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Effect of Glyphosate on the Generation Parent and Four Successive Generations to *Daphnia magna*

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Abstract

This present study focuses on assessing the toxicity of glyphosate on *Daphnia magna*. The first part of this work was devoted to evaluate the acute and chronic toxicity of the herbicide studied according to the Moroccan standards ISO 6341 and ISO 100706. Analysis of the test results revealed that glyphosate is toxic to *D. magna*. This effect is marked by short-term responses at high concentrations, immobility of juveniles (24h), and chronic disturbances (21d) onthe growth and reproduction of this species. Then, the second part was dedicated to evaluating the toxicity of glyphosate over four successive generations from the F0 mother generation to the F4 daughter generation. The results show that the toxic effect of glyphosate was more pronounced in the F4 daughter generation than in the F0 parent generation.

Keywords: Acute Toxicity, Chronic Toxicity, *D. magna*, Four Successive Generations

1. Introduction

Pesticides are introduced by humans into the environment through different industries (chemical, agrochemical) to increase agricultural production and achieve global food sufficiency. Given its status as a final receptacle, the aquatic environment includes the ecosystems most affected,either directly or indirectly, by the harmful effects of these substances[14,25].

Over the past decade, glyphosate-based pesticides have been the most widely used agricultural chemicals on earth2.Since the introduction of genetically modified seeds in 1996, the global use of pesticides has increased almost 15-fold amounting to 826,000 tons[3]. Due to its non-selective nature, it is used in a variety of crops such as corn, cotton, soybeans, cereals, legumes as well as in pasture, forestry, vegetation control on industrial land, and for domestic maintenance[3,5,12,28,30,22].

In response to the dangerous potential that glyphosate represents for wildlife and human health[4], several studies in the risk assessment field, have shown the toxic effect of this herbicide. However, adverse effects of glyphosate have been observed in living organisms at different levels of the food chain (bacteria, algae, crustaceans, amphibians and fish)[1,6,8,23,27]. This pesticide can inhibit specific enzymes in invertebrates such as phosphoenolpyruvate[32], and phosphatase[17]. In addition, it can also alter the expression of stress-sensitive genes[13]. Exposure to glyphosate can cause oxidative stress, inhibition of acetylcholinesterase and increases susceptibility to parasitic infections[1]. Such pesticide has been the subject of a major international issue concerning the carcinogenic risk of its active ingredient on human health. In January 2015, the administrative court of Lyon canceled the marketing authorization of Roundup Pro 360, which contains glyphosate.

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In Morocco, the approval procedure and the marketing of pesticides for agricultural use is governed by laws and regulations. This legislation aims to evaluate physio-chemical, analytical, toxicological, and eco-toxicological data prior to placing the pesticide on the Moroccan market. Despite this strict regulation and the classification in 2015 of glyphosate as a carcinogen by the World Health Organization, products based on this pesticide for agricultural use, with the exception of the one combining glyphosate with the Co-formulant POE-Tallowamine[24], are still used in Morocco market under the control of ONSSA.

The aim of this work is the evaluation of the acute and chronic toxicity of the herbicide glyphosate using *Daphnia magna* as a biological model. This invertebrate represents the most sensitive taxon among aquatic organisms[19] and has been widely used in bioassays to assess aquatic toxicity.

2. Material and Methods

2.1 Conditions for the Cultivation of *Daphnia magna* in the Laboratory

The *D. magna* used in the bioassays is maintained in the ONEE-water branch laboratory accredited according to NM ISO 17025[11] since 2005. The Daphnia of clone 5 was obtained from a culture from the MEDZ toxicology and ecotoxicology laboratory. The breeding was carried out in a room temperature controlled to adjust at 20 °C ± 2 °C. The water culture has a pH of 7.8 ± 0.2, a total hardness of 250 ± 25mg /L and a concentration of dissolved oxygen greater than 80% of the saturation in the air. *D. magna* were fed daily with a synthetic food (Tetramin) and natural food containing a mixture of algal culture (9.106 cells of *Chlorella pyrenodoisa/day/Daphnia* and 1.25.106 cells of Scenedesmus subspicatus/day/Daphnia). The breeding environment of daphnia was completely renewed three times a week.

2.2 Acute Toxicity Test of *Daphnia magna*

The stock solution of glyphosate used in this work is an aqueous solution of herbicide that contains 480 g/L of glyphosate IPA salt. Before each renewal of the test medium, dilutions were prepared from this solution.

In this study, we performed acute tests according to NM ISO 6341[34] to evaluate the short-term toxicity of the herbicide. It is a test to determine the concentration causing immobility in D.magnaafter 48 hours of exposure. Five neonates (less than 24 hours old) are placed in a 15 mL glass tube containing 10 mL of the water culture and the desired concentration of toxicant. For each concentration, four replicates were prepared in addition to the control. Daphnia was not fed and the test takes place in a static system (no renewal).This exhibition will take place under a day/night cycle of 16h/8h.After 24 and 48 hours of exposure, the immobility was observed. The concentration range used in the test: 0,8,10,11,12,14,16,18 (mg/L).

