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A review on advances and perspectives of glyphosate determination: challenges and opportunities

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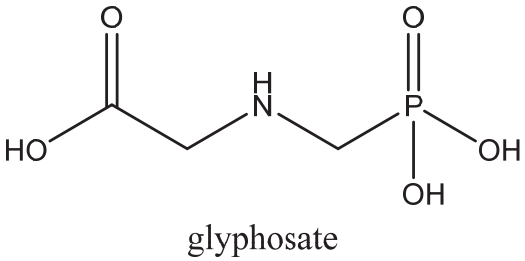
Abstract: Glyphosate is an inhibitor of the shikimate pathway in plants and the most widely used broad-spectrum herbicide. Due to the abundance of its use, there exists a necessity to measure the levels both in humans and in the environment to control the nefarious outcomes of its use. The appropriateness, selectivity, and the specificity of the employed analytical methods are crucial for the reliability of the resultant deductions when conducting biomonitoring studies on possible exposure to chemicals, whether the samples are biological or environmental in nature. The aim of this study is to evaluate the analytical techniques used to monitor glyphosate levels in human and environmental samples. A detailed web-based literature search was conducted to gather data on the analytical techniques used for glyphosate determination. The most preferred authentic samples are blood, urine, and milk. Environmental samples include plants, soil, and water. Among widely used analytical techniques used to detect glyphosate are High Performance Liquid Chromatography, Liquid Chromatography with tandem mass spectrometry, Gas Chromatography – Tandem Mass Spectrometry, and enzyme-linked immunosorbent assay. Depending on the sample and study, the most suitable analytical method has been selected. A critical evaluation and publication of pre-existing literature on analytical methods in glyphosate-based herbicide detection will thus aid all relevant researchers in the determination of an appropriate, selective, and specific methodology.

Introduction

Glyphosate, N-(phosphonomethyl) glycine (Figure 1), is an organophosphate herbicide that inhibits the key enzyme in the shikimate biosynthetic pathway in plant (Jensen et al. 2016). After its entry to market, glyphosate became the most widely used herbicide in the world. It is used to eliminate unwanted plants in agricultural areas, as well as public areas such as parks, gardens, and even paved surfaces. Glyphosate based herbicides (GBHs) greatly increase crop yields by reducing weeds. Nevertheless, according to extensive studies conducted on the carcinogenicity of GBHs, International Agency for Research on Cancer (International Agency for Research on Cancer, 2015), determined to classify glyphosate in "Group 2A – probably carcinogenic to humans". After this decision, GBHs gained both public and regulatory agencies' attention as a probable public health threat. Pesticide residues on foodstuff can gain entry into the body by ingestion. Acceptable Daily Intake (ADI) value estimates the amount of residue in food that can be consumed on a daily basis without any hazardous endpoints over a lifetime (EFSA, n.d.). European Food Safety Authority set ADI value of GBHs as 0.5 mg/kg bodyweight per day (mg/kg bw/day) and Acceptable Occupational Exposure Level (AOEL) of GBHs was set to 0.1 mg/kg bw/day (EFSA, nd).

According to studies conducted on GBHs, it was observed that exposure to GBHs is associated with several health conditions including reduction of sperm motility in men, kidney damage, mental and neurological diseases, and miscarriages in women occupationally exposed to glyphosate (Anifandis et al. 2018, Van Bruggen et al. 2018). Since GBHs are associated with several health problems including carcinogenesis, the monitoring of GBHs becomes an important consideration. Biomonitoring is the most effective manner of



glyphosate

Fig. 1. Chemical structure of Glyphosate

exposure monitoring in cases of environmental exposure and exposure via foodstuffs. It is an exposure assessment tool, and it is extremely useful in the analysis of pesticides with known pharmacokinetic parameters. Biomonitoring studies involve the measurement of a biomarker, or the xenobiotic under consideration itself, in a biological sample such as blood or urine (Connolly et al. 2017).

The reliability of a biomonitoring study depends on several factors including the study design, the type of biomarker, suitability of the sample with the biomarker being analyzed, collection, storage, and pre-treatment of the samples, as well as the detection techniques employed (Ladeira and Viegas, 2016, Manno et al. 2010). The method used should meet the regulatory needs and be able to detect the biomarker in question. There are many validated methods used to detect GBH and aminomethylphosphonic acid (AMPA), glyphosate's major metabolite, in different biological matrices such as blood, urine, or milk (Bienvenu et al. 2021, Bressán et al. 2021, Mcguire et al. 2016, Ruiz et al. 2021, Steinborn et al. 2016, Zouaoui et al. 2013).

