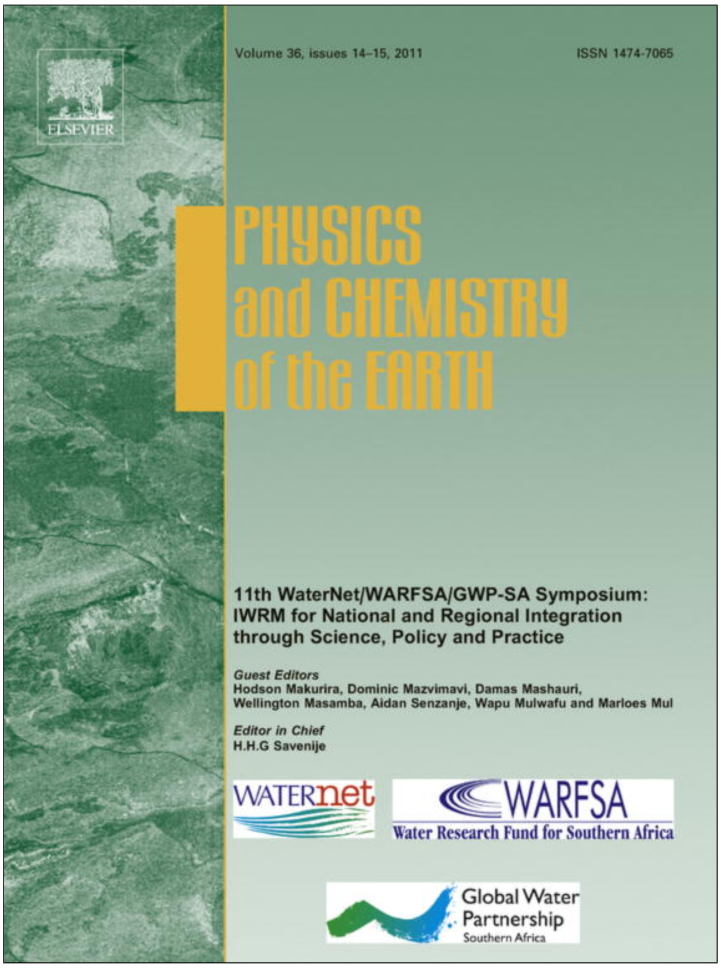
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Acute toxicity of Roundup® herbicide to three life stages of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae)

P.K. Mensah *, W.J. Muller, C.G. Palmer

Unilever Center for Environmental Water Quality, Institute for Water Research, Rhodes University, Box 94, Grahamstown 6140, South Africa

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ABSTRACT

Glyphosate based herbicides, including Roundup®, are frequently used in the chemical control of weeds and invading alien plant species in South Africa. These herbicides ultimately get into water courses directly or indirectly through processes such as drifting, leaching, surface runoff and foliar spray of aquatic nuisance plants. Despite their widespread use, no water quality guideline exists to protect indigenous South African freshwater organisms from the toxic effects of these herbicides. The toxicity of the herbicide Roundup® was assessed using three different life stages of the freshwater shrimp *Caridina nilotica*, a prevalent species in South African freshwater ecosystems. Neonate (<7 days post hatching (dph)), juvenile (>7 dph and <20 dph) and adult (>40 dph) shrimps were exposed to varying concentrations (1.5–50 mg/L acid equivalence (a.e.)) of the herbicide in 48 and 96 h acute toxicity tests in order to determine the most sensitive life-stage. The results showed neonates to be more sensitive to Roundup® than both juveniles and adults with mean 96 h LC_{50} values of 2.5, 7.0 and 25.3 mg/L a.e. respectively. The estimated 96 h LC_{50} of neonates is much lower than the application rate (20–30 mg/L a.e.), although the application's impact will depend on the dilution rate of the applied concentration in the environment. All three life-stages of unexposed animals exhibited active and coordinated movement but exposed shrimps were erratic and slow in their movements, with neonates showing most of these behavioral irregularities. This study shows that low levels of the herbicide Roundup® may adversely affect *C. nilotica* health and survival. Thus, the herbicide should be carefully managed to minimize any negative impact on non-target freshwater organisms.

