Glyphosate-based phospho-organic herbicides – an outline of action, metabolism and the selected effects on humans and other organisms

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Abstract

Glyphosate (GP) is an active substance of the most common group of herbicides, applied to large cultivation areas as well as small allotments, home and industrial greeneries, those along traffic routes and around public areas. GP is highly valued for its lack of direct risks to humans and animals or no tendency to bioaccumulate in trophic networks. Over the past four decades of its use, GP was found to be extremely effective. However, controversies over its applications have been growing; therefore, it is increasingly implied that its effects on the environment, living organisms and people residing in it should be verified.

Key words: glyphosate, herbicides, plant pesticides

Glyphosate (GP) came onto the market 40 years ago [1]. This glycine analogue was first synthesised in the laboratories of Cilag in 1950, yet no pharmacological applications of GP were expected. The compound in which the amine group was combined with two acid groups (carboxyl and phosphate) was rediscovered, tested and patented in the first half of the 60ties and considered a chelating agent. Shortly after, its effects on plant physiology were appreciated in the Monsanto Company laboratories (USA). GP was found to be an extremely efficient, broad-spectrum and non-selective systemic herbicide, which sealed its marketing [2, 3]. Biochemically, N-(phosphonomethyl) glycine-based agents affect the synthesis of tryptophan, phenylalanine and tyrosine by impairing the conversion of shikimic acid into chorismic acid. In plants, fungi and numerous microorganisms, thanks to the activity of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS, EC2.5.1.19), shikimate 3-phosphate (S3P) is converted into 5-enolpyruvylshikimate-3-phosphate (ESSP) mediated by phosphoenolpyruvate (PEP). Glyphosate binds more strongly to the EPSPS active centre than the right intermediate substrate, which leads to shikimic acid accumulation in cells and deficiency of aromatic amino acids [4-7]. As a result, the plant cannot produce the dyes and flavone compounds essential for its functioning; after several-several dozen minutes, the plant dies together with underground elements. Effects of glyphosate on other pathways of organic polycyclic compounds are less known [8].

Considering the way of penetrating the plant through green parts and phloem transport,

the target group for the phospho-organic herbicides discussed is herbaceous plants, many of which are considered troublesome weeds (couch, knotgrass, buttercup). Once genetically modified cultivated plants resistant to glyphosate effects were produced, the value of this group of preparations became higher. Soya, wheat, maize, rape, cotton, sugar beet, alfalfa resistant to phosphonomethylglycine preparations are known as Roundup Ready (RR), (Roundup Ready 2 -RR2 in the case of varieties of improved crop yielding) or Glyphosate-resistant Crops (GRCs). Thanks to them, skilful use of GP enables the removal of redundant plants, both during pre- and post-sprout procedures. The resistance of plants resulted from the introduction of the gene encoding EPSP synthase resistant to glyphosate, isolated from the soil *Agrobacterium tumefaciens* sp. strain CP4 or from selection aiming at overproduction of EPSPS. The most recent solution is to provide the plants with a modified gene of soil *Bacillus licheniformis*, encoding glyphosate N-acetyltransferase (GAT). Joining the acetyl-group glyphosate molecule, GAT enables binding of a synthetic analogue to EPSP synthase. Recently, the possible use of class II EPSPS found in *Ochrobactrum anthropi* has also been considered. [9-13]. At present, after almost 20 years of their presence in agronomy, transgenic varieties constitute the essential profile of continent-wide cultivation. [14, 15]. However, the GRC rice form of global importance has not come onto the market, despite the availability of its appropriate varieties and competitive solutions [16-18].

From the ecological perspective, the use of glyphosate should be regarded as another factor for plant selection. Thus, the appearance of the varieties of several wild plant species of various GP resistances was not surprising [19-21].