2.3 Chronic Toxicity Test of *Daphnia magna*

To evaluate the long-term toxicity of the pesticide on *D. magna*, we performed the chronic test according to NM ISO 10706[33].This test evaluates the effect of a toxic effluent on the reproduction and growth rate in the population of the species over a 21-days exposure period. The neonates (less than 24 hours old) were individually placed in a glass containing 50 mL of the water culture, the food and the concentration of the desired toxic element. For each concentration, 10 replicates were made in addition to the control. The medium was renewed 3 times per week throughout the test. The exposure takes place under a day/night cycle with a 16 h photoperiod. The concentration range used in the test: 0, 1.5, 2.5, 3.5, 4.5, and 5.5 (mg/L).

The introduction of pesticides into aquatic environments can have chronic effects on individuals and the population as a whole, leading to a disruption in the health of these ecosystems. Our results underline the interest of using multi-generation tests to study the long-term effects of these substances on a key freshwater species *Daphnia magna*. For this reason, the chronic toxicity of the herbicide glyphosate was evaluated across five generations of the crustacean. Individuals from the parental generation (F0) and the offspring (F1, F2, F3, F4) are exposed to glyphosate at concentrations of 1.5 to 5.5 mg/L. Survival and reproduction are monitored to measure the effects on the different generations.

These tests follow the same conditions as the chronic tests except the exposure time that is reduced to 14 days for the daughter generations (F1, F2, F3).

2.4 Statistics

For an interpretation of the results of the statistical tests, two software packages were used;Probit (Probit version 1.5, USEPA, OH, USA); and Statistica (STATISTICA version 6 for Windows, Statsoft, Tulsa, OK, USA).The determination of significant differences between the control groups and the different glyphosate concentrations tested varied according to the data. If the conditions are met, a single-factor ANOVA followed by Dunnett's post-hoc test is used. However, if one of the conditions is not established, a non-parametric method, the Kruskal-Wallis test followed by the

Mann-Whitney U-test, is used. Statistical significance is established at $p<0.05$.

The comparison between the different generations is performed by Fisher's exact test (one-tailed $P<0.05$).

3. Results

3.1 Lethal Effect of Glyphosate on *Daphnia magna*

Acute exposure has shown that glyphosate has a lethal effect on *D. magna*. Compared to controls, the percentage of immobility, increases significantly with the increase inconcentration levels of the pesticide, reaching 100% at the maximum concentration of 18 mg/L (Figure 1). Indeed, the time of exposure influences the toxicity of glyphosate with respect to *D. magna*. It was observed that the EC50 value decreased from 9.53 mg/L after 24 hours to 8.61 mg/L after 48 hours.

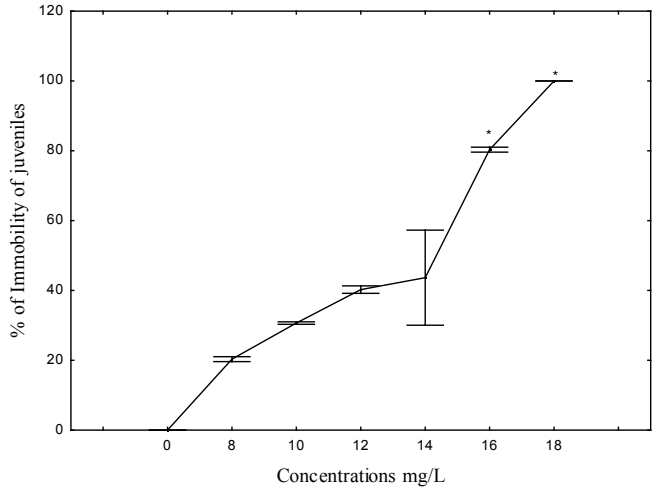


Figure 1. Effect of glyphosate on survival of *Daphnia magna* after 48h of exposure.

To assess the short-term risk of this herbicide, we defined the concentration inhibiting 10% of the Daphnia population (48h-EC_{10}= 6.59 (4.96 – 7.49) mg/L), the lowest observed effect concentration (LOEC= 4.45 mg/L), and the highest no observed effect concentration (NOEC= 3.13 mg/L).

It appears that the chronic (21 days) exposure to the herbicide has a toxic effect on the survival of the species studied. This effect is significant for the three concentrations tested 3.5; 4.5 and 5.5 mg/L. The percentage of inhibition increases significantly with the increase in glyphosate concentrations, reaching 100% for the maximum concentration 5.5 mg/L. The mothers died massively during the last 6 days of exposure with this concentration. Compared to controls, daphnia's mortality was not significant at low concentrations (Figure 2).