In addition to their biological impact, GBHs are also an environmental concern. They might act as soil and water pollutants after their application. It is established that glyphosate adsorbs to clay (Glass, 1987). This will lead to a reduction in the rate of its degradation by soil microorganisms, making accumulation of GBH in soil inevitable. Studies show that GBH and its metabolite AMPA may persist in clay-rich soils more than a year but not in sandy soils depending on pH (Banks et al. 2014, Cassigneul et al. 2016, Okada et al. 2016, Sidoli et al. 2016, Van Bruggen et al. 2018, Zhang et al. 2015). In addition, GBH in soil may dissolve in surface and ground water. As a result, soil and water ecosystems might be negatively affected by GBH contamination. Thus, environmental monitoring of glyphosate is as significant and important as biological monitoring. Furthermore, the determination of GBHs in different matrices allows scientists to understand the links between environmental contamination and human exposure, monitor the levels and force stakeholders to take actions where appropriate.

There are many analytical techniques and methods used to monitor GBH in different biological or non-biological samples. These techniques are subject to change depending on the type of study as well as the sample type being studied. The most widely used techniques can be listed as High--Performance Liquid Chromatography (HPLC), Liquid Chromatography with tandem mass spectrometry (LC-MS/MS), Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) and Enzyme-Linked Immunosorbent Assay (ELISA) (Jayasumana et al. 2015, Steinborn et al. 2016, Tsao et al. 2016, Valle et al. 2019). Our review summarizes these validated techniques that are used during the analysis of GBH and glyphosate's major metabolite (AMPA) in biological and non-biological samples.

Methods

A detailed web-based literature search was conducted to gather data on the analytical techniques used for glyphosate determination. Taking the above statement into account, our review updates and evaluates the data on analysis of glyphosate in different samples. The following reputable sources were used:

- Pubmed
- Google Scholar
- ATSDR website
- EFSA website
- European Commission website
- U.S Food and Drug Administration website

Analysis of glyphosate in biological samples

Glyphosate is classified as a probable carcinogen to humans (International Agency for Research on Cancer, 2015). In addition to its carcinogenic properties, there are other, multiple health problems associated with GBH exposure. Even though the animals lack shikimate pathway, the main pathway through which GBH exerts its effects on plants, GBH can still cause several distinct toxicities through several different mechanisms. A study showed that GBH exposure results in the reduction of sperm motility in men (Anifandis et al. 2018). Furthermore, there is a correlation in miscarriages in women who are occupationally exposed to GBHs during their pregnancies (Avila-Vazquez et al. 2018). GBHs and AMPA are believed to interfere with normal neurotransmission, resulting in alterations to the balance between cell proliferation and apoptosis (Van Bruggen et al. 2018). Consequently, it may cause Alzheimer's and Parkinson's diseases. Moreover, exposure to GBHs is correlated with attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (Fluegge and Fluegge, 2015, Von Ehrenstein et al. 2019). In regard to these facts, it is crucial to monitor the fate of GBHs in humans. Human biomonitoring is the most widely used and most effective way of controlling the effects of GBHs. In the biomonitoring of glyphosate, the most preferred biological samples are blood, urine, and milk (Table 1).

Analysis of Glyphosate in Blood Samples

Blood is a highly useful, well-established, and important biological sample. In the case of GBH intoxication, blood samples are extremely useful to detect the presence of GBH in blood. Such samples are used to analyze GBH in such cases where GBH exposure is caused by accidental or suicidal ingestion and the patient is hospitalized. Blood is also a particularly significant forensic tool which enables post-mortem evaluation. Even though blood has multiple advantages, one drawback is that it needs to be collected invasively by a specialist under suitable conditions. Therefore, even though it is the first choice of sample for hospitalized intoxication cases, for biomonitoring studies, its use is uncommon.