Thanks to their easy use, even over huge areas, high efficacy of action and broad spectrum of applications, the agents containing glyphosate derivatives have been classified high in the rankings of production, sale and usage worldwide for many years [22, 23]. The patent protection expired at the beginning of the 20th century; therefore, many individual producers started their production of glyphosate-based herbicides (GBHs). Currently, more than 70 generic preparations are approved for sale in Poland (the list of the Ministry of Agriculture and Rural Development of 13.02.2015). At the same time, the list of formulae using qualitatively and/or quantitatively different adjuvant substances (in many cases not fully divulged by manufacturers) has lengthened. Currently, these herbicides are widely available in retail and wholesale trade. In 2015, the situation in the European Union countries is going to change due to the 128/2009 directive, imposing the selection of preparations available only for professional use. The most widely offered preparations include liquid concentrates based on markedly better soluble isopropyl salt, less frequently in the form of ammonium or potassium salt, most commonly containing 360g xenobiotic per 1000mL of preparation. To improve their usability, the agents are enriched with a variety of adjuvant substances, which do not show phytotoxic properties yet are indispensable for the active factors to permeate into the plant interior. Adjuvants prevent too quick flow of the preparation down leaves and shoots or its evaporation from their surface, facilitate permeation of the active substance through the stomata or individual anatomical barriers of

plants. In the case of N-(phosphonomethyl) glycine-base preparations, the surfactant applied most commonly is polyethoxylated tallowamine (POEA), which constitutes about 10-25% of preparation mass. Moreover, the addition of inorganic salts prevents inactivation of glyphosate by bivalent and trivalent cations [24, 25]. The recommended dose of the herbicides in question for the majority of applications, i.e. weed and redundant flora control, is 5-6 L preparation/ hectare; in well-grounded cases- removal of perennial plants, shrubs and trees, it is recommended to use the concentrate in the ratio of 1:1. No pre-harvest intervals or prevention periods for people, animals, and bees are determined; however, there are limit norms for glyphosate concentrations in the individual crops (table 1).

The resistance of C-P bond in the GP molecule to physical and chemical destructive factors is substantially hindered by its distribution. The action of C-P-lyase enzyme and glyphosate oxyreductase, occurring in bacteria and fungi, is essential. Microbiological, aerobic and anaerobic transformation of the xenobiotic into aminomethylphosphonic acid (AMPA), sarcosine and glyoxylic acid subsequently leads towards carbon dioxide, ammonium salts and phosphates [26-28]. Furthermore, another possible mechanism of degradation of glyphosate and AMPA is the pathways involving manganese oxide present in the soil [29]. The availability of glyphosate compounds to microflora can be limited by its ability to form complexes with bi- and trivalent metal ions, thus strong binding to the soil sorption complex. One of the consequences is limited availability of microelements to plants. Likewise, multi-molecular conglomerates can be formed. In extreme cases, a several dozen-day half-time of the xenobiotic increases to several years [30-32].

Table 1. Maximum residue levels (MRLs) of glyphosate in the individual groups of food products according to the EU Directive No. 293/2013 of March 20, 2013

No.	Groups of food products	MRLs [mg/kg]
1	citrus fruits	0.1-0.5
2	walnuts	0.1
3	pome fruits	0.1
4	stone fruits	0.1
5	berries and small fruits	0.1-0.5
6	various fruits	0.1
7	root and tuberous vegetables	0.1-0.5
8	onion-vegetables	0.1
9	fruit-vegetables	0.1*
10	cruciferous vegetables	0.1
11	leafy vegetables and fresh herbs	0.1
12	leguminous vegetables (fresh)	0.1
13	stalk vegetables	0.1
14	mushrooms	0.1-50.0
15	seaweeds	0.1
16	leguminous seeds	0.1-10
17	oil seeds	0.1-20
18	oil fruits	0.1-1
19	cereals	0.1-20
20	tea, coffee, herb infusions, cocoa, hops	0.1-2
21	spices	0.1
22	sugar-yielding plants	0.1-15
23	meat and products of meat, offal, blood, animal fats, - fresh, cooled, frozen, brined, dried or smoked, processed to flour or flour powders, and other processed products, such as cured meat products and food products based on these ingredients	0.05**
24	milk and sour cream (non-condensed and unsweetened), butter and other fats produced of milk, cheese and cottage cheese	0.05
25	bird's eggs – fresh or boiled, without eggshells or yolks, dried, moulded, frozen or preserved otherwise, with or without sugar or other sweeteners	0.05
26	honey	0.05
27	other land animal products	0.05

