Acute and Chronic Effects of a Glyphosate and a Cypermethrin-Based Pesticide on a Non-Target Species *Eucypris* sp. Vavra, 1891 (Crustacea, Ostracoda)

Arsène Mathieu Houssou 1*, Daniel Cocan 2, Camelia Maria Răducu 2, Eric Joslin Daguégué 3, Vioara Miresan 2 and Elie Montchowui 1

1 School of Aquaculture, National University of Agriculture, Porto-Novo BP 55, Benin; arnsheous@yahoo.fr (A.M.H.); e.montchowui@yahoo.fr (E.M.)
2 Faculty of Animal Science and Biotechnologies, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Calea Mănăștur 3-5, 400372 Cluj-Napoca, Romania; Daniel.cocan@usamvcluj.ro (D.C.);
vmiresan@yahoo.com (V.M.)
3 Laboratory of Hydrobiology and Aquaculture, Faculty of Agricultural Sciences, University of Abomey-Calavi, Cotonou BP 526, Benin; eric2001@yahoo.com

* Correspondence: author: craducu2001@yahoo.com

Abstract: Ostracoda species are indicators of their current and past environment (paleoenvironment). The study aims to evaluate the acute and chronic sensitivities of a freshwater ostracod species (*Eucypris* sp.) to agricultural pesticides (a cypermethrin-based insecticide and a glyphosate herbicide-based formulation). Lethal concentrations (*LC*50) of each pesticide for the species at 24 and 48 h were determined. The chronic exposure allowed assessing the effects of low concentrations of both pesticides; firstly, on the parthenogenetic reproduction of *Eucypris* sp., and, secondly, on its population growth. Then, individuals of *Eucypris* sp. were exposed to 0.536 ppb and 1.072 ppb of cypermethrin and 4.51 ppm and 9.03 ppm of glyphosate. These concentrations are respectively the 10%, and the 20% of the 48-h *LC*50 (median lethal concentration) of both pesticides for the species. The estimated 24-h *LC*50 of cypermethrin was 7.287 ppb. At 48-h, it was 5.361 ppb. For glyphosate, the 24-h *LC*50 was 50.521 ppm, while at 48-h it was 45.149 ppm. After 10 days of exposure to low concentrations of cypermethrin, only 30% of females reproduced parthenogenetically with 10% and 20% of *LC*50 -48-h. For the control treatment, reproduction in 80% of females was observed. *Eucypris* sp. population growth after 28 days of exposure to low concentrations of cypermethrin showed significant retardation. Regarding glyphosate chronic exposure, 60%, 50%, and 90% of individuals were able to reproduce at 10% of *LC*50-48-h, 20% *LC*50-48-h, and the control treatment, respectively. The population growth was also affected by the tested low concentrations of glyphosate. The study showed high sensitivity of *Eucypris* sp. to cypermethrin compared to glyphosate. However, low concentrations of both pesticides affected the species at individual and population level.

Keywords: chronic effects; cypermethrin; *Eucypris* sp.; glyphosate; lethal doses

1. Introduction

Ostracods are present in all surface waters, in great depths, as well as in shallow temporary pools. Most of them live in contact with sediments, which play a very important role for water quality, as they serve as habitat and store pollutants [1]. Benthic organisms in contact with these pollutants have a life history that bears witness to the geochemistry of the environment. Sensitivity is found to be highly variable between groups of organisms and between species [2]. This makes it possible to use these aquatic organisms as bio-indicators of changes undergoing or having taken place in their environment [1,3]. Thus, ostracods sensitive to the quality of their environment [4] can be excellent indicators of water quality [5]. Thus, the chemical composition of their shells is an excellent tool for evaluating changes in dissolved ions in their environment [6]. They allow reliable detection...
of pollutants in sediment [1]. Therefore, ostracods constitute a real tool for paleolimnology in the reconstruction of paleohydrological conditions [7,8].

Ostracods can determine the toxicity of sediments without the need of continuous culture methods, which are as expensive as they are demanding. This is due to their ability to reproduce in a non-sexual way, usually through encysted eggs. These drought-resistant eggs can be stored dry and then inundated and incubated as needed, avoiding the constraints of maintaining continuous cultures [1]. Thus, the importance of ostracods for ecologists, paleolimnologists, as well as ecotoxicologists is clear. Despite all this and even though ostracods are present in all aquatic environments, they have received very little interest in scientific studies in comparison to other microcrustaceans such as copepods and cladocerans [9].

