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# Effective dose of ionic liquids with glyphosate

**Katarzyna Marcinkowska[1], Agnieszka Łacka[2]**

[1]Department of Weed Science and Plant Protection Techniques, Institute of Plant Protection – National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland,
e-mail: k.marcinkowska@iorpib.poznan.pl
[2]Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland

SUMMARY

The effective dose of six herbicidal ionic liquids containing glyphosate [*N*-(phosphonomethyl)glycine] was investigated. Varied biological activity of the tested compounds was observed depending on the type of cation and targeted plant species. In the case of common lambsquarters, the lowest effective dose was obtained for compounds containing didecyldimethylammonium and di(hydrogenated tallow)dimethylammonium cations. In the case of white mustard, the lowest $ED_{50}$ and $ED_{90}$ values were obtained for the reference compound, which contained glyphosate isopropylamine salt. These parameters were determined using dose efficiency curves based on log-logistic models with three or four parameters. The study indicates that ionic liquids with glyphosate may be used as a new form of this herbicide in the future.

**Key words:** dose-response, herbicidal ionic liquids, glyphosate, weed control, efficacy

## 1. Introduction

The use of chemicals is currently the most effective, cheapest and most commonly used method of weed control. Approximately 130 chemical compounds are used for this purpose as selective herbicides. Additionally, approximately 30 other chemical compounds are used as non-selective herbicides (Müller et al., 2014). Glyphosate [*N*-(phosphonomethyl)glycine] is the most widely used non-selective herbicide worldwide, and successfully combats a wide range of mono- and dicotyledonous weed species, both annual and perennial. The cultivation of genetically modified plants towards resistance to this herbicide is a relatively new area of application of glyphosate in some countries. The global area of cultivation of such crops is consistently growing, especially in such countries as the USA, Brazil, Argentina and China. In the preparations currently available on the market, glyphosate is most commonly present in the form of an isopropylamine salt, but other forms such as ammonium, sodium, potassium and trimethylsulphonium salts are also known.

The results of studies regarding the synthesis and biological activity of new forms of glyphosate as ionic liquids have been recently published by Choudhary et al. (2017) and Pernak et al. (2014).

The purpose of the present study was to determine the relationship between the glyphosate dose and the fresh weight of plants to estimate the effective dose ($ED_{50}$, $ED_{90}$) of six compounds with various cations, which are mostly of natural origin. The studies are a follow-up to research regarding the effectiveness of ionic liquids containing the glyphosate anion.

## 2. Materials and methods

### 2.1. Characteristic tested herbicidal ionic liquids with glyphosate

Six herbicidal ionic liquids containing glyphosate (*N*-(phosphonomethyl)glycine) with the chemical formula presented in Figure 1 were used in the studies. Their synthesis was described by Pernak et al. (2014). The commercial herbicide Roundup 360 SL, containing 360 g of glyphosate in the form of the isopropylammonium salt per 1 L of preparation (Monsanto Europe SA/NV, Antwerp, Belgium) was selected as the reference product. The tested compounds were synthesized at Poznań University of Technology, and their structures were confirmed by means of proton, carbon and phosphorous magnetic resonance spectroscopy. The cations of the tested compounds are listed in Table 1.

Despite the fact that glyphosate is a solid with a high melting point ($T_m$ = 200 °C) (Senseman et al., 2007), only one compound among the six synthesized salts was a solid. [Arq 2HT][Glyph], which possessed two long

