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The concentration of glyphosate in the tap water in Greater Poland Region

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ABSTRACT: The harmfull effects of glyphosate (N-(phosphonomethyl) glycine) on animal and human health was stated by many researchers. The studies on such effects concerned mainly the people exposed to herbicides. In the environment, glyphosate remains relatively stable, with half-life ranged between a few days to several months or even a year in field studies, depending on soil composition. As this herbicide the widely used all over the world, the monitoring its concentration in everyday food becomes necessary. The aim of the study was to estimate the glyphosate levels in tap water samples collected from different Water Treatment Plants in Greater Poland region. The concentration of glyphosate was measured in 66 randomly collected drinking water samples from separate Water Treatment Plants. Measurements were done using two analytical techniques: enzyme-linked immunosorbent assay and high-performance liquid chromatography technique. Levels of glyphosate in the tested samples were low (0.15±0.07 µg/L). Both assays have been found well suited to the analysis of glyphosate concentrations in the drinking water. The concentration of glyphosate in the tap water is very low, and could be discarded in estimation of daily intake of this herbicide in Great Poland region. So, it is unlikely that drinking water from Water Treatment Plants can be important source of glyphosate contamination in urbanized populations compared to vegetables, fruit and other possible sources.

Keywords: Glyphosate; Water; Water Treatment Plants; Poland.

1. INTRODUCTION

The herbicide glyphosate (N-(phosphonomethyl) glycine) is a non-selective, systemic chemical agent. It is used for non-selective control of broad-leaved weeds and grass from agriculture to wine production and forestry. It also has found applications in non-food crop use, including weed control in aquatic habitats and that in non-cultivated areas [1]. It is the dominant herbicide in the world (constituting almost 72% of global pesticide burden [2]). In Poland, as in other parts of the world, the use of glyphosate has been growing. It is possible to estimate the growth based on the statement of the representative of Ministry of Agriculture and Rural Development. According to the statement, the sale of glyphosate increased from 4.4

million Kg in 2015 to 5.4 million Kg in 2016 [3]. However, in the last decade a significant number of papers indicated the harmful effect of glyphosate on animals, even at very low concentrations [4-6]. Moreover, the possibility of carcinogenic effects of glyphosate to humans [7, 8] caused a passionate debate, crossing from research to politics [9-12].

Glyphosate has a relatively short environmental half-life (up to about 70 days), being inactivated in soil by adsorption and microbial degradation, so its use is claimed to be safe for humans [13, 14]. Thus, it was believed only individuals in the occupational settings can be exposed to glyphosate [15, 16]. The thorough analysis of the available data showed that persistence of glyphosate in soil is highly variable, ranging from a few days up to two years. Moreover, the pesticide can be transported off-site by wind and water erosion [17], so many populations could be exposed to it's effects [18]. The increased contamination of human surroundings is confirmed by increased urinary excretion levels of glyphosate over the last 20 years [19, 20]. The widespread use of glyphosate has raised a concern with regard to its presence in ground water and, hence, in drinking water, the more so that this herbicide is suspected to have negative effects on mammalian health even at ultra-low concentration [21].

The aim of our study was, therefore, to estimate the glyphosate levels in tap water samples derived from different Water Treatment Plants facilities in Greater Poland.

2. MATERIALS AND METHODS

2.1. Water samples

The concentration of glyphosate was measured in 66 drinking water samples randomly collected from separate Water Treatment Plants facilities within Greater Poland (except of Poznań region) (Fig. 1) in the period October-December 2017. Samples from Soil and Water Survey Laboratory of Provincial Sanitary-Epidemiological Station were collected in dark brown glass bottles (200 mL), preserved with sodium thiosulphate to a final concentration about 0.002%, and stored at 4ºC before glyphosate estimations (up to two weeks).

2.2. The enzyme-linked immunosorbent assay (ELISA)

A 96-well ELISA kit (Glyphosate ELISA Plate Kit PN5000086 Abraxis Inc., PA, USA) was used for estimation of glyphosate concentrations in tap water. All the samples, at least in duplicates (in few cases in triplicates) were processed according to the manufacturer's instructions. Results of tests were read with an ELISA reader (EPOCH, BioTek). The concentrations of glyphosate in the samples was determined based on the standard curve plotted in each test run.

