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Short communication

The effect of glyphosate, its metabolites and impurities on erythrocyte acetylcholinesterase activity

Marta Kwiatkowska[a,*], *Hanna Nowacka-Krukowska*[b], *Bożena Bukowska*[a]

[a] *University of Lodz, Faculty of Biology and Environmental Protection, Department of Environmental Pollution Biophysics, Lodz, Poland*
[b] *Institute of Industrial Organic Chemistry, Warsaw, Poland*

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ABSTRACT

Glyphosate [N-(phosphonomethyl)glycine] is used all over the world to protect agricultural and horticultural crops. According to initial reports, glyphosate has been considered to be safe for humans and animals; nevertheless, recent investigations had proven its toxicity. Extensive use of glyphosate and the conviction of its low toxicity leads to a situation in which it is used in excessive amounts in agriculture. That is why, we have investigated the effect of the most commonly used pesticide: glyphosate, its metabolites and impurities on acetylcholinesterase (AChE) activity (in vitro) in human erythrocytes, which is biochemically similar to acetylcholinesterase present in neural synapses.

The analysis of noxious effects of metabolites and impurities of pesticides seems to be very important to evaluate toxicological risk that is associated with the effect of pesticide formulations (requirement of the EU regulations 1107/200/EC).

The erythrocytes were incubated with xenobiotics at concentrations range from 0.01 to 5 mM for 1 and 4 h. Statistically significant decrease in AChE activity (about 20%) was observed only at high concentrations of the compounds (0.25–5 mM), which enter body only as a result of acute poisoning. There were no statistically significant differences in the effect of the investigated compounds, while the changes caused by them were similar after 1 and 4 h incubation. The investigated metabolites and impurities did not cause stronger changes in AChE activity than glyphosate itself.

It may be concluded that the compounds studied (used in the concentrations that are usually determined in the environment) do not disturb function of human erythrocyte acetylcholinesterase.

Abbreviations: AChE, acetylcholinesterase; AMPA, aminomethylphosphonic acid; PMIDA, N-(phosphonomethyl)iminodiacetic acid; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid).

* *Corresponding author*. Tel.: +48 426354453.
E-mail address: m.n.kwiatkowska@wp.pl (M. Kwiatkowska).

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1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is a herbicide used in 75% of all genetically modified crops (GMOs), which tolerate high concentrations of this compound. Glyphosate-based formulations are used all over the world to protect agricultural and horticultural crops (Clive, 2009). Widespread presence of glyphosate in the environment and human surrounding make necessity to analysis the effect of this compound on living organisms. According to literature reports, glyphosate can enter living organisms, including humans and exhibit various toxic effects (Sandrini et al., 2013; Cavalli et al., 2013; Paganelli et al., 2010). Glyphosate was detected at low concentrations in human blood (73.6 ng/ml) (Aris and Leblanc, 2011).

Worst case scenario provides even ten-fold increase in glyphosate use in the next years (Benbrook, 2012). The exposure to glyphosate concerns both agriculture workers and persons who do not have direct contact with this herbicide, e.g. consumers of products containing glyphosate (Chen et al., 2013; Bøhn et al., 2014).

The European Commission (EU) planned to verify the toxicity of glyphosate in 2012, but at the end of 2010, EU decided not to perform this verification up to 2015 (European Commission Directive 2010/77/EU).

Furthermore, in accordance with the Regulation of the European Parliament and Council Regulation 1107/2009/EC on 21st of October 2009, it is necessary to identify metabolites and impurities present in technical formulation of pesticides and undertake toxicological researches concerning these substances. Analysis of noxious effects of metabolites and impurities of pesticides seem to be very important during evaluation of toxicological risk that is exerted by pesticide formulations (Regulation of the European Parliament and Council Regulation 1107/2009/EC). There have been many experiments conducted in which it was observed that metabolites and impurities of pesticides revealed stronger toxicity than their parent compounds (Pohjanvirta and Toumisto, 1994; Sosnowska et al., 2013). Taking into consideration the overall spread and expanding use of glyphosate in agriculture, the risks concerning glyphosate utilization should be monitored.

