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THE ROLE OF GLYPHOSATE AND ITS EFFECTS ON HUMAN HEALTH AND LIFE®

Rola glifosatu i jego wpływ na zdrowie oraz życie człowieka®

Key words: glyphosate, toxicity, public health, food safety.

It is estimated that agricultural losses due to competition of crops mainly for water and nutrients with weeds can be as high as 50%. For this reason, herbicides are commonly used to control weeds. One of these, considered to be the most widely used, is Roundup, whose main active ingredient is glyphosate. Glyphosate is a systemic, wide-spectrum herbicide. The use of glyphosate has increased and is now the most widely used herbicide in the world. The increased use of glyphosate has also led to increased concerns about its possible toxicity and human health consequences. However, there is currently no consensus among the scientific community and the safety and health consequences of glyphosate are controversial. As glyphosate is mainly used in fields and can persist in the soil for several months, concerns have been raised about the impact that its presence in food may cause in humans. ***This work, aims to review the use, toxicity and occurrence and methods of detection of glyphosate in various food matrices, which in some cases is present in amounts exceeding acceptable standards.***

Słowa kluczowe: glifosat, toksyczność, zdrowie publiczne, bezpieczeństwo żywności.

Oszacowuje się, że straty rolnicze wynikające ze współzawodnictwa roślin uprawnych głównie o wodę i składniki pokarmowe z chwastami mogą stanowić aż 50%. Z tego względu powszechnie do ograniczania zachwaszczenia wykorzystuje się herbicydy. Jednym z nich, uznawany za najczęściej stosowany jest Roundup, którego główną substancją czynną jest glifosat. Glifosat jest herbicydem systemicznym, o szerokim spektrum działania. Stosowanie glifosatu wzrosło i obecnie jest on najczęściej stosowanym herbicydem na świecie. Wzrost zużycia glifosatu spowodował również wzrost obaw o jego możliwą toksyczność i konsekwencje dla zdrowia ludzi. W chwili obecnej nie ma konsensusu wśród społeczności naukowej, a bezpieczeństwo i konsekwencje zdrowotne glifosatu są kontrowersyjne. Ponieważ glifosat jest stosowany głównie na polach i może utrzymywać się w glebie przez kilka miesięcy, pojawiły się obawy o wpływ, jaki jego obecność w żywności może wywołać u ludzi. ***Niniejszy artykuł ma na celu dokonanie przeglądu stosowania, toksyczności i występowania oraz metod wykrywania glifosatu w różnych matrycach żywnościowych, który w niektórych przypadkach występuje w ilościach przekraczających dopuszczalne normy.***

INTRODUCTION

Glyphosate is an organophosphate herbicide [11]. The herbicidal nature of glyphosate was discovered in 1970 by John Franz, a chemist at Monsanto® company (USA), who produced the first glyphosate-based herbicide (GBH), Roundup®, a few years later [14]. Today, there are hundreds of GBHs commercialised worldwide under various brand names in more than 100 countries [42, 53]. Currently, glyphosate is the most widely used herbicide in the world [2]. This herbicidal substance belongs to the group of glycine-derived compounds (aminophosphonates). From a chemical point of view, it blocks the proper functioning of the plant enzyme (EPSP) in the shikimic acid pathway and the biosynthesis of amino acids (phenylalanine, tryptophan and tyrosine), which are essential for protein building. It exhibits hydrophilic properties (easily soluble in water) [4, 26, 33, 52]. Glyphosate-based herbicides penetrate through the leaves and other above-ground parts of plants. The time at which symptoms are visible on plants after application is variable and depends mainly on the species to be controlled, the development stage, the dose applied and the weather conditions [4]. The presence of glyphosate on plants reveals stunted growth and chlorosis starting from their youngest parts. The whole plant generally dies 2–3 weeks after application of the chemical. Currently, over 80 herbicides containing glyphosate have been registered in Poland, and new ones are still being registered [4]. One of the examples worth mentioning is Halvetic, a new herbicide offered by CIECH Sarzyna [4]. According to the manufacturer's assurance, the patented formula ensures high effectiveness of weed control at a reduced dose of glyphosate, which fits in well with European

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environmental protection initiatives [4, 26, 33, 52]. The Bayer concern, which took over Monsanto a few years ago, has also announced research aimed at indicating the safest way of using glyphosate and probably subsequent herbicides based on this substance [4, 26, 33, 52]. The rapid increase in the use of glyphosate over the years has also raised concerns about its possible toxicity and possible consequences for human health [9]. Consequently, concerns about the potential impact of this herbicide and its metabolites on the environment and humans have increased in the scientific community. Therefore, the distribution of glyphosate formulations is strictly regulated and there are maximum residue limits (MRLs) for glyphosate in food [42]. The aim of this review is to assess the sources and occurrence of glyphosate in different food matrices and its effects on the environment and human health.