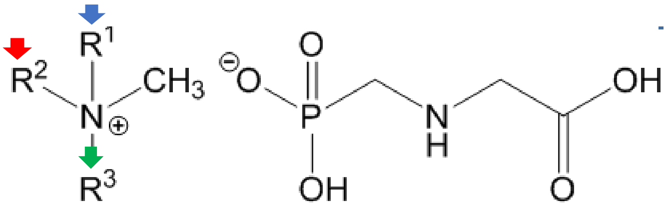


**Figure 1.** Chemical structure of glyphosate-based herbicidal ionic liquids

**Table 1.** Cations used in the tested HILs

| HILs | Cation | | |
|---|---|---|---|
| | $R^1$ | $R^2$ | $R^3$ |
| [DDA][Glyph] | $CH_3$ | $C_{10}H_{21}$ | $C_{10}H_{21}$ |
| [DecEtMor][Glyph] | $CH_3$ | $C_{10}H_{21}$ | - |
| [DADMA][Glyph] | $CH_3$ | $CH_2CH{=}CH_2$ | $CH_2CH{=}CH_2$ |
| [Etq O-12][Glyph] | $CH_2CH_2OH$ | $CH_2CH_2OH$ | Alkyl[a] |
| [Etq C-12][Glyph] | $CH_2CH_2OH$ | $CH_2CH_2OH$ | Alkyl[b] |
| [Arq 2HT][Glyph] | $CH_3$ | Alkyl[c] | Alkyl[c] |

[a]Oleyl – mixture of unsaturated alkyl substituents $C_{12}$–5%, $C_{14}$–1%, $C_{16}$–14%, $C_{18}$–80%. [b]Coco – mixture of saturated alkyl substituents $C_8$–5%, $C_{10}$–6%, $C_{12}$–50%, $C_{14}$–19%, $C_{16}$–14%, $C_{18}$–10%. [c]Hydrogenated tallow – mixture of saturated alkyl substituents $C_{12}$–1%, $C_{14}$–4%, $C_{16}$–31%, $C_{18}$–64%.

chains containing a mixture of (hydrogenated tallow) alkyl substituents, was found to have a melting point of 45 °C and a crystallization point of 38 °C during cooling. The remaining HILs were liquids with high viscosity, which exhibited only a glass transition temperature, as shown by Pernak et al. (2014). Those authors also reported that the lowest glass transition temperature was observed in case of [Etq C-12][Glyph], which had two hydroxyethyl groups in the cation; the highest glass transition temperature was recorded for [DDA][Glyph], which lacked hydroxyethyl groups in its structure. Pernak et al. (2014) also showed that all synthesized herbicidal ionic liquids containing glyphosate were characterized by high thermal stability ($T_{onset}$ > 200 °C).

### 2.2. Greenhouse experiments

The greenhouse experiments were carried out at the Institute of Plant Protection – National Research Institute in Poznań, Poland. Common lambsquarters and white mustard seeds were sown in 0.5 L plastic pots filled with a commercial medium for cultivation of plants with a slightly acidic pH (pH 6.0). The pots with plants were then placed in a greenhouse, in which the following environmental conditions were maintained: temperature 20 ± 2 °C, photoperiod 16/8 hours (day/night), humidity 60%. After germination the plants were thinned, leaving 5 plants in each pot. The plants were watered as needed. The ionic liquids and reference product were dissolved in water, or in a mixture of water and ethanol with a volume ratio of 1:1 for [Arg 2HT][Glyph], in 7 doses of active ingredient corresponding to 22.5, 45, 90, 180, 360, 720 and 1440 g $ha^{-1}$. After the six-leaf stage was achieved (BBCH 16), the plants were treated with spray solution (100 mL) containing the tested compounds, using a cabin sprayer (APORO, Poznań, Poland) equipped with a TeeJet® VP 110/02 nozzle (TeeJet Technologies, Wheaton, IL, USA). The pressure of the liquid in the atomizer was equal to 2.105 Pa, and the liquid flow was calculated as 200 L per 1 ha. The sprayer moved above the plants (40 cm from their tops) at a constant speed of 3.7 m $s^{-1}$. Tests of 7 compounds at 7 doses and two control samples (water and water + ethanol) were performed in a completely randomized system with four replicates for the two tested plant species. After treatment, the plants were placed in the greenhouse again for a period of three weeks, and after that time their weight was determined.

### 2.3. Statistical methods

The effect of glyphosate was investigated on the basis of measurements of fresh plant mass. In order to determine the relationship between the glyphosate dose and the fresh weight of the plants, a method based on dose response curves was used. The reduction of plant mass with increasing glyphosate dose was described using an appropriate non-linear regression model. In cases where the assumption of homogeneity of variance was not satisfied, the Box–Cox transformation with the λ parameter was used. The optimal λ value was determined using a profile likelihood approach: for each lambda value the dose-response regression model was fitted and the lambda value (and corresponding model fit) resulting in the largest value of the log likelihood function was chosen (Carroll and Ruppert, 1988; Ritz et al., 2015).