GBH analysis in blood samples is conducted using different analytical techniques. The first and the most widely used is Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). LC-MS/MS is an extremely sensitive and technologically advanced apparatus that has limit of detection (LOD) and limit of quantification (LOQ) of 0.1 µg/mL. A recently developed and validated technique by Tsao et al. (2016), uses acetonitrile during the pre-treatment of the sample and without the need for expensive solid-phase extraction cartridges, is able to detect GBHs in small volumes of blood. Furthermore, the determination process takes only 10 minutes,

which makes it exceptionally fast and useful during situations where an urgent result is needed (Tsao et al. 2016). A recent study that developed an alternative sample pre-treatment procedure including dilution and evaporation procedure aimed to validate a method for GLY and AMPA analysis in post-mortem samples. In this study, plasma and urine were selected as sample matrices and the LOD and LOQ values were 0.2 and 0.5 µg/mL respectively for both analytes (Ohara et al. 2021). Recently, an ion chromatography-tandem mass spectrometry (IC-MS/MS) method for GLY and AMPA in serum has been developed by (Zhang et al. 2021).

Nuclear magnetic resonance (NMR) is another technique that is used to detect GBH in blood samples. NMR assesses the magnetic properties of atomic nuclei and gives detailed data about the molecular structure. Throughout the GBH determination, there is no need to separate and/or derivatize the relevant constituents. The major merits of using NMR to detect GBH are as follows: its duration is between 10–20 minutes, it requires a small sample size, and it needs no sample pre-treatment. The main limitation of this method are relatively inferior quantification capabilities. Thus, in situations where only detection is necessary, it is suitable to use NMR (Cartigny et al. 2004, Steinborn et al. 2016).

Analysis of Glyphosate in Urine Samples

Glyphosate itself is known to be ionic and water-soluble which makes it difficult to analyze (Nagatomi et al. 2013). Nevertheless, the aforementioned properties allow glyphosate to be excreted unchanged in urine (Brewster et al. 1991). As urine has ease of collection and the collection method is noninvasive, it is the most preferred sample in GBH biomonitoring studies. As a result, there are multiple methods and techniques developed to detect GBH in urine samples (Bressán et al. 2021, Curwin et al. 2007, Jayasumana et al. 2015).

Immunoassays are another class of test methods used in the detection of GBH in urine samples. They are relatively cheap compared to other methods, they require small sample sizes, and produce results comparatively faster. The first immunoassay used in GBH measurement in urine is Fluorescence Covalent Microbead Immunoassay (FCMI). The test relies on the competition of pesticide with a bead-bound conjugate for the fluorescently labelled anti-pesticide antibodies. Once the concentration of pesticide, such as GBH, increases in urine, this leads to a reduction of fluorescence signals. The limit of detection (LOD) of this immunoassay is 0.9 µg/L (Curwin et al. 2007).

ELISA is another immunoassay currently used in the detection of GBH in urine (Grau et al. 2022). Simplicity and high specificity promote the use of ELISA (Rendón-Von Osten and Dzul-Caamal, n.d.). Commercial kits specifically designed to detect GBH in liquid samples are available. Urine samples can be used with or without dilution depending on the kit. The assay relies on the competition, similar to FCMI, between glyphosate and glyphosate-horseradish peroxidase conjugate for binding to a specific antibody. ELISA has a LOD value of 0.6 µg/L. The main drawback of the test is the need for validation of the results in general with GC-MS (Jayasumana et al. 2015).

HPLC is one of the analytical method devices used in the detection of GBH. There are several methodologies that can be followed using HPLC. One main modification of these methodologies includes the quantification of GBH using HPLC with post-column reaction and fluorescence detection (Acquavella et al. 2004). This modification has a LOD of 1 µg/L for a 100 mL sample The main disadvantages of using HPLC are high costs and the need to operate and maintain the apparatus by trained personnel (Habekost, 2017).

GC-MS/MS and LC-MS/MS are technologically advanced method machines and are used as part of GBH analysis. GC-MS/MS requires only 50 µL sample volume, which is a relatively small amount, and the method has a LOQ of 0.1 µg/L with high selectivity. On the other hand, LC-MS/MS has a LOQ value of 0.5 µg/L for 50 µL of urine. Both devices require extensive sample pre-treatment including solid phase extraction with expensive cartridges (Connolly et al. 2017, Nagatomi et al. 2013). A recent study also used LC-MS/MS for GLY and AMPA determination and the LOQ for glyphosate and AMPA was 0.05 and 0.1 ng/mL, respectively, and the LOD was 0.02 and 0.04 ng/mL, respectively (Zoller et al. 2020). Another study conducted with LC-MS/MS had a LOQ value of 1 µg/L for both analytes (Ruiz et al. 2021). A study validated a method for the detection of GLY and AMPA in urine samples using UPLC-MS/MS. In that particular study, the LOD and LOQ values were 0.5 and 1 µg/L for GLY and 0.1 and 0.5 µg/L for AMPA, respectively