CHEMICAL AND PHYSICAL PROPERTIES OF GLYPHOSATE

Glyphosate (M_W 169.1 g/mol) in terms of chemical structure is a zwitterion [13] with phosphonate, carboxylate and amine functions (Figure 1). The zwitterionic structure of glyphosate gives the ability to chelate trivalent and tetravalent metals [34, 51, 55]. The covalent bond between carbon and phosphorus atoms, which is characteristic of these organophosphorus compounds, gives glyphosate a number of specific chemical and physical properties, such as high solubility in water, compatibility with other chemicals and high adsorption [51]. Glyphosate is a highly polar molecule, which contributes to its high solubility in water and insolubility in organic solvents [53]. The particular chemical and physical properties of glyphosate, such as low volatility and high hydrophilicity, lack of chromophore or fluorine group, lack of absorption in the ultraviolet region, low ionization [40], require the use of complex analytical methodologies for the detection and quantification of this herbicide to achieve the required sensitivity and accuracy [9, 16, 25, 48, 54]. Glyphosate is a widely-used foliage-applied herbicide. Glyphosate forms the aminomethylphosphonic acid (AMPA) when metabolized in soil or water [25, 48].

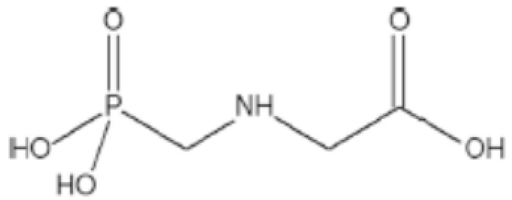

Fig 1. Glyphosate chemical structure.
Rys. 1. Chemiczna struktura glifosatu.
Source: Own elaboration based on source Soares et al. [42]
Źródło: Opracowanie własne na podstawie Soares i in. [42]

Glyphosate in salt form is more soluble than in acid form, hence GBHs consist of glyphosate in its salt form (isopropylamine, ammonium, sodium, potassium and trimethylsulfone). Of all these glyphosate salts, isopropylamine is the most commonly used in agriculture [51]. Components of GBH (besides glyphosate) are polar surfactants such as polyoxyethyleneamide (POEA), sulphuric acid and phosphoric acid [1, 49]. They enhance the herbicidal effect of glyphosate by increasing its water solubility and also promote its penetration and absorption in the plant [14, 55].

THE USE OF GLYPHOSATE

Herbicides containing glyphosate are classified as total herbicides, destroying mono- and dicotyledonous plants with some exceptions such as field horsetail [4]. They have a systemic effect, which means that the active substances, after penetrating plant tissues, move within them and also reach non-sprayed parts, including underground organs, which are also destroyed. They are used in the destruction of all kinds of unwanted vegetation in agricultural fields and other areas not used for agricultural purposes. Used responsibly, in accordance with the label, it allows farmers to obtain higher yields by minimising weed infestation [4, 26, 33, 52]. This, in turn, can also contribute to reducing the expenses incurred in protecting plants from diseases and pests, which are often spread to crops from weeds. In addition, it has a desiccating effect on plants, which is why it is also used for pre-harvest desiccation of rape and cereals in order to make them mature faster [4, 26, 33, 52]. The versatility of glyphosate has meant that its popularity has steadily increased since it was synthesised and patented by the US agrobiotechnology company Monsanto Company in the 1970s. This fact was exacerbated by the fact that this corporation also patented agricultural plant varieties resistant to Roundup, the so-called Roundup Ready® varieties. As reported by Soares et al. [42] with regard to glyphosate consumption in the EU, there is limited information available. Europe Direct (EDCC) and the European Statistical Office (EUROSTAT) were requested electronically for data on glyphosate consumption in the EU, but were informed that they did not have this data. The last available data from the EU, which dates back to 2003 [32]. It appears that in that year glyphosate was the most widely used herbicide in the EU. However, the amount used was reported as confidential.