1. Introduction

Glyphosate, N-(Phosphonomethyl)glycine, is an organophosphonate, non-selective and post-emergent herbicide widely used the world over. Roundup® is the major formulation in which glyphosate is formulated as isopropylamine (IPA) salt, polyoxyethylene amine (POEA) (a surfactant that enhances its efficacy) and water. Glyphosate inhibits the activity of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is found in the shikimate pathway of plants and promotes the production of aromatic amino acids such as phenylalanine, tryptophan and tyrosine. Thus, inhibition of EPSPS by glyphosate causes deficiency in the production of these essential substances that are necessary for the organism's survival and propagation (Stenersen, 2004). The shikimate pathway is absent in animals, which may account for glyphosate's low toxicity to animals. However, high glyphosate doses have been thought to alter mitochondrial activity of animals, possibly, by uncoupling of oxidative phosphorylation during cellular respiration (WHO, 1994). Roundup® may also be an endocrine disrupting chemical (EDC) as it has been reported to interfere with steroidogenic acute regulatory (StAR) protein expression and serum growth hormone thereby inhibiting steroidogenesis and growth respectively in animals (Walsh et al., 2000; El-Shebly and El-kady, 2008).

The exposure of non-target aquatic organisms to glyphosate formulations is of great concern to ecotoxicologists because of the high water solubility of such chemicals and their extensive use in the environment, especially in shallow water systems (Tsui and Chu, 2003). The POEA is thought to be responsible for the relatively high toxicity of Roundup® to several freshwater invertebrates and fishes although IPA salt of glyphosate is also thought to contribute its share of the toxicity (Giesy et al., 2000; Tsui and Chu, 2003). Although glyphosate herbicides are thought to reach concentrations of 2.8 mg/L acid equivalence (a.e.) in aquatic habitats due to accidental overspray, a concentration of 1.7 mg/L a.e. has been reported in a pond in the USA (Giesy et al., 2000).

Although glyphosate has not been cited often in South African literature, it has long been found since the 1990s in the Hex River Valley of the Western Cape (Maharaj, 2005). In recent years, the use of glyphosate herbicides has increased tremendously in South Africa and manufacturers normally recommend between 2 and 4%

* Corresponding author. Tel.: +27 722814190.
E-mail address: kojomens2@hotmail.com (P.K. Mensah).

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of the active ingredient (i.e. glyphosate) as the application rate. The recommended rate by Working for Water (WfW) programe of the South African national Department of Water and Environmental Affairs (DWEA) guide for foliar spray treatment for adults of some aquatic plants with glyphosate herbicides is 3% active ingredient (Bold, 2007; DWEA, 2009). Working for Water uses glyphosate formulated herbicides such as Mamba, Tumbleweed, Ecoplug, Touchdown and Roundup® to control aquatic alien plant species. Roundup® was selected as the toxicant model for this study because: (1) it is a good representative of glyphosate-based herbicides by the virtue of it being the most popular and widely used herbicide in South Africa and most parts of the world (Bold, 2007; Romero et al., 2011); (2) though glyphosate is generally regarded as having a low potential of contaminating surface waters because it is believed to rapidly dissipate from surface waters and strongly adsorbs to soils and sediments, it has been detected in surface waters long after used to kill aquatic weeds (Glusczak et al., 2007); (3) there is a growing concern among aquatic ecotoxicologists regarding its potential impact on the environment due to increase cultivation of genetically modified glyphosate-resistant crops in recent years (Kolpin et al., 2006); (4) glyphosate's mode of action was designed to affect only plants (Stenersen, 2004), but various studies in recent years have reported adverse impact on non-target animals (Giesy et al., 2000; Tsui and Chu, 2003; El-Shebly and El-kady, 2008).

