g<sup>-1</sup>) [49, 66, 67], whereas the only paper on fish does not report any information on LOQ [68].

A communication dealing with the determination of GLYP and GLUF in animal feeds shows linearity more than 0.999, instrumental detection limits (IDLs) of 8.3 µg kg<sup>-1</sup> and 1.1 µg kg<sup>-1</sup>, respectively, accuracy between 102 and 112%, and precision below 6% in both matrices [69].

Finally, about the GLYP determination in cheese or, basically, in milk-based foods, the authors just discussed above the only paper present in the literature [58]. Please note that the milk as beverage will be discussed in other section.

### 2.4 Vegetables

Some papers dealing with the GLYP determination in such food matrices are just discussed previously [46, 49, 56, 58, 61]: here the attention is focused on papers showing novelty or improvements in the analytical methodology or large studies on the herbicide content. The first interesting paper is dated in 1992: Tanaka and coauthors developed a very easy method employing routinely available instrumentation, that is, HPLC with a fluorescence detection [70]. The analytical parameters are quite weak (recoveries >68% and >88% for GLYP and AMPA, respectively,

and LOD 0.05 ppm for both), but it is to be appreciated the use of common equipment. In 1996, two papers investigated the GLYP presence in green lentils, fresh beans [71], and carrot [72]. The first paper introduced a post-column reaction, a denitroization, for obtaining a N-nitroso-GP (NGP) derivate to be analyzed by HPLC coupled with thermal energy analyzer (TEA), that is, a chemiluminescence detector. Over vegetables, the authors also analyzed beverages (water and beer) and cereals (rice flour, corn, barley, and rye). They obtained recoveries between 83 and 97% for vegetables, 70–100% for beverages, and 67–100% for cereals with LODs ranging between 0.005 and 1  $\mu\text{g g}^{-1}$ . On the other hand, the second paper presents a GC analysis coupled with flame photometric detection (FPD) for analyzing GLY, AMPA, and GLU. The use of instrumentation commonly present in each laboratory is to be appreciated also in this case. The three compounds were derivatized with N-isopropoxycarbonyl (isoPOC) for obtaining the relative isoPOC methyl ester derivatives: 0.5–1  $\mu\text{L}$  of this solution was injected in the GC-FPD. The authors determined the LODs (12, 8, and 20 pg injected for GLY, AMPA, and GLU, respectively), the recoveries (91–104, 94–104, and 91–100%, respectively), and the correlation coefficients ( $R^2$  0.9992, 0.9982, and 0.9992, respectively) in a linearity range of 5–200 ng.

Hooijschuur and coauthors explored the possibility to use the microcolumn liquid chromatography with FPD detection ( $\mu\text{LC-FPD}$ ) and compared these results with those obtained by capillary electrophoresis (CE) with FPD (CE-FPD) [73]. They used a silica column (25 cm  $\times$  320  $\mu\text{m}$  ID, 450  $\mu\text{m}$  OD) with 5  $\mu\text{m}$  LiChrosorb RP-1 bonded silica. Although CE-FPD was faster than  $\mu\text{LC-FPD}$ , this is more sensitive for the GLYP and AMPA analysis: LODs are 15 and 7.5  $\text{ng mL}^{-1}$ , respectively, versus LOD of 1.0  $\mu\text{g mL}^{-1}$  for both compounds by CE-FPD. Grey et al. applied the LC-ESI/MS analysis after the derivatization with FMOC-Cl of GLYP and AMPA [74]. They evaluated the use of isotope-labeled compounds: their conclusions were positive in the GLYP determination (LODs 0.11  $\mu\text{g g}^{-1}$  and 0.06  $\mu\text{g L}^{-1}$  for lettuce and water samples, respectively), whereas they did not find any contribution for the accurate AMPA analysis (LODs 0.53  $\mu\text{g g}^{-1}$  and 0.3  $\mu\text{g L}^{-1}$ , respectively). Finally, the recoveries increased from 23.2 to 98.4% for GLYP and from 33.8 to 99.4% using the isotope dilution mass spectrometry (IDMS)-based glyphosate analytical method. Finally, Takahashi et al. determined GLYP and GLUF in cabbage, Chinese cabbage, carrot, onion, strawberry, lemon, kiwi fruit, over soybean, corn, and brown rice after derivation with FMOC-Cl and analysis by HPLC with fluorescence detection [75]. Another interesting paper came from Japan in 2004: Watanabe set up a rapid method for determining GLYP, GLUF, and 3-MPPA in vegetables (cucumber and spinach) and fruits (apple, mandarin, and orange) using an anion exchange resin and elution with acetic acid, followed by derivatization with trimethyl orthoacetate and clean-up on SPE Florisil cartridge and GC-FPD analysis [76]. The method allows to reach LODs of 0.01, 0.01, and 0.005  $\mu\text{g g}^{-1}$  and recoveries of 83.5–89.8, 77.9–92.2, and 75.0–87.2% for GLYP, GLUF, and 3-MPPA, respectively.