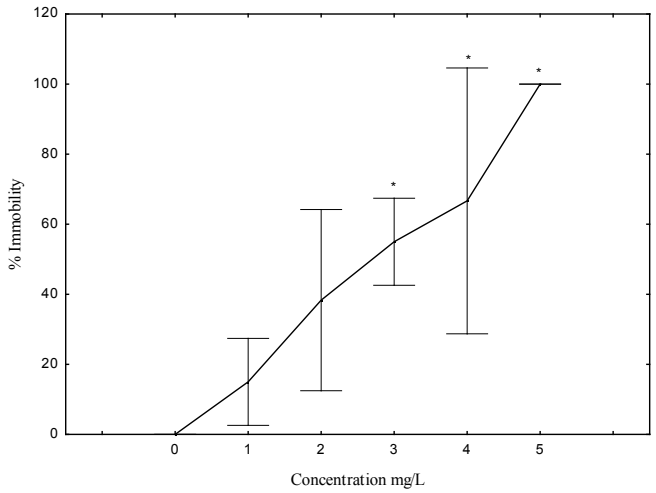


Figure 2. Effect of glyphosate on survival of *Daphnia magna* after 21 days of exposure.

The performance of the chronic tests for the survival parameter allowed us to define concentrations important for the evaluation of glyphosate toxicity. The effective concentration inhibiting 50% of the *D. magna* population during glyphosate exposure was 3.13 mg/L (2.45–3.84, the NOEC concentration is 1.40 mg/L and the LOEC concentration was 1.65 mg/L.

3.2 Effect of Glyphosate on Daphnia Reproduction

The production was significantly reduced from the 3.5 mg/L concentration, but at the low concentrations (1.5 mg/L and 2.5 mg/L) it was more or less similar to the controls (Table 1). This reduction was marked by a significant decrease in the number of offspring per mother (Figure 3) and the number of eggs laid (Figure 4) for concentrations 3.5, 4.5, 5.5 mg/L. According to Figure 5, a significant decrease in egg-laying size was observed at the two maximum concentrations 4.5 and 5.5 mg/L.

In addition, a significant delay in maturity was observed at these two concentrations 4.5 and 5.5 mg/L. Compared to the controls, the first brood occurred on approximately day 9 for the 4.5 mg/L concentration and day 10 for the 5.5 mg/L concentration (Table 1). In comparison with the controls, malformations were observed in juveniles at the highest glyphosate concentration (5.5 mg/L) in the form of a curvature of the apical spine.

Table 1. Positive controls used for each tester strain in presence and absence of metabolic activation system

Glyphosate mg/L	Longevity in days	Days to first brood	Number of neonates per female	Brood size	Number of broods
0	21±0	8.1±0.3	97.2±2.0	19.7±4.0	5.3±0.9
1.5	19.4±1.5	8.5±0.7	100.0±4.3	19.6±4.6	5.2±0.9
2.5	18.7±1.3	8.2±0.6	98.0±1.8	18.2±5.6	4.8±0.9
3.5	18.0±1.3*	8±0	79.5±13.9*	17.0±2.4	4.0±0.6*
4.5	17.0±1.4*	9.1±0.8*	46.0±9.3*	12.5±1.4*	2.4±0.7*
5.5	13.9±0.8*	10±0*	20.6±10.0*	7.7±1.4*	2.0±0.6*

Values are means ± standard deviation; *p<0.05.

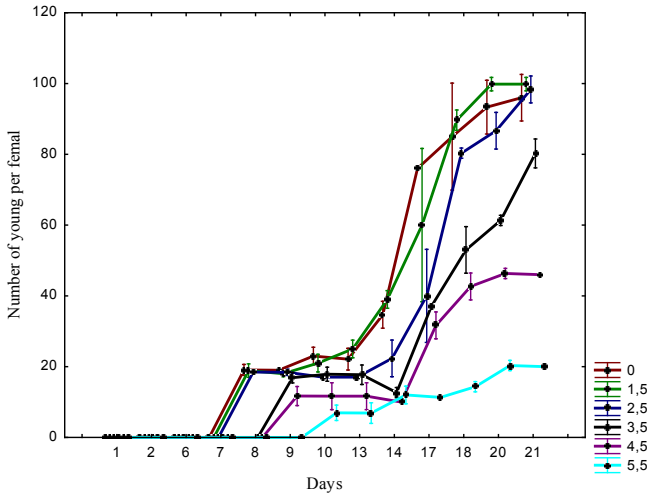


Figure 3. Effects of glyphosateon the number of neonates per female of *Daphnia magna* exposed for 21 days(n=10/concentration).

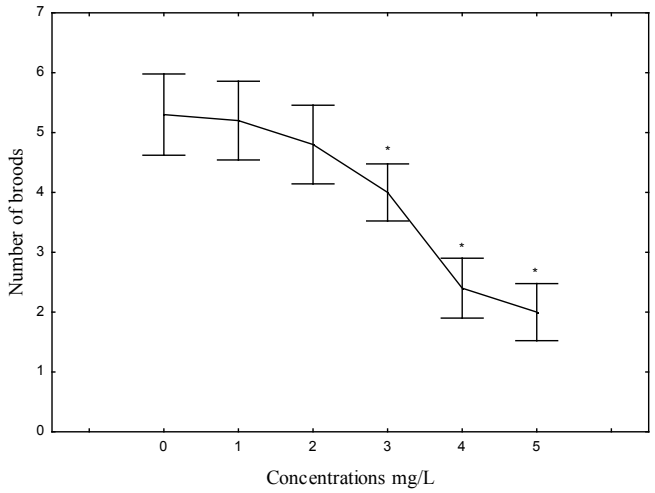


Figure 4. Effects of glyphosate on the number of broods exposed for 21 days (n=10/concentration).

