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The toxicity of carbofuran to the freshwater rotifer, *Philodina roseola*

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Abstract In this study, the effects of exposing the rotifer *Philodina roseola* to the pesticide carbofuran were investigated. Its range of sensitivity to potassium dichromate, the acute toxicity of active ingredient carbofuran and of carbofuran dosed as its commercial form, Furadan® 350 SC were determined. Chronic toxicity of carbofuran dosed as Furadan® 350 SC on *P. roseola* survival and fecundity were also studied. The sensitivity of *P. roseola* to $K_2Cr_2O_7$ ranged from 29.52 to 64.67 mg L^{-1}, averaging 47.10 mg L^{-1}. The 48-h EC_{50} were 13.36 ± 2.63 mg L^{-1} for carbofuran and 89.32 ± 6.52 mg L^{-1} for commercial form. Chronic toxicity tests showed that the survival of this rotifer was not affected by the carbofuran dosed as Furadan® 350 SC at the concentrations tested and that at 1.56 and 3.12 mg L^{-1} their fecundity was higher than in the absence of this commercial product, characterizing the hormesis phenomenon. The sensitivity profile of several species to carbofuran indicated that *P. roseola* is more susceptible to this pesticide than the fish *Clarias batrachus*, the bacterium *Vibrio fischeri*, the protozoan *Paramecium caudatum* and the rotifer *Brachionus calyciflorus*, although the acute toxicity of carbofuran dosed as Furadan® 350 SC to *P. roseola* is much lower than that of active ingredient carbofuran. The results also imply that the exacerbated use of pesticides and the constant, accelerated expansion of agricultural activity will make aquatic non-target species even more vulnerable. Furthermore, the relevant role of benthic organisms in aquatic environments justifies the inclusion of *P. roseola* and other benthic species in toxicity screening for risk assessment, regarding this environmental compartment.

Keywords Furadan® 350 SC · Potassium dichromate · Species sensitivity distribution (SSD) · Hormesis

Introduction

Tropical aquatic ecosystems constitute great reservoirs of biodiversity. However, they are currently under various kinds of threat arising from their role as terminal or intermediate receiving water bodies for a wide variety of pollutants that may be discharged into the air, the soil or directly into the water (Aguilar-Alberola and Mesquita-Joanes 2012). On reaching the aquatic environment, pollutants can suffer various fates, depending on the chemical and physical properties of the extraneous compounds, the chemical, physical and biological characteristics of the receiving ecosystem and the rate of entry of the compound into the environment (Rand 1995). With these considerations in mind, the considerable risk of contamination of the water environment is quite evident, especially where an agricultural ecosystem and water bodies are in close proximity (Abhilash and Singh 2009).

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The expansion of agriculture has been described as one of the biggest threats to the conservation of biodiversity in continental waters around the world (Lacher and Goldstein 1997). Apart from the long list of problems directly related to the destruction of natural vegetation, the adoption of intensive agricultural methods has led to an exponential growth in the use of pesticides, compounds that in general are highly toxic to the natural environment (Carvalho 2006; Henriques et al. 1997).

The carbamates, and among them the pesticide carbofuran, are being used in increasing amounts on fields of rice, cotton, coffee, sugarcane, beans and corn (Ehler 2004). A major problem, however, is that most of these chemicals are not ecologically selective and can represent a threat to the survival of non-target species within or outside the agricultural area to which they are applied. In the specific case of carbofuran, it is known to bind irreversibly to the enzyme acetyl cholinesterase, inhibiting its action on the important metabolite acetylcholine (Trotter et al. 1991; Gupta 1994; Heath et al. 1997). Carbofuran is considered highly toxic to birds, bees and aquatic animals, such as fish (Collective SPA 2002).

An in-depth appraisal of the consequences of the presence of carbofuran for aquatic life would require, evidently, a more systematic investigation, involving a variety of test organisms. A review article (Breitholtz et al. 2006) listed a series of challenges to the improvement of ecotoxicological testing in environmental risk assessment. The authors pointed out that an ecotoxicity test employing a model organism should be validated on the basis of cost, ecological relevance, reliability (reproducibility) and sensitivity, reflecting earlier recommendations made by Rand et al. (1995).

The minute pseudocoelomate invertebrates belonging to the phylum Rotifera (once known as "wheel animalcules", on account of a ciliated corona on the head resembling a rotating wheel) are readily found in the vast majority of freshwater ecosystems, yet they are seldom used as test organisms, despite displaying a number of biological attributes that recommend them for this purpose: small size, simple body structure (no separate circulatory or respiratory system), parthenogenetic reproduction, high fecundity, short life-cycle and many species with both sexual and asexual reproduction (Snell and Janssen 1995). Their ecological and taxonomic representativeness, wide distribution, high density in natural water bodies and easy culturing argue strongly in favor of employing several species of rotifer in lab studies.