TOXICOKINETICS AND EFFECTS ON HUMAN HEALTH

In both plants and animals, glyphosate is poorly metabolised [57]. It is excreted mostly unchanged and only less than 1% is metabolised, by hydrolysis to form glyphosate's main metabolite, aminomethylphosphonic acid (AMPA) [8, 19]. The main route of elimination of glyphosate in rats is faeces; approximately 60-70% of the administered dose is eliminated by this route [53]. The remaining 20 to 30% is rapidly eliminated via the urinary route [19, 50]. Excretion via the bile and lungs is residual [19]. The half-life of glyphosate is estimated to be between 6 and 12 h. The vast majority of glyphosate and its metabolites are excreted after 48 h, and virtually all are eliminated from the body after 7 days [16, 19].

As reported in the literature, glyphosate, as the main, most popular active substance of currently used herbicides, is not inert for animal organisms and humans. The consumption of its residues in food has been shown to correlate with the occurrence of, among others, hypertension, stroke, kidney failure, Alzheimer's disease, diabetes and cancer (mainly non-Hodgkin's lymphoma and breast cancer) [4]. It affects the reproductive capacity of organisms. Hormonal balance is also disturbed and the risk of foetal damage and birth defects increases. In this context, it is worth quoting the information on the use by women of intimate hygiene products made from cotton, in the cultivation of which glyphosate-based herbicides are used [4, 22]. Several studies have also shown that glyphosate and GBHs can cause oxidative stress and damage certain organs, particularly the liver, due to increased oxygen free radicals [22, 31, 42]. Assessment of the cytotoxicity of glyphosate and GBHs was made through a recent study on human cells. A study using human erythrocytes showed that GBHs caused morphological changes in these cells [28]. A meta-analysis published in 2019 showed an increased risk of non-Hodgkin lymphoma (NHL) in individuals heavily exposed to GBHs [56], while a review of epidemiological studies published in 2020 reveals an absence of association between glyphosate exposure and the occurrence of NHL [15]. Although several entities do not consider glyphosate to be toxic, several studies have nevertheless reported the effects of its toxicity, so until this issue is clarified, the precautionary principle is recommended [42]. The risk is compounded by information on the harmful effects of substances associated with glyphosate, such as surfactants (substances which facilitate penetration of the active substance into plant tissue) in herbicides and genetically modified varieties (GMOs). It is therefore important to remember that it is the herbicide itself which is far more toxic than the active substance – glyphosate – contained in it [4, 26, 33, 36, 52].

Due to increased concerns about glyphosate's toxicity, the European Food Safety Authority (EFSA) carried out in 2015 a review on the risk associated with the use of glyphosate, and the following toxicological endpoints were defined or reviewed based on laboratory studies in rabbits:

- Acceptable Daily Intake (ADI) of 0.5 mg/kg of body weight per day.
- Acute Reference Dose (ARfD) of 0.5 mg/kg of body weight per day.
- Acceptable Operator Exposure Level (AOEL) of 0.1 mg/kg body weight per day
- No Observable Adverse Effect Level (NOAEL) of 100 mg/kg body weight per day [16, 42].

LEGISLATION IN FOOD

Concerns about the possible toxicity of glyphosate and glyphosate-based formulations in recent decades have brought an increase in GBHs consumption [4, 26, 33, 52]. In 2016, the renewal of the marketing authorisation for GBHs was debated by the European Parliament (EP) and the resolution was rejected [47]. In 2017, the European Commission (EC) overturned the decision made earlier by the EP and decided to renew the approval for the sale of glyphosate in the European Union (EU) for a period of 5 years, until December 2022. However, due to growing concerns about the safety of POEA, a surfactant present in several GBHs, the EC banned the commercialisation of GBHs containing this ingredient in all its Member States [46]. The MRL corresponds to the maximum legal residue amount of a contaminant in food for human consumption. In the EU, the EC is responsible for setting MRLs acceptable in foodstuffs, which range from 0.1 µg/L in water to 20 000 µg/kg in oat cereals [45]. In 2019, at the request of the EC, a review of glyphosate MRLs was conducted by EFSA, the highest food safety authority at European level [17]. However, although EFSA has already published this review, to date the MRLs for glyphosate have not been updated by the EC [44].

