PANAMJAS
Pan-American Journal of Aquatic Sciences

Prediction of a low-risk concentration of diflubenzuron to aquatic organisms and evaluation of clay and gravel in reducing the toxicity.

CLAUDIO M. JONSSON[1*], MARIANA S. G. MOURA E SILVA[2]; VINICIUS S. DE MACEDO[1]; DARLENE D. DANTZGER[3]; JOSÉ H. VALLIM[1]; ANA L. S. MARIGO[2]; HIROSHI AOYAMA[3]

[1]*Laboratório de Ecotoxicologia e Biossegurança, Embrapa Meio Ambiente, Jaguariúna, 13820-000, São Paulo, Brazil.*
[2]*Laboratório de Ecossistemas Aquáticos, Embrapa Meio Ambiente, Jaguariúna, 13820-000, São Paulo, Brazil.*
[3] *Universidade Estadual de Campinas, Instituto de Biologia, Departamento de Bioquímica, Campinas, 13083-970, São Paulo, Brazil.*

**Corresponding author:* claudio.jonsson@embrapa.br

Abstract: Diflubenzuron is an insecticide that, besides being used in the agriculture, has been widely used in fish farming. However, its use is prohibited in this activity. Diflubenzuron is not in the list of Brazilian legislation establishing maximum permissible limits in water bodies for the protection of aquatic communities. In this paper, according toxicity data of diflubenzuron in non-target organisms, it was calculated an hazardous concentration for only 5% of the species (HC5) of the aquatic community. This parameter value was estimated to be about 7 x 10^{-6} mg L^{-1}. The low value is due to the extreme high toxicity of diflubenzuron to daphnids and to the large variation in sensitivity among the species tested. Two relatively low cost and inert materials were efficient in removing the diflubenzuron from solutions containing this compound. Among these materials, expanded clay shown to promote reduction of approximately 50% of the toxicity of a solution containing diflubenzuron. The results may contribute to the establishment of public policies in Brazil associated to the definition of maximum permissible limits of xenobiotics in the aquatic compartment. This study is also relevant to the search of low cost and inert materials for xenobiotics removal from aquaculture or agricultural effluents.

Key words: insecticide, aquaculture, hazardous concentration, non-target organisms, toxicity removal

Resumo. Predição da concentração de baixo risco de diflubenzuron para organismos aquáticos e avaliação da argila e brita na redução da toxicidade. O diflubenzuron é um inseticida que além de ser usado agricultura, tem sido amplamente empregado na piscicultura, apesar do seu uso ser proibido nesta atividade. Este composto não consta na lista da legislação brasileira que estabelece limites máximos permissíveis em corpos de água para a proteção das comunidades aquáticas. No presente trabalho, a partir da toxicidade do diflubenzuron em organismos não-alvo, foi calculada a concentração de risco para somente 5% das espécies (HC5). O valor deste parâmetro foi estimado em aproximadamente 7 x 10^{-6} mg L^{-1} . Este baixo valor é devido à extremamente alta toxicidade do diflubenzuron para dafnídeos e à grande variação de sensibilidade entre as espécies testadas. Dois matérias de relativamente baixo custo se mostraram eficientes na remoção da toxicidade do diflubenzuron de soluções contendo este composto. Dentre esses materiais, a argila expandida promoveu a redução em aproximadamente 50% da toxicidade de uma solução contendo diflubenzuron. Os resultados podem contribuir

para políticas públicas no Brasil relacionadas ao estabelecimento de limites máximos permissíveis de xenobióticos no compartimento aquático. Também, para a pesquisa de matérias inertes e de baixo custo com potencial de remoção de xenobióticos presentes em efluentes da aqüicultura ou da agricultura.