In this 21st century, when the use of chemical pesticides has widely developed in Africa, as everywhere in the world, to fight both against the vectors of major endemic diseases and crop pests [10], a chronic contamination of all ecosystems compartments is observed, whether they are aquatic, atmospheric, or terrestrial [11,12]. The balance of ecosystems is therefore affected, leading even to the extinction of some species [13–16]. Thus, it is necessary to put all the tools that can contribute in the effective monitoring of ecosystems.

Pesticides of the pyrethroid family are known to be toxic to non-target aquatic organisms [3,17,18]. Aquatic invertebrates are extremely sensitive to cypermethrin and pyrethroids in general [19–21]. Herbicides, although they are used to control pests, present some risks to aquatic environments. They also affect the biological community. Among herbicides, glyphosate is the most widely used in agriculture [22] in different formulations. Its effects in the aquatic environment have been reported for several species [3,23,24]. It induces a reduction in reproduction in the water flea Daphnia magna [25].

The interspecific variability of the responses of organism to pollutants makes it necessary to take into account as many species as possible in the assessment of the toxicological risks of biocides. The present study investigated the acute and chronic effects of the insecticide cypermethrin and the herbicide glyphosate on Eucypris sp., an ostracod species of the cosmopolitan genus.

2. Materials and Methods
2.1. Test Species

A plankton sample (100 mL) was collected from the Oueme River (Benin) at Bétérôu (9°11'56.4'' N and 2°16'04.0'' E), with a plankton net of 50 µm mesh size. Since most ostracod species are benthic, the sampling was conducted during low flow by trailing the net on the substratum of the river. The sample was subdivided in two parts. A subsample was cultured in laboratory conditions, with a natural fertilizer (chicken droppings) at 0.6 g/L [26]. The temperature of the culture medium was 26.9 ± 0.1 °C, the pH was 7.1 ± 0.0, and the dissolved oxygen was 4.2 ± 0.3 ppm. The second subsample was fixed with formalin for ostracod species identification (just one ostracod species was identified in the sample, Eucypris sp.). The ostracod was identified at genus level, according to the description in the existing literature [27–29]. After two weeks of culture, 10 specimens of Eucypris sp. were isolated and placed in a phytoplankton (Scenedesmus sp.) culture to allow the development of a monospecific culture, which was subcultured every two weeks. Individuals used for the present study were of the fifth generation. They may be considered as non-contaminated individuals by environmental pollutants.

Culture monitoring showed that the asexual female of Eucypris sp. reached sexual maturity with the deposit of the first eggs between 15 and 17 days of age, under the conditions presented above. Thus, neonates of 24-h of age, obtained from several females maintained individually, were grouped and bred to 14 days of age, when they were used in the experiment.

2.2. Chemicals Properties

The test solutions were obtained by dissolving known concentrations of commercial formulation of both pesticides in water. Cyperforce® (SBM Development, Marseille
CEDEX 11, France) was used as cypermethrin-based pesticide. It contained cypermethrin at 10% of emulsifiable concentration. Regarding glyphosate, Kumark® 480 g/L (Kumark Company Limited, Kumasi, Ghana) was used. Cypermethrin is a synthetic pyrethroid with a level II toxicity (moderately hazardous). Its chemical formula is C_{22}H_{16}ClNO_3 and its molecular weight is 416.3 g/mol. Cypermethrin is not likely to volatilize (vapor pressure = 1.7 × 10^{-9} mm Hg at 20 °C). It is lowly persistent in water, with a half-life of 7 days in aerobic condition. The molecular structure of cypermethrin is presented below (Figure 1, E_1).

Its molecular weight is 169.07 g/mol. Its degradation in water by photolysis is of 20 to 36 days (half-life). In regards to glyphosate, it is a non-selective systemic herbicide, with the chemical formula C_3H_8NO_3P. Its molecular weight is 169.07 g/mol. The structure is presented below (Figure 1, E_2). Glyphosate is not likely to volatilize (vapor pressure = 1.31 × 10^{-2} mPa). Its half-life in water is 9.9 at 20 °C.

![Molecular structure of cypermethrin (E1) and glyphosate (E2).](image)

Figure 1. Molecular structure of cypermethrin (E_1) and glyphosate (E_2).