* except for maize, whose MRL is 3 mg/kg

**except for land animal kidneys, whose MRL is 2 mg/kg

The toxicological literature often raises the issue of additive effects of preparations containing glyphosate and POEA. Generally, reports regard model aquatic organisms and tissue cultures. A considerable proportion of studies discuss epidemiological findings of individual professional and social groups. In many cases, a common denominator of the papers mentioned is transgenic plants. However, GMO issues often concern not only the possibilities of uncontrolled spread of a modified genome in the natural environment. The use of large amounts of chemical pesticides over these cultivations, hence a high proportion of agricultural produce containing considerable xenobiotic residues is disquieting, as well [33]. An additional source of toxic exposure can be pre-harvest use of these herbicides as desiccants [34, 35].

Pre-harvest spraying of crops can contribute to relatively easy and uncontrolled spread of individual components of herbicides in the environment. The literature findings have confirmed possible transport of glyphosate with air movements (wind and rain) [36].

Ecotoxicologically, effects of N-(phosphonomethyl) glycine on humans and animals can be

considered from three aspects: a) direct contact with the preparations sprayed in agrocenoses, b) transfer of individual components of herbicides to surface waters, and c) penetration of herbicide residues to the food chain. Irrespective of the way of xenobiotic penetration to organisms, its effects can vary. Besides acute toxicity, the following issues should be considered: various changes occurring at subacute or chronic exposure, abnormalities in individual metabolic pathways, teratogenicity, disorders of the cell cycle, genetic dysfunctions, endocrine pathophysiology. As far as endocrine pathophysiology is concerned, possible inability to reproduce due to dysregulation of sexual functions should be taken into account. Widespread occurrence of endocrine disrupting compounds (EDCs) has been confirmed in various environmental elements [37]. Table 2 compiles the basic data regarding studies on pathogenicity of glyphosate and adjuvant agents. The remaining herbicide components present in traded preparations can enhance or widen their effects (e.g. on hormonal metabolism); therefore, less harmful substitutes have been searched for [38].

Table 2. Glyphosate (GP), polyethoxylated tallowamine (POEA) and their preparations (GP+POEA) as the factors modifying vital functions

Xenobiotic	Effects	Reference
Test organisms (laboratory conditions):		
Daphnia magna (GP, POEA)	LD50 (48 h) at concentrations of 690-900 µg/mL water for GP and 3.1 µg/mL water for POEA	[39]
Daphnia magna (GP, POEA)	LD50 (48 h) at concentrations of 4.2 mg/L water for isopropylamine salt; 38.1 mg/L and 48.9 mg/L for preparations containing POEA or otherwise, respectively	[40]
Vibrio fischeri (GP, POEA)	EC50 (disappearance of luminescence after 30-minute exposure) in the presence of 7.5 mg of isopropylamine salt in 1 litre of water; 5.4 mg/L and 7.6 mg/L for preparations with or without POEA, respectively	[40]
Escherichia coli MG1655 (GP, POEA)	EC50 (inhibition of colony growth after 5-hour incubation) for isopropylamine salt in the concentration of 506 mg/L medium, 548mg/L and 706mg/L for preparations with and without POEA, respectively	[40]
Pseudomonas putida KT2440 (GP, POEA)	EC50 (inhibition of colony growth after 5 hours) at the concentration of 343 mg/L medium for isopropylamine salt; 340 mg/L and 515 mg/L for preparations with surfactant or otherwise, respectively	[40]
rat (GP)	35-40% of 10 mg/kg b.w. of oral xenobiotic is absorbed from the gastrointestinal tract; about 1% of the dose applied was still present one week later, predominantly in the skeletal system	[41]
rat (GP)	100 mg/kg b.w. intravenously and 400 mg/kg b.w. orally - half-life from circulation 10 and 15 h, respectively; between post-administration hour 2 and 3, peak AMPA levels were observed, constituting about 10 % of maximum GP concentration	[42]
rat (GP)	animals receiving 0.5% and 1% solution of GP in water during pregnancy showed lower weight increases (which did not concern foetal body weight); visible differences in the activity of cytoplasmic enzymes of NADPH metabolism (glucose-6-phosphate dehydrogenase, malate and isocitrate dehydrogenase) of the maternal and foetal liver, myocardium, and brain	[43]