Although it is true that all species in a given habitat play significant parts in the ecosystem, the place occupied by rotifers in the food chain in fresh or brackish water is of crucial importance. They are important primary consumers that feed on microorganisms such as microalgae, bacteria, yeasts and protozoa (Hyman 1951; Miller and Harley 2002) and usually reach high population densities and extraordinary rates of production (Starkweather 1987; Walz 1997; Wallace 2002; Wallace and Snell 2010). The position of rotifers in the food chain of water bodies is also relevant to energy flow, as they take an active part in the transfer of energy between trophic levels (Armengol 1980; Park and Marshall 2000; Wetzel 2001). They are active members of the microbial loop; while directly consume suspended organic particles (Pourriot 1965), or indirectly assimilate dissolved organic substances as they consume bacteria or protozoa (Arndt 1993).

The general aim of this study was to assess the acute and chronic toxic effects of the pesticide carbofuran, employing the rotifer *Philodina roseola* Ehrenberg, 1830 (Rotifera, Bdelloidea), a representative benthic organism as the test species, whereas most of the toxicity tests use monogonont rotifers of the genus *Brachionus*. The reason we use the species *P. roseola* as test organism is that we believe that ecotoxicological studies with rotifers should include and standardize procedures for many other species considering that toxicity responses are toxicant and species specific (Dahms et al. 2011). Another reason is that species of the genus *Philodina* besides having the biological characteristics recommended for a test organism (Buikema et al. 1974; Hagen et al. 2009, 2010; Allinson et al. 2011) are present in a wide variety of water bodies and also in semi-terrestrial habitats, litter or soil, and even in station tanks of sewage treatment (Wallace and Snell 2010). In tropical reservoirs of Southeast Brazil they are frequent and develop very dense populations (Souza-Soares et al. 2011; Garraffoni and Lourenço 2012). Furthermore, the functional role of benthic rotifers in lotic and lentic aquatic environments justifies its inclusion in the battery of tests for risk assessment, thus covering another environmental compartment, as pointed out by Vidal et al. (2014) when addressing the importance of using the benthic diatom *Navicula libonensis* for toxicity testing, a relevant component of microphytobentos, that had been until now overlooked.

The specific aims were: (1) To determine the range of sensitivity of this rotifer to a reference substance, with a view to its eventual routine use in test laboratories; (2) To determine the 48-h EC_{50} of the carbofuran dosed as Furadan® 350 SC, commercial pesticide (FMC, Brazil; 350 g active ingredient per L) and its active compound (carbofuran); (3) To assess the effects of prolonged (4-days) exposure to the commercial product on the variables survival and fecundity (egg production).

Materials and methods

Stock maintenance and culture of rotifers

Philodina cf. *roseola* Ehrenberg, 1830 (Rotifera, Bdelloidea) was collected from experimental tanks of capacity

10,000 L, maintained at the Aquaculture Station of the Hydrobiology Department at the Federal University of São Carlos, São Carlos, SP, Brazil (21°98′–31°25′ S by 47°87′–81°21′ W). Throughout the manuscript this species will be referred as *P. roseola* for the easy of readability. The rotifers were collected with 68 μm mesh plankton net and stored in polyethylene flasks. Those of the species *P. roseola* were isolated and identified on the basis of description by Koste and Terlutter (2001) and Koste and Shiel (1986). Micrograph records of speciments can be found as supplementary material.

The culture medium was reconstituted water, prepared by the methods recommended by the American Society for Testing and Materials ASTM (2001). In accordance with the recommendations of the Brazilian Technical Standards Society (ABNT 2004). The water had pH 7.0–7.8, hardness 40–48 mg $CaCO_3$ L^{-1} and electrical conductivity 160 μScm^{-1}.

Having reached a high numerical density (116 ind mL^{-1} on average), the stock cultures of *P. roseola* were maintained in 50–250 mL beakers, incubators set at 25 ± 1 °C, with a photoperiod of 16 h light:8 h dark. To prevent evaporation of water from the medium, the beakers were covered with cling film. Food and water were replaced every 76 h using the method proposed by Hagen et al. (2009). The rotifers were fed on a suspension of live microalgae of the species *Raphidocelis* (formerly *Pseudokirchneriella subcapitata*), grown in CHU-12 medium (Müller 1972), added to the rotifer culture at a final density of 1×10^5cel mL^{-1}.

The juvenile rotifers used in the experiments were selected from the stock culture initiated from ovigerous females collected from experimental tanks. All observations of the rotifers and the manual separation of the juveniles were done under a Zeiss Stereoscopic microscope, individuals being handled carefully with Pasteur pipettes. The life-cycle of the rotifers of the genus *Philodina* is around 20 days, at 22 °C (Ricci and Fascio 1995). The juvenile stage, which lasts until just before the first clutch of eggs is produced, is less than 3 days old. Despite this very limited duration, juveniles (mean size 198.77 ± 25.88 μm) and adults (mean size 429.96 ± 28.12 μm) can be clearly distinguished by their size. Thus, the smaller rotifers (size ≤198.77 μm) were collected and distributed randomly into the test containers in all the experiments. A juvenile, or non-egg-laying, rotifer is less than 3 days old. There is a clear distinction in size between adults and juvenile rotifers, albeit only for a limited period of time. Thus, rotifers that were much smaller in size in comparison to full-fledged adults up to 3 days old were collected and used directly in the tests. This procedure complies with the ASTM's recommendation that test organisms should be as young as possible (less than 3 days old) (ASTM 1996), although it does not guarantee that the rotifers are neonates, i.e., less than 24-h old. Hence, the generic term juvenile was adopted.