ANALYTICAL METHODOLOGIES

Due to the fact that pesticides are a large variety of substances, with many residues belonging to different classes, their analysis is a difficult task. It may require liquid chromatography or SFC tandem mass spectrometry (LCMS/MS or SFC-MS/MS) or gas chromatography tandem mass spectrometry (GC-MS/MS) [35]. These triple quadrupole mass spectrometers are most commonly used in pesticide testing due to their high acquisition speed in selected reaction monitoring (SRM), which allows simultaneous testing of hundreds of pesticides in a single run with high sensitivity, selectivity and wide linear range [35]. The physicochemical properties of glyphosate, namely low molecular weight, high polarity, high solubility in water, low ionisation, low volatility and lack of ultraviolet absorption, make it a compound difficult to detect by conventional analytical methods [13, 29, 40, 54]. On the other hand, the absence of a chromophore group in the structure of glyphosate makes its direct detection by photometer coupled chromatography difficult and derivatisation is necessary to increase the sensitivity of the method [51]. Therefore, many alternative analytical methods have been developed for the detection and quantification of glyphosate in food [5, 6, 7, 8, 9, 10, 23, 24, 30, 38, 39, 42, 48, 54]. The method recommended so far by the European Union Reference Laboratory for Pesticide Residues is the HPLC-MS/MS method, which has the highest sensitivity and selectivity for assessing glyphosate in food [10, 25]. However, there are other methodologies with good sensitivity, namely UHPLC-MS/MS or FASI-MEKS with LOQ values, which are about 30 and 100 times lower than the specified MRLs (Maximum Residue Levels), respectively [42]. Because of the risks, MRLs have been set in the European Community for any food or feed where pesticides are used correctly, in accordance with the principles of GAP (Good Agricultural Practice), in order to ensure the lowest possible consumer exposure. Commission Regulation (EC) No 396/2005 lists 320 specific commodities for which more than 152,000 MRLs have been established [36]. Maximum residue levels for pesticides are published by the EU Commission and regularly updated, such as Regulation (EU) 2019/90 of 18 January 2019 [12].

OCCURRENCE IN FOOD

Studies have been conducted in several countries to assess human exposure to glyphosate through the analysis of different food categories (Table 1).

Studies in Canada [48] detected the presence of glyphosate in almost all honey samples. A multinational study conducted by EFSA revealed that in 186 honey samples, 24 contained glyphosate, 8 of which were higher than legally permitted [17]. Baby food has also been analyzed in several studies (Table 1). Studies in Switzerland [57] and France [27] did

Table 1. Occurrence of glyphosate in different kind of food
Tabela 1. Występowanie glifosatu w różnych rodzajach żywności

Country/Product	Number of Samples	Detection Frequency (%)	Reference
Honey			
Canada	200	98.5	[48]
USA	85	28.2	[3]
Several European Countries	186	12.9	[18]
Baby food			
Switzerland	11	0	[57]
Italy	15	13.3	[39]
France	71	0	[27]
Animal products			
Switzerland -Milk	3	0	[57]
Switzerland -Egg	1	0	[57]
Switzerland -Meat and fish	13	23,1	[57]
Water			
Switzerland -Surface water	151	0	[20]
Switzerland -Groundwater	29	89.7	[37]
Several European Countries -Surface water	50 805	28.9	[21]
Several European Countries - Groundwater	36 298	1.3	[21]
Fruit and vegetables			
China - Vegetables	35	0	[7]
France - Vegetables	14	0	[27]
Italy - Vegetables	83	18.1	[39]
Switzerland - Fruit juice	11	100	[57]
France - Fruit	6	0	[27]
Portugal - Orange	11	0	[41]
Cereal and cereal products			
Several European Countries – Wheat	676	9.0	[18]
Several European Countries – Rye	534	3.4	[18]
Switzerland – Breakfast Cereals	10	80	[57]
Switzerland – Wheat	18	88.9	[57]

Source: [42]
Źródło: [42]

not detect the presence of glyphosate, while an Italian study detected the presence of glyphosate in 2 samples, but none had levels above the MRL of 10 µg/kg defined [17]. A study in Switzerland did not detect the presence of glyphosate in the surface water samples analyzed [20]. In a European study, thousands of surface water and groundwater samples from several countries were analyzed. Glyphosate residues were detected in about 30% of surface water samples. In 80% of these samples, the values were much higher than the MRL, including a sample that was 500 times higher than allowed. Only 1% of the groundwater samples contained glyphosate, of which more than half had values that exceeded the MRL [21].

Due to the use of glyphosate in agricultural fields, several studies have determined levels of glyphosate in various cereals as well as in cereal-based foods (Table 1). Several samples of major cereals grown in Europe were analysed in a multinational study conducted by EFSA in 2017. The results revealed that glyphosate residues were present in a small percentage of samples [18]. In a study conducted in Switzerland, the presence of glyphosate residues was detected in several samples, with approximately 90% of wheat samples and 80% of breakfast cereal samples containing glyphosate residues [43, 57]. In recent years, studies evaluating glyphosate levels in vegetables and fruits have been conducted in several countries (Table 1). Studies from France [27] and China [7] did not detect the presence of glyphosate in several vegetables, while in Italy it was identified in 18.1% of samples.