Palavras chave: inseticida, aquacultura, concentração de risco, organismos não- alvo, despoluição

Introduction

According to the latest FAO report (FAO 2014), aquaculture is demonstrating a substantial growth, with the increment of an average annual growth rate equal to 6.1%. Therefore it is necessary that the downstream impacts of aquaculture activities be minimal. That is, the quality of effluents must be the best possible in order that the changes be minimized on water bodies of surrounding areas (Ruiz-Zarzuela *et al.* 2009). In Brazil, fish farming has been developed a lot as an economic activity; on the other hand, it was observed an increment of problems associated with ectoparasites diseases (Zago *et al.* 2014).

The pesticide most commonly used in fish farming in Brazil is diflubenzuron (Figure 1). Its use is also beneficial in agriculture farming against several insect species. This insecticide belongs to the benzoyl-urea group and has an inhibitory action of chitin synthesis during the immature stage of the insect. Despite its not allowed to be used in aquaculture, it has been applied by several fish farmers to control ectoparasites like the crustaceans *Lernaea cyprinacea* and *Dolops carvalhoi* (Maduenho & Martinez 2008).

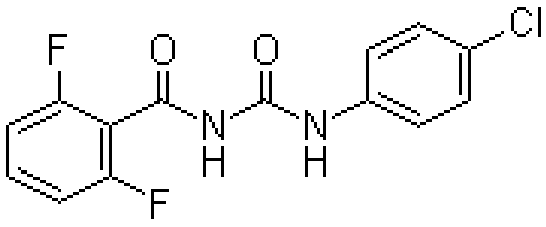

Figure 1. Molecular structure of diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea; N-(((4-chlorophenyl)amino)carbonyl)-2,6-difluorobenzamide).

The legislation establishing maximum permissible limits of chemicals in water bodies in Brazil (BRASIL 2005) reports a small number of agrochemicals, although a large amount of compounds are currently used. The diflubenzuron, among many other agrochemicals, are not included in the list of this legislation. So, is necessary to collect data upon its adverse effects to implement public policies regarding the establishment of maximum permissible levels in water bodies. The distribution of species sensitivity against a pollutant is an approach used in risk analysis to predict harmful concentrations ("Hazardous Concentrations" - HC) that affect a certain percentage of species in a community. Typically, this approach is used to determine a preliminary concentration level with 5% uncertainty (HC5), that is, the concentration in the environment that would protect 95% of the species (Aldenberg & Slob 1993, OECD 1995). In this context, the use and determination of toxicological endpoints in several representative species of different trophic levels is an important tool in risk analysis. In order to estimate the HC5 from small number of toxicity data, procedures were developed to correct for uncertainty due to small sample size (Aldenberg & Slob 1993). Such methods can be applied if toxicity data are available for at least five different species (OECD 1995).

The microalgae *Pseudokirchniella subcapitata* and the microcrustacean *Daphnia similis* are two bioindicators organisms listed in the "Manual of tests for evaluation of ecotoxicity of chemical agents" (IBAMA 1988), used in studies for the registration of chemicals in regulatory Brazilian agencies.

The microcrustacean *Artemia salina* is another phytoplanktonic arthropod which effectively responds to the insecticidal action of several compounds (Gartenstein *et al.* 2006, Hassarangsee *et al.* 2015). Although this organism is representative of marine ecosystems, it has been used together with freshwater organisms in HC predictions (Nunes *et a*l 2014, Minguez et al. 2014).

The aquatic insect *Chironomus sancticaroli* (Diptera: Chironomidae), in its larval stage, is a native Brazilian freshwater organism that has been used to assess the toxicity of sediments (Morais *et al*. 2014, Rebechi *et al.* 2014). Despite the fact that this organism is a benthic invertebrate, HC predictions included toxicity parameters of the genus Chironomus together with organisms living in the water column (Heijerick *et al*. 2012, Rodrigues *et al* 2013). Insecticides have been shown to be extremely

toxic to Chironomus (Le Blanc *et al*. 2012).