2.3. Acute Test

Twenty-eight (28) glass cups composed the experimental design for each pesticide. Six acute concentrations were tested for each pesticide and one control (water only) was used. Each treatment had four replications. Seven adults of *Eucypris* Vavra, 1891 (14 days old) were placed in each glass cup immediately after distribution of the test solutions (28 individuals per treatment, and a total of 168 per test). To identify the nominal concentration to be tested for both pesticides, preliminary exposure tests with randomly selected concentrations were carried out (data not presented). Definitive nominal concentrations tested for the cypermethrin were 0 (control), 1, 2, 4, 6, 8, and 10 ppb. For the glyphosate, concentrations of 0 (control), 6, 12, 24, 36, 48, and 60 ppm were used in the definitive acute tests. Each test lasted 48 h. The test solutions were not renewed and *Eucypris* sp. individuals were not fed [30]. Considering the chemicals properties, the loss of active ingredient concentrations in the 48-h was considered insignificant. During the experiment, the photoperiod was set at 16/8-h (light/darkness). Tests with cypermethrin and glyphosate were performed at temperatures of 26.6 ± 0.4 °C and 27.0 ± 0.3 °C, pH of 6.7 ± 0.2 and 7.2 ± 0.1, and dissolved oxygen of 4.0 ± 0.9 ppm and 4.6 ± 0.1 ppm, respectively. The mobility of individuals and mortality were monitored at 1-h, 24-h, and 48-h of exposure. Individuals were declared dead, when a lack of movement was observed after a mild stimulus.

2.4. Chronic Test

During chronic tests, the parthenogenetic reproduction, the survivorship of neonates, and population growth of *Eucypris* sp. under low concentration of cypermethrin and glyphosate conditions were tested. To assess the reproduction and neonate survival, three treatments (two pesticide concentrations and one control) were conducted in glass containers. Each treatment was carried out in ten replications with a total of 30 containers. The tested concentrations were 10% of the estimated 48 h LC_{50} and 20% of the estimated 48 h LC_{50} for each pesticide. These concentrations were 0.53 ppb and 1.07 ppb of cypermethrin and 4.51 ppm and 9.03 ppm of glyphosate, respectively. Twenty milliliters of the prepared test solutions were immediately placed in each glass container. One adult female of *Eucypris* sp. was placed in each cup. Individuals were fed ad libitum with phytoplankton (*Scenedesmus* sp.). The experiment lasted 10 days and the test solution was renewed every 96-h. The reproduction and survivorship of neonates were controlled daily.

Regarding the population growth test, it was conducted in polyethylene aquaria of 3 L each. The same concentrations were tested as in the reproduction test. Three replications were used with a total of 9 aquaria. One liter of test solutions enriched with phytoplankton (*Scenedesmus* sp.) was used in each aquarium and 10 *Eucypris* sp. individuals were dropped.
The test solution was renewed every 96-h. Once a week, 20 mL of the culture medium of each treatment was collected after homogenization and fixed with formalin in a pillbox. These were used for individual enumeration under a light microscope. The tests lasted 28 days. The photoperiod was 16/8-h (light/darkness), the temperature was 25.0 ± 0.3 °C, the pH 6.5 ± 0.3, and the dissolved oxygen 3.6 ± 0.8 ppm.

2.5. Data Analysis

The mortality values were used to estimate the median lethal concentrations (LC$_{50}$) at 24-h and 48-h for each pesticide. The LC$_{50}$ were estimated using the probit method in the computing program PoloPlus v.1.0 (LeOra Software LLC, Northampton, UK). Reproduction rates and survival of neonates were calculated for each treatment. The one way analysis of variance (ANOVA) was used to check differences among treatments (Statistica v.7 Software (StatSoft, Tulsa, OK, USA) was used). Before the ANOVA test, normality of data was tested with the Kolmogorov–Smirnov (K-S) test, while the homogeneity of variance was assessed with the Levene’s test.

3. Results

3.1. Swimming Ability

*Eucypris* sp. individuals exposed to acute doses of glyphosate and cypermethrin showed a perturbation of their swimming ability shortly after exposure. They became motionless with an erratic movement following a soft needle stimulus. This steady state preceded individual death in most of the cases, while some remained alive with the imperfection until the end of the study (48 h of acute exposure). Thus, after one hour of exposure to cypermethrin, immobilization ranged from 22% to 56% (Figure 2). In 24 h, the percentage was higher, ranging from 42% to 100%. These proportions were increased to 100% for each tested dose after 48 h of exposure. For the three timeframes, the induced immobilizations by cypermethrin acute concentrations are significantly different for the first four concentrations ($p < 0.05$). In regards to the glyphosate acute exposure, immobilization ranged from 13% to 82% after one hour (Figure 3). In 24 h, it ranged from 45% to 82%, while in 48 h, it was from 63% to 96%. The first four concentrations of glyphosate also showed significant temporal variations of individual immobilization ($p < 0.05$).