Test substances and solutions

The three chemical products tested in the acute toxicity assays were: potassium dichromate ($K_2Cr_2O_7$), from Labsynth (Brazil), carbofuran (2.3-dihydro-2.2-dimethyl-7-benzofuranyl *N*-methylcarbamate), from Sigma-Aldrich, and Furadan® 350 SC, from FMC (Brazil).

The first compound was chosen because is universally employed as a reference toxic substance, used routinely in sensitivity tests, and was used here to establish the sensitivity range of *P. roseola* (Environment Canada 1990), since to our knowledge there is no sensitivity range already established with a reference substance for this species.

Regarding the pesticide, although the acute toxicity tests were conducted using both the active ingredient and the commercial formulation, chronic tests covered only the latter. This option encompasses the requirements of higher level risk assessment procedures where reproduction assays fit adequately, and where commercial formulations should be considered rather than the active ingredient. In fact, although pesticide approval regulation focuses mostly on the active ingredient (e.g. U.S. EPA 2010) the adjuvant added to formulate the pesticides have already been found to play an important role in the ecotoxicological outcome (Cox and Surgan 2006; Hagen et al. 2011; Nobels et al. 2011).

The levels of purity of the potassium dichromate, carbofuran and Furadan® 350 SC were: 99, 98 and 35 %, where in the last case, the remaining 65 % consisted of inert ingredients added to the product formulation. All three products are highly soluble in water and stock solutions were prepared in water at the following concentrations: 500 mg L^{-1} $K_2Cr_2O_7$; 100 mg L^{-1}carbofuran and 100 mg L^{-1} of carbofuran dosed as Furadan® 350 SC. Test concentrations of each product were prepared by diluting the stock solution in culture medium (reconstituted water).

Although chemical analysis of test solutions, to confirm the actual nominal concentrations of the toxicants, was not performed, it should be noted that the major factor influencing the fate and persistence of carbofuran is the water and soil pH. Carbofuran is very mobile and persistent in acidic environments, but dissipates more rapidly in pHs that are more basic. Carbofuran is stable to hydrolysis at pHs <6, but becomes increasingly susceptible to hydrolysis as the pH increases, hydrolyzing rapidly in alkaline pHs (half-lives of less than a day). The half-life for carbofuran is on the order of weeks at pH 7 (28 days), days at pH 8 (3 days), and hours at pH 9 (0.8–15 h) (Tarkowski 2004). In the present study, because the pH was kept around 7.0, degradation was probably not an interfering factor.

Acute and chronic toxicity tests

For the acute toxicity tests, we incorporated changes in the standard guidelines available for the rotifer *Brachionus* (ASTM 2004) and for the chronic toxicity tests the standard guidelines available for *Daphnia magna* (OECD-211 2008). The modifications made for both are described in the Table 1.

In each acute test, 10 juveniles per replicate were exposed to the nominal concentrations: 21.22, 29.71, 41.59, 58.23, 81.52 and 114.13 mg L^{-1} of potassium dichromate ($K_2Cr_2O_7$); 0.78, 1.56, 3.12, 6.25, 12.5, 25 and 50 mg L^{-1} of carbofuran and 48.22, 57.86, 69.43, 83.52 and 100 mg L^{-1} of carbofuran dosed as Furadan® 350 SC.

For the acute toxicity test, the test concentrations used were as follows: 6 plus a control ($K_2Cr_2O_7$), 7 plus a control (carbofuran) and the 5 plus a control (Furadan® SC 350). For the acute toxicity test 7 plus a control. For both the acute and chronic toxicity test a minimum of 5 plus a control (OECD 2008; ASTM 2004) are recommended, although it is not required to limit the treatments to this number of concentrations. The range of concentrations tested was established based on a series of preliminary tests.

The potassium dichromate acute toxicity tests were repeated 20 times, at intervals varying between 35 and 60 days, to establish the range of sensitivity of *P. roseola* to this compound. For carbofuran and Furadan® 350 SC, these tests were repeated 5 times, to determine the acute toxicity (48-h EC_{50}) of those products to this species.

Acute toxicity tests were conducted in 10 cm watch-glasses kept individually inside 11 cm plastic Petri dishes, for 48 h—the 24 h readings produced higher variability in the EC_{50} estimates, hence setting the exposure period to 48 h was found more adequate. A group of juvenile *P. roseola* (n = 10) was transferred from the stock culture to each watch-glass, by means of a glass micropipette (capillary), under a Leica MZ6 stereo microscope, at ×50 magnification. The culture medium carried over with the rotifers was drained from the test-glass with the capillary pipette, to prevent it from diluting the test solution, and the latter was then added to the rotifers.

