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SHORT COMMUNICATION

Acute toxicity of a glyphosate herbicide, Roundup®, to two freshwater crustaceans

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Abstract: The acute toxicity of a glyphosate herbicide Roundup®, contaminating freshwater ecosystems in Sri Lanka was investigated with two species of the most common freshwater crustaceans: calanoid copepod (*Phyllodiaptomus annae* Apstein) and decapod shrimp (*Caridina nilotica* P. Roux). LC50 values in adult males were determined using standard toxicity assays under static conditions. The 48h LC50 value for *P. annae* was estimated as 1.06 mg/L and the values of 72 h and 96 h LC50 for *C. nilotica* were 107.53 and 60.97 mg/L, respectively. The present study reveals that Roundup® may cause a significant impact on native non-target organisms. Further studies are needed to establish the toxic effect of Roundup® to the very important structural group in the aquatic ecosystems in Sri Lanka.

Keywords: Acute toxicity, glyphosate, mortality, Roundup®, shrimp, zooplankton.

INTRODUCTION

Glyphosate is one of the most widely used toxic herbicides in the world. When formulated as its monoisopropylamine salt (N-phosphonomethyl glycine), glyphosate is commonly sold under the trade name Roundup® (France *et al*., 1997; Greenpeace, 1997). According to the most recent data on pesticide usage, glyphosate was the most widely used herbicide in agriculture. The active ingredient in Roundup® is classified by the U.S. Environmental Protection Agency (EPA) as category IV (Class I chemicals are the most toxic in a scale of I-IV) for its acute oral and dermal toxicity, based on tests conducted on laboratory animals.

Several authors have concluded that under normal conditions Roundup® did not present a hazard to the aquatic environment because both the glyphosate and the surfactant in the herbicide would be diluted sufficiently in large bodies of water or in lotic aquatic environments (Hildebrand *et al.*, 1982; Mitchell *et al*., 1987). Therefore, glyphosate has been hailed as the most important herbicide ever developed (Neskovic *et al*., 1996). However, at normal application dosages the concentration of surfactant may reach toxic levels in shallow, lentic or ephemeral water bodies, although this scenario has yet to be addressed (Beyond Pesticides, 2001). Many authors have already concluded that glyphosate directly impacts on a variety of non-target animals, and indirectly on birds and small mammals. Nugegoda *et al*. (2002) have concluded that the recommended agricultural dosage for Roundup® may pose an ecological risk to Australian native crustaceans; the daphnid *Daphnia carinata* King, freshwater shrimp *Parataya australiensis* Kemp and postlarvae of freshwater crayfish *Cherax destructor* Clark, if sprayed too close to inland aquatic systems. However, very little research has been carried out on the effects of glyphosate on aquatic micro-organisms or invertebrates (Austin *et al*., 1991, Alberdi *et al*., 1996; Neskovic *et al*., 1996). Herbicides used in Sri Lanka are not routinely tested on Sri Lankan species before widespread use. It is vital that research must be conducted on both the acute and chronic toxic effects of these herbicides on non-target organisms. Therefore, static bioassay tests were performed to assess the acute toxicity of the herbicide on the two most prevalent crustacean species in Sri Lanka.

METHODS AND MATERIALS

Adult male calanoid copepod *Phyllodiaptomus annae* Apstein (total length 1.19±0.02 mm, excluding caudal

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setae) and adult male decapod shrimp, *Caridina nilotica* P. Roux (total length 1.74±0.23 cm and wet weight 0.047±0.008 g) were selected for the acute toxicity bioassay. They were acclimatized to laboratory conditions for 48 h before being used in the bioassay. Roundup® (360 g/L, purity 98%) was purchased from a local retail outlet, and dosing solutions were prepared by diluting with water to give the nominal concentrations. Prior to the definitive test, preliminary screening with a wide concentration series was carried out to determine the definitive exposure ranges for testing the chemical. Tests were conducted in accordance with the EPA guidelines (US EPA, 1993c).