Caridina nilotica (Decapoda: Atyidae) is the most common of four indigenous freshwater caridean species found in the South Africa (Hart, 2001). It thrives in temperatures between 10 and 30 °C and is considered an important role player in the freshwater ecosystems as it forms part of most food webs. Its omnivo-detritivorous mode of feeding is useful in enhancing aquatic macrophyte photosynthesis and recycling of organic matter (Hart, 2001). It has been identified as a potential standard toxicity test organism for producing ecologically relevant toxicity test data. Other factors that make *C. nilotica* suitable organism for this study include the fact that it is indigenous, freshwater and widely distributed species; it breeds throughout the year in the wild and in captivity; and can easily be cultured and maintained in the laboratory (Hart et al., 2001). *C. nilotica* acute toxicity test methods have been developed for neonate, juvenile and adult stages, while chronic toxicity test methods for embryo, partial life-cycle and full life-cycle are currently under development by the Unilever Center for Environmental Water Quality (UCEWQ) of the Institute for Water Research (IWR), Rhodes University, Grahamstown. This study investigated the ecotoxicological effect of Roundup® herbicide on three life stages of *C. nilotica* by examining the acute lethal and behavioral activities of the shrimps after exposure to the herbicide.

2. Materials and method

2.1. Test organism

Shrimps were obtained from a culture maintained at the UCEWQ and acclimated for 24 h in a controlled environment of temperature 24 °C ± 1 and 14:12 h light:dark regime. After acclimation, shrimps were individually transferred into experimental vessels using a glass pipette.

2.2. Test chemical

Liquid Roundup®, active ingredient: 360 g glyphosate (glycine) a.e./L (contains 480 g isopropylamine salt of glyphosate/L, registered and distributed by Monsanto South Africa (Pty) Ltd.), was purchased from a local chemical shop in Grahamstown, South Africa. Since the manufacturers recommend 2–4% application rate, a 2% stock solution was prepared by dissolving 20 mL Roundup® (in a 1000 mL Schott Duran bottle) with distilled water to the 1000 mL mark to obtain a concentration of 7200 mg/L a.e. Appropriate dilutions of the stock solution were made with dechlorinated tap water to obtain the desired nominal exposure concentrations just before start of the experiments. Thus, all units for Roundup® concentrations reported in this study were in mg/L a.e., just as the unit of the stock solution.

2.3. Acute toxicity tests

This study employed a static experimental method to determine the 48 and 96 h median lethal concentration values of Roundup® for neonate, juvenile and adult shrimps. Concentrations used were 0, 1.7, 2.6, 4.1, 6.4 and 8 mg/L for the neonates (<7 days post hatching (dph)); 0, 1.7, 2.6, 4.1, 6.4, 8 and 10 mg/L for juveniles (>7 dph and < 20 dph); and 0, 5.4, 8.4, 13.1, 20.5, 32 and 50 mg/L for adults (>40 dph). Each concentration contained 10 shrimps and replicated three times. Dead shrimps were recorded twice daily and removed from experimental vessels. The cumulative number of mortality were recorded at the end of 48 and 96 h and used to estimate the herbicide's median lethal concentration (LC_{50}) for *C. nilotica*. Shrimps were not fed during the experimental period (Gola and Muller, 2008). All experiments were repeated using different concentrations as follows: 0, 1.3, 2.1, 3.3, 5.1 and 8 mg/L for neonates; 0, 1.3, 2.1, 3.3, 5.1, 8 and 10 mg/L for juveniles; and 0, 4.3, 6.7, 10.5, 16.4, 25.6 and 40 mg/L for adults. Swimming behavior of all three life stages of the shrimp due to the 48 and 96 h short-term acute exposure tests were observed in both experiments and recorded.

Water quality parameters including temperature, electrical conductivity (EC), hydrogen ion concentration (pH) and dissolved oxygen (DO) were recorded daily. The experiments were performed in accordance with institutional ethical guidelines.