A Chinese group proposed an original method for determining GLYP in apple samples [77]: after clean-up with SPE-C18, a derivatization step was performed using 4-chloro-3,5-dinitrobenzotrifluoride (CNBF). The quantification occurred by reverse ion-pair liquid chromatography using cetyltrimethylammonium bromide (CTAB) as ion-pair reagent. The strengths of the method are the formation of a stable derivative (5% degradation after 7 storage days at room temperature) and the easy pretreatment procedure. LOD of 0.01  $\mu\text{g g}^{-1}$ , recoveries from 86.0 to 99.5%, and RSDs from 1.43 to 6.32 were achieved applying this method to apple samples.

Rembisz and coauthors started from a different idea: GLYP (as well GLU) is an aminophosphonic acid, analogous of the amino acid. So, they proposed a derivatization with phenyl isothiocyanate (PITC) for obtaining



phenylthiocarbamyl derivatives (PTC derivatives): a thin-layer chromatography (TLC) with iodine-azide detection allowed to detect such compounds in parsley and lettuce samples [78]. The method was sensitive, accurate, and inexpensive showing recoveries between 95 and 104%, LODs 0.99–4 µg per spot, LOQs 1.78–8.45 µg per spot, and RSDs <7.7 for both compounds.

A fast routine analysis was developed by Boušová et al. for routinely determining the polar pesticides, including GLYP, AMPA, GLUF, and 3-MPPA, in lettuce, orange, and flour samples [79]. The coupling of ion chromatography to a triple quadrupole mass spectrometer allowed the authors to reach very good LODs and LOQs (1–10 µg kg<sup>-1</sup> and 10–20 µg kg<sup>-1</sup>), recoveries ranging between 71 and 116% according to the matrix, and RSD < 18%. Rajski et al. implemented this procedure using an orbitrap detector and validating the method for aubergine, zucchini, cabbage, orange, and watermelon samples [80], achieving good recoveries (70–120%) and LOQ (0.01 mg kg<sup>-1</sup>) for GLY, AMPA, and GLU. Melton et al. still used the ion chromatography but coupled with the tandem mass spectrometry (IC-MS/MS) for determining highly polar pesticides (including GLY, AMPA, and GLU) in 288 samples of melon, peas without pods, and pineapple [81]. Finally, a paper by Savini et al. worth to be mentioned: the authors used the UHPLC coupled with a orbitrap detector for analyzing GLPY, AMPA, GLUF, and other polar pesticides in 98 samples (83 processed fruits and vegetables and 15 infant foods) [82]. Using the developed method, the authors obtained LOQ of 0.003 mg kg<sup>-1</sup> for all three compounds, recoveries 75–113% in all matrices, RSDs below 18.5%, and a *R*<sup>2</sup> between 0.9954 and 0.9998 in the linear range of 0.001–0.1 mg L<sup>-1</sup>. Another important advantage of this method is the simultaneous determination of six polar pesticides (i.e., AMPA, glyphosate, phosphonic acid, chlorate, fosetyl-Al, and perchlorate) in 25 min.

