

Comparative Acute Toxicity of Two Herbicides, Paraquat and Glyphosate, to *Daphnia magna* and *D. spinulata*

J. L. Alberdi, M. E. Sáenz, W. D. Di Marzio, M. C. Tortorelli

Ecotoxicology Research Program, Department of Basic Sciences, National University of Luján, cc 221 (6700) Luján (B), Argentina

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Glyphosate (N-(phosphonomethyl)glycine) (GLY) and Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichlorine) (PQ) are broad spectrum, non selective herbicides used extensively for vegetation control (deep-rooted perennial species of annual and biannual species of grasses, sedges and broad-leaved weeds) and management in many nonagricultural settings.

Glyphosate is the active ingredient (a.i.) of *RON-DO*® and Paraquat is the active ingredient of *OSAUAT*® formulations. Both herbicides are commercialized by OSA-ARGENTINA S.A.. The use of herbicides and other chemical agents may result in accidental introduction into the waters (Gersich et al. 1985). Even though they are immobilized by soil adsorption in terrestrial applications, when they are applied as post emergence spray, herbicides may reach aquatic systems by leaching, run-off or by adsorption to particulate matter (Goldsborough and Brown 1988; Lokhart et al. 1989). GLY and PQ are soluble in water. GLY is moderately toxic to several aquatic organisms; PQ is very toxic to the aquatic fauna (Tortorelli et al. 1989). Both are potentially used near or in aquatic environments (Hartman and Martin 1984; Haley 1979).

Paraquat has been used for aquatic weed control in Argentina. The application rate in agricultural practice is 4 L PQ formulation/Ha (Instituto Nacional de Tecnología Agropecuaria, Buenos Aires Province, Argentina, personal communication); in water, for aquatic weed control, is 0.1 to 2 mg PQ/L (Claderbank 1972). Following application, the concentration decreased to about the half of the initial level within 36 h in water, and in less than two weeks, the concentration was below 0.001 to 0.02 mg PQ/L (WHO 1984). In a treated pond with a initial concentration of 1.14 mg PQ/L, concentrations of 0.2 mg PQ/L were determined in the water after 4 d and no residues were detected after 16 d (limit of detection was 0.01 mg/L) (Earnest 1971).

GLY may exhibit toxicological properties to constituent aquatic flora and fauna (Goldsborough and Beck 1989). It has extreme stability in sterile water in the

Correspondence to: J. L. Alberdi

laboratory, but it has a rapid dissipation in aquatic environments in the field (Bronstad and Friestad 1985). Glyphosate can move considerable distances in canal or stream water (Duke 1988). Fifty-eight percent of applied GLY was detected at distances 8 and 14.4 Km downstream from sites of introduction (Comes et al. 1976). The rate of recommended use ranges with the grazing, and varies about 0.25 and 2 Kg a.i./Ha. Approximately 1 or 3 % of applicated GLY may be recupered in aquatic environments (Urban and Cook 1986) or less than 1 % (Edwards et al 1980).

Species of the genus *Daphnia* (Crustacea-Cladocera), and specifically *Daphnia magna*, are some of the most sensitive organisms used in toxicological bioassays (Lewis and Weber 1985; Gersich et al. 1986; Lewis and Horning II 1991), and have been utilized to determinate the toxicity of herbicides in several occasions (Van Leewen and Maas, 1985; Marchini et al 1988; Heisig-Gunkel and Gunkel 1982). The information about the effects of GLY, PQ and its formulations on aquatic animals (Wan et al 1989) and specifically on *Daphnia* (Hartman and Martin 1985; Servizi et al. 1987) is scarce.

D. spinulata, a native argentine cladoceran, is a widespread zooplankton organism in freshwater environments of the Buenos Aires province (Olivier 1960). This area has most of the urban concentrations and the greatest number of industries, agricultural and cattle centers of Argentina. *D. spinulata* was used successfully as a biological model in aquatic bioassays (Alberdi et al. 1990).