The control solution consisted of reconstituted water alone. For each concentration of the test product and the control in each of the repeated tests, four replicates were carried out, and 2 mL of test solution and 10 juveniles were used in each replicate. The experiments were maintained at 25 ± 1 °C, with no food or light. The pH, electrical conductivity, temperature, hardness and dissolved oxygen content were measured in the test and control solutions at the beginning and end of each test. For each complete test, non-toxic plastic cups containing 100 mL of each test solution and the control solution were prepared before the test. The 2 mL aliquots used in each test were taken from these 100 mL volumes and the remaining volume, which was sufficient for the variables to be measured, was placed in the incubator together with the tests and controls.

At the end of the 48-h exposure, the number of immobile rotifers in the 4 replicates was counted under the stereo microscope. The criterion of immobility used in this study was the absence of any internal or external motion in the organism when it was exposed to incident light for 10 s. The toxic effect was defined as immobilization because a motionless animal (even though it may not be dead) is functionally removed from the community, given that it neither eats nor reproduces.

For chronic toxicity tests two complete tests (control plus all pesticide concentrations) were performed. The assays were carried out with seven concentrations of the commercial pesticide, in a series of twofold dilutions: 0.04, 0.09, 0.19, 0.39, 0.78, 1.56 and 3.12 mg L^{-1}. These levels were chosen on the basis of the preliminary acute toxicity

Table 1 Summary of the methodological modifications made to adapt the acute and chronic toxicity test developed with the rotifer *Philodina roseola* from the standard acute toxicity test with *Brachionus* (ASTM 2004) and the standard chronic toxicity test with *Daphnia magna* (OECD 2008), respectively

Test conditions	ASTM (2004)	Present study acute toxicity test	OECD (2008)	Present study chronic toxicity test
Duration	24 h	48 h	21 days	4 days
Endpoint	LC_{50}	EC_{50}	survival and reproduction	survival and reproduction
Temperature (°C)	25	25 ± 1	18 a 22	25 ± 1
Photoperiod	continuous darkness	continuous darkness	16 h light:8 h dark	16 h light:8 h dark
Test chamber size (mL)	2.5	5	50–100	5
Test solution volume (mL)	1.0	2	50–100	2
Age of test animals	0–2 h	<3 days	<24 h	<3 days
Number of neonates per replicate	10	10	1	1
Feeding	No	No	Yes	Yes

tests for this product: the highest tested concentration at which 100 % of the organisms survived was taken as the highest concentration to be tested in the chronic toxicity assay, from which the series of six dilutions was prepared. The tests were held under semi-static conditions, the test solutions and the food being replaced every 2 days. The tests lasted 4 day, which is the normal period allowing the release of the third brood by the females of the control treatment. Rotifers were fed and cultured as described above during the test period.

During the experiments, the pH, temperature, electrical conductivity, dissolved oxygen concentration and hardness of the test solutions were measured when they were replenished, to check whether the medium might affect the biological response, using the same procedure as for the acute toxicity tests. Possible alterations in reproduction caused by the pesticide were assessed by monitoring the fecundity of the rotifers (total number of eggs produced per female) during the exposure. Neonates and eggs were counted and removed with a glass micropipette, under the stereo microscope. The survival of each test organism was also recorded throughout the chronic toxicity test.

Although the genus *Philodina* includes species that are primarily benthic creeping forms, some species are also semi-pelagic thriving in the water column for food, heavily relying on its ciliate corona to swim freely (Hochberg and Litovaitis 2000). Laboratory toxicity tests using the species of this genus may not include the sediment phase in the treatments, as argued by Hagen et al. (2011).

Data treatment and statistical analysis

In the acute toxicity tests, at the end of the 48-h period of exposure, the immobile organisms were counted. If the proportion of immobile rotifers in the control group were to exceed 10 %, the test would be declared invalid, but this did not happen in the present study. The results were used to calculate EC_{50}, the median effective concentration, which causes the defined toxic effect (in this case immobilization) in 50 % of the organisms within the period of exposure (ABNT 2004), and the 95 % confidence interval. The Trimmed Spearman–Karber statistical method was used in these calculations (Hamilton et al. 1977).

The potassium dichromate sensitivity range of *P. roseola* was established by means of a model developed by the USEPA (1985). The control chart consisted of a plot of the 48-h EC_{50} calculated for each of the 20 assays. The upper and lower limits of the range are shown as two lines, which correspond roughly to two standard variations above and below the mean of the 20 values for the 48-h EC_{50}.

Data from the chronic toxicity test were analyzed for significant differences for adult female survival by Fisher's exact test using the program TOXSTAT 3.3 (Gulley et al. 1991). Reproduction data were first checked for normality (with the Chi squared test) and homocedasticity (with Bartlett's test). Because they did not comply with homoscedasticity they were log $(x + 1)$ transformed prior and then subjected to one-way ANOVA followed by the Dunnett's multicomparison test to discriminate significant differences between toxicant concentrations and the control treatment. The statistical analysis was performed using Statistica software version 10 (Statsoft 2010). All the reproduction data were analyzed for significant differences and, where applicable, these were indicated. In all the tests, the difference was considered significant when $P \leq 0.05$.