Table 1. The most frequently used techniques during the analysis of GLY in Biological Samples

Biological Sample	Technique	Sample Preparation	LOD/LOQ	Reference
Blood	LC-MS/MS	LLE	0.1 µg/mL (LOD, LOQ)	(Tsao et al. 2016)
	NMR	No pretreatment	Structure identification	(Cartigny et al. 2004, Valle et al. 2019)
Urine	FCMI	Fortification with metolachlor mercapturate	0.9 µg/L (LOD)	(Curwin et al. 2007)
	ELISA	Dilution	0.6 µg/L (LOD)	(Jayasumana et al. 2015)
	HPLC	Chelation	1 µg/L (LOD)	(Acquavella et al. 2004)
	LC-MS/MS	SPE	0.5 µg/L (LOQ)	(Nagatomi et al. 2013)
Milk	LC-MS/MS	Ultrafiltration	0.92 µg/L (LOD) 10 µg/L (LOQ)	(Jensen et al. 2016, Mcguire et al. 2016, Steinborn et al. 2016)
	GC-MS/MS	LLE		(Steinborn et al. 2016)

(Martin-Reina et al. 2021). These devices are extremely sensitive, yet the cost of use is high.

Analysis of Glyphosate in Milk Samples

Milk is not always the first choice of sample in GBH biomonitoring studies. Nevertheless, after GBH toxicity became an important topic of consideration, it became necessary to monitor breast milk as infants might be exposed during breastfeeding. Milk consists of carbohydrates, proteins, and fat. Thus, being complex in nature, it is challenging to work with milk. For this reason, methods conducted on watery matrices cannot be used without major modifications (Mcguire et al. 2016, Steinborn et al. 2016).

LC-MS/MS and GC-MS/MS are used for GBH analysis of milk. LC-MS/MS requires 150 µL of milk sample. Both analytical techniques have their own pre-treatment, however, these pre-treatment methods are subject to change according to method validated. For instance, pre-treatment of milk sample before LC-MS/MS analysis conducted by Steinborn et al. (2016), involved an ultrafiltration then chromatography on an anion-exchange column (Steinborn et al. 2016). LOD and LOQ values of GBH analysis with LC-MS/MS also vary between the methodologies. The study conducted by Jensen et al. had LOD and LOQ values of 0.92 µg/L and 10 µg/L, respectively (Jensen et al. 2016). On the other hand, McGuire et al. had a different LOD value (1 µg/L) but the same LOQ (Mcguire et al. 2016). The lowest LOQ value of GBH is 1 ng/L in the method developed by Steinborn et al. (Steinborn et al. 2016).

GC-MS/MS is another analytical instrument that is preferred for GBH determination in milk. To conduct GBH analysis, milk sample needs to be extracted and then cleaned-up on a cation exchange column. Afterwards, there is a derivatization step which includes heptafluorobutanol and trichloroacetic acid anhydride (Steinborn et al. 2016).

Regardless of its complex nature, these highly sophisticated devices with validated methods allow for the detection of GBH in milk.

Analysis of glyphosate in environmental samples

GBHs are not only used in agriculture but also in public locations, for instance common parks, gardens and even roadsides. Thus, they were detected in a variety of environmental matrices such as soil, water and even in air (Philipp Schledorn, 2014). This may lead human and animal exposure to GBH not only through ingestion but also environmental exposure. GBHs have the ability to adsorb onto clay which in turn leads to their accumulation in clay-rich soil. Therefore, glyphosate and its major metabolite, AMPA, are able to persist in clay-rich soil for over a year. On the other hand, glyphosate is expected to be washed out rapidly in sandy soils (Van Bruggen et al. 2018).

Glyphosate degradation in the environment depends on both biotic and abiotic mechanisms. The major pathway of glyphosate degradation is via microbial degradation in the soil. Photodegradation occurs less commonly (Alexa et al. n.d., Sviridov et al. 2015). Regardless of the degradation pathway, AMPA is the major metabolite (Meftaul et al. 2020), which is a known phytotoxin that affects the biosynthesis of chlorophyll (Marcelo et al. 2004). Thus, environmental monitoring of glyphosate, as well as AMPA is very significant in order to preserve both human and ecological health.