The aim of this study was to evaluate and compare the acute toxicity of GLY and PQ formulations on *D. magna* and *D. spinulata*, in order to expand the South American toxicity database of these herbicides.

MATERIALS AND METHODS

The test materials used were the *RON-DO*® and *OSAQUAT*® formulations both provided by OSA-ARGENTINA S.A.. *RON-DO* formulation contains 48 % of GLY as isopropylamine salt and 15 % of surfactant (oxide-coca-amide-propyl dimethyl-amine). *OSAQUAT* commercial formulation contains 27.6 % of PQ and 5 % of unknown surfactant.

Two acute toxicity tests (48 h) under static conditions were carried out with newborn (< 24 h old) of *D. magna* and *D. spinulata* in a chamber set at constant temperature (20°C ± 1) without photoperiod (24 h total darkness). The organisms were not fed during the test.

The acute tests consisted in exposing groups of ten (10) organisms of two Cladocera species, at similar dilutions of both *OSAQUAT* and *RON-DO* formulations. The test solutions were prepared taken into account the concentration of active ingredient in the formulations. Appropriate dilutions used in *RON-DO* bioassays were the same for *D. magna* and *D. spinulata*: 18, 32, 54,

90, 150 and 250 mg GLY a.i./L.. The dilutions of *OS AQUAT* were prepared by the same procedure for both species; the test concentrations were: 2, 3.7, 5.9, 9.6, 15.5 and 24.5 mg PQ a.i./L. All test dilutions were prepared using artificial pond water (APW) (pH 7.8 ± 0.3 ; dissolved oxygen: 8.4 ± 0.4 mg/L; total hardness: 95.8 ± 6.5 mg CO₃Ca/L; conductivity: 475.5 ± 46.3 μ hos/cm; alkalinity: 189.3 ± 14.5 mg CO₃Ca/L). APW was prepared based on the methods of U. S. Environmental Protection Agency (USEPA 1989). All concentrations and the control were set in triplicate. Physicochemical parameters (pH, hardness, conductivity, dissolved oxygen and alkalinity) were measured in the higher dilutions of two herbicides.

The 24 and 48 h EC50, 95 % confidence intervals and χ^2 were estimated using the probit analysis (Finney 1971).

RESULTS AND DISCUSSION

Our studies indicated that *OS AQUAT* formulation were more toxic than *RON-DO* formulation to *Daphnia magna* and *D. spinulata*. At 24 h of exposure to *RON-DO*, all individuals of both species presented immobility at the highest concentration (250 mg Gly/L). At 48 h of exposure to *RON-DO* all organisms treated with concentrations above 150 mg Gly/L became immobilized. In *OS AQUAT* experiments with *D. spinulata*, at 24 h, all organism were immobils at 32 mg PQ/L. At 48 h all organisms of *D. magna* and *D. spinulata* exposed to 16 mg PQ/L and 85 % of organisms of *D. spinulata* exposed to 8 PQ/L became immobilized, in the totality of experiments.

Table 1 summarizes the 24 and 48 h EC50 values of *D. spinulata* in both experiments with 95 % confidence intervals and χ^2 values. It shows, in all cases, that the toxicity of PQ formulation was higher than GLY formulation.

Table 1. 24 and 48 h EC50 values of exposed *D. spinulata* to *OS AQUAT* and *RON-DO*, the 95 % confidence intervals (a) and χ^2 values.

Time	24 h		48 h	
	<i>OS AQUAT</i>	<i>RON-DO</i>	<i>OS AQUAT</i>	<i>RON-DO</i>
EC50	9.91	94.87	2.57	66.18
a	8.9-10.8	89.1-101	2.2-2.9	61.1-71.8
χ^2	3.61	5.28	6.93	6.47

Table 2. 24 and 48 hs EC50 values of *D. magna* exposed to *OSAQUAT* and *RON-DO*, the 95 % confidence intervals (**a**) and χ^2 values.