Data on the toxicity of this pesticide to other species were taken from the ecotoxicity database available at http://cfpub.epa.gov/ecotox/, from which values of LC_{50} and EC_{50} (mg L^{-1}) were selected for bacteria, algae, protozoa, amphipods, cladocerans, rotifers, decapods, insects and fish. Where the test was applied to the commercial product, the effective lethal concentration of the active ingredient was used. The species sensitivity distribution (SSD) curve was constructed with the program ETX 2.0 (Van Vlaardingen et al. 2004). This program also includes the Anderson–Darling test for goodness of fit of log-normality.

Results

Abiotic variables: validity of the toxicity tests

During the tests of acute toxicity to *P. roseola*, the average pH readings in the test solutions were in the range 7.0–7.7 and did not vary by more than 1.0 unit in any assay, while the chronic toxicity tests, the pH kept within the range 7.2–7.6. The water temperature varied between 24.0 and 25.0 °C throughout the periods of the acute and chronic toxicity tests. The electrical conductivity varied between 156.8 and 162.3 μS cm^{-1} during the acute toxicity tests and between 154.6 and 159.4 μS cm^{-1} during the chronic toxicity tests. Water hardness varied between 40 and 48 mg $CaCO_3$ L^{-1} throughout all acute and chronic tests. Dissolved oxygen content remained in the range 5.9–7.2 mg L^{-1} in all the acute toxicity tests and within 6.2–7.9 mg L^{-1} in the chronic toxicity tests.

Acute toxicity

The mean effective concentration of the reference substance potassium dichromate ($K_2Cr_2O_7$) (48-h EC_{50}) and its 95 % confidence limits was 47.10 mg L^{-1} with confidence interval range of 29.52–64.67 mg L^{-1}. The mean values of 48-h EC_{50} and their respective 95 % confidence intervals, for the carbofuran dosed as pesticide Furadan® 350 SC and for its active principle, the carbofuran, are also presented in Fig. 1.

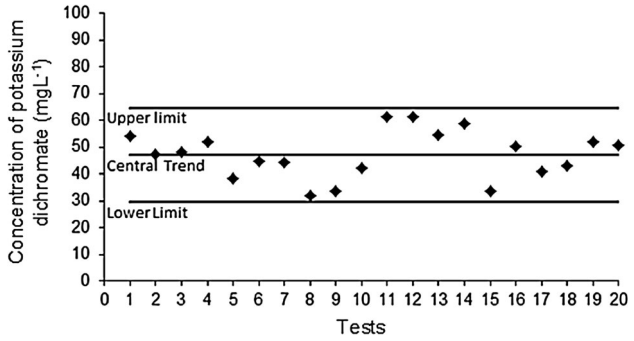

Fig. 1 Range of sensitivity of *P. roseola* to the potassium dichromate based on the results of 20 acute toxicity tests. The *upper* and *lower limits* (95 % confidence intervals) were 29.52 and 64.67 mg L^{-1} of potassium dichromate and the tests were performed at intervals varying between 35 and 60 days

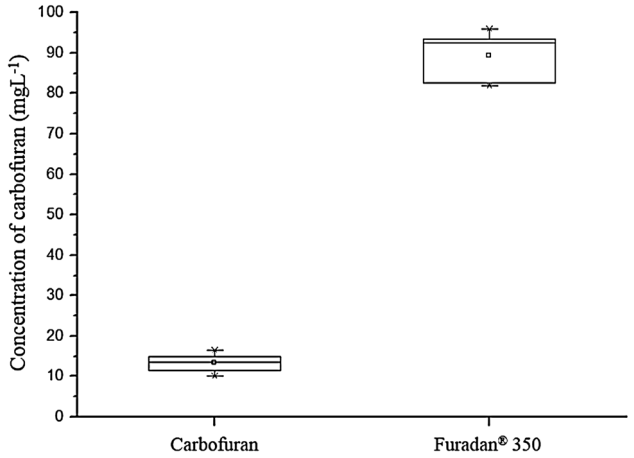

Fig. 2 Box-plots representing mean values of 48-h EC_{50} of the pure active ingredient carbofuran and the carbofuran dosed as pesticide Furadan® 350 SC for the rotifer *P. roseola*

The mean value of 48-h EC_{50} for the carbofuran dosed as Furadan® 350 SC and its active principle, for carbofuran, were computed separately for each of the 5 tests carried out. The results for EC_{50} are present in Fig. 2. From these data, it is clear that the active ingredient, carbofuran, was more toxic to the rotifers than the carbofuran dosed as commercial product Furadan® 350 SC.