## 2.5 Olives and olive oil

Two papers dealt with the determination of GLYP in olives and olive oil [83, 84]. Both papers deal the difficulty of analyzing such matrices, and there is strong matrix effect. In the first paper, two different methods were developed, that is, UHPLC-TOFMS and UHPLC-MS/MS using HILIC separation: in this way, the authors reached LOQ of 0.3 µg kg<sup>-1</sup> and 0.1 µg kg<sup>-1</sup>, respectively, and recoveries between 57.2 and 117.6% with a linearity >0.99 and an RSD < 3.9%. The two different LOQs were calculated using time-of-flight mass spectrometry (TOFMS) and triple quadrupole instruments: as expected, the MS/MS shows lower quantifiable levels. The second paper presented a green fast-analytical method based on vortexing (1 min with acidified water) and centrifugation (10 min at 3700 rpm) and extract injection in UHPLC-MS/MS for determining GLYP, AMPA, and GLUF in different olive oils, that is, extra virgin olive oil, virgin olive oil, olive pomace oil, and soy oil. Particularly, the paper reported the determination with no internal standards nor matrix-matched calibration. The authors tested the linearity in the concentration range of 5–250 µg L<sup>-1</sup>: they fixed LOQs at 5 µg kg<sup>-1</sup> for AMPA and at 10 µg kg<sup>-1</sup> for GLYP and GLUF and determined recoveries between 81.4 and 119.4% with intra and inter-day precision lower than 19%.

## 2.6 Honey

During the past few years, the important question has emerged about GLYP contamination in natural honey samples. Different papers have been published dealing this issue. Some of them have already been discussed previously [49, 65].

A first interesting paper dealing with such of matrix was this of Karise and coauthors [85]. They set up a multiresidue method for analyzing GLYP along with other



47 pesticides in 33 honey samples collected from beehives of Estonia. The paper was focused on the detection of the pesticide concentration and the relative maximum residue levels and the possible impact of the agriculture on the product. In any case, the authors largely used the analytical methodology based on using QuEChERS (acronym of Quick, Easy, Cheap, Effective, Rugged and Safe) extraction methodology followed by detection using GC-MS and ultra-high-performance liquid chromatography-MS/MS (UHPLC-MS/MS): the method shows recovery between 78 and 115%, repeatability from 3.0 to 16%, LOQ for GLYP of  $0.050 \text{ mg kg}^{-1}$  (and  $0.010 \text{ mg kg}^{-1}$  for the other pesticides), and correlation coefficients  $>0.990$  for all compounds.

In 2018, Zoller et al. found GLYP at very low levels in 15 of 16 honey samples analyzed; in addition, they also analyzed pulses (tofu and soy sauce), breakfast cereals (corn flakes and pops), durum wheat, pastry and snacks (crisps, etc.), bread, flour and baking mixtures, and beverages (beer, wine, milk, fruit juices, and mineral water) for a total of 243 samples [86]. The authors applied a well-tested analytical method based on solvent extraction with methanol and LC-MS/MS analysis for determining GLYP and AMPA (LODs  $0.2\text{--}0.4$  and  $0.5\text{--}1 \text{ } \mu\text{g kg}^{-1}$ , respectively; LOQs  $0.5\text{--}1$  and  $1\text{--}2.5 \text{ } \mu\text{g kg}^{-1}$ ; recoveries  $92\text{--}103$  and  $92\text{--}115\%$ ; RSDs  $<9.5$  and  $<13.9\%$ ). Further, in this paper, the authors assessed a dietary risk of each food for a child of 15 kg body weight and for an adult of 60 kg body weight. The first findings of this work were that the GLYP maximum residue levels did not exceed more than the legally tolerated ones ( $0.1 \text{ mg kg}^{-1}$  for plant products and  $0.05 \text{ mg kg}^{-1}$  for animal products). So, the scores reported by authors for the risk assessment highlighted a low exposure only for the pulses (5% of the acceptable daily intake, ADI, and acute reference dose, ARfD), whereas in all the other cases, honey samples included, the exposure to GLYP is less than 1% of the ADI/ARfD, meaning there is no any human health issue in all samples. Further, the authors, simulating a daily ingestion of the different investigated foods, estimated the probable GLYP content in urine. They found levels in agreement with those found by other authors in German [3, 17] and Swiss [87] populations, whereas some differences could be expected in AMPA concentration comparison [17].

A pilot study for monitoring GLYP and AMPA in 32 honey samples was set up by Pareja et al. based on IC coupled to a Q-Orbitrap accurate high-resolution mass spectrometry [88]. It is still confirmed that the use of IC simplifies the polar pesticide determination, whereas the use of an orbitrap detector allows to reach a GLYP LOQ of  $5 \text{ } \mu\text{g kg}^{-1}$  ( $20 \text{ } \mu\text{g kg}^{-1}$  for AMPA), less than the allowed EU MRL ( $50 \text{ } \mu\text{g kg}^{-1}$ ) and recoveries ranging between 80 and 110% with RSDs  $<20\%$  in the linearity range of  $5\text{--}500 \text{ } \mu\text{g kg}^{-1}$ .