Time	24 h		48 h	
	<i>OSAQUAT</i>	<i>RON-DO</i>	<i>OSAQUAT</i>	<i>RON-DO</i>
EC50	16.47	95.96	4.55	61.72
a	14.4-19.3	91.5 - 101.1	4.1-5.1	58.8 - 64.2
χ^2	2.42	3.17	7.53	6.94

Table 2 reports the 24 and 48 h EC50 values of *D. magna* in both assays with 95 % confidence intervals and χ^2 values. *D. magna* treated individuals had similar response than *D. spinulata* to two herbicide, PQ formulation was more toxic than GLY formulation.

Both tables (Tables 1 and 2) show similar GLY formulation toxicity on exposed Cladoceran species at 24 and 48 h. In all the experiments with *OSAQUAT*, the native Cladoceran specie presented higher sensitivity to PQ formulation than *D. magna*. These results support the use of *D. spinulata* as a good test organism in aquatic toxicological assays.

Analyzed water quality parameters at beginning of bioassays, indicated that *OSAQUAT* and *RON-DO* formulations didn't alter them in comparison with APW values. At the end of bioassays was registered a significant decrease of dissolved oxygen concentration at the highest dilutions of *OSAQUAT* (APW: 6.7 ± 0.5 mg O₂/L; 24.5 mg PQ/1: 2.9 ± 0.7 mg O₂/L)

In *RON-DO* experiments, the pH showed a slight decrease in comparison with the APW. Also there was a slight increase in the alkalinity parameter with respect to the APW values. The presence of surfactant and others additives in *RON-DO* and *OSAQUAT* composition would altered the water quality parameters with respect to the APW.

Data reported by U.S. Fish and Wildlife Service (Eisler 1990) about the toxicity of PQ on *D. magna* and *D. pulex* (LC50-48h: 6 mg PQ/L and 2.7 - 4 mg PQ/L respectively) showed a similar toxicity obtained in comparison with our experiments (Tables 1 and 2).

Related with the toxicity of GLY, Hartman and Martin (1984) reported results about the effect of *Rondoup*® formulation (Monsanto Company) on *D. pulex*.

They found a 48 h EC50 of 7.9 mg Gly/L. This result is different with respect to the 48 h EC50 obtained for *RON-DO* formulation in our studies on *D. magna* (Table 2) and *D. spinulata* (Table 1), indicating a higher toxicity of *Rondoup* than *RON-DO* in these Cladoceran species.

Different 48 h EC50 of *D. magna* exposed to GLY formulation were reported by The World Health Organization (1994). They show a higher toxicity with respect to the results obtained in the experiments reported here (in a range of 24-42 mg/L).

Servizi et al. (1987) reported the effect of *Rondoup* and its surfactant MON0818 on the immobility of *D. pulex*. They obtained a 96 h EC50 of 25.5 mg Gly/L for formulation and 3.8 mg/L for surfactant, demonstrating the highest toxicity of the last one. The formulation data were almost similar to those obtained in our experiences considering the time of exposure (96 h). They reported the 96 h EC50 value instead of the recommended 48 h EC50 in standardized international protocols.

Results obtained indicate a moderate toxicity of *RON-DO* on newborn *Daphnia spinulata* and *D. magna* (Tables 1 and 2). These data rose in comparison with the possible Glyphosate concentration that it could be found in the aquatic environment, whereas the recommended application rates for terrestrial weed control (0.25-2 kg Gly a.i./Ha). If approximately 1 or 3 % or less than 1 % of the application dose may be recovered in aquatic environments, so the possible Glyphosate concentrations found would be 0.7 to 4.8 mg GLY/L.

In contrast, *OSAQUAT* was very toxic for both Cladoceran species; the 48 h EC50 obtained were 2.57 mg PQ/L to *D. spinulata* and 4.55 mg PQ/L to *D. magna*. These values were near the used range of PQ in aquatic environments (0.1 to 2 mg PQ/L) and may be dangerous for the chronic exposure of natural populations of the native Cladoceran specie.

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