2.4. Statistical analysis

Probit statistical software version 1.5 (USEPA, 1990) was used to estimate the LC_{50} values and their 95% confidence limits. One-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison post hoc tests were used to compare mean 48 h LC_{50} values and mean 96 h LC_{50} values of all three age groups. Student's t-test was used to compare mean 48 h LC_{50} and mean 96 h LC_{50} values of separate age groups. Statistics were performed using Statistica Version 9 and all statistical decisions were made at alpha = 0.05 a priori.

3. Results

The mean water quality parameters for Roundup® 48 and 96 h acute toxicity tests are shown in Table 1. In both experiments for all three life stages, treatment groups and control pH ranged from 8.34 to 8.55, DO from 5.89 to 5.99 mg/L, EC from 0.90 to 0.97 mS/cm and temperature from 24.00 to 24.50 °C. These ranges were all within the acclimated conditions of the culture maintained in the laboratory.

Table 1
Mean water quality parameters with standard deviations for the neonate, larva and adult *Caridina nilotica* Roundup 48 and 96 acute toxicity tests.

Shrimp life stage	pH	DO (mg/L)	EC (mS/cm)	Temperature (°C)
Neonate	8.55 ± 0.03	5.98 ± 0.32	0.92 ± 0.01	24.45 ± 0.34
Larva	8.34 ± 0.21	5.99 ± 0.32	0.97 ± 0.05	24.00 ± 0.20
Adult	8.41 ± 0.16	5.89 ± 0.10	0.90 ± 0.01	24.50 ± 0.41

Table 2
Mean 48 and 96 h LC_{50} values and 95% confidence limits of Roundup for different ages of *Caridina nilotica*.

Duration	Age (days)	Experiment number	LC_{50} (mg/L)	95% confidence limits (mg/L)	
				Lower	Upper
48	7	Experiment 1	3.673	3.099	4.303
		Experiment 2	5.231	4.408	6.546
	20	Experiment 1	8.898	8.138	10.163
		Experiment 2	9.896	8.888	12.155
	40	Experiment 1	36.810	27.114	62.603
		Experiment 2	37.438	28.085	62.298
96	7	Experiment 1	2.448	2.068	2.800
		Experiment 2	2.842	2.524	3.190
	20	Experiment 1	6.768	6.198	7.320
		Experiment 2	7.166	6.449	7.986
	40	Experiment 1	27.785	19.988	48.021
		Experiment 2	22.825	18.688	29.625

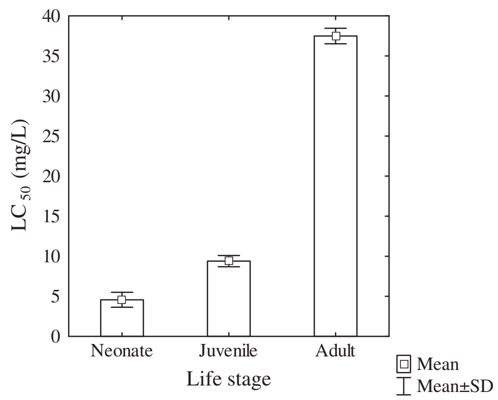

Fig. 1. Mean 48 h LC_{50}s (mg/L) ±0.95 standard deviation of Roundup for neonate, juvenile and adult *Caridina nilotica*.

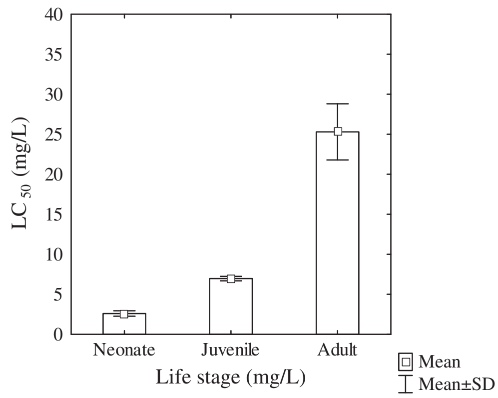

Fig. 2. Mean 96 h LC_{50}s (mg/L) ±0.95 standard deviation of Roundup for neonate, juvenile and adult *Caridina nilotica*.