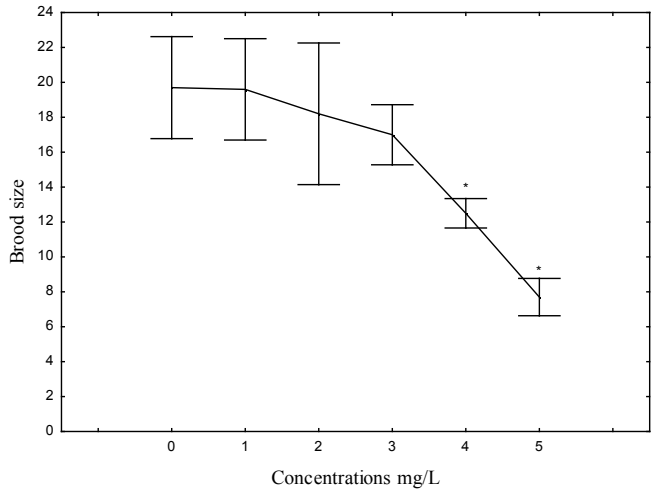


Figure 5. Effects of glyphosate on brood size exposed for 21 days (n=10/concentration).

In addition, a significant delay in maturity was observed at these two concentrations 4.5 and 5.5 mg/L. Compared to the controls, the first brood occurred on approximately day 9 for the 4.5 mg/L concentration and day 10 for the 5.5 mg/L concentration (Table 1). In comparison with the controls, malformations were observed in juveniles at the highest glyphosate concentration (5.5 mg/L) in the form of a curvature of the apical spine.

The NOEC concentration of glyphosate tested on all parameters studied in *D. magna* after 21 days of exposure compared to controls was 1.5 g/L. The most sensitive parameter in reproduction is the number of eggs laid. The 21–day EC_{10} for this parameter is 2.30 (0.33–3.13) mg/L (Table 2).

Table 2. EC_{10} and EC_{50} of several parameters (mg/L) estimated at *Daphnia magna* following exposure to glyphosate during 21 days. (Values represent EC_x with 95% confidence intervals)

Parameters	EC10	Confidence intervals	EC50	Confidence intervals
Number of broods	2.30	0.33-3.13	4.39	3.29-9.18
Brood size	3.16	2.23-3.65	5.02	4.45-6.27
Number of neonate per female	3.65	1.77-4.18	4.53	3.75-5.44

3.3 Multi-generation Test

For the F0 parental generation, the results of 21–day exposure to glyphosate were similar to the results of acute and chronic tests (see chapters 1 and 2). The pesticide has a toxic effect on survival and fertility (Figures 6 and 7).

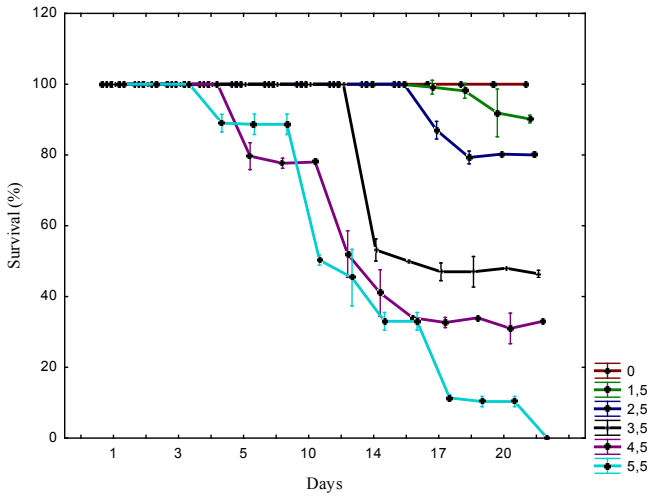


Figure 6. Effects of glyphosate on the survival of *Daphnia magna* (F0) exposed for 21 days (n=10/ concentration).

Comparing with the F0 generation, the results show that survival was more affected in the daughter generation than in the parent generation. In effect, survival decreased significantly (p≤0, 05) starting 1.5 mg/L for the F2, F3 and F4 daughter generations (Figure 8). This effect is more pronounced for the maximum concentration of 5.5 mg/L in which the percentage of survival decreases to 0% for the F2, F3, and F4generations. For the F1 daughter generation, survival was similar with the F0 parental generation.

The difference in the production parameter between the parental generation and the daughter generations was significant starting from the 1.5 mg/L concentration (Figure 9). The decrease in reproduction (number of juveniles per female) was similar among the F0 generation and the two F1 and F2 generations for the maximum concentration (5.5 mg/L). In comparison with controls, however, this decrease becomes significant starting from 3.5 mg/L for F0 parents and 1.5 mg/L for F1, F2, F3, F4 offspring.

As for the survival parameter, the time of exposure and the dose of the pesticide increases the toxicity of glyphosate towards *D. magna*. Reproduction is totally stopped at high concentrations for the F3 (from 4.5 mg/L) and F4 (from 2.5 mg/L) generations.