Bioassay with *P. annae* was conducted in 50 mL acid-washed glass beakers filled with 40 mL of source water filtered through Whatman GF/C glass microfibre filters. Some characteristics of this water were: temperature, 28±1 ºC; dissolved oxygen, 8.4–9.2 mg/L; pH, 7.3–7.8; and conductivity, 200 –215 mS. Groups of test organisms, each consisting of 20 individuals were selected at random and placed into test chambers indiscriminately arranged on a bench in the laboratory. Different concentrations of Roundup® (0.1, 0.2, 0.4, 0.8, and 1.6 mg/L) were added to the experimental beakers and mortality was assessed at the end of the test (48 h). Organisms with no response at all were considered dead. The toxicity test consisted of a control and 5 concentration groups with 6 replications per concentration group, resulting in a total of 120 individuals for each concentration. Test solutions were neither renewed nor aerated.

The bioassay with *C. nilotica* was performed in glass aquaria (23x23x35 cm) with a 4 L capacity. Test chambers were filled with 2 L of dechlorinated tap water having the following physicochemical characteristics: temperature, 27.5 ± 0.5 ºC; pH, 6.8 - 7.3; dissolved oxygen, 8.3–8.9 mg/L; and conductivity, 167–229 mS. Groups of experimental animals, each consisting of 20 individuals, were selected at random and placed into aerated aquaria. The toxicity test consisted of a control and four concentration groups with four replications per group, resulting in a total of 80 individuals for each concentration. The different concentrations of Roundup® (20, 40, 80 and 160 mg/L) were added to the experimental aquaria. Mortality was assessed at 24, 48, 72 and 96 h after the start of the test. Test solutions were renewed every day, so the animals were transferred to new aquaria containing fresh medium every 24 h. Animals were not fed during the 48 h adaptation period and throughout the experiment. Controls were treated in the same way but without adding of pesticide to the test water.

All data were expressed as arithmetic means ± SD. Statistical analysis was performed using SPSS for Windows (Version 10.0.1). Mortality rates among exposure groups and controls were compared using the ANOVA least significant difference (LSD) test. Median lethal concentrations (LC50) and their respective confidence intervals (95%) were calculated by means of the EPA probit analysis programme (US EPA, 2003).

RESULTS

The percentage mortality of copepods in each concentration group became progressively higher as the concentration of exposure increased, and the mean mortality in all of the five concentration groups exposed to Roundup® was significantly higher than that of the controls. Similarly, the percentage mortality in the shrimp exposed to glyphosate showed a progressive increase from the lowest to the highest concentration group. Mean mortality rates in the shrimp exposed to all concentrations at 72 and 96 hours were significantly higher than that of controls (Table 1). Control mortality was zero. Within each group, the mean mortality rate became progressively higher as duration of exposure increased. A statistically higher mean mortality rate in animals exposed to the lowest concentration was not observed until the end of third day (72 hours). Similarly, in another concentration group (40 mg/L), a significantly higher mean mortality rate was not observed until the end of the second day (48 hours). The calculated 48 hour acute LC50 value (95% confidence limits) of Roundup®, using static bioassay of adult male *P. annae* was 1.059 mg/L (0.985 - 1.134), and the calculated LC50 values at 72 and 96 hours for Roundup® in adult male *C. nilotica* were 107.53 and 60.97 mg/L, respectively (Table 2).

DISCUSSION

Based on the ecotoxicological risk categories for aquatic organisms (US EPA, 1993a) and derived 48 and 96 hours LC50 values, Roundup® appears to be moderately toxic to *P. annae* and slightly toxic to *C. nilotica*. The LC50 values for these two aquatic invertebrates have not been reported in the available literature. However, the United States Department of Agriculture (1997) reported that the LC50 value of Roundup® in aquatic invertebrates was 4.0-37.0 mg/L. A concentration of 11 mg/L was responsible for 48 hour LC50 value in standards ecotoxicology laboratory species *Daphnia magma* Straus (Monsanto Canada, 2002) and 13.2 mg/L in *Daphnia carinata* King (Nugegoda *et al*., 2002). Moreover, the 48 hour LC50 value for Roundup® in Australian native freshwater shrimp, *Parataya australiensis* Kemp, was >700 mg/L (Nugegoda *et al*., 2002), and the 96 hours LC50 value

Table 1: Mean mortality of *P. annae* Apstein (a copepod) and *C. nilotica* P. Roux (a shrimp) exposed to different concentrations of glyphosate in Roundup®