There were no mortality in the control group for all three shrimp life stages but there were significant differences between control and treatment groups ($p < 0.05$). Mortalities at 48 and 96 h were used to estimate the median lethal concentration (LC_{50}) and their 95% confidence limits of Roundup® for *C. nilotica* different age groups, which are presented in Table 2. The ANOVA of mean 48 h LC_{50} values across all age groups were found to be significantly different ($p < 0.05$), while Newman-Keuls post hoc test performed to determine specific pairwise differences showed significant difference between any two age groups (Fig. 1). The ANOVA of mean 96 h LC_{50} values across all age groups were significantly different ($p < 0.05$), while Newman-Keuls post hoc test revealed that adult LC_{50} values were significantly different from neonates and juveniles, just as values between neonates and juveniles were significantly different (Fig. 2).

There was no statistical difference ($p > 0.05$) between mean 48 h LC_{50} (4.502 ± 1.172) and mean 96 h LC_{50} (2.545 ± 0.139) values for neonate (Fig. 3). However, there were statistical differences ($p < 0.05$) between mean 48 and 96 h LC_{50} values for juvenile and adults, although the mean 48 h LC_{50} values for juvenile (9.397 ± 0.706) and adults (37.124 ± 0.44) were higher than the mean 96 h LC_{50} values for juveniles (6.967 ± 0.281) and adults (25.305 ± 3.507) respectively (Figs. 4 and 5).

Swimming behavior of all three life stages of *C. nilotica* were haphazard within few hours of exposure, but organisms latter became less active and movement reduced. Swimming reduction decreased with increased concentration and, mortality of organisms was preceded by 'slow motion', organism becoming stationary and finally, not responding to a gentle prod.

4. Discussion

The current study has shown that Roundup® herbicide adversely affects all ages of *C. nilotica* as observed in the mortality of shrimps when exposed to the herbicide. Although a few studies exist on the toxicity of Roundup® and other glyphosate-based herbicides to freshwater organisms, especially fishes and crustaceans (Folmar et al., 1979; Hartman and Martin, 1984; Alberdi et al., 1996; Servizi et al., 1987; Tsui and Chu, 2003, 2004), no study has examined the toxicity of Roundup® to *C. nilotica*. It is therefore necessary to compare the LC_{50}s obtained from this study to already reported LC_{50}s of other crustaceans exposed to Roundup® and other pesticides, bearing in mind that these reported values were mostly that of the adult organism.

Folmar et al. (1979) reported Roundup® 48 h LC_{50} for the scud *Gammarus pseudolimnaeus* as 62 mg/L, while Tsui and Chu (2004) reported it for the daphnid *Ceriodaphnia dubia* to be 5.7 mg/L. From the present study, Roundup® 48 h LC_{50} for *C. nilotica* ranged from 4.5 to 37.1 mg/L, which falls within the reported values for other crustaceans as stated above. Folmar et al. (1979) reported Roundup® 96 h LC_{50} for *G. pseudolimnaeus* as 43 mg/L, while Mayer and Ellersieck (1986) reported it as 7 mg/L for the crayfish *Orconectes nais*. The Roundup® 96 h LC_{50} for *C. nilotica* from the current study ranged from 2.6 to 25.3 mg/L, which falls within that reported above for other crustaceans. Roundup® LC_{50} values in this study decreased as the test duration increased from the 48 h to the 96 h in all shrimp life stages (Table 2; Figs. 3–5), which confirms previous studies including Tsui and Chu (2003, 2004). This study also showed that *C. nilotica* neonate, which has the lowest mean LC_{50}s values, is most sensitive to Roundup®, followed by the juvenile and then the adult.