Chronic toxicity

The analysis of survival of the adult females fulfilled the requirements for the validity of the chronic toxicity test, as laid down in the OECD guidelines (2008), with mortality in the control group being lower than 20 %, since none of the control animals died during these tests. Detailed information on the biology of *P. roseola* was collected in a parallel study (data to be published later), in which it was observed

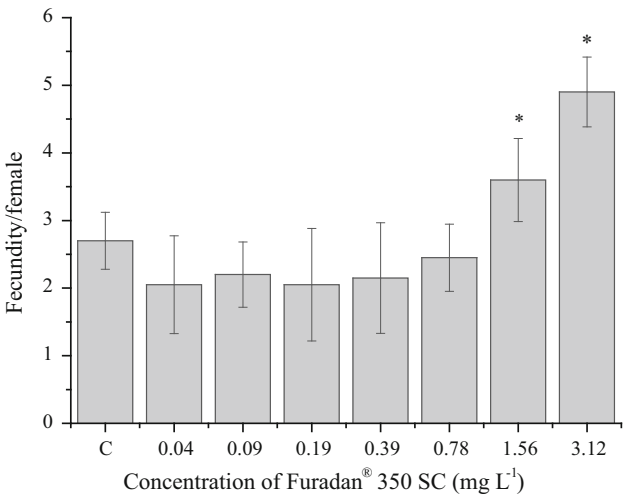

Fig. 3 Fecundity of *P. roseola* (mean ± SD number of eggs per female after 96-h of exposure) to Furadan® 350 SC, at the nominal concentrations of: 0.04, 0.09, 0.19, 0.39, 0.78, 1.56, 3.12 mg L^{-1}. *Asterisk* indicates significant difference from control, by the Dunnet test ($P \leq 0.05$)

that the time from birth to fully developed adulthood (production of third brood of offspring) was 4–5 days. In the present chronic toxicity tests, this was confirmed among the females in the control group.

With regard to the rate of survival of the *P. roseola* females in the groups exposed to the carbofuran dosed as Furadan® 350 SC at the various concentrations and those in the control, no significant differences were detected by Fisher's exact test. The Fisher's critical value was (10.10.10) ($P = 0.05$) ≤ 6, b = 10.

The effects of the carbofuran dosed as Furadan® 350 SC on the fecundity of *P. roseola*, at the end of the 4-days bioassays are shown in Fig. 3. It can be seen that, the concentrations 1.56 and 3.12 mg L^{-1} increased the fecundity significantly, as evidenced by the results of the Dunnett test (F = 14.90; MS = 0.01; df = 71.0; $p = 0.000029$). For the variable fecundity, the highest concentration of the pesticide without any observable effect (NOEC) was 0.78 mg L^{-1}, while the lowest concentration causing an observable effect (LOEC) was 1.56 mg L^{-1}.

Discussion

The comparison of the sensitivity of *P. roseola* with that of a wide variety of species tested elsewhere revealed that this rotifer has a rather low sensitivity to potassium dichromate; only the rotifer *Brachionus plicatilis* and the fishes *Danio rerio* and *Poecilia reticulata* are less resistant than *P. roseola,* as can be observed in Table 2. While some

Table 2 Literature values of acute toxicity (LC_{50} or EC_{50}) of potassium dichromate (reference substance) to a broad spectrum of species, as compared to that for the rotifer *Philodina roseola*, from the present study

Test organism	Parameter	LC_{50}/EC_{50} (mg L^{-1})	References
Rotifera			
Philodina roseola	Immobilization—48 h	47.1	Present study
Philodina acuticumis	Mortality—48 h	29.0	Cairns et al. (1978)
Brachionus calyciflorus	Mortality—24 h	5.2	Crisinel et al. (1994)
Brachionus plicatilis	Mortality—24 h	146.0	Persoone et al. (1989)
Other taxonomic groups			
Raphidocelis subcapitata	Growth inhibition—72 h	0.59	Halling-Sorensen (2000)
Paramecium caudatum	Mortality—24 h	2.567	Madoni et al. (1994)
Tetrahymena pyriformis	Growth inhibition—9 h	5.6	Bogaerts et al. (2001)
Pseudosida ramosa	Mortality—48 h	0.029	Freitas and Rocha (2011)
Navicula libonensis	Growth inhibition—96 h	0.0421–0.0798	Vidal et al. (2014)
Daphnia pulex	Mortality—48 h	0.180	Wu et al. (2007)
Daphnia similis	Mortality—48 h	0.025–0.042	Coelho and Rocha (2010)
Daphnia carinata	Mortality—48 h	0.140	Wu et al. (2007)
Daphnia magna	Mortality—48 h	0.154	Martínez-Jerónimo et al. (2008)
Simocephalus vetulus	Mortality—48 h	0.270	Wu et al. (2007)
Acartia tonsa	Mortality—48 h	10.0	Andersen et al. (2001)
Gammarus aequicauda	Immobilization—48 h	9.520	Cesar et al. (2002)
Hyalella curvispina	Mortality—96 h	0.550	Peluso et al. (2011)
Danio rerio	Mortality—96 h	89.1	Oliveira-Filho and Paumgartten (1997)
Poecilia reticulata	Mortality—96 h	114.6	Oliveira-Filho and Paumgartten (1997)

differences in the sensitivity observed for this metal may arise from small deviations in the test conditions, the values in Table 2 probably reflect largely the intrinsic characteristics of the species.

Potassium dichromate is widely used to check the sensitivity of the lab-cultured invertebrates employed in ecotoxicological studies (Environmental Canada 1990; USEPA 2002). The main concern is to assess the state of health of the animal at the start of the test, which is strongly influenced by the conditions of culture, such as the temperature, pH and hardness of the water and the numerical density, type and quantity of food supplied to the stock cultures used in the bioassays.