Analysis of Glyphosate in Edible Plants

Glyphosate is a broad-spectrum herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (ESPS) which is part of the crucial shikimate pathway in plants. Glyphosate is transported through the phloem. During transportation, it leads to the death of the roots and as well as the reproductive parts of plants (Brito et al. 2018). In order to expand efficient usage, genetically modified crops have been produced that are resistant to glyphosate. Through this, glyphosate gained selectivity and its application increased significantly. Nevertheless, during application, non-target crops are contaminated with drift-off. Consuming contaminated crops is the major pathway through which humans are exposed to pesticides. To minimize the effects of exposure, regulatory authorities set limit values for each pesticide that is allowed to be present on or in a foodstuff. Maximum residue level (MRL) is the highest value that is permitted to exist in or on food or animal feed and it is calculated conservatively to protect the most vulnerable consumers. MRL values are subject to change depending on the foodstuff that is under consideration (Łozowicka and Kaczyński, 2011). MRL value of glyphosate is 0.1 mg/kg for most foodstuffs (European Commission, nd). On the other hand, the acceptable daily intake (ADI) is the estimated safe value of a compound that can be taken daily throughout the lifetime with no harmful effects (FDA, 2016). ADI of glyphosate is calculated as 0.5 mg/kg bodyweight per day (European Commission, n.d.). Even though these levels are claimed to be the safe limits, there are some studies which show that exposure to low levels of glyphosate may lead to toxic effects (Van Bruggen et al. 2018). Thus, it is critical to analyze glyphosate levels in foodstuff with highly sensitive and reliable techniques (Table 2).

There are multiple analytical techniques being used and claimed to be safe, efficient, and easy to use. The first technique is Field Amplified Sample Injection and Sweeping Micellar Electrokinetic Chromatography (FASI sweep MEKC). Gotti et al. (2019) studied this technique with solid-phase extraction (SPE) and they were able to produce LOQ values of 5 and 2.5 ng/mL for glyphosate and AMPA, respectively (Gotti et al. 2019). Capillary electrophoresis (CE) with electrochemiluminescence detection is another method for glyphosate analysis. This technique gained attraction due to its duration as it takes less than an hour including sample preparation. This methodology has an LOD of 1 µg/g with soybean samples (Chiu et al. 2008). Liquid chromatography with quadrupole mass spectrometry is also used for the analysis of glyphosate. Depending on the type and species of the sample, LOD and LOQ values are subject to change. For instance, LOD values for coffee beans, rice and black beans are 12, 28 and 91 µg/kg respectively. LC-MS/MS, on the other hand, is one of the most sensitive and sophisticated devices that can be used to detect glyphosate (Jansons et al. 2021, Marek and Koskinen, 2014). The main disadvantage of using LC-MS/MS is that it might not be possible to access the device when it is needed, and an experienced person should run the entire procedure. Moreover, nanostructured CuO and ZnO electrochemical sensors were utilized for the detection of glyphosate in rye (Gerbreders et al. 2021).

In summary, it is compulsory to control the levels of glyphosate residues on foodstuff to predict their possible ecological and public endpoints. Furthermore, it is crucial to select the most appropriate device and technique depending on the type of the sample.