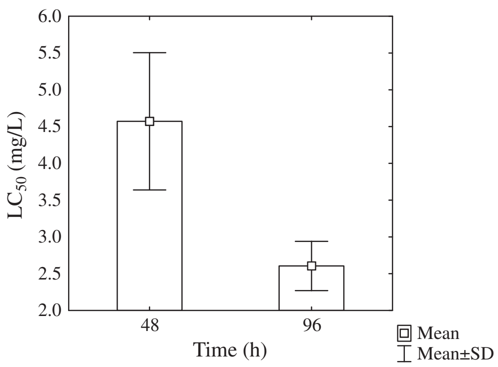

Fig. 3. Mean 48 and 96 h LC_{50}s (mg/L) ±0.95 standard deviation of Roundup for neonate *Caridina nilotica*.

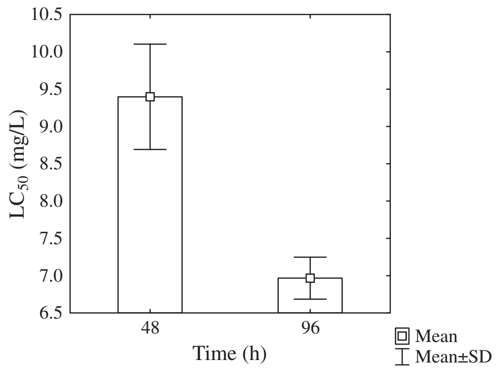

Fig. 4. Mean 48 and 96 h LC_{50}s (mg/L) ±0.95 standard deviation of Roundup for juvenile *Caridina nilotica*.

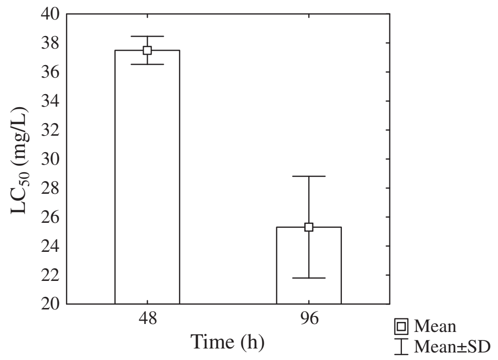

Fig. 5. Mean 48 and 96 h LC_{50}s (mg/L) ±0.95 standard deviation of Roundup for adult *Caridina nilotica*.

Roundup® adversely affected swimming behavior of *C. nilotica* all three life stages. All three life-stages of unexposed animals exhibited active and coordinated movement but exposed shrimps were erratic and slow in their movements, with neonates showing most of these behavioral irregularities, which confirms their lowest LC_{50} values. Gola and Muller (2008) made similar observations when all three life-stages of *C. nilotica* of similar ages as those used in the current study were exposed to deltamethrin. They observed erratic and decreased swimming activities in exposed shrimps in lower concentrations, while higher concentration caused organisms to lay motionless on their backs or sides at the bottom of the experimental vessels. The abnormal behavior of *C. nilotica* from the current study may either be attributed to hypoxia due to interruption of cellular respiration or interference of the endocrine system (WHO, 1994; Walsh et al., 2000; El-Shebly and El-kady, 2008) or synergic function of both phenomena.

5. Conclusion

This study shows that low levels of the herbicide Roundup® may adversely affect *C. nilotica* health and survival as was evidenced in some levels of mortalities in all three life stages and the abnormal swimming behavior of the shrimp. The neonates of *C. nilotica* were found to be most sensitive with a mean 96 h LC_{50} of 2.5 mg/L, which is more than 1.7 mg/L reported in the USA. However, 2.5 mg/L is much lower than the application rate of 20–30 mg/L, although the application's impact depends on the dilution rate of the applied concentration in the environment. Although under field conditions Roundup® is considered to pose less risk due to high adsorption of glyphosate to soil, the herbicide should be carefully managed to minimize any negative impact on non-target freshwater organisms. Moreover, it is necessary to include early life stages of toxicity test organisms such as *C. nilotica* in experimental work in order to obtain accurate measure of glyphosate formulated herbicides potential toxic effects on survival in terms of ecological risk assessment.

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