	Percentage mortality (mean values)			
	24 h	48 h	72 h	96 h
P. annae				
(adult males)				
Control	-	0	-	-
Glyphosate exposed				
0.1 mg/L	-	1.33*	-	-
0.2 mg/L	-	1.67*	-	-
0.4 mg/L	-	2.67**	-	-
0.8 mg/L	-	4.00***	-	-
1.6 mg/L	-	19.00***	-	-
C. nilotica				
(adult males)				
Control	0	0	0	0
Glyphosate exposed				
20 mg/L	1.25	3.75	8.75*	8.75*
40 mg/L	3.75	8.75*	16.25***	16.25***
80 mg/L	8.75*	21.25***	38.75***	38.75***
160 mg/L	12.50***	30.00***	53.75***	53.75***

For a specific test species, mortality data are significantly different from the respective control group (* $p<0.05$, ** $p<0.01$, *** $p<0.001$)

Table 2: Acute toxicity of glyphosate in Roundup® for adult males of the copepod, *P. annae* Apstein and the shrimp, *C. nilotica* P. Roux

Test species	LC50 (mg/L)	95% confidence limits	Slope ± SE	Intercept ± SE
P. annae				
48 h	1.059	0.985 - 1.134	8.96 ± 0.89	4.77 ± 0.14
C. nilotica				
72 h	107.53	88.04 - 140.49	1.88 ± 0.25	1.18 ± 0.46
96 h	60.96	53.21 - 70.04	2.71 ± 0.27	0.17 ± 0.48

of glyphosate in Grass shrimp and Mysid shrimp was recorded as 281 mg/L and >1000 mg/L, respectively (Monsanto Technology LLC, 2002). The acute toxicity of Roundup® in the two species of crustaceans tested here was found to be much lower than the values available from standard ecotoxicology laboratory testing.

Roundup® is one of the most commonly used pesticides in Sri Lanka. There is increasing evidence that formulations based on glyphosate are highly toxic to invertebrates. However, pesticide toxicity is highly dependent on the duration, frequency and intensity of exposure, as well as the susceptibility of target organisms, which can be influenced by age, sex, state of health and genetic variation (Landis & Yu, 1985). Therefore, in the present work the acute toxicity of Roundup® in two species of adult male freshwater crustaceans was investigated. In Sri Lanka, *P. anne* is a highly prevalent zooplankton, hence it is one of the main links in tropic chains in freshwater ecosystems. Likewise, *C. nilotica* is the commonest species of freshwater shrimp, found in all lentic and lotic environments throughout the low country, where pesticide contamination is currently unavoidable. It is a dominant macro invertebrate, occurring in very large numbers, and thus forms an important constituent of the food of fishes (Fernando, 1990). The habitat of the two tested species extends from water column to bottom, providing them with the potential of being useful for water and sediment quality assessment. The two species tested in the present study are not in the species list recommended for acute toxicity testing, using the static test system. Derived LC50 values for the two species tested here are very low compared with the available values given for standard laboratory testing.

Generally, glyphosate is highly soluble in water. If it reaches surface water, it is not broken down readily by water or sunlight (USEPA, 1993b). The half-life of glyphosate in pond water ranges from 70 to 84 days (ETN, 1996). Technically, it is extremely difficult to measure the contamination levels of glyphosate in environmental samples. Therefore, data is often lacking on residue levels in food and in the environment, and existing data may not be reliable. According to Frank (1990), glyphosate had been detected in a watershed four months after application. Even after a year, 0.1 mg/L of glyphosate was still found in sediments of a farm pond (US EPA, 1993a). Therefore, it is to be expected that use of glyphosate-containing herbicides may result in long-term presence of residues in aquatic environments. The World Health Organization (WHO) (1994) has reported that glyphosate could be detected in most aquatic systems, including streams (up to 150 mg/L), ponds (up to 250 mg/L), as well as surface water (up to 25 mg/L). Based on our results and the above glyphosate concentrations found in aquatic systems (WHO, 1994), given long persistence of such residues, invertebrates living in water bodies located near or around the agricultural areas in Sri Lanka may face an ecological risk, indirectly affecting the tropic dynamics of the water bodies.