Some ecotoxicological studies on pesticides have demonstrated that the commercial product is frequently more toxic to non-target species than the equivalent concentration of active ingredient (Cedergreen and Streibig 2005; Pereira et al. 2000, 2009). Commercial pesticides are generally a mixture of an active ingredient and a variety of other chemical compounds (usually referred to as inert ingredients, or adjuvants) that aid in the mixing and dilution of the product and in its application and stability (Cox and Surgan 2006). A number of authors (e.g. Oakes and Pollak 2000; Krogh et al. 2003; Solomon and Thompson 2003) have already cast doubt on the use of ecotoxicological data on pesticides obtained by testing the active ingredient alone. These authors demonstrated that the so-called inert ingredients can be responsible for a large part of the toxicity of the formulation, whether by exerting their own toxic activity or by promoting that of the active ingredient. For this reason, we tested the acute toxicity of both the carbofuran dosed Furadan® 350 SC and its active principle, carbofuran, to the rotifer under study, *P. roseola*, but our results did not follow the frequently-observed trend, in that this commercial pesticide was not more toxic to this non-target species than the pure active compound. The sensitivity range of *P. roseola* to carbofuran (48-h EC_{50}) was 6 times lower than that to of carbofuran dosed as Furadan® 350 SC, suggesting that the lower toxicity of the commercial product resulted from antagonistic effects of the "inert" ingredients on the carbofuran.

When the sensitivity data of *P. roseola* to carbofuran, recorded in this study, are plotted on the SSD curve together with data from the literature for the acute toxicity of carbofuran to other species (Fig. 4), it can be seen that *P. roseola* is more sensitive than the fish, *C. batrachus*, the bacterium *Vibrio fischeri* and the protozoan *Paramecium caudatum*, but less sensitive than the other 9 species, in particular the daphnids and chironomids. Rotifera exhibit several special characteristics that make them typical opportunists or r-strategists, highly adaptable to unstable

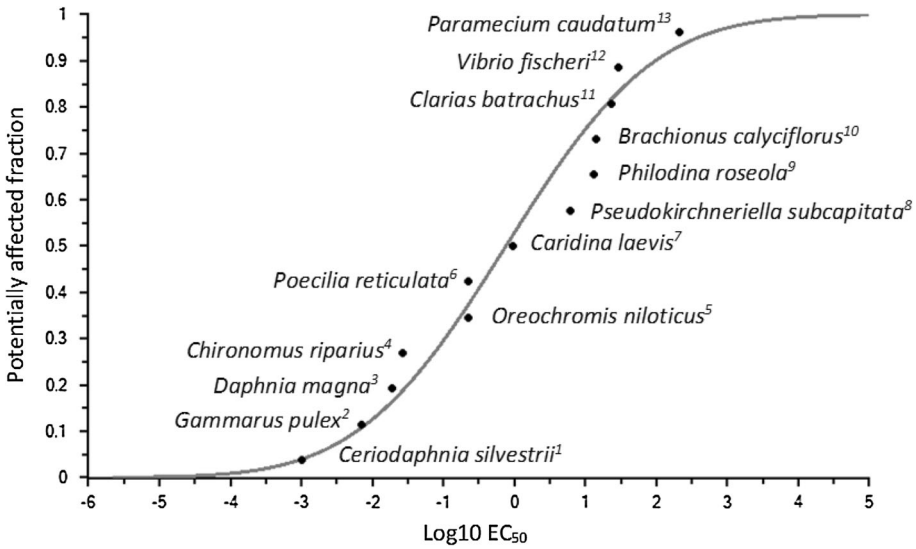

Fig. 4 Species sensitivity distribution (SSD) of organisms from various taxonomic groups, based on the values of LC_{50} or EC_{50} (mg L^{-1}) for the pesticide carbofuran.Source: *1 Ceriodaphnia silvestrii*, Mansano et al. (2013), *2 Gammarus pulex*, Ashauer et al. (2010), *3 Daphnia magna*, Dobšíková (2003), *4 Chironomus riparius*, Ibrahim et al. (1998), *5 Oreochromis niloticus*, Pessoa et al. (2011), *6 Poecilia reticulata*, Dobšíková (2003), *7 Caridina laevis*, Sucahyo et al. (2008), *8 Pseudokirchneriella subcapitata*, Ma et al. (2006), *9 P. roseola*, Present study, *10 Brachionus calyciflorus*, Iesce et al. (2006), *11 Clarias batrachus*, Begum (2008), *12 V. fischeri*, Fernández-Alba et al. (2002), *13 Paramecium caudatum*, Hussain et al. (2008)

habitats. Such characteristics include, for instance, a less specialized diet, high fecundity and frequently parthenogenetic reproduction (Allan 1976; Matsumura-Tundisi et al. 1990). Several studies on the effects of pesticides on the field zooplankton community have evidenced the lower susceptibility of rotifers to toxicant effects (Havens and Hanazato 1993; Peither et al. 1996; Friberg-Jensen et al. 2003; Chang et al. 2005; López-Mancisidor et al. 2008; Golombieski et al. 2008).