2.3. The high-performance liquid chromatography technique (HPLC)

Standard glyphosate solutions at concentrations between 100 pmol/L and 50 mmol/L were prepared in bidistilled water from standard stock glyphosate (CAS# 1071-83-6) (99.8, w/w, Instytut Przemysłu Organicznego, Warsaw, Poland). Analytical grade sodium borate (POCh, Poland), sulfuric acid (POCh, Poland), sodium hydroxide (POCh, Poland), potassium hydroxide (POCh, Poland), potassium dihydrophosphate (POCh, Poland), phosphoric acid (Fluka, Switzerland, HPLC grade) were used. As the derivatising agents, 9-fluorenylmethyl chloroformate (FMOC-Cl) (98%, w/w, Sigma-Aldrich) and 1-(hydroxymethyl) pyrene chloroformate were employed. Acetonitrile (HPLC grade, J.T. Baker), analytical grade dichloromethane (DCM) (POCh, Poland), chloroform (POCh, Poland), diethyl ether (Lachema,

Czechoslovakia) and bidistilled water were used as solvents. All analytical grade solvents were purified according to Purification of Laboratory Chemicals [22].

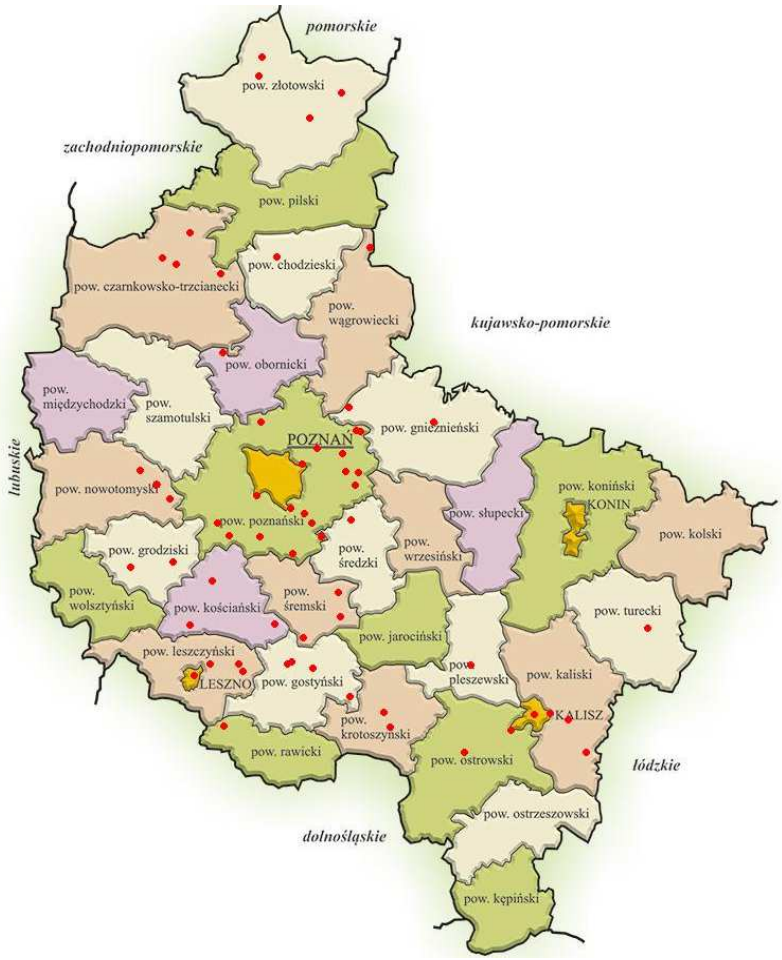

Figure 1. The localization of places (red dots) in Great Poland where water samples were taken (pow. - district).