The mean mortality rates in tested *P. anne* and *C. nilotica* have revealed that even a very low dose of Roundup® (0.1 mg/L and 20 mg/L, respectively) can severely affect both species, which are fundamental links in the dynamics of the tropic chain in Sri Lankan water bodies. According to WHO (1994) an even higher concentration of Roundup® than that tested here on *C. nilotica* may possibly be found in Sri Lankan water bodies, especially those located near or around the paddy fields, as most of the farmers do not follow the label directions (NARESA, 1991). In a lotic system where pesticide can be concentrated and accumulated downstream, existing fauna may be at risk, or suffer an undue disturbance. For instance, an investigation of the impacts of aerial application of Roundup® in a creek watershed has found that watershed drift densities of aquatic invertebrates were not unduly disturbed; but two populations of organisms (*Gammarus* sp. and *Paraleptophlebia* sp.) downstream from the watershed showed a herbicide induced disturbance (Kreutzweiser *et al*., 1989). Therefore, it is possible that sediment dwelling organisms and organisms associated with the bottom, such as shrimps, which spend most of their time digging in the bottom of water bodies, may be at risk from the contamination of Roundup® in lentic and lotic systems. Repeated field application and the long half-life of glyphosate in water would deleteriously impact on the existing aquatic invertebrate fauna and may finally affect the entire ecosystem of the contaminated water bodies.

The present study indicates that even low concentration levels of Roundup® may have a deleterious effect on a very important structural group of aquatic ecosystems in Sri Lanka. These results become more important when considering that standard ecotoxicity laboratory species like *D. magna* and *Daphnia pulex* De Geer neonates, recognized by their high sensitivity to many substances, are at least ten degrees of magnitude less sensitive than *P. annae* adults. Therefore, larval forms should be even more susceptible than mature *P. anne*, as previously observed in water fleas (Haretman & Martin, 1984). In a previous study (Soto *et al*., 2003), another cyclopoid copepod, *Eucyclops neumani neumani* Pesta, highly sensitive to organic and inorganic compounds, was found to be more resistant than *C. nilotica* to Roundup®. This leads to the conclusion that *C. nilotica* is more sensitive to Roundup® than other freshwater shrimps already tested for glyphosate herbicide resistance. The results of the present study emphasize that toxicity testing on multiple species is often required, because different species have different tolerances to Roundup®. It has been shown that sensitivity to Roundup® herbicide in conspecific freshwater crustaceans was different from one hemisphere to another (USEPA, 1993b). Therefore, it is crucial that selected bioassay organisms can provide a clear picture on differential sensitivity levels in different organisms. Roundup toxicity cannot be expressed using only standard laboratory species and without making a thorough investigation of its effect on local fauna. The toxic hazards to non-target organisms exposed to Roundup® herbicide in Sri Lanka could be extreme. However, this remains speculative because toxicological assessments of the effects of Roundup® on non-target terrestrial invertebrates are still lacking, and further studies are required.

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References

1. Alberdi J.L., Saenz M.E., Di Marzio W.D. & Tortorelli M.C. (1996). Comparative acute toxicity of two herbicides, Paraquat and Glyphosate, to *Daphnia magna* and *D. spinulata. Bulletin of Environmental Contamination and*

Toxicology **57**(2):29-235.