Still in 2019, a Canadian group developed an easy method for analyzing GLYP, AMPA, and GLUF at low  $\mu\text{g kg}^{-1}$  levels based on both the derivatization with FMOC-Cl in acetonitrile solution and online SPE(C18)-LC-MS/MS analysis [89] and the use of isotopically labeled internal standards (as just evidenced previously). In particular, for all the investigated compounds, the authors obtained accuracies ranging between 95.2 and 105.3% (intraday precision 1.6–7.2%) and LOQ  $1 \text{ } \mu\text{g kg}^{-1}$ . By this method, 200 honey samples were analyzed: GLYP was found in 196 samples at maximum level of  $49.8 \text{ } \mu\text{g kg}^{-1}$  with a 95th percentile of  $14.2 \text{ } \mu\text{g kg}^{-1}$ , evidencing no risks for the consumers. Further, the authors performed a survey between their data with others from worldwide studies (the United States, Estonia, Switzerland, some just cited in this review) [85, 86, 90–92].

A 2020 paper evaluated the exposure risk of bees and humans to GLYP and AMPA residues in three different bee matrices, that is, beebread, wax, and paired samples of wax/honey collected from 379 Belgian apiaries using an analytical method based on clean-up on SPE-C18 followed by derivatization step with FMOC-Cl and

HPLC-ESI-MS/MS analysis [93]. LOD and LOQ of  $1 \text{ ng g}^{-1}$  and  $10 \text{ ng g}^{-1}$ , respectively, were achieved for both compounds in all matrices with recoveries ranging between 72.2 and 112.9% and RSDs from 0.1 to 4.5%. The authors stated that the GLYP levels were below the EU regulation in all samples. In any case, they suggest particular attention because recent studies deal the effects of GLYP [94] and AMPA [95] below the allowed concentrations.

## 2.7 Beverages

This last matrix is really important considering the large use of beverages in the daily dietary intake. Beverages such as water, beer, milk, and fruit juices are under strict attention by the different national authorities. For instance, in 2019, a study by Cook of the CalPIRG Education Fund (available at [https://uspirg.org/sites/pirg/files/reports/WEB\\_CAP\\_Glyphosate-pesticide-beer-and-wine\\_REPORT\\_022619.pdf?\\_ga=2.33097086.1581849178.1551185850-857148262.1551185850](https://uspirg.org/sites/pirg/files/reports/WEB_CAP_Glyphosate-pesticide-beer-and-wine_REPORT_022619.pdf?_ga=2.33097086.1581849178.1551185850-857148262.1551185850)) reported that 19 of wine (5) and beer (14) brands contained GLYP at levels ranging between 4.8 and 51.4 ppb. Several papers have been published in recent years, some of which have already been mentioned in this review [49, 58, 71, 73, 86].

The first interesting paper by Hao et al. describes a method for analyzing GLYP, AMPA, and GLUF in drinking water, surface water, and groundwater samples [96], that is, a LC-MS/MS method with reversed-phase and weak anion-exchange mixed-mode Acclaim® WAX-1 column. Good analytical parameters were obtained: LODs of 1.5, 3.9, and  $1.7 \mu\text{g L}^{-1}$  for GLYP, AMPA, and GLUF, respectively; LOQs of 4.5, 11.6, and  $5.3 \mu\text{g L}^{-1}$ ; and recoveries between 62 and 102%. The main aspect is the analysis by direct injection of aqueous samples without derivatization or clean-up procedures with the risk of artifacts.

In 2015, a Chinese group developed a procedure for analyzing GLYP and GLUF in tea samples by means of FMOC-Cl derivatization and UPLC-MS/MS analysis [97]. The method shows good linearity ( $r > 0.990$ ) in the range of  $0.003\text{--}0.1 \text{ mg L}^{-1}$ , LODs of  $0.03 \text{ mg kg}^{-1}$  for both compounds, and recoveries between 81.4 and 99.1% with RSDs  $< 2.3\%$ .