Analysis of Glyphosate in Soil and Water

One of the main concerns during pesticide application is the drift--off. While the application is ongoing, it is practically inevitable to contaminate soils in and around the area of application. Soil is an important matrix of concern in the case of glyphosate. Glyphosate shows a persistent profile, especially in clay-rich soils due to the aforementioned ability for adsorption. This slows down its metabolism by soil microorganisms. Depending on the soil pH and content it may persist for more than a year (Van Bruggen et al. 2018). Thus, glyphosate is said to be 'pseudopersistent' due to its high accumulation in agricultural soils. In addition, glyphosate is a concern for the water bodies around the affected area. During application, glyphosate may drift off to surface waters, may runoff from the surface and even be transported with the wind as particles (Bento et al. 2017, Okada et al. 2019). Therefore, it is not only important to analyze foodstuffs for possible human exposure, but it is also vital to determine and control the levels in soil and water bodies (Figure 2). A major problem is finding the most appropriate methodology to use. There are multiple developed methods in the literature (Table 2). A Linker-Assisted Enzyme Linked Immunosorbent Assay (L'ELISA) is easy to carry out and a cheaper option compared to alternatives. For soil samples, the LOD value of this technique is 0.8 ng/g (El-Gendy et al. 2018). HPLC coupled with electrospray tandem mass spectrometry has also been developed for glyphosate analysis in water samples. The main advantage of this method is that it requires only 150 µL of sample volume. The limit of detection ranges from 0.02 to 0.05 µg/L and the limit of quantification is 0.1 µg/L (Guo et al. 2018). Hottes et al. (2021) also developed a method to detect GLY in water (Hottes et al. 2021). LC-MS/MS is the most preferred methodology where the necessary conditions such as the equipment and experienced oversight and control are present. For water, the LOD 0.25 µg/L and LOQ is 0.5 µg/L (Okada et al. 2019, Poiger et al. 2017). Delhomme et al. (2021) described a method using LC-MS/MS following the extraction and purification of soil samples. The LOQ values were 0.030 µg/g and 0.025 µg/g for GLY and AMPA, respectively. On the other hand, UPLC-MS/MS is also preferred. LOD values are 0.1 µg/L and 0.5 µg/kg and LOQ values are 0.5 µg/L and 10 µg/kg for water and soil, respectively (Aparicio et al. 2013). Furthermore, a universal platform with a twin-sensing structure method was developed to detect GLY and atrazine in aqueous environments (Dhamu et al. 2021). Yadav and Zelder, (2021) developed an optical detection system with an immobilized Cu(II)-pyrocatechol violet complex for the detection of GLY. Efforts are ongoing to develop alternative methods for detecting GLY in different matrices, especially in drinking water (Scandurra et al. 2022).

Table 2. The most frequently used techniques during the analysis of GLY in Environmental Samples

Sample Medium	Technique	Sample Preparation	LOD/LOQ	Reference
Plants	FASI Sweep MEKC	SPE	5 ng/mL (LOQ)	(Gotti et al. 2019)
	*CE	LLE	1 µg/g (LOD)	(Chiu et al. 2008)
	LC-MS/MS	Acidification	12 µg/kg (LOD – soybean samples)	(Marek and Koskinen, 2014)
Soil and Water	L'ELISA	LLE	0.8 ng/g (LOD-soil samples)	(El-Gendy et al. 2018)
	UPLC-MS/MS	LLE	0.5 µg/kg (LOD-soil samples) 10 µg/kg (LOQ-soil samples) µg/L (LOD-water samples) 0.5 µg/L (LOQ-water samples)	(Aparicio et al. 2013)
	HPLC	Filtration	µg/L (LOD) 0.1 µg/L (LOQ) water samples	(Guo et al. 2018)
	LC-MS/MS	Filtration	0.25 µg/L (LOD-water samples) 0.5 µg/L (LOQ-water samples)	(Okada et al. 2019, Poiger et al. 2017)

* Capillary Electrophoresis with electroluminescence detection.

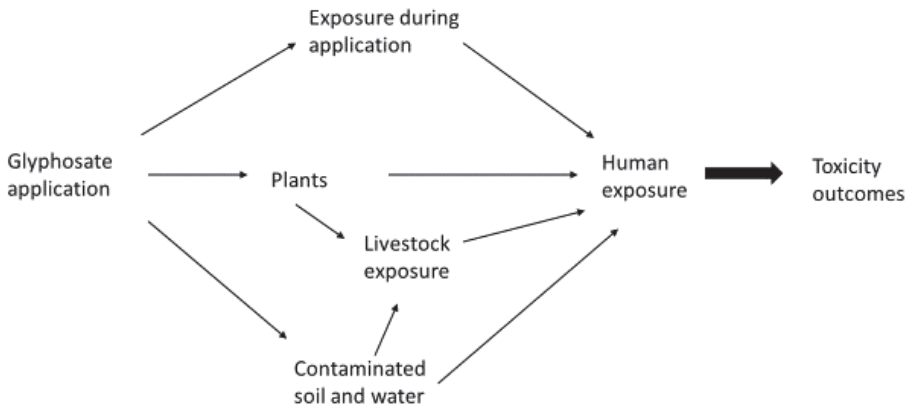


Fig. 2. Potential sources of toxic exposure to glyphosate

The applicability of these methods depends on the expected outcomes. Therefore, the most suitable method should be selected in accordance with the samples and the available opportunities.