Some studies have suggested that glyphosate may reduce acetylcholinesterase (AChE) activity (El-Demerdash et al., 2001; Menendez-Helman et al., 2012; Sandrini et al., 2013), therefore we have investigated the effect of glyphosate, its metabolites and impurities on AChE activity present in human erythrocytes membrane (in vitro).

There are two cholinesterases: the proper, also called acetylcholinesterase (E.C. 3.1.1.7), which is mainly contained in erythrocytes (including mammals) and neurons. The second is called butyrylcholinesterase (EC 3.1.1.8), and it is present in blood plasma (Li et al., 2006). Cholinesterases are also present in some organs (Carlock et al., 1999).

Determination of cholinesterase activity has a great application in diagnostic of poisonings with reversible and irreversible inhibitors of this enzyme, such as phosphororganics and drugs used in myasthenia gravis treatment. There are differences in diagnostic value of the results obtained during examination of AChE activity. Plasma cholinesterase is more susceptible to factors that are not associated with poisoning like infections or liver damage. The activity of this enzyme shows high interindividual differences, reflecting minor disturbances in nervous system.

Erythrocytes AChE is biochemically identical with the enzyme that is present in nervous tissue. It is characterized by lower interindividual differences and it is resistant to external factors. That is why measurement of its activity reflects dynamics of inhibitor absorption and disturbances in nervous system (Danysz, 2002). In many earlier studies, correlation between inhibition of AChE activity in blood and its inhibition in target tissues has been shown (Yang and Dettbarn, 2002; Kale et al., 1999). Rendon von Osten et al. (2004) studied AChE activity present in blood of farmers with symptoms of poisonings with organophosphate pesticides. The results of the above study showed that inhibition of erythrocyte AChE activity is useful biomarker of the exposure of workers to organophosphate pesticides (field studies).

Glyphosate concentrations tested in this study are within the range of the concentrations determined in blood of people who are not directly exposed to this pesticide (>0.05 mM) (Aris and Leblanc, 2011) or may enter human organism only as a result of acute poisoning (0.05–5 mM) (Zouaoui et al., 2013). We have studied the effect of glyphosate, two of its metabolites: aminomethylphosphonic acid (AMPA) and methylphosphonic acid and its four impurities: *N*-(phosphonomethyl)iminodiacetic acid (PMIDA), *N*-methylglyphosate, hydroxymethylphosphonic acid and bis-(phosphonomethyl)amine on erythrocytes membrane acetylcholinesterase activity (Fig. 1).

2. Experimental

2.1. Materials

The investigated compounds i.e. aminomethylphosphonic acid (AMPA) (purity 98%), methylphosphonic acid (purity 98%), *N*-(phosphonomethyl)iminodiacetic acid (PMIDA) (purity 98%), *N*-methylglyphosate (purity 98%), hydroxymethylphosphonic acid (purity 98%) and bis-(phosphonomethyl)amine (purity 97%) were provided by the Institute of Industrial Organic Chemistry, Warsaw, Poland. Glyphosate [*N*-(phosphonomethyl)glycine] (purity 95%), 5.5′-dithio-bis-2-nitrobenzoic acid (DTNB) and acetylthiocholine iodide were bought from Sigma–Aldrich, USA.

Human erythrocytes were isolated from human blood taken from healthy donors in the Blood Bank of Lodz, Poland. The erythrocytes were centrifuged (3000 rpm/min) and washed twice with phosphate-buffered saline (PBS; 150 mM NaCl, 1.9 mM NaH_2PO_4, and 8.1 mM Na_2HPO_4, pH 7.4).

The investigated compounds were dissolved in phosphate-buffered saline (pH = 7.4). The erythrocytes at 5% hematocrit were incubated at 37 °C (with continuous mixing) with solutions of the compounds used in the concentration from 0.01 to 5 mM for 1 and 4 h. Samples with the erythrocytes and sodium phosphate buffer without herbicide, its metabolites and impurities were used as controls.