Daphnia magna (GP, GP+POEA)	acute toxicity (EC50 48h) was found in lower ranges of GP concentrations compared to both substances present together; reduced rate of organisms growth was observed at similar exposures to given doses with the tendency for quicker action of higher GP+POEA concentrations. At GP+POEA doses exceeding 1 mg/L, the majority of animals reached the reproductive age yet produced infertile gametes or gametes unable to develop embryos	[44]
Tadpoles of 4 species of North American tailless amphibians (GP, POEA, GP+POEA)	visible differences in mortality (LC50 24h), depending mainly on the presence of surfactants or otherwise; effects on larva development evidenced as prolonged time for reaching the final stage of metamorphosis in the presence of GP, particularly POEA, with markedly reduced numbers of surviving animals and their sizes	[45]
North American tadpoles of Rana cascadae (GP+POEA)	significant differences in subacute toxicity (average larva survival at concentrations of 0.1 and 2 ppm of xenobiotic in water) and the time of metamorphosis onset between experimental animals and controls	[46]
Nile tilapia (GP+POEA)	chronic toxicity (1-3-month exposure at concentrations of 5 and 15 ppm) observed based on markedly increased activity of aspartate aminotransferase and alkaline phosphatase in the most severe experimental system; moreover, histopathological changes in the branchiae and liver	[47]
pregnant female rats (GP)	glyphosate administered during pregnancy (in the form of 1% drinking solution) substantially modified the activity of hepatic lipid peroxidation, both in mothers and fetuses	[48]
rats of both genders expose to herbicides during embryonic life and M=maternal milk feeding	males demonstrated the tendency to accelerated sexual development; females – to delayed sexual development; in maturing males - reduced levels of testosterone; in sexually mature males – anomalies in spermatogenesis and sperm quality	[49]
rat foetuses developing under conditions of pregnant mother exposure (GP+POEA)	no statistically significant changes in embryo body weight and ratio of foetal genders; in several cases, foetal oedema, significantly increased incidence of skeletal developmental abnormalities, predominantly within the cranium (15.4%, 33.1%, 42.0% and 57.3%, respectively, as compared to the control group at doses of 500, 750, and 1000 mg GP/kg b.w. of mother)	[50]
embryos of clawed frogs and of hens (GP, GP+POEA)	intracellular injection of xenobiotic to embryos resulted in disorders of neural crest differentiation at the neurula stage, increased activity of retinoids and decreased activity of SHH; in exposed hen embryos, weaker development of rhombomeres and optic vesicles as well as microcephaly were observed	[51]

Tissue cultures, cell fractions:		
human JEG3 line placental cells (GP)	LD50 48h was found for the GP concentration of 1% medium volume	[52]
human HepG2, JEG3 and HEK293 line cells (GP, POEA, GP+POEA)	GP and all its preparations showed mitochondrial toxicity in each type of cells, manifested as reduced activity of succinate dehydrogenase, particularly in the presence of POEA alone (LC50 24h 1-2 ppm); damage to cell membranes (release of adenylate kinase) and cell apoptosis (activation of caspases) induced with POEA and GP, respectively	[53]
rat hepatic mitochondria (GP, GP+POEA)	significantly reduced intensity of mitochondrial respiration and mitochondrial membranous potential were only observed for a full-composition herbicide (concentration 0.5-5 mM), whereas GP was a neutral factor even at the highest concentration; inhibitory effects of GP+POEA on the activity of succinate dehydrogenase (complex II) and cytochrome c reductase (complex III)	[54]
Effects on the endocrine system:		
rat Leydig cells (GP, GP+POEA)	damage to cell membranes observed already after one-hour incubation in the presence of GP+POEA, 1000 ppm, decreased production of testosterone at concentration of 1 ppm	[55]
HEK 293 and JEG3 line cells and extracts from human and equine placental and nuclear cells (GP+POEA)	decreased activity of microsomal aromatase at a concentration of 0.5% of both chemical factors in all experimental systems	[56]
human HepG2 and MDA-MB453-kb2 line cells (GP+POEA)	substantial differences in cell reactions to individual types of preparations used as xenobiotics; markedly increased incidence of hepatocyte DNA damage (shuttle test) at concentrations of 5-10 ppm GP, in the presence of POEA - disorders of conversion of androgens to estrogens combined with a 130-250% increase in the number of aromatase-specific mRNA	[57]
cross male gonads (GP+POEA)	15-day exposure to both doses of GP+POEA (5 and 100 mg/kg b.w. of an adult animal caused an about 50% reduction in the androgen level at the lower dose of herbicide accompanied by a decrease in blood estrogen concentration; histopathological picture of gonads and efferent ducts with distinct morphological changes; reduced expression of androgen receptors in Sertoli cell nuclei yet no such changes within the epididymis ducts	[58]
rat males exposed to GP+POEA by the end of foetal life and as suckling with maternal milk	in the exposed group – higher expression of gonadotropin genes, elevated LH levels in the peripheral blood and pituitary and over two-fold higher levels of plasma testosterone and estradiol	[59]