The fish *Oreochromis niloticus* (Nile tilapia) is a species of economic importance and is widely disseminated in aquaculture (Santos *et al.* 2007). It inhabits national water resources (Miranda et al, 2010;. Sarmento-Soares *et al.* 2008) and has been used in ecotoxicological tests (Assis *et al*. 2012, Jordaan *et al*. 2013).

Natural treatment systems using filters filled with low-cost materials are increasingly used to treat effluents from agro-industry and pesticide run-off (Augusto 2011, Al Hattab & Ghaly 2012). Thus, materials like clay and gravel, has been studied due its effectiveness in reducing the degree of toxicity of effluents contaminated with various pollutants (Stearman *et al.* 2003, 2012, Nkansah *et al.* 2012).

The clays are natural materials with earthy texture and low granulometry. Its basic unit has surfaces charged negatively which are neutralized by cation exchange. This is where adsorption occurs by the adsorbed water molecules or exchange of cations, such as Ca^{2+}, Mg^{2+}, Na^{+}. Or toxic cations such as Pb^{2+} contained in industrial effluents (Tomasella 2013). However, the literature has also reported about the use of clays in the removal of organic compounds (Dordio & Carvalho 2013, Tahar *et al.* 2014). Thus, for example, Suciu *et al*. (2011) found a high yield (~96%) at removing the fungicides penconazole and cyazofamid by a commercial clay.

The gravel is a material classified as an basaltic aggregate, widely used in the manufacture of concrete, highway ballast and other constructions. This material has also been used in the removal of toxic compounds of wastewater from agricultural sources (Stearman *et al.* 2012, Ibrahim *et al.* 2015).

The Resolution Nº 357 of the National Council of Environment (Brasil 2005), besides establishing maximum concentration limits for some chemicals , considers the need to reformulate the classification of waters by a better specification of the required quality standards . Also it considers the pollutant launch control on harmful levels for the aquatic life and the establishment of treatment systems for this release by the entrepreneurs . According to the Resolution , these arguments are based on the Article 90 of the Federal Law Nº 6938 on the National Environmental Policy (Brasil 1981), which promotes the establishment of environmental quality standards and the implementation of technologies to improve the environmental quality.

Therefore, on the basis of the exposed above, this study evaluated the toxicity of diflubenzuron in different species of aquatic organisms in order to obtain toxicological endpoints that were used to estimate an HC5 for this compound. For that, it was used the criterion of species sensitivity distribution. Because of the need to develop technologies for the efficient treatment of aquaculture wastewater at low cost, it has also been studied the effectiveness of gravel and expanded clay as potential materials for use in reducing the toxicity of effluent containing diflubenzuron.

Materials and Methods

Test-material: It was used a commercial formulation (Dimilin®, Chemtura Indústria Química do Brasil Ltda.; wettable powder) of diflubenzuron . The product contained 25% of the active ingredient and 75% of inert excipients, according the label information.

Effect on the algal growth: The unicellular algae chloroficea *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was used as test-organism. It was grown in NPK medium (Sipaúba-Tavares & Pereira 2008) under controlled temperature at 20 ± 2 ° C and light intensity of approximately 1,300 lux. The test was performed as described by Becaro *et al.* (2015). The algal suspension was distributed in Petri dishes so that the final volume of 15 ml per dish had an algal concentration of ~10^5 cells ml^{-1}. Algae suspensions were exposed to the concentrations of the active ingredient equals to 0.0, 13.0; 20.0; 30; 45.0; 67.0 and 100 mg L^{-1} These test-concentrations were prepared in NPK medium. These concentrations were chosen with basis on a preliminary range finding test. At 0, 24, 48, 72 and 168 hours, aliquots were taken from the algal suspensions for evaluate the algal growth. This was accomplished by measuring the absorbance (750 nm) using a Shimadzu spectrophotometer Model UV-1650 PC, since the absorbance is proportional to cell concentration. The calculation of the effective concentration that inhibited the growth rate by 10% and 50% for 168 h (EC10-7d and EC50-7d) was based on the exposure to different concentrations of the active ingredient. In calculating the growth rates, we used the slope of the linear regression values of the respective growth curves in function of time (Grillo *et al.* 2015). For this, the absorbance values were previously processed in the respective natural logarithms. The data were analysed by the Simple Regression module contained in the Statgraphics Plus version 5.1 program (Manugistics 2001).