The best fit was obtained in case of a log-logistic curve with four or three ($c = 0$) parameters, based on the function:

$$f(x,(b,c,d,e)) = c + \frac{d-c}{1+\exp\{b(\log(x)-\log(e))\}} \quad (1)$$

where the parameter $e$ corresponds to the $ED_{50}$ value and denotes the dose for which the function assumes a value which is half the distance between its upper limit $d$ and lower limit $c$ (Streibig et al., 1993). The relevance of the model (lack-of-fit) was examined. A classical lack-of-fit test (Bates and Watts, 1988) was used to compare the dose-response model with a more general ANOVA model using an approximate F-test. Based on the fitted models, the average effective dose ($ED_{50}$) and 90% effective dose ($ED_{90}$) were determined for each compound. The analyses were performed using the DRC package (Ritz and Streibig, 2005) for the R environment (The R Core Team, 2013).

## 3. Results

The dependence of fresh plant weight on the glyphosate dose was determined for both plant species – common lambsquarters and white mustard. The control (dose "0") was treatment with water/ethanol in the case of [Arq 2HT][Glyph], and treatment with water in the case of the remaining HILs. The curves were determined only for compounds which exhibited activity in the range of tested glyphosate doses.

In the case of white quinoa a log-logistic model (1) with four parameters was fitted for the compounds [DDA][Glyph], [DecEtMor][Glyph], [Etq O-12][Glyph] and [Etq C-12][Glyph]. In the case of Glyph-IPA, the analogous model at $c = 0$ was appropriate. Since, as already mentioned, the control sample for the ionic liquid [Arq 2HT][Glyph] was an aqueous-ethanolic solution, this compound was analysed separately. An appropriate model (1) with four parameters was used. The determined curves are shown in Figure 2, and the significance of their fitting and values of the parameter λ are listed in Table 2.

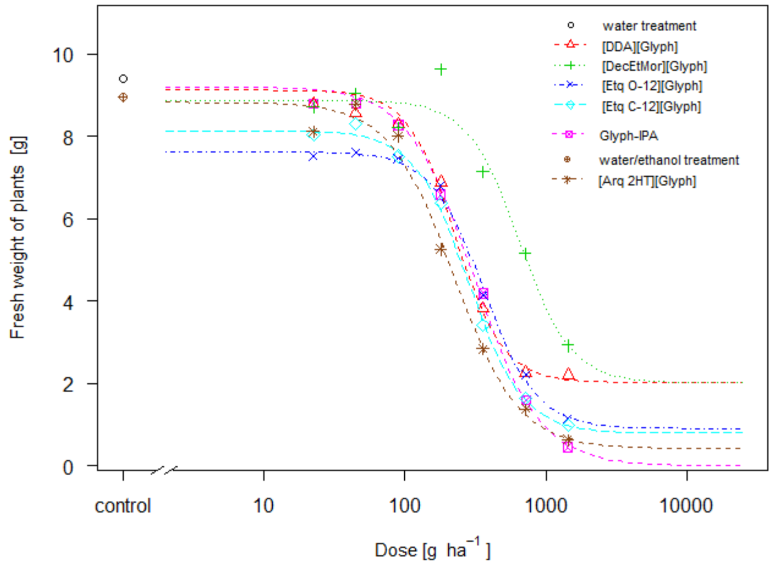


**Figure 2.** Dependence of fresh weight of common lambsquarters on glyphosate dose

**Table 2.** Significance of fitting of the dose-response curves (common lambsquarters)

| | Model Df | RSS | Df | F | p |
|---|---|---|---|---|---|
| | [DDA][Glyph], [DecEtMor][Glyph], [Etq O-12][Glyph], [Etq C-12][Glyph] | | | | |
| ANOVA | 98 | 15.07 | - | - | - |
| DRC model (λ=0.465) | 111 | 16.26 | 13 | 0.59 | 0.85 |
| | Glyph-IPA | | | | |
| ANOVA | 35 | 4.46 | - | - | - |
| DRC model (λ=0.545) | 40 | 4.71 | 5 | 0.38 | 0.86 |
| | [Arq 2HT][Glyph] | | | | |
| ANOVA | 24 | 3.87 | - | - | - |
| DRC model (λ=0.505) | 28 | 4.30 | 4 | 0.67 | 0.62 |

The effective doses of the new forms of glyphosate with respect to common lambsquarters, and other parameters of the log-logistic curve, are given in Table 3.