The results show that survival and reproduction were significantly reduced with increasing pesticide concentration in parents and offspring. The toxic effect of glyphosate was more pronounced in the F4 daughter generation than in the F0 parent generation.

The determination of glyphosate toxicity was dependent on the dose and the exposure time in the parental F0 generation and the F1, F2, F3 and F4 daughter generations. After 14 days of exposure to the herbicide, the results obtained show that a short period (two weeks) was already indicative of the chronic toxic effects of glyphosate in the different generations. The observed effects of glyphosate on the offspring reveal that the four daughter generations F1, F2, F3 and F4 are more sensitive than the parental generation F0. Reproduction and survival decreased significantly starting from the concentration 3.5 mg/L for the parents. In the F2, F3 and F4 generations, both parameters were affected starting from the 1.5 m/L concentration. While for the F1 daughter generation, survival was significantly decreased starting from the 3.5 mg/L concentration but reproduction was observed to be affected from the 1.5 mg/L concentration.

The sensitivity of Daphnia fertility to glyphosate is most pronounced in the last two daughter generations F3 and F4. Reproduction is totally stopped at high concentrations for the F3 (from 4.5 mg/L) and F4 (from 2.5 mg/L) generations.

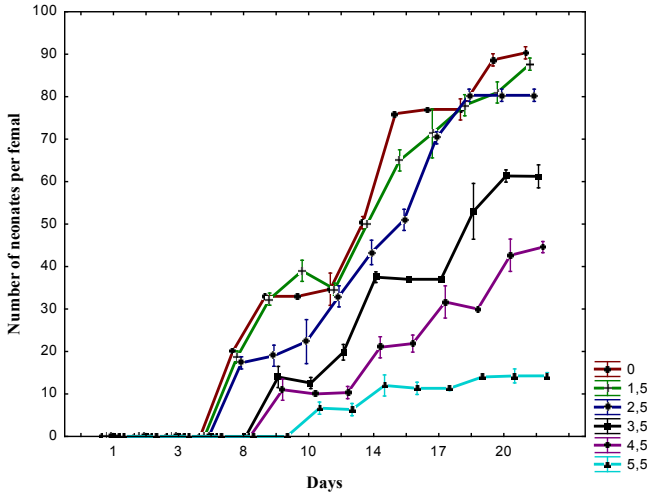


Figure 7. Effects of glyphosate on the number of young per adult of *Daphnia magna* (F0)exposed for 21 (n=10/concentration).

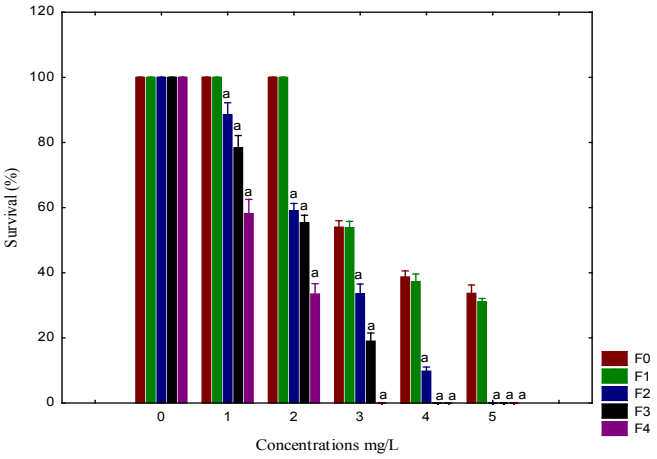


Figure 8. Effect of glyphosate on survival of *Daphnia magna* (F0, F1, F2, F3, F4) during 14 days of exposure. ([a]p<0.05 in comparison to F0 values).

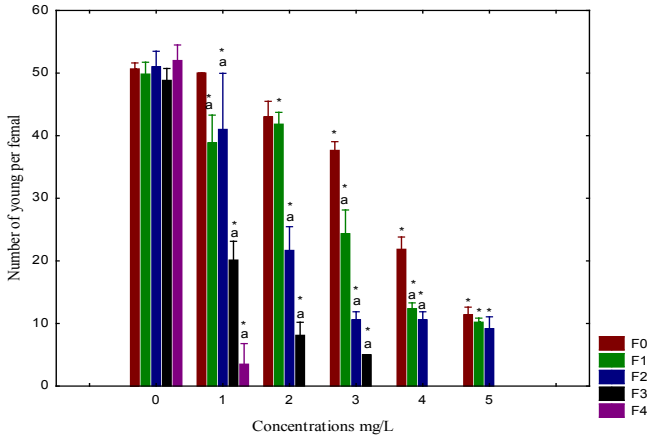


Figure 9. Effect of glyphosate on reproduction of *Daphnia magna* (F0, F1, F2, F3, F4) during 14 days of exposure.*p<0.05 in comparison to control; [a]p<0.05 in comparison to F0 values.

4. Discussion

According to the classification Persoone[20], the short-term toxicity of glyphosate belongs to the 4th class and shows high toxicity for the species studied (TU-24h= 10.49 and TU-48h= 11.61).