Advantages and limitations of analytical techniques used for determination of gbh and ampa

LC-MS/MS consists of different instrumentation to allow for various sorts of experiment. It consists of an atmospheric pressure ionization source coupled with ion-inlet and focusing component, the first mass-filtering device, a collision chamber, the second mass-filtering device and the ion--impact detector. Thus, a LC-MS/MS allows to perform five different experimental designs. These are listed as Full Scan, Product Ion Scan, Precursor Ions Scan, Neutral Lass Scan and Selective (or Multiple) Reaction Monitoring (SRM or MRM). The first three experiments are mostly conducted in method development studies. In clinical practice, SRM is the most widely used LC-MS/MS experiment. SRM mode combines high analytical sensitivity and specificity, leading to short chromatography run-times relative to other experiments. The main advantages of LC-MS/MS are (i) easy application to low molecular weight compounds that act as a limitation for immunoassays, (ii) relatively simpler workflow and higher output than its alternatives, and (iii) relatively lower cost of equipment in comparison to the alternatives. Despite its many advantages, there are drawbacks and limitations of conducting experiments with LC-MS/MS. First of all, it has many manual workflows which gives a complexity to operate and maintain the device. Furthermore, sample output is limited and for some analytes it may give insufficient detection sensitivity (Grebe and Singh, 2011). LC-MS/MS is a leading apparatus that is preferred to detect GLY and its metabolite AMPA in a wide range of sample matrices. Similar to other methodologies, it may need derivatization depending on the nature of sample pre-treatment. However, Guo et al. 2018 used QuPPe method as a blood sample pre-treatment and skipped the time-consuming derivatization step(Guo et al. 2018). Conducting LC-MS/MS analyses is not limited to blood samples, it may also be used to detect GLY and AMPA in several sample matrices including biological and environmental samples (Jensen et al. 2016, Marek and Koskinen, 2014, Mcguire et al. 2016, Nagatomi et al. 2013, Okada et al. 2019, Poiger et al. 2017, Steinborn et al. 2016, Tsao et al. 2016). The derivatization step is crucially important while performing chromatography. The efforts are ongoing for developing methodologies to bypass or optimize this step. To do so, Fontàs and Sanchez (2020), studied derivatization and chromatographic conditions and they found that in the case of GLY and AMPA, pH is the most significant parameter (Fontàs and Sanchez, 2020).

NMR exploits the principle of using radiofrequency waves to gather data from magnetic nuclei. There are three main features of NMR which make it different from other types of spectroscopy. Firstly, NMR focuses on the specificity of nuclei and distribution of chemical elements throughout the sample. This leads to a wider target range than most of the other techniques. Being sensitive to local surroundings of the nuclei under consideration is the second important feature of NMR. Thus, it is able to collect more information from the chemical and physical environment of an atom compared to other spectroscopy methods. Lastly, it has more penetrating power and is damaging to a lesser extent. On the other hand, NMR's working principle, radiofrequency, uses low energy radiation which makes it less sensitive compared to other spectroscopy techniques. It's complicated working principle and data derivation are another drawback (Bothwell and Griffin, 2011). NMR spectroscopy gains importance when the experiments are being used to diagnose acute poisonings. NMR does not need separation and/or derivatization procedures prior to the experiment which makes it suitable as a diagnostic tool. Thus, it is used in GLY and AMPA analyses mostly in biological fluids (Cartigny et al. 2004).

FCMI assays (FCMIA) are combinations of several methodologies, featuring immunoassay, microsphere, and flow cytometry characteristics. During FCMIA, immunoassays are conducted on solid support microspheres with internal fluorophores. This makes it possible to analyze numerous analytes at the same time. FCMIAs need smaller sample sizes, they cost less and have increased ranges in comparison to ELISA. They have also fast running times (Biagini et al. 2004).

ELISA is an assay that uses the principle of antigen--antibody reactions. Thus, it has high sensitivity, as well as high selectivity of quantitative/qualitative antigen analysis of the material under consideration. Advantages of ELISA can be summarized as follows: simple and easy to perform procedure, high specificity, selectivity, and efficiency, no need for complicated pre-treatment, safety, eco-friendliness, and low cost. This technique has however some disadvantages: some antibodies might be expensive to prepare, in some cases, expensive culture media may be required, because of the nature of antigen-antibody binding, there is a high risk of false negatives or false positives, as it works with antibodies, they should be preserved in conditions that promote and maintain protein stability, and, finally, the techniques requires refrigerated transport and storage conditions (Sakamoto et al. 2018). In comparison to other analytical methods, ELISA does not need sophisticated and expensive equipment. Therefore, it can be run by most laboratories. In the case GLY, ELISA is a good monitoring tool with high sensitivity, fast working principle and low cost. These features make it ideal for routine monitoring of GLY but not for AMPA (Curwin et al. 2007, El-Gendy et al. 2018).