**Table 3.** Effective dose of new forms of glyphosate with respect to common lambsquarters, and other parameters of the log-logistic curve

| Treatment | *e* - $ED_{50}$ | $ED_{90}$ | *b* | *c* | *d* |
|---|---|---|---|---|---|
| [DDA][Glyph] | 229.50 | 539.98 | 2.57 | 2.03 | 9.13 |
| [DecEtMor][Glyph] | 651.01 | 1648.48 | 2.36 | 2.01 | 8.88 |
| [Etq O-12][Glyph] | 364.51 | 939.57 | 2.32 | 0.91 | 7.63 |
| [Etq C-12][Glyph] | 282.47 | 744.91 | 2.27 | 0.79 | 8.14 |
| [Arq 2HT][Glyph] | 222.93 | 710.63 | 1.90 | 0.42 | 8.83 |
| Glyph-IPA | 308.92 | 1005.31 | 1.86 | 0 | 9.21 |

*b* – curve slope factor; *c* – lower limit; *d* – upper limit

In the determination of the effective dose of the compound, the parameter *c* (the lower limit of the curve) is crucial, providing insight regarding the values to which plant weight can be reduced using the proper dose of the given compound. For this experiment, the ionic liquids [DDA][Glyph] and [DecEtMor][Glyph] reduced the mass of white quinoa plants to approximately 2 g, and the $ED_{50}$ for the first compound was already equal to a dose of 229 [g a.c. $ha^{-1}$]. However, in order to achieve a 50% reduction with [DecEtMor][Glyph], this compound should be used at a dose corresponding to 651 g of active substance per hectare. The best result for the average effective dose was achieved using [Arq 2HT][Glyph] ($ED_{50}$ = 223). The $ED_{90}$ values for ionic liquids were found to lie in a range from 540 to 1005. The tested compounds may be ranked from lowest to highest $ED_{90}$ as follows:

$ED_{90}$ [DDA][Glif] < $ED_{90}$ [Arq 2HT][Glif] < $ED_{90}$ [Etq C-12][Glif] <
< $ED_{90}$ [Etq O-12][Glif] < $ED_{90}$ Glif-IPA < $ED_{90}$ [DecEtMor][Glif]

The average reductions of fresh mass of white quinoa by the tested compounds are given in Table 4. [DDA][Glyph] begins to work at a dose of 90 g glyphosate per hectare. However, at doses of 720 and 1440 [g $ha^{-1}$], clear effects were already observed (Figure 3). In contrast, [DecEtMor][Glyph] exhibited much lower performance: the first effects were visible only at a dose of 720 [g $ha^{-1}$].

**Table 4.** Mean fresh weight reduction of common lambsquarters for the tested compounds

| Treatment | Mean fresh weight reduction (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 22.5 g $ha^{-1}$ | 45 g ha-1 | 90 g $ha^{-1}$ | 180 g $ha^{-1}$ | 360 g $ha^{-1}$ | 720 g $ha^{-1}$ | 1440 g $ha^{-1}$ |
| [DDA][Glyph] | 6.43 | 8.93 | 35.89 | 26.83 | 59.38 | 75.88 | 76.62 |
| [DecEtMor][Glyph] | 7.28 | 3.77 | 27.60 | -2.50 | 24.10 | 45.09 | 68.86 |
| [DADMA][Glyph] | 20.24 | 24.79 | 30.00 | 21.25 | 28.08 | 30.00 | 44.58 |
| [Etq O-12][Glyph] | 20.03 | 19.13 | 53.48 | 27.79 | 55.87 | 76.51 | 87.70 |
| [Etq C-12][Glyph] | 14.43 | 11.64 | 50.64 | 32.20 | 63.58 | 82.49 | 89.40 |
| [Arq 2HT][Glyph] | 9.27 | 1.82 | 55.92 | 41.12 | 68.24 | 84.58 | 92.85 |
| Glyph-IPA | 6.62 | 6.46 | 44.00 | 29.97 | 55.47 | 82.94 | 95.19 |