Evaluation of the acute effect and prolonged

exposure in D. similis: As test-organism it was used the fresh water microcrustacean *Daphnia similis* with age of approximately 24 hours. The *D. similis* culture and exposure media were prepared using tap water that had been previously filtered for 48 h using a filter containing activated carbon (Clemente *et al* 2014). The invertebrates were previously cultured in aquariums of 40 x 25 x 15 cm containing the dechlorinated and filtered water with the following physical-chemical characteristics: total hardness = 53.58 mg L^{-1} $CaCO_3$; pH 7.5 and conductivity = 111.4 µS cm^{-1}. The organisms were kept under controlled temperature room at 20 ± 2 ° C, light intensity about 1,000 lux and were fed with the algae *P. subcapitata*. Acute toxicity immobilization tests were performed in accordance with OECD Guideline Part I (OECD 1984, Becaro *et al.* 2015). The daphnids were exposed for 48 hours to diflubenzuron at concentrations of 0.0; 9.0 x 10^{-5}; 1.7 x 10^{-4}; 3.0 x 10^{-4}; 5.5 x 10^{-4}; 1.0 x 10^{-3} and 1.8 x 10^{-3} mg L^{-1} of the insecticide prepared in water with the physical-chemical characteristics described above . These concentrations were chosen based on preliminary range finding test. The results of immobility of the organisms towards different concentrations of the chemical were subjected to probit analysis in order to determine the EC50-48h and its confidence interval 95%. It was performed using the Statgraphics Plus Version 5.1 software (Manugistics 2001).

Based on the EC50-48h value, it was designed an exposure for 7 days in which a neonate of *D. similis* was placed in a beaker containing 30 ml of diflubenzuron solution. The experiment was carried out in 10 replicates for each test-concentration: 0.0; 1.1 x 10^{-5}; 3.3 x 10^{-5}; 1.1 x 10^{-4}; 3.3 x 10^{-4} and 1.0 x 10^{-3} mg L^{-1}. The solutions were renewed in periods of 48 hours for 7 days and the daphnid of each container was taken periodically to assess the total length of the individual (Johnson & Delaney 1998). In this case it was considered the length from the head to the end of the carapace, disregarding the apical spine (Van Dam *et al*. 1995). The measurement was performed using a stereomicroscope which was coupled to a digital camera. The images analysis were performed using the MB-Ruler® program (Software Solutions Markus Bader, Iffezheim, Germany; http: // www. markus-bader.de/MB-Ruler/). The body measures in function of time allowed to calculate the growth rate for each concentration tested (Clemente *et al*. 2014). Growth rates were compared by the "Kruskal-Wallis Test" contained in the "One Way ANOVA" module of the Statgraphic Centurion XVII Version 1.17.04 (StatPoint Technologies 2014). Bonferroni test was used for post hoc comparison between the control group (0.0 mg L^{-1}) and the exposed groups.The adopted statistically significance level was $p<0.05$. It was determined the highest concentration that did not manifest significant effect on the growth rate compared to the control (No Observable Effect Concentration - NOEC).