Effects of genetic material, genetic information reading, cell cycle:		
bovine lymphocytes (GP)	17-170 uM GP at 72-hour incubation in vitro; substantially higher incidence of chromosomal aberrations and decreased mitotic index	[60]
mouse bone marrow cells(GP)	25 and 50 mg GP/kg b.w. at 24, 48 and 72-hour in vivo incubation; markedly higher percentage of cells with chromosomal aberrations, whose number increases with time, a reverse tendency for mitotic index	[61]
erythrocytes and branchial epithelial cells in <i>Prochilodus lineatus</i> (GP, POEA)	10 mg of preparation for one litre of aquarium water; 6-, 24 and 96-hour exposure, in the same experimental variants – a significant increase in the incidence of cell nucleus injuries yet no differences in a shuttle test	[62]
sea urchin eggs (GP+POEA)	increasing delay in cell cycle (time of the first embryo division) with an increase in water xenobiotic concentration (0-30 mM GP)	[63]
sea urchin embryos (POEA, GP+POEA)	effects of mini-molar amounts of GP on genetic material transcription expressed as reduced amounts of chorionase transcript and delayed larva hatching (abolished after xenobiotic removal), as well as dose –dependent reduction in the number of cell divisions	[64]
Epidemiological tests:		
Individuals involved in agriculture and their families (USA)	GP present in urine on exposure day in 60% of farmers and in 4-12% of family members, including children and teenagers assisting in field works, in the range of several to several hundred ppb (relatively easy method to limit the extent of contamination of farmers)	[65]
Children and teenagers below the age of 16 years from the families involved in farming (66 individuals) or otherwise (52 individuals)		
Individuals monitored for the presence of the most popular pesticides in urine	in the case of GP – no significant differences between both populations; the estimated daily dose below the EPA limit	[66]
monitoring of GP in human urine and in dairy cattle (intravitaly – urine, post-slaughter – internal organs)	marked differences in GP concentrations in urine of animals bred in various European regions (Germany vs. Denmark and traditional agricultural regions vs. regions considered GMO-free); xenobiotics present in all internal organs (muscles, lungs, liver, kidneys, spleen, intestinal wall). In humans, a similar relation was found for traditional- ecological diet as well as healthy individuals and those chronically ill	[67]
dairy cattle (Denmark)	100% GP exposure (10 – over 1000 ng/mL urine) coexisting with non-physiological values of enzymatic markers of cytotoxicity (creatine kinase, aspartate aminotransferase, glutamate dehydrogenase, alkaline phosphatase) and of nephrotoxicity (urine, creatine)	[68]

To sum up, the following should be emphasised: a) importance of glyphosate-based preparations for global economy, b) increasingly well defined qualitative and quantitative effects on living organisms, including individual vital functions, c) widespread and mass use, which combined with easy migration, even for long distances, can pose a widespread hazard of chronic exposure.

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