**0 22,5 45 90 180 360 720 1440** [g $ha^{-1}$]

**Figure 3.** The herbicidal effects of [DDA][Glyph] against common lambsquarters depending on the dose of glyphosate

In the case of the herbicidal ionic liquid [DADMA][Glyph] no effective herbicide dose was determined, since this compound showed very poor efficacy of action against common lambsquarters. [Etq C-12][Glyph] and [Arq 2HT] [Glyph] exhibited an effect at a dose of 180 [g $ha^{-1}$], whereas for [Etq O-12]

[Glyph] the first clear symptoms were observed at a dose of 360 [g ha$^{-1}$]. For the commercial preparation, the average effective dose was equal to 309 [g ha$^{-1}$] (Table 3).

The dependence of fresh weight of white mustard plants on the dose of the active ingredient is presented in Figure 4. The model (1) with four parameters was adapted for both the test compounds and the reference preparation Glyph-IPA. The significance of model fitting is presented in Table 5. In this experiment, there was no significant difference between the tested compounds (Table 6 and 7). The average plant mass values for the compounds [DDA][Glyph] and [DADMA][Glyph] were slightly higher than in the control sample only at the lowest dose. The lower limits of most curves for tested salts assumed similar values (ranging from 1.1 to 1.7). The exception was [DecEtMor][Glyph], for which the parameter *c* took the value 2.73 (Table 6).

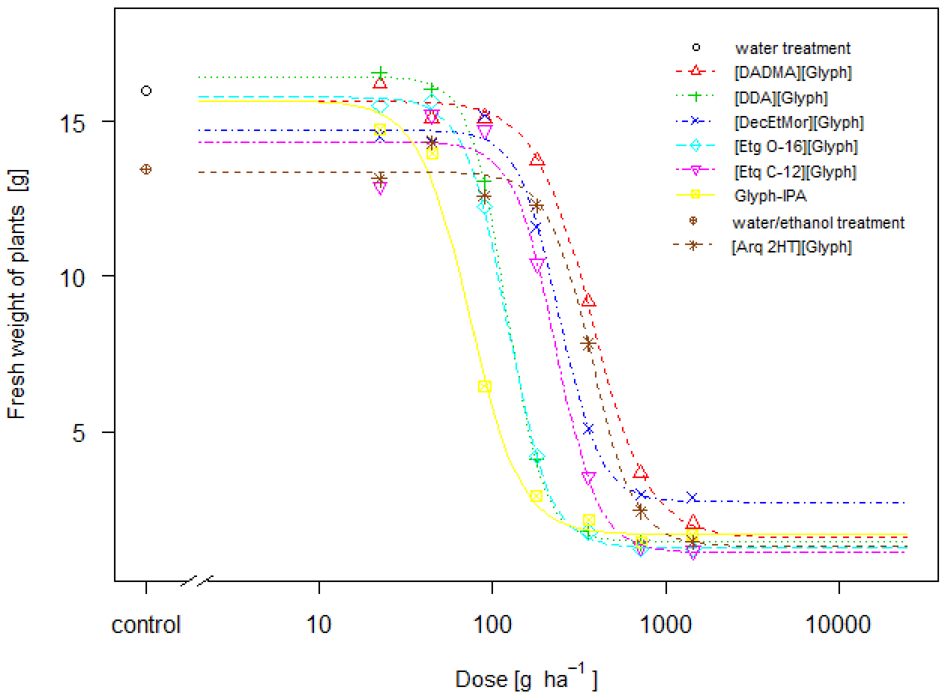


**Figure 4.** Dependence of fresh weight of white mustard plants on glyphosate dose

**Table 5.** Significance of fitting of the dose-response curves (white mustard)

| | Model Df | RSS | Df | F | p |
|---|---|---|---|---|---|
| | [DDA][Glyph], [DecEtMor][Glyph], [DADMA][Glyph], [Etq O-12][Glyph], [Etq C-12][Glyph], Glyph-IPA | | | | |
| ANOVA | 129 | 24.329 | - | - | - |
| DRC model (λ=0.465) | 148 | 26.477 | 19 | 0.5994 | 0.9013 |
| | [Arq 2HT][Glyph] | | | | |
| ANOVA | 24 | 3.124 | - | - | - |
| DRC model (λ=0.545) | 28 | 3.690 | 4 | 1.0867 | 0.3853 |