Various studies have reported the acute effect of glyphosate on Daphnia. This toxicity is defined by the significant immobility of juveniles during 48 hours of exposure to the pesticide. In our study, the 24h-EC50 is 9.53 mg/L and the 48h-EC50 is 8.61 mg/L. Comparing our results with those of the Hansen and Roslevstudy[10], we find a 48h-EC_{50} of 8.9 mg/L similar to the one defined in our work. Similarly, the study by Cuhra *et al.,*[6] shows that three different *Daphnia magna* clones tested for Roundup (active ingredient glyphosate) have a 48h EC_{50} value that is within this range of 3.7 to 10.6 mg/L.

Whereas in other studies Folmar *et al.,* and Sarigul and Bekcan[8,26], 48h-EC_{50} was not the same found in this study. This can be explained by the formula of the glyphosate studied and its purity.

Similarly, exposure time increases the toxicity of glyphosate to *D. magna*. In the Sarigul and Beckanstudy, the effective concentration inhibiting 50% of *D. magna* decreased significantly, as noted in our work, from 0.019 mg/L after 24 hours to 0.012 mg/L after 48 hours.

According to the study by Zhang *et al.,*[35] the cyanobacterium *Microcystis aeruginosahas* sensitivity towards glyphosate (95% purity) similar to daphnia with a 48h-EC_{50} of 8.1 mg/L.Other species, such as saltwater invertebrates appeared more sensitive to Roundup (41% glyphosate) than daphnia[31] with a 48h-IC_{50} of 1.77 mg/L for *Acartia tonsa* and 48h-IC_{50} of 5.00 mg/L for *Euplotes vannus*. The fish *Clarias gariepinus* with a 96h-CL_{50} of 300 mg/L[7], the crustacean *Gammarus pseudolimnaeus* with a 48h-EC_{50} of 62 mg/L and the larva of *Chironomus plumosus* with a 48h-EC_{50} of 18 mg/L[8] are more resistant to glyphosate than *D magna*.

This work has identified a chronic value for freshwater invertebrates (EC_{10} 21d of 2.30 (0.33-3.13) mg/L) which can be used to refine the guideline values for the protection of aquatic life. Based on European regulations, a PNEC (predicted no effect concentration) of 15 µg/L could be obtained by applying a safety factor of 100 to the reproduction NOEC (1.5 mg/L). A more protective value of 3.3 µg/L was obtained based on the lower limit of EC_{10} 21j.By comparing this value with that the one required

by the French National Agency for Environmental and Occupational Health and Safety[21] (PNEC = 60 µg/L), the value of this study can be integrated to reduce the PNEC recommended by this agency.

The Cuhra *et al.*[6] study show that chronic 15-day exposure to glyphosate has adverse effects on the survival and reproduction of Daphnia. This was marked by a significant reduction in survival and reproduction as early as 4.05 mg/L glyphosate concentration. The same effect was noted in our study, in which mortality and decreased fertility parameters of daphnia were significant at 4.5 mg/L concentration.

Taking into account the above, the US EPA[22] has set the limit for glyphosate concentrations in surface water at 0.7 mg/L and the limit of the environmental guideline specific to the state of California is 1.0 mg/L. In the present study, a chronic value of 3.3 µg/L was obtained based on the lower limit of EC_{10} 21j reproduction. Comparing this value with those mentioned by both guidelines, the toxicity of the glyphosate used in this work may not be sufficiently restrictive to ensure the viability of populations of *D. magna* and other aquatic species.

Roundup solutions at concentrations of 25 and 50 mg/L of glyphosate during 15 days of exposure demonstrate a lack of adaptation to the herbicide in four successive generations of *D. magna*[18]. For the parental generation, analyses of reproductive parameters (number of eggs laid, clutch size and number of juveniles per female), showed that there was a significant decrease in these parameters for both 25 and 50 mg/L concentrations. In comparison with the controls (80.3±2.8), the number of juveniles decreased by 46.0±7.0 for the 50 mg/L concentration. Similarly, a significant reduction in the number of eggs laid (2.6±0.2) and the size of the clutch (17.1±2.1) was observed in daphnia for the maximum concentration. These 15-day experiments revealed that Roundup solutions at concentrations of 25 and 50 mg/L reduced reproduction as early as the first generation. Based on the results of our multi-generation tests, the effect of glyphosate on the parental generation for reproductive parameters is similar for the high concentrations tested in our study.

Comparing the results of the Papchenkova *et al.*[18] study with those obtained from our multi-generation tests, the effect of glyphosate on the parental generation for reproductive parameters is similar for the high concentrations tested. In addition, a comparison between successive generations revealed a decrease in herbicide resistance observed for the fourth generation compared to the maternal generation in this study. Contrary to our results, glyphosate toxicity is more pronounced in the parental generation than in the 4th daughter generation.