Immediately prior to analysis, the derivatising solution was prepared by dissolving 26 mg of FMOC-Cl (or 29.5 mg of 1-(hydroxymethyl)pyrene chloroformate) in 50 mL acetonitrile (2 mmol/L, stored at refrigerator for maximum 24 hours). Next, at least a sixtyfold molar excess of derivatising reagent to all amino group was taken. Sodium borate buffer was prepared by dissolving 7.15 g of sodium borate in water (500 mL) and adjusted to pH 9.0 using sodium hydroxide or sulphuric acid.

2.4. Derivatisation

To 4 mL of the standard solution of glyphosate (100 pmol/L up to 33 mmol/L) or analysed tap water sample (unconcentrated or concentrated in rotary evaporator), 2 mL of sodium borate buffer was added, pH (9.0) was adjusted by acid/alkali when necessary, and 4 mL of freshly prepared FMOC-Cl (or 1-(hydroxymethyl)pyrene chloroformate) in acetonitrile was added. The test tubes were stirred for 30 min at 45-50ºC and cooled to 5-8ºC. To remove the excess of the derivatising agent, the samples were washed three times with 5 mL of DCM (or chloroform, or diethyl ether) (15 mL = 3 x 5 mL in total), then 2 mL of the aqueous layer was added from a Teflon syringe filter (0.22 µm).

2.5. Instrumentation

An auto-analytical HPLC system was made with degasser (ERCATECH ERC Solvent Degasser Model 310SP), pumping system consists of one Gilson 307 master pump (with manometric module and touchpad (used to programming the pumps)) and one Gilson 306 slave pump (equipped with 5 mL/min Stainless steel and 10 mL/min Ti heads, respectively), connected to a dynamic mixer (Gilson 811 C 1,5 mL) and a Gilson 231-402 auto-sampler with controller - keypad. Separation was performed on MetaChem Polaris NH_2 column (2 x 150 mm, 3 microns) (with guard column C18 packing) at 25ºC. Analog signal (voltage) from Dionex AD20 UV-Vis detector was converted by A/D HP 35900 interface module connected by GPIB cable to computer with Agilent PCI GPIB 82350 card and HP ChemStation 7.0. Starting signal (injection to the column) from the Gilson 231-402 auto-sampler induced (i) reading data from the detector and creating chromatogram from collected data, and (ii) appropriate pumps program.

High-performance liquid chromatograph capable of injecting 10-50 µL aliquots and utilising a pumping system with a constant flow rate of 0.45 or 0.50 mL/min was employed. Mobile phase 1:1(v/v): acetonitrile and 0.05 mol/l solution of KH_2PO_4 (adjusted to pH 5.0 by phosphoric acid or potassium hydroxide) was then filtered through a 0.22 µm Teflon filter. All tested water samples were analysed at least twice in the HPLC system.

2.6. Statistical data processing

Data distribution pattern was evaluated using the Kolmogorov-Smirnov criterion. The significance of the differences between the HPLC and ELISA findings were assessed by paired Student's test. Correlation between the HPLC and ELISA findings was evaluated by Pearson's correlation coefficient. The data were analysed with the Medcalc® (Belgium) statistical processing software.

3. RESULTS

Overall, concentrations of glyphosate in the tested samples were relatively low (M±m: 0.15±0.06 µg/L as shown by the HPLC and 0.15±0.07 µg/L for the ELISA). Both assays have been found well applicable to the analysis of glyphosate level in the drinking water. The HPLC method detected glyphosate in the range of 0.07-0.31 µg/L (95% CI for the mean: 0.12 to 0.18 µg/L), whereas the ELISA indicated its values from below the detection limit (0.1 µg/L) to 0.332 µg/L (95% CI for the mean: 0.12 to 0.18 µg/L).

The Kolmogorov-Smirnov test confirmed the acceptance of normality of the data distribution, so the Student's test was therefore applicable to the assessment of the differences between the two data cohorts. The differences between the HPLC and ELISA appeared to be non-significant ($p>0.2$), which showed that the two methods provide similar results.

Correlation between the data provided by both methods appeared to be high ($r = 0.97$) as shown by the regression analysis (Fig. 2). For all samples analysed in duplicates, the coefficient of variation for the parallel tests was in the range of 3.2% (for higher concentrations of glyphosate) to 14.3% (for lower concentrations).