![Figure 2. Distribution of swimming ability perturbation according to tested cypermethrin concentrations and time. Error bars indicate average ± standard deviation. For each concentration, different heading letter indicate significant difference (ANOVA 1, $p < 0.05$).](image-url)
3.2. Individual Mortality

The acute exposure of *Eucypris* sp. to cypermethrin has induced 7.1% and 89.3% mortality in 24 h, with the lowest (1 ppb) and the highest (10 ppb) tested concentrations, respectively. In 48 h, the mortality for the respective lowest and highest concentration was 7.4% and 96.4%.

The estimated 24 h median lethal concentration (24-h LC$_{50}$) of cypermethrin for *Eucypris* sp. was 7.287 ppb, with a 95% confidence interval of 6.394–10.023 ppb (Figure 4). At 48 h, the LC$_{50}$ was 5.361 ppb, with a 95% confidence interval of 4.115–6.851 ppb. Regarding glyphosate, the median 24 h lethal concentration to cypermethrin was 50.525 ppm (Figure 4). The interval of confidence was 45.351–58.204 ppm. For an exposure of 48 h, the glyphosate LC$_{50}$ was estimated at 45.149 ppm (40.082–51.296 ppm).

Figure 3. Distribution of swimming ability perturbation according to tested glyphosate concentrations and time. Error bars indicate average ± standard deviation. For each concentration, different heading letter indicate significant difference (ANOVA 1, p < 0.05).

Figure 4. Cont.
The acute exposure of Eucypris sp. to cypermethrin has induced 7.1% and 89.3% mortality in 24 h, with the lowest (1 ppb) and the highest (10 ppb) tested concentrations, respectively. In 48 h, the mortality for the respective lowest and highest concentration was 7.4% and 96.4%.

The estimated 24 h median lethal concentration (24-h LC₅₀) of cypermethrin for Eucypris sp. was 7.287 ppb, with a 95% confidence interval of 6.394–10.023 ppb (Figure 4).

At 48 h, the LC₅₀ was 5.361 ppb, with a 95% confidence interval of 4.115–6.851 ppb. Regarding glyphosate, the median 24 h lethal concentration to cypermethrin was 50.525 ppm (Figure 4). The interval of confidence was 45.351–58.204 ppm. For an exposure of 48 h, the glyphosate LC₅₀ was estimated at 45.149 ppm (40.082–51.296 ppm).

Figure 4. Acute lethal concentrations (24 and 48 h) of cypermethrin and glyphosate to Eucypris sp.

3.3. Reproduction and Neonate Survival

An obvious effect of low concentrations of cypermethrin to the parthenogenetic reproduction of Eucypris sp. individual was observed after 10 days of exposure (Table 1). With 10%LC₅₀ (0.53 ppb) and 20%LC₅₀ (1.07 ppb), only 30% of females have reproduced against 80% in the control treatment (0.0 ppb). In these cases, 100% of the obtained neonates were alive in the control and 0.53 ppb conditions. The survival was reduced to 67.2 ± 0.01%, with the exposure to 1.07 ppb. As regards the glyphosate (10 days exposure), 60%, 50%, and 90% of parthenogenetic reproduction was obtained with the 10%LC₅₀ (4.51 ppm), 20%LC₅₀ (9.03 ppm), and the control (0.0 ppm), respectively. The survival of neonates was 98 ± 0.1%, 83.7 ± 0.5%, and 95 ± 0.3%, respectively (Table 1).