Nevertheless, our results indicate that the rotifer *P. roseola*, while it may be less sensitive to carbofuran than most aquatic organisms it is not immune to its toxic effects, so that in the face of the continual and accelerating expansion of agriculture in many countries, this non-target freshwater species will probably become subject to adverse side effects of this pesticide. Although *P. roseola* was not the species most susceptible to this pesticide, Rotifera as a whole is one of the main groups among the zooplankton in most freshwater ecosystems (Segers et al. 1993; Rocha et al. 1995; Bozelli 2000; Sharma and Sharma 2012), and ecotoxicological studies already revealed that a particular species may be less sensitive to one compound, but extremely sensitive to other. Considering that an accurate assessment of toxicity in aquatic environments requires a battery of species representing a variety of ecological niches, the use of *P. roseola*, a benthic species, mainly exposed to sediment environment is of relevance since the most frequently used species of rotifer are planktonic. In light of these considerations, the use of *P. roseola* in toxicity evaluations might give a closer assessment of toxicant effects in whole ecosystem.

The number of *P. roseola* females surviving the 4-days chronic test wasnt affected at all by the exposure to the carbofuran dosed as Furadan® 350 SC throughout that period, at any of the tested concentrations. However, at the two highest concentrations, the fecundity (number of eggs per female) was enhanced significantly. Some studies focusing on the effects of toxic agents on the developmental phases of rotifers life-cycles have found evidences of their role as endocrine disruptors (Snell and Carmona 1995; Preston et al. 2000; Preston and Snell 2001; Radix et al. 2002; Xi et al. 2007) indicating that sexual reproduction and egg production are the most sensitive features of the cycle. It is also possible that the apparently unexpected result for the two last concentrations in the chronic test is related to the fact that rotifers actually are less sensitive to the mode of action of the Furadan® 350 SC, mainly related to the cholinesterase enzyme (Gupta 1994), not affecting this rotifer at the maximum concentration established. Thus, the increased fecundity could be related to a favorable effect of the inert ingredients which are not fully described in the commercial formulation. Another possibility is that the duration of the test of chronic toxicity was not sufficient to show any chronic effect, because the 4 days we had chosen for the duration of the experiment was based on the time required for the third brood production found in our laboratory cultures of *P. roseola*.

Our results exhibit the phenomenon of hormesis, which is when small amounts of a stressor or toxic agent produce

a stimulatory effect, qualitatively different from the effect of the agent at higher concentrations (Towsend and Luckey 1960). The prevalence of this phenomenon as a dose response to environmental contaminants has been subjected to a wide-ranging review by Calabrese and Blain (2005). It has been reported for many species of zooplankton (Calabrese and Baldwin 2003; Gama-Flores et al. 2007; Guo et al. 2012; Rumengan and Ohji 2012), and also, for other species of rotifers. Huang et al. (2014) tested the pesticide azadirachtin at sublethal doses and noted a rise in the population density of *Brachionus plicatilis* at the lowest concentrations tested.

However, in the case of *P. roseola* in this study, differently from pattern of hormesis normally observed by the authors referred to above, we observed the hormetic response only at the highest concentrations of the carbofuran dosed as Furadan® 350 SC. The same type of hormetic profile was recorded recently by Huang et al. (2013), who found that the secondary production of the rotifer *B. calyciflorus* was greater at one of the highest concentrations of the pesticide aldrin than at lower concentrations.

The underlying causes of these types of hormesis are poorly understood and there is still controversy about their ecological significance (Forbes 2000). Nonetheless, it can be inferred that the high fecundity observed at the highest sub lethal concentrations of pesticide used in the chronic toxicity tests would probably have adverse effects on the future reproductive performance of the female rotifer and on its own survival, as consequence of allocating so much energy to reproduction under the stress caused by the pesticide. In a study of the lifespan and fecundity of the rotifer *Asplanchna brightwelli*, Snell and King (1977) found that there was a trade-off between longevity and reproductive performance in these rotifers. The females that exhibited a high fecundity tended not to survive as long as those that had a lower rate of reproduction.

The reasons behind the distinctive dose–response of *P. roseola* to carbofuran, particularly the special pattern of hormesis, need to be investigated in more depth, taking into account that, in nature, organisms exhibit compensatory mechanisms and, also, that it is hard to simulate the multifactorial complexity of the natural habitat in the laboratory. Experiments in mesocosms (more complex and realistic set-ups) might provide the conditions for a deeper understanding of the effects of this pesticide and of its interactions with other environmental factors in the aquatic habitats.

Conclusions

It may be concluded that the rotifer *P. roseola* is less susceptible to the toxicity of both potassium dichromate and carbofuran than most other aquatic species and that the carbofuran dosed as Furadan® 350 SC, the commercial pesticide, is less toxic to this rotifer than its pure active ingredient, the carbofuran, at equivalent nominal concentrations. Furthermore, in this study it has been found that the dose response of *P. roseola* to prolonged exposure to sublethal concentrations of this pesticide displays an unusual form of hormesis, whose underlying mechanism is not yet understood and should be further investigated.

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Conflict of interest The authors declare that they have no conflict of interests.

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