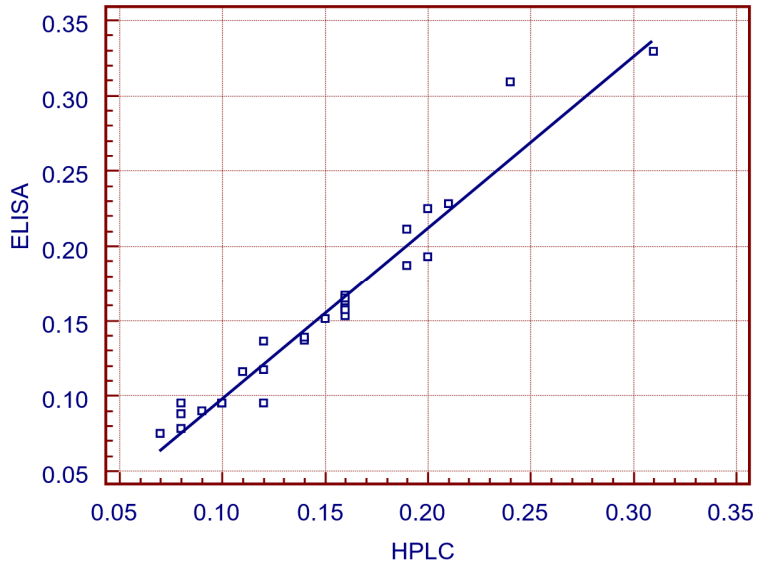

Figure 2. Regression chart for the glyphosate levels (µg/L) in drinking water samples detected by the HPLC (X) and ELISA (Y). The regression equation is Y = -0.0148 + 1.1373 X.

4. DISCUSSION

Glyphosate has been formally registered in more than 130 countries. It is the most heavily used herbicide in the world [2]. In the period from 2019 to 2024, the glyphosate market is expected to have the compound annual growth rate (CAGR) of at least 4.5% [23]. The use of the herbicide is currently regulated to protect human health and the environment, the more so that glyphosate has already been detected in various quantities in different human body fluids [18]. In the EU acceptable daily intake (ADI) levels of glyphosate-herbicide exposures for humans has been set at 0.5 mg/kg body weight per day [24].

Glyphosate was found in many groundwater samples collected all over the world [25, 26]. Its presence seems to be mainly influenced by major agricultural areas [27, 28], however non-agricultural uses may significantly contribute to the overall loads of glyphosate in surface waters [29, 30]. Because of glyphosate's polar structure, weak volatility and low molecule mass, measurement of glyphosate in drinking water (where it is present at low levels) is difficult [31]. Furthermore, all the aforementioned characteristics of the molecule and the lack of chromophore groups are the primary reasons for the analysis employing derivatisation [32]. A review [33] focussed on derivatisation in the glyphosate analyses concluded that, despite of its drawbacks, it is a necessary step to achieve high sensitivity of the assay. We decided to use the two analytical techniques, both included derivatisation of the measured agent, but based on different principles: ELISA and HPLC. The results obtained in both methods are consistent what is in agreement with earlier publications [34, 35]. The concentration of glyphosate in the tested samples was relatively low, not exceeding 0.33 µg/L, whereas, in the European Union, the maximum permitted limit is 0.1 g/L [36]. The concentration of glyphosate in one liter of the tap water in Great Poland region is very low, at least thousand times lower than ADI. So, it is unlikely that drinking water from Water Treatment Plants can be an important source of glyphosate in urbanized populations compared to vegetables, fruit and other possible sources [37, 38].

Glyphosate has been shown to pose danger for multicellular organisms. Its cytotoxicity, genotoxicity, nuclear aberration, chromosomal aberrations and DNA damage have been registered in humans [39]. Moreover, glyphosate is able to influence genetic information via modifying the epigenome [40]. There is

also data indicating that this herbicide can seriously affect the human endocrine [41], immune [42] and nervous systems [43, 44], either directly or via the microbiome. What is important, the adverse health effects may be occur at extremely low glyphosate concentration [21]. Thus, at least until all aspects of the potential harmful action of this agent on human health are deciphered, the monitoring of glyphosate in human food seems to be necessary.

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