Two papers published in 2015 reported the GLYP, AMPA, and GLUF determination in milk and milk-based products. Ehling and Reddy carried out a derivatization with FMOC-Cl followed by means of LC-MS/MS in different nutritional milk matrices such as cow's milk, human breast milk, soy milk, and whole milk powder [98]. This study is important because the reported analytical method does not require any analytical treatment such as clean-up, evaporation, or concentration; so, the possible artifact formation is drastically reduced. Further, the importance of the use of a triple-quadrupole mass spectrometry is still confirmed in terms of selectivity and fragment analysis. This occurrence gives good analytical parameters:  $R^2 > 0.99$  in the entire investigated linearity range ( $5\text{--}500 \text{ ng mL}^{-1}$ ); recoveries between 91.1 and 115.2%; LODs of 0.012 and  $0.01 \mu\text{g g}^{-1}$  for GLY and AMPA, respectively; LOQ of  $0.05 \mu\text{g g}^{-1}$  for both; high intra-day ( $< 4.0$  and  $< 7.7\%$  for GLYP and GLUF, respectively) and inter-day ( $< 8.4$  and  $< 3.8$ , respectively) precision. The second paper investigates the direct injection of milk extract after deproteinization and SPE on Oasis cartridge [99]: the LC-MS/MS analysis under the negative ion-spray ionization mode allowed to reach low method detection limits (MDLs), that is, 0.3, 1.4, and  $0.4 \text{ ng mL}^{-1}$  for GLYP, AMPA, and GLUF, respectively, and low method quantification limits (MQLs), 1, 4, and  $1 \text{ ng mL}^{-1}$ , respectively, with recoveries ranging between 81 and 107% and RSDs 2.04–8.36%. A LC-MS/MS method (6 min chromatographic run) was successfully applied to a sample of fortified milk with a very low herbicides concentration ( $0.025 \mu\text{g mL}^{-1}$ ). Further, the use of negative mode ion spray offers high sensitivity and selectivity. According to the study's

authors (and these authors agree), this methodology could be competitive with the enzyme-linked immunosorbent assay (ELISA) method.

Steinborn et al. reported of a survey on the GLYP content in 114 breast milk samples collected in Bavaria and Lower Saxony, Germany, by comparing the data obtained by LC-MS/MS and GC-MS/MS analyses [100]. The two analyses required (a) an ultrafiltration and chromatography on an anion exchange column for LC-MS/MS and (b) a clean-up step on a cation exchange column and derivatization with trifluoroacetic acid anhydride (TFAA) and heptafluorobutanol (HFB) for the GC-MS/MS. The authors deeply investigated the difference between the chromatograms obtained with the two methods, especially for evaluating parameters such as precision, accuracy, LOD, and LOQ. Basically, GC-MS/MS allowed to reach instrumental detection limit (IDL) lower than that found in LC-MS/MS (0.02 vs. 0.5 ng mL<sup>-1</sup>), but they detected an interference on a GLYP peak, which they did not manage to identify (all reagents, ultrapure water, all components were tested). Therefore, they fixed the LOQ at 1 ng mL<sup>-1</sup>, the same concentration determined by LC-MS/MS (whose LOD is 0.5 ng mL<sup>-1</sup>). The recoveries ranged between 83 and 128% with RSD < 17% for LC-MS/MS and between 71 and 102% with RSD < 13% for GC-MS/MS. Resuming, the GC-MS/MS is powerful at lower concentrations, but it simultaneously gives more bias than LC-MS/MS; both methods manage to investigate concentration above 1 ng mL<sup>-1</sup> with high precision and accuracy.