**Table 6.** Effective dose of new forms of glyphosate with respect to white mustard, and other parameters of the log-logistic curve

| Treatment | $e$ – $ED_{50}$ | $ED_{90}$ | $b$ | $c$ | $d$ |
|---|---|---|---|---|---|
| [DDA][Glyph] | 120.75 | 207.04 | 4.08 | 1.47 | 16.43 |
| [DecEtMor][Glyph] | 243.64 | 443.10 | 3.67 | 2.73 | 14.72 |
| [DADMA][Glyph] | 375.53 | 865.60 | 2.62 | 1.59 | 15.64 |
| [Etq O-12][Glyph] | 121.86 | 226.74 | 3.54 | 1.25 | 15.77 |
| [Etq C-12][Glyph] | 232.42 | 430.76 | 3.56 | 1.10 | 14.34 |
| [Arq 2HT][Glyph] | 373.68 | 713.00 | 3.40 | 1.30 | 13.37 |
| Glyph-IPA | 74.19 | 155.93 | 2.96 | 1.69 | 15.67 |

*b* – curve slope factor; *c* – lower limit; *d* – upper limit

The lowest values of the lower limit of curves were observed for herbicidal ionic liquids containing Ethoquad O-12 in their structure. In the case of ionic liquids with Ethoquad C-12 and Arquad 2HT at high concentrations (doses) up to 90%, reduction of fresh plant weight was achieved (Table 7). The lowest efficacy among the tested compounds was observed for [DecEtMor][Glyph], which reduced the plant weight by an average of 82.6% at the highest tested concentration. The lowest $ED_{50}$ and $ED_{90}$ values were obtained for the commercial agent and for [DDA][Glyph] and [Etq O-12][Glyph]. The worst result for both parameters was observed in case of [DADMA][Glyph].

**Table 7.** Mean fresh weight reduction of white mustard for the tested compounds

| Treatment | Mean fresh weight reduction (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 22.5 g ha$^{-1}$ | 45 g ha-1 | 90 g ha$^{-1}$ | 180 g ha$^{-1}$ | 360 g ha$^{-1}$ | 720 g ha$^{-1}$ | 1440 g ha$^{-1}$ |
| [DDA][Glyph] | -0.47 | 2.79 | 20.54 | 75.14 | 89.22 | 91.38 | 91.15 |
| [DecEtMor][Glyph] | 11.78 | 13.04 | 7.94 | 29.52 | 69.10 | 81.85 | 82.61 |
| [DADMA][Glyph] | 1.70 | 8.33 | 8.21 | 16.67 | 44.35 | 77.82 | 87.69 |
| [Etq O-12][Glyph] | 6.00 | 5.07 | 25.70 | 74.38 | 89.31 | 92.61 | 92.73 |
| [Etq C-12][Glyph] | 21.63 | 7.67 | 10.64 | 36.87 | 78.40 | 92.15 | 93.00 |
| [Arq 2HT][Glyph] | 2.39 | -6.39 | 6.42 | 8.72 | 41.80 | 81.87 | 89.24 |
| Glyph-IPA | 10.46 | 15.23 | 60.72 | 82.17 | 86.92 | 91.29 | 90.00 |

## 4. Conclusions

Varied efficacy of glyphosate-based ionic liquids was established depending on the type of cation and the species of the targeted plant. The compounds [Arq2HT][Glyph], [DDA][Glyph] and [Etq C-12][Glyph] exhibited higher efficacy with respect to common lambsquarters than the isopropylamine salt of glyphosate. In the case of white mustard, the isopropylamine salt of glyphosate was found to have a higher efficiency than the tested ionic liquids. Glyphosate-based ionic liquids may be an interesting solution for new glyphosate formulations.

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