Table 1. Parthenogenetic reproduction and neonate survival of Eucypris sp. exposed to low concentrations of cypermethrin and glyphosate. Survival of neonates is presented as average ± standard deviation.

|                      | Control | 10% LC₅₀ | 20% LC₅₀ |
|----------------------|---------|----------|----------|
| Percentage of females able to reproduce (%) | Cypermethrin | 80       | 30       | 30       |
|                      | Glyphosate | 90       | 60       | 50       |
| Neonates survival (%) | Cypermethrin | 100      | 100      | 67.2 ± 0.01 |
|                      | Glyphosate | 95.0 ± 0.3 | 98.2 ± 0.1 | 83.7 ± 0.5 |

3.4. Population Growth

The temporal growth of Eucypris sp. according to cypermethrin and glyphosate concentrations is presented in Figure 5. After 28 days of exposure to low doses of cypermethrin, a slowdown of the population growth was observed compared to the control in which an exponential profile was obtained. From 10 individual inoculations, the control population reached a maximum of 2871 ± 11.2 ind./L in 28 days. With 0.53 ppb (10%LC₅₀) of cypermethrin, the maximum density in 28 days was 1066.7 ± 23.5 ind./L. In the case of 1.07 ppb of cypermethrin, only 701.3 ± 9.7 ind./L were obtained. For the glyphosate exposure, a slowdown was also observed. A maximum density of 1271.7 ± 7.6 ind./L (28th day) and 651.8 ± 8.0 ind./L (21st day) was observed with 4.51 ppm (10%LC₅₀) and 9.03 ppm (20%LC₅₀), respectively, against 2671.7 ± 13.9 ind./L (28th day) in the control.
Figure 5. Temporal growth of *Eucypris* sp. density under low concentrations of cypermethrin and glyphosate conditions.

4. Discussion

Both pesticides appear to cause paralysis before the death of *Eucypris* sp. individuals. This is the known mode of action of cypermethrin, which is a synthetic pyrethroid. In fact, the activity of most pesticides from the pyrethroid family, and cypermethrin in particular, affects the sodium channel (Na+) by modifying their opening time or by increasing the frequency of nervous stimuli [31]. This disturbance in the transmission of nervous impulses associated with a disturbance in the exchange of calcium and magnesium ions ends up causing an imbalance in the body. Therefore, the infected individual presents a physiological disorientation with an incoordination of movements [31,32]. This manifestation observed in target species, in particular terrestrial insects, appears to be the same in non-target organisms such as zooplankton, in particular zooplanktonic crustaceans. These are generally very sensitive to cypermethrin [33]. In the present study on the ostracod *Eucypris* sp., the phases of disorientation, tetanization followed by death, are well observed in infected individuals. The same observations were made on the copepod *Acanthocyclops robustus* [3]
and on marine copepods [20]. Christensen et al. [21] also reported that cypermethrin at concentrations above 0.1 ppb causes immobilization in zooplanktonic crustaceans in general and in *Daphnia magna* in particular from 6 h of exposure. Immobilization is an irreversible symptom [20]. Observations on *Eucypris* sp. revealed that the most sensitive individuals are significantly affected from the first hour of exposure to the minimum concentration of 1 ppb. However, most of the infected individuals with this concentration did not die after 48 h. It, therefore, emerges that *Eucypris* sp. has a greater tolerance than *D. magna*, which is a reference species for toxicological risk assessments. Concentrations of cypermethrin up to 0.07 ppb have also been shown in the past to be harmless to ostracods [34]. However, the environmental concentration of cypermethrin in surface water in Benin could well reach or exceed the minimum tested in this study (1 ppb). Gbaguidi et al. [35] detected concentrations of total dissolved pyrethroid ranging from 0.75 to 4.450 ppb in Agbado River during the rainy season.

Glyphosate also affects the mobility of the species before death. Individuals of *Eucypris* sp. presented the same symptoms (immobilization, incoordination of movements after stimulus, tetanization, and death) as in the case of cypermethrin. The difference in action is therefore not physically visible, but could be observed at the physiological level. Since glyphosate is an herbicide, its mode of action on animals remains little known since it could vary from one organism to another. Studies taking into account the physiology of different animal organisms appear important for a good understanding of the effects of this pesticide, which could be dangerous for aquatic ecosystems. It is also recognized that many herbicides remain in water for a long time and negatively affect zooplankton [36]. However, *Eucypris* sp. appears to be more resistant to glyphosate than to cypermethrin. Indeed, it was necessary to reach a relatively high concentration of 6 ppm of glyphosate to have the first effects on the species. Similar effects have also been observed in the copepod *A. robustus* [3]. Resistance of ostracods to glyphosate has also been demonstrated in the past. Gardner and Grue [37] and Linz et al. [38] reported that the treatment of the vegetation covers of wetlands with the glyphosate Rodeo® had no effect on ostracod populations.