2. Austin A.P., Harris G.E. & Lucey W.P. (1991). Impacts of an organophosphate herbicide (glyphosate) on periphyton communities developed in experimental streams. *Bulletin of Environmental Contamination and Toxicology* **47**(1):9-35.
3. Beyond Pesticides/National Coalition Against the Misuse of Pesticides (2001). Pesticides and You. *Chemical Watch Factsheet.* Available at *http://www.beyondpesticides.org/infoservices/pesticidesandyou/Spring%2001/Chemical%20Watch%20Factsheet%20on%20Glyphosate.pdf,* Accessed15 June 2008.
4. Extension Toxicology Network (ETN) (1996). Pesticide Information Profiles: Glyphosate. Available at *http://ace.orst.edu/cgi-bin/mfs/01/pips/glyphosa.htm*, Accessed 15 June 2008.
5. Fernando C.H. (1990). *Freshwater Fauna and Fisheries of Sri Lanka*. pp. 1-442. Natural Resources, Energy and Science Authority of Sri Lanka, 47/5, Maitland Place, Colombo 7.
6. Frank R. (1990). Contamination of rural ponds with pesticide, 1971-1985, Ontario, Canada. *Bulletin of Environmental Contamination and Toxicology* **44**(3): 401- 409.
7. Franz J.E., Mao M.K. & Sikorski J.A. (1997). *Glyphosate, a Unique Global Herbicide*, pp. 65-97. American Chemical Society, Washington DC, USA.
8. Greenpeace (1997). *Glyphosate Factsheet.* 1436, U St., NW, Washington DC, USA.
9. Haretman W.A. & Martin D.B. (1984). Effects of suspended bentonite clay on the acute toxicity of glyphosate to *Daphnia pulex* and *Lemna minor*. *Bulletin of Environmental Contamination and Toxicology* **33**(1):355-361.
10. Hildebrand L.D., Sullivan D.S. & Sullivan T.P. (1982). Experimental studies of rainbow trout populations exposed to field application of Roundup herbicide. *Archives of Environmental Contamination and Toxicology* **11**(1): 93-98.
11. Kreutzweiser D.P., Kingsbury P.D. & Feng J.C. (1989). Drift responses of stream invertebrates to aerial application of glyphosate. *Bulletin of Environmental Contamination and Toxicology* **42**(3): 331-338.
12. Landis W.G. & Yu M.H. (1985). *Introduction to Environmental Toxicology: Impacts of Chemicals upon Ecological Systems*, pp. 328. Lewis Publishers, Boca Raton, Florida, USA.
13. Mitchell D.G., Chapman P.M. & Long T.J. (1987). Acute toxicity of Roundup and Redeo herbicides to rainbow trout, Chinook and Coho salmon. *Bulletin of Environmental Contamination and Toxicology* **39**(6): 1028-1035.
14. Monsanto Canada (2002). *Material Safety Data Sheet* (MSDS). Monsanto Canada **1.2**: 1-8.
15. Monsanto technology LLC (2002). *Roundup Pro Herbicide Technical Factsheet.* pp. 1-5. Monsanto Technology, LLC**,** 800, Lindbergh Blvd, St. Louis, Missouri, USA.
16. National Resources, Energy and Science Authority of Sri Lanka (1991). *Natural Resources of Sri Lanka, Conditions and Trends*, pp. 280. NARESA, 47/5 Maitland Place, Colombo 7.
17. Neskovic N.K., Poleksic V., Elezovic I., Karan V. & Budimir M. (1996). Biochemical and histipathological effects of glyphosate on carp, *Cyprinus carpio* L. *Bulletin of Environmental Contamination and Toxicology* **56**(2): 295-302.
18. Nugegoda D., Hayes K., Thompson L. & Thomassen K. (2002). The toxicity of a herbicide to Australian freshwater crustaceans, RMIT University, Australia. Available at *http://safe2use.net/ca-ipm/02-09-19d.htm*, Accessed 15 June 2008.
19. Soto E., Oyarce G., Inzunza B. & Bay-Schmith (2003). Acute toxicity of organic and inorganic compounds on the freshwater Cyclopoid copepod *Eucyclops neumani neumani* (Pesta, 1927). *Bulletin of Environmental Contamination and Toxicology* **70**(5): 1017–1021.
20. United States Department of Agriculture (1997). *Glyphosate: Herbicide Information Profile,* pp. 16. USDA Forest Service, Pacific North Western Region, Portland, Oregon, USA.
21. United States Environment Protection Agency (1993a). *EPA registration eligibility document, Glyphosate*, pp. 259. Office of Prevention, Pesticides and Toxic Substances, USEPA, Washington, DC, USA.
22. United States Environment Protection Agency (1993b). Glyphosate Registration Eligibility Division. Available at *http://www.epa.gov/REDs/old-reds/glyphosate.pdf*, Accessed 15 June 2008.
23. United States Environment Protection Agency (1993c). *Methods of Measuring the Acute Toxicity of Effluents and Receiving Water to Freshwater and Marine Organisms*, 4th edition, pp. 273. USEPA, Washington, DC, USA.
24. United States Environment Protection Agency (2003). *EPA Probit Analysis Program, USED for calculating LC/EC values*, version 1.5. Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory, USEPA, Ohio, USA.
25. World Health Organization (1994). Glyphosate, *Environmental Health Criteria* No. 159. The International Program on Chemical Safety (IPCS), WHO, Geneva, Switzerland.