In the environment, glyphosate hydrolyzes in half-lives greater than 30 days at pH 5.7 and 9 and is not readily biodegradable. But several studies have shown that it can form complexes with metals present in aquatic environments. The carboxylic and phosphonic groups of glyphosate can react to form covalent bonds with divalent metal ions[9,10,16,29]. Such complexes are considered very stable in water and may alter toxicity profiles compared to glyphosate alone15, 31. In the Hansen and Roslev study[10], complexes of glyphosate with copper appear to be more toxic to Daphnia than glyphosate alone. The 48h-EC50 mobility parameter is 52.8 µM and 25.5 µM for glyphosate and glyphosate-Cu, respectively.

5. Conclusion

At the end of this eco-toxicological study, we were able to evaluate several aspects of acute and chronic toxicity of the herbicide glyphosate on the *Daphnia magna* population. The results of the acute tests show that the pesticide has a significant toxic effect on the survival of the *Daphnia* population with a 48h-EC_{50} of 8.61 mg/l (7.62, 9.30). The toxicity effect of these substances is marked by the immobility (mortality) of juveniles exposed to the herbicide especially at high concentrations.

Also, the results obtained from long-term toxicity tests show that glyphosate has a disruptive effect on the reproduction of the species studied. This effect is marked by a significant delay in sexual maturity and a significant decrease in the number of neonate per mother at a concentration of 3.5 mg/l. This can be explained by the probable presence and long-term accumulation of endocrine disruptors that affect the hormonal system regulating the physiological functions of *D. magna*.

Finally, the contamination of aquatic environments by glyphosate can disrupt the entire aquatic ecosystem and thus affect its biodiversity. The chronic value of 3.3 µg/L obtained in this study remains well above the international guidelines for the protection of aquatic fauna. At the limit of the obtained results in this study, it is desirable to revise these values on one hand and to conduct impact and risk assessment studies to define more robust concentration limits for these chemicals.

6. Acknowledgement

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A big thank you to my sister Imane Berrebaane for all the tips and the hours she has dedicated to help me in my work.

7. References

1. Annett R, Habibi HR, Hontela AH. Impact of glyphosate and glyphosate based herbicides on the freshwater environment. J Appl Toxicol. 2014; 34(5):458-479. https://doi.org/10.1002/jat.2997. PMid:24615870
2. Baylis AD. Why glyphosate is a global herbicide: Strengths, weaknesses and prospects. Pest Manag Sci. 2000; 56(4):299-308. https://doi.org/10.1002/(SICI)1526-4998(200004)56:4<299::AID-PS144>3.0.CO;2-K
3. Benbrook CM. Trends in glyphosate herbicide use in the United States and globally. Enviro Sci Eur. 2016; 28(1):3. https://doi.org/10.1186/s12302-016-0070-0. PMid:27752438. PMCid:PMC5044953
4. Classification OMS recommandée des pesticides en fonction des dangers qu'ils présentent et lignes directrices pour la classification. Genève:Organisationmondiale de la Santé; 2020.
5. Cox C. Glyphosate fact sheets: Part 1 and Part 2. Journal of Pesticide.1995; 15:3–4.
6. Cuhra M, Traavik T, Bohn T. Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*. Ecotoxicology. 2012; 22(2):251–62. https://doi.org/10.1007/s10646-012-1021-1 PMid:23224423. PMCid:PMC3572389
7. Erhunmwunse NO, Ogbeide OS, Tongo II, Enuneku AA, Adebayo PO. Acute toxicity of glyphosate-based isopropylamine formulation to juvenile African catfish (Clarias gariepinus). Nig J Basic Appl Sci. 2018; 26:97–101. https://doi.org/10.4314/njbas.v26i2.14
8. Folmar LC, Sanders HO, Julin AM. Archives of environmental contamination and toxicology toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch Environ Contam Toxicol. 1979; 8:269–78. https://doi.org/10.1007/BF01056243. PMid:507937
9. Franz JE, Mao MK, Sikorski JA. Glyphosate: A unique global herbicide. Washington DC: American Chemical Society;1997.
10. Hansen LH, Roslev P. Behavioral responses of juvenile *Daphnia magna* after exposure to glyphosate and glyphosate-copper complexes. Aquat Toxicol. 2016; 179:36–43. https://doi.org/10.1016/j.aquatox.2016.08.010. PMid:27564378
11. General requirements for the competence of testing and calibration laboratories. ISO 17025; 2017.
12. Jaskulski D, Jaskulska I. The effect of pre-harvest glyphosate application on grain quality and volunteer winter wheat. Rom Agric Res. 2014;31:283–9.
13. Le TH, Lim ES, Lee SK, Choi YW, Kim YH, Min J. Effects of glyphosate and methidathion on the expression of the Dhb, Vtg, Arnt, CYP4 and CYP314 in *Daphnia magna*. Chemosphere. 2010; 79(1):67–71. https://doi.org/10.1016/j.chemosphere.2009.12.067. PMid:20096918
14. Montiel-León JM, Munoz G, Duy SV, Do DT, Vaudreuil MA, Goeury K, Guillemette F, Amyot M, Sauvé S. Widespread occurrence and spatial distribution of glyphosate, atrazine, and neonicotinoids pesticides in the St. Lawrence and tributary rivers. Environ Pollut. 2009; 250:29–39. https://doi.org/10.1016/j.envpol.2019.03.125. PMid:30981933
15. Morillo E, Undabeytia T, Maqueda C. Adsorption of glyphosate on the clay mineral montmorillonite: Effect of Cu(II) in solution and adsorbed on the mineral. Environ Sci and Technol. 1997; 31:3588–92. https://doi.org/10.1021/es970341l
16. Morillo E, Undabeytia T, Maqueda C. FTIR study of glyphosate–copper complexes. Environ Sci and Technol. 2002; 50(7):1918–21. https://doi.org/10.1021/jf010988w. PMid:11902933
17. Ørsted M, Roslev P. A fluorescence-based hydrolytic enzyme activity assay for quantifying toxic effects of Roundup® to *Daphnia magna*. Environ Toxicol Chem. 2015; 34(8):1841–50. https://doi.org/10.1002/etc.2997. PMid:25809520
18. Papchenkova GA, GolovanovaIL, Ushakova NV. The parameters of reproduction, sizes, and activities of hydrolases in *Daphnia magna* Straus of succesive generations affected by roundup Herbicide. Inl Wat Biol. 2009; 2(3):286–91. https://doi.org/10.1134/S1995082909030158
19. Parrish P, Schimmel S, Hansen D, Patrick J, Forester J. Chlordane: Effects on several estuarine organisms.