HPLC combines speed, reproducibility, and sensitivity in a single device. It allows for rapid and precise quantitatively analysis. The device is highly sensitive and enables quantitative sample recovery. The cost and complex working conditions are the primary disadvantages of HPLC. However, due to its selectivity, sensitivity, resolution and high data capacity, it became one of the most widely used equipment in the determination of many pesticides in environmental samples. HPLC coupled with ESI-MS/MS allows for direct analysis of GLY and AMPA in ultra-trace concentrations. According to Guo et al, 2016, HPLC-ESI-MS/MS method does not need derivatiszation steps and has a simple sample preparation process. The method can be applied to many environmental sample matrices (Guo et al. 2016).

GC-MS/MS is another apparatus used to detect a wide variety of chemicals. Because of its nature, GC-MS/MS works perfectly well with volatile compounds, but it needs extra derivatization steps with non-volatile compounds. It is highly

sensitive and accurate (Sadkowska et al. 2019). GC-MS/MS has a good dynamic range and with the help of mass spectral library it offers compound identification. It also provides additional data, such as retention time, which can be useful for further experiments. Furthermore, it can identify stereoisomers. However, there are limitations of using GC-MS/MS. First of all, samples must be volatile (or derivatization is needed), thermally and energetically stable. Prior to analysis, a suitable sample preparation must be performed since it is hard to work with large and highly polar metabolites. It requires trained staff to run the experiment (de Villiers and Toit Loots, n.d.).

Capillary Electrophoresis can be used to analyze a wide range of chemicals including cationic, anionic, and neutral species. It is also suitable to work with a diverse range of separation modes and detection techniques. This technique is preferred due to its short working time, high resolution and efficiency, as well as cheaper reagents and small sample requirement (El Deeb et al. 2016, Masár et al. 2020, Phillips, 2018). Despite its valuable features, low detection sensitivity compared to other analytical techniques is the main disadvantage of CE (Masár et al. 2020).

Glyphosate's physicochemical properties are an analytical challenge. GLY is highly soluble in water, insoluble in organic solvents and has high polarity and low volatility (PubChem, n.d.). Due to these properties, regardless of the sample matrix, samples need to be processed via a suitable sample pre-treatment procedure prior to analysis.

During the selection of a sample preparation method, there are some independent factors to be considered. Some of these factors can be listed as follows: the aim of the analysis, sample characteristics, the constituents of the analyte, and the chromatographic technique that is going to be used. Depending on these factors, either an already described sample pre-treatment technique can be used or, when there is no alternative, a new technique might be developed (Moldoveanu and David, 2015). Here, two important sample pre-treatment procedures are going to be discussed.

Liquid-Liquid Extraction (LLE) is one of the most preferred sample pre-treatment methods prior to qualitative and quantitative analysis. One of the greatest advantages of LLE is that it allows for large linear sample capacities. Furthermore, the organic extract can be used directly to conduct quantitative or qualitative analysis without any need for further procedures. Although there are many different types of LLE procedures, conducting LLE with separatory funnels produces large amounts of organic waste (Cantwell and Losier, 2002). When used prior to GLY analysis, LLE is oftentimes problematic due to GLY's physicochemical properties.

SPE, on the other hand, is also a preferred method due to its convenience, simplicity, and reduced consumption of organic solvents. SPE has been widely used for the treatment of many samples prior to GLY and AMPA analysis. Despite its efficiency, SPE cartridge to be utilized, should be selected with care depending on the sample matrix (Gotti et al. 2019).

Future aspects and conclusion

This review summarizes commonly used techniques in the analysis of glyphosate. Each developed method has its distinct advantages and disadvantages. This review enlightens the way of future studies which will focus on the biomonitoring of GBH in exposed farmers and horticulture and will aid in the monitoring of their occupational health. The most applicable method should be selected depending on the type of the sample, the cost, and the availability of the device or the kit. Nevertheless, more precise and advanced methods, such as Quadrupole Time-of-Flight LC-MS (LC/Q-TOF MS), might be more suitable to detect very low amounts of glyphosate in a diverse variety of samples. Thus, it will be useful to develop a methodology that focuses on LC/Q-TOF MS where applicable. Nonetheless, cheap, easy to apply, robust and sensitive methods are yet to be developed.

Declarations

Conflict of Interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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