Two papers investigated the GLYP and AMPA content in human milk and urine samples. In the first, a high-throughput LC-MS/MS method using stable isotope labeled internal standard and clean-up with methylene chloride allowed to reach very low LODs (0.92 and 1.2 for GLYP and AMPA in human milk samples and 0.023 and 0.033 µg mL<sup>-1</sup> in human urine samples) and LOQs (10 µg mL<sup>-1</sup> for both in breast human milk samples and 0.1 µg mL<sup>-1</sup> in human urine samples), high recoveries (GLYP ranging between 92 and 107% in both matrices, AMPA between 89 and 107%) with low RSDs (<7.4 and <11.6% in human milk and urine samples, respectively) [101]. The authors also studied the matrix stability over a storage in 5°C (refrigerator) and at -20°C (freezer): in the first case, the recoveries were acceptable also after 24 hours, whereas in the second case, they were good also after 3 months. On the other hand, the second paper investigated the presence of GLYP and AMPA in milk (41 samples) and urine (40 samples) from healthy lactating women from Russia and the United States [102]. The authors used the same analytical procedure as reported above (i.e., LC-MS/MS, the use of stable isotope labeled internal standard and two fragments, such as precursor and product ion transitions, for the quantification) for the analysis, that is, the same analytical parameters. The results showed GLYP and AMPA in milk samples at levels below the LODs, whereas at low concentrations (<LOD and 1.93 µg mL<sup>-1</sup> and <LOD and 1.33 µg mL<sup>-1</sup>, respectively, in urine samples). The authors extrapolated the maximum intake of milk containing 1 µg mL<sup>-1</sup> of GLYP for a 5-kg infant: their conclusions were that the expected levels should be 12,000 times lower than the health concern.

The presence of MRLs for GLYP in barley, wheat, rye, and hops is regulated by EU Regulation (EC) No. 396/2005 (i.e., 20, 10, and 0.1 mg kg<sup>-1</sup>) [37, 38]. These are the raw agricultural commodities for beer beverage. Jansons et al. (2018) analyzed 100 beer samples from 24 different producers and distributors in Latvia with LC-MS/MS method ( $R^2 > 0.999$  in the range of 0.2–25 µg kg<sup>-1</sup>; LOD 0.2 µg kg<sup>-1</sup>; LOQ 0.5 µg kg<sup>-1</sup>; RSD < 4.1%) [103]. Among the numerous samples analyzed, 8 samples showed levels below the LOD and 9 samples below the LOQ, whereas 80 samples reported a GLYP concentration below 15 µg kg<sup>-1</sup> and 1 sample reached a GLYP content of 150 µg kg<sup>-1</sup>. The authors pointed out the attention on beer brands of “undisclosed” origin, that is, no country production reported on the labeling (it sounds strange to the authors of this review considering the restrictions on food



labeling in the EU, but we reported the authors' considerations), which could have higher GLYP content than the locally produced beer. Further, they also compared beers by malt type (barley or combined/other), color (light or dark), packaging (canned or bottled), the presence of precipitate (precipitate or no precipitate), filtration (filtered or not filtered), and pasteurization (pasteurized or not pasteurized), finding no significant differences in these cases.

Over these papers, it should be underlined two other paper dealing the GLYP determination in river water and soil samples. This particular occurrence regards the analytical protocol used by authors. In the first paper, Kudzin et al. developed a procedure based on derivatization with TEA-trifluoroacetic anhydride (TFAA)-trimethyl orthoacetate reagent and analyses by GC-CI(or EI)/MS (LOD 2.5–5.0 pmol) and GC-flame ionization detection (GC-FID; LOD 30–80 pmol, recovery 97%) [104]. In the second paper, Hu et al. investigated the performance of a method based on GC with nitrogen-phosphorus detector (GC-NPD): they estimated a LOD of  $9 \times 10^{-12}$  g and a LOQ of 0.01 mg kg<sup>-1</sup> in samples, recoveries between 84.4 and 94.0%, and RSDs between 8.1 and 13.7% [105]. These two papers deserve to be mentioned for having introduced the possibility to analyze GLYP by two very easy, cheap, and worldwide available detectors such as FID and NPD.

### 3. Conclusion

This long *excursus* wanted to cover the novel or advanced methodologies based on chromatographic analysis reported in the literature. The GLYP determination in foods is a really important issue, even if the different international agencies still do not totally agree on the human health concern. The importance of a continuous monitoring of such compound (and its main metabolite, AMPA), and GLUF as well, is well known by scientists and politics worldwide due to its large use in agriculture. The suggestion is to continuously develop new methods, more accurate and sensitive, based on GC-MS/MS or LC-MS/MS analysis but also routine method based on inexpensive or use-friendly detectors (FID, FPD, or NPD).

In any case, the fear for the future is that the refinement of analytical methods increasingly leads to alarmist attitudes based on the discovery of very low quantities of GLYP, which is possible for a very wide range of products, even extremely toxic, without forgetting that in nature the zero residue does not exist.

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### Conflict of interest

The authors declare no conflict of interest.



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