J Toxico Enviro Health. 1976; 1: 485–94. https://doi.org/10.1080/15287397609529347. PMid:1246088

20. Persoone G, Marsalek B, Blinova I, Törökne A, Zarina D, Manusadzianas L, Nalecz-Jawecki G, Tofan L, Stepanova N, Tothova L, Kolar B. A practical and user-friendly toxicity classification system with microbiotests for natural waters and waste waters. Enviro Toxicol. 2004; 18(6):395–402. https://doi.org/10.1002/tox.10141. PMid:14608609
21. Phytopharmacovigilance -Synthèse des données de surveillance- Appui scientifique et technique n°2017-glyphosate. France : Agence national de sécurité sanitaire de l'environnement et du travail; 2019 July.
22. Prevention Pesticides and Toxic Substances - Glyphosate EPA-738-F-93-011. United States: United States Environmental Protection Agency (US-EPA); 1993.
23. Reno U, Doyle SR, Momo FR, Regaldo L, Gagneten, AM. Effects of glyphosate formulations on the population dynamics of two freshwater cladoceran species. Ecotoxicology. 2018; 27(7):784–93. https://doi.org/10.1007/s10646-017-1891-3. PMid:29404864
24. Retrait des pesticides à usage agricole à base de glyphosate en association avec le Co-formulant la POE-Tallowamine [Internet]. Agadir : Office National de Sécurité Sanitaire des produits Alimentaires (ONSSA); 2018. Available from: http://www.hortitecnews.com/wp-content/uploads/2018/03/501.pdf.
25. Robichaud CD, Rooney RC. Low concentrations of glyphosate in water and sediment after direct over-water application to control an invasive aquatic plant. Water Res. 2021; 188:116573. https://doi.org/10.1016/j.watres.2020.116573. PMid:33152590
26. Sarigül Z, Bekcan S. Acute toxicity of the Herbicide Glyphosate on *Daphnia magna*. Tarim Bilimleri Dergisi. 2009; 15(2):204–8. https://doi.org/10.1501/Tarimbil_0000001092
27. Sihtmäe M, Blinova I, Kunnis-Beres K, Kanarbik L, Heinlaan M, Kahru A. Ecotoxicological effects of different glyphosate formulations. Appl Soil Ecol. 2013; 72:215–24. https://doi.org/10.1016/j.apsoil.2013.07.005
28. Soltani N, Blackshaw RE, Gulden RH, Gillard CL, Shropshire C, Sikkema PH. Desiccation in dry edible beans with various herbicides. Can J Plant Sci. 2013; 93(5):871–7. https://doi.org/10.4141/cjps2013-061
29. Subramaniam V, Hoggard P. Metal complexes of glyphosate. J Agric Food Chem. 1988; 36(6):1326–9. https://doi.org/10.1021/jf00084a050
30. Tomlin CDS. The Pesticide Manual, a World Compendium. 14th Edition. Alton: British Crop Protection Council; 2006. https://doi.org/10.1016/S0045-6535(03)00306-0
31. Tsui MT, Chu LM. Aquatic toxicity of glyphosate-based formulations: Comparison between different organisms and the effects of environmental factors. Chemosphere. 2003; 52(7):1189–97.
32. Tu M, Hurd C, Randall JM. Weed Control Methods Handbook: Tools and Techniques for Use in Natural Areas. The Nature Conservancy; 2001.
33. Water quality-Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea), Standards catalogue. Morocco: ISO10 706; 2014.
34. Water quality -Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test, Standards catalogue. Morocco: International standard organization (ISO) 6341; 2014.
35. Zhang Q, Qu Q, Lu T, Ke M, Zhu Y, Zhang M, *et al.* The combined toxicity effect of nanoplastics and glyphosate on *Microcystis aeruginosa* growth Part B. Environ Pollut. 2018; 243:1106–12. https://doi.org/10.1016/j.envpol.2018.09.073. PMid:30253301