In France, six samples were analyzed and no glyphosate residues were detected in any of the samples [27]. Another study, conducted in Switzerland, detected the presence of glyphosate in fruit samples [57]. In Portugal, in all the products of vegetable origin tested, no glyphosate residues were detected and the glyphosate MRL was not exceeded [41].

RISKS

Farmers cultivating genetically modified crops (with a gene that makes them resistant to Roundup or that breaks down glyphosate), especially in the form of large-scale maize and soya crops, are spraying this herbicide also during the growing season, leading to the creation of fields exclusively with arable crops [4]. It has emerged that, as a result of the continuous use of glyphosate over many years, weeds have also developed a tolerance to this substance over time. Their current forms have become more troublesome and more difficult to control with chemical means. Thus, weed control with herbicides in arable crops, including Roundup Ready® varieties, became even more difficult initially in the fields of US farmers. The abandonment of indigenous varieties and cultivation technology in favour of Roundup Ready® varieties and herbicides has contributed to the dependence of farmers on the corporation as a result of having to purchase seed and Roundup every year [4]. As a result, monoculture (continuous cultivation of the same varieties on the same site using glyphosate) leads to the depletion of plant and animal biodiversity, including soil microorganisms. Varieties that are well adapted to local conditions are being forgotten. Unfortunately, an attempt to return to traditional cultivation is associated with many difficulties resulting from, inter alia, rapid takeover of agricultural land not protected by herbicides by weeds which are difficult to control, including invasive ones [4].

CONCLUSION

It is observed that there has been an increase in the use of glyphosate in recent decades. At European level, there are only data on herbicide consumption in the EU and no information on the consumption of glyphosate itself. There is currently no scientific consensus on the toxicity of glyphosate and further independent research is needed to assess the toxicity of glyphosate as an active substance. The European Commission is responsible for setting the MRLs allowed in food in Europe, and these limits are reviewed periodically. EFSA reviewed the MRLs for glyphosate in 2019 at the request of the EC, but they are not sufficiently respected [42]. If the EC does not accept EFSA's findings, the national control reports on pesticide residues from each Member State will not adequately identify samples that may pose a risk to public health. In addition, studies conducted to assess human exposure to glyphosate through food in several countries have indicated glyphosate residues in a large number of samples, sometimes at values above the legal limits, which may pose a risk to vulnerable populations such as children and the elderly. It is necessary to increase the number of studies as well as the number of samples analysed in each study to get an accurate picture of glyphosate residues in food.

PODSUMOWANIE

Obserwuje się, że w ostatnich dziesięcioleciach nastąpił wzrost stosowania glifosatu. Na poziomie europejskim istnieją jedynie dane dotyczące zużycia herbicydów w UE i nie ma informacji na temat zużycia samego glifosatu. Obecnie w środowisku naukowym nie ma jednomyślnego stanowiska, co do toksyczności glifosatu, dlatego potrzebne są dalsze niezależne badania w celu oceny toksyczności glifosatu jako substancji czynnej. Komisja Europejska jest odpowiedzialna za ustalanie NDP dozwolonych w żywności w Europie, a limity te są okresowo poddawane przeglądowi. EFSA przeprowadziła przegląd NDP dla glifosatu w 2019 r. na wniosek KE, które wydają się nie być jednak wystarczająco respektowane [42]. Jeśli KE nie przyjmie ustaleń EFSA, krajowe sprawozdania kontrolne dotyczące pozostałości pestycydów z każdego państwa członkowskiego nie będą odpowiednio identyfikować próbek, które mogą stanowić zagrożenie dla zdrowia publicznego. Ponadto przeprowadzone badania w celu oceny narażenia ludzi na glifosat poprzez żywność w kilku krajach wskazały na pozostałości glifosatu w dużej liczbie próbek, czasami w wartościach przekraczających prawnie dozwolone limity, co może stanowić zagrożenie dla najbardziej narażonych populacji, takich jak dzieci i osoby starsze. Konieczne jest zwiększenie liczby badań, jak również liczby próbek analizowanych w każdym badaniu, aby uzyskać dokładny obraz pozostałości glifosatu w żywności.

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