*Eucypris* sp. resistance to glyphosate is confirmed by the mean lethal dose (*LC*$_{50}$), which is estimated at 50.525 ppm in 24 h and 45.149 ppm in 48 h. These concentrations are much higher than those of cypermethrin, estimated at 7.287 ppb in 24 h and 5.361 ppb in 48 h. Thus, cypermethrin is more toxic to the species, confirming the observation of Golombieski et al. [39], who reported that insecticides are generally more toxic to live organisms than other pesticides. Ostracods also appears to be more resistant to cypermethrin than other planktonic crustaceans such as the copepod *Mesocyclops leuckarti*, which exhibits a 24 h *LC*$_{50}$ of 0.63 ppb and 0.38 ppb in 48 h [40]. The marine copepod *Oithona similis* is also more sensitive to cypermethrin with a 48 h *LC*$_{50}$ of 0.24 ppb [20]. The interspecific variability of the sensitivity of aquatic organisms to biocides is therefore demonstrated, as observed by Zhou et al. [41] and Wendt-Rasch [2]. As for glyphosate, *Eucypris* sp. is even more resistant than the freshwater copepod *A. robustus*, for which the *LC*$_{50}$ is 19 ppm and 13 ppm in 24 h and 48 h, respectively [3]. This herbicide in its Roundup® formulation also showed greater toxicity on the copepod *Pseudodiaptomus annandalei* (*LC*$_{50}$ 11.7 ppm and 10.23 ppm in 24 and 48 h, respectively) [24] than on the ostracod observed in the current study. On the other hand, *Eucypris* sp. appears to be more sensitive to glyphosate than the decapod *Caridina nilotica*, for which the *LC*$_{50}$ of Roundup® in 72 h is 107.53 ppm [23].

As the reproduction and the survival of neonates are two parameters determining the sustainability of a species in its living environment, pesticides most often affect these two parameters in aquatic organisms. Hanazato [42] has demonstrated that pesticides can affect the dynamics of freshwater zooplankton populations by reducing their survival rate, impairing egg hatching and reducing their specific wealth and diversity. The present study showed that in the presence of low doses relative to the tolerance observed in the ostracod species, cypermethrin (0.53 ppb) and glyphosate (9.03 ppm), can affect its population dynamics. Indeed, chronic exposure of *Eucypris* sp. to both biocides has shown a reduction
in reproduction, juvenile survival, and, consequently, a decrease in population growth over time. These observations confirm those of Wendt-Rasch et al. [2], who reported that the direct effect observed following exposure of certain species of zooplanktonic crustaceans to cypermethrin is the rapid reduction of populations. They also confirm the observations of Wendt-Rasch et al. [2], who after 11 days of exposure to doses greater than 0.13 ppb of cypermethrin, observed a reduction in the zooplankton population. Cypermethrin is therefore very toxic to zooplanktonic crustaceans. As for glyphosate, the chronic effects observed are less comparable to those of cypermethrin, in particular for the parthenogenetic reproduction and for the survivorship of neonates. Exposure of the water flea *D. magna* to concentrations of 0.38 ppm showed reduced reproduction within 21 days [25]. The high tolerance of *Eucypris* sp. is also observed at a chronic level, as reproduction was affected only from 9.03 ppm. Since ostracods are mainly benthic, they are prone to contamination from the accumulated pesticides in the sediments [4]. This led to physiological adaptations or genetic mutations allowing species to reduce their sensitivity. This could well justify the observed tolerance of *Eucypris* sp., the species being not only benthic, but frequently found in the planktonic environment.

5. Conclusions

The freshwater ostracod *Eucypris* sp. showed a relative tolerance to cypermethrin with the median lethal concentration of 7.287 ppb in 24 h and 5.361 ppb in 48 h of exposure. Its tolerance to glyphosate appears also greater with 24 h LC$_{50}$ of 50.525 ppm and 45.149 ppm in 48 h. Sub-lethal concentrations such as 0.53 ppb of cypermethrin and 9.03 ppm of glyphosate may affect the parthenogenetic reproduction of the species. These may also affect the survivorship of neonates and, consequently, the population growth over time.

In developing countries where there is an absence of monitoring devices for aquatic ecosystems and the use of pesticides is experiencing a rapid increase, high concentrations of the two biocides can quickly occur in aquatic environments. The risk of ecological disturbances would, therefore, be great with the corollary of a considerable reduction in services.

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