Evaluation of the acute effect on Artemia salina: Brine shrimp nauplii were obtained after 48 h of incubation of commercial Artemia cysts (INVE Aquaculture Inc., Ogden, Utah) in 30 g L^{-1} saline solution "Sera Premium®" (Sera GmbH, Heinsberg) prepared in a similar water that was used for *D. similis*. An intense aeration was applied to this suspension cysts through a porous rock, at temperature of 25±1°C and light intensity about 6,300 lux . The test was carried out in beakers with 50 ml capacity containing 20 ml of test-solution and 10 nauplii in each container. The test-solutions containing the insecticide were prepared in saline solution as described above. The test -concentrations evaluated were 0,00; 0.05; 0.09; 0.153; 0.291; 0.53 mg L^{-1}, in triplicate, under the light intensity of 1,000 lux and temperature of 20 ± 2 °C (USEPA 1991, Becaro *et al.* 2015). After 48 h of exposure to diflubenzuron, the number of organisms was recorded to calculate the concentration that affected 50% of mobility (EC50-48h), together with the 95% confidence interval (Becaro *et al.* 2015). For this, it was used the Statgraphics Plus Version 5.1 software (Manugistics 2001).

Assessment of the toxicity in Chironomus sancticaroli: Clutches of *C. sancticaroli* were allocated in plastic trays (30 x 37 x 8cm) containing ~1cm height of sieved sand that was washed and sterilized at 121°C for 30 minutes. The organisms were grown in 2 L of water from an artesian well with the following physico-chemical characteristics: pH = 7.5; conductivity = 111 µS cm^{-1} and dissolved oxygen = 5.5 mg L^{-1}. The containers remained in temperature controlled room providing a water temperature of 22± °C and photoperiod and 12 h light (~500 lux) / 12h dark. The water column was aerated through a porous stone and a mini compressor. The newly hatched larvae were initially fed with algae (first 48 hours) and then fed once a day with Tetramin® until they reach the last larval stage (4th instar) (Fonseca & Rocha 2004). A total of 30 larvae (4th instar) were allocated in crystallizers in a total volume of 300 ml (Lee & Choi 2006, Rebechi *et al*. 2014). The test-concentrations of

diflubenzuron evaluated were 0,0; 1,0; 10,0 and 100,0 mg L^{-1}. These were prepared in the same water used for the culture of the organisms. The total number of larvae per test-concentration was divided in triplicate. After 96 h exposure to the toxic agent, the mobility of the organisms was quantified to determine the concentration that is effective to 50% of the population (EC50-96h), as described by Rebechi *et al.* (2014).

Assessment of toxicity in fish: Fingerlings of tilapia (*O. niloticus*), (average measure and weight, 3 cm and 6g, respectively) were purchased from a local supplier (Aquaculture Brumado, Mogi Mirim, SP). The organisms were acclimated in plastic tanks during one week in a volume of 175 L of dechlorinated water with the following characteristics: conductivity = 160 µS cm-1; pH = 7.2 and hardness = 50 mg L^{-1} $CaCO_3$. They were subjected to a photoperiod of 16h light (~1,000 lux) / 8h dark and temperature of 28±2 °C. During the acclimation period, the fish were fed with commercial feed TetraMin Plus® (Tetra Holding US Inc.) and remained under observation in order to check for possible signs of infection, behavioral changes and mortality. After the acclimation , fish were transferred to glass tanks containing 10 L of the test-solution. Ten organisms were placed into each container that remained under static system. The test-concentrations of diflubenzuron evaluated were 0.0; 0.1; 1.0; 10.0 and 100.0 mg L^{-1} (OECD 1992a).

The test-solutions were prepared in a similar water used for fish acclimatation. The total period of exposure to diflubenzuron was 96 hours, during which the fish were not fed and were maintained under the same environmental conditions mentioned above. At the end of the exposure period, the number of dead individuals was recorded in order to determine the lethal concentration that affect 50% of the population (LC50-96h). The study followed the Ethical Principles in Animal Experimentation adopted by the Brazilian Society of Laboratory Animal Science (SBCAL). It was approved by the Ethics Committee on the Use of Animals of the State University of Campinas (CEUA/Unicamp) under the registration Nº 2756-1 (Law Nº.11794/2008).

Determination of the harmful diflubenzuron concentration to 5% of the species (HC5-50%) of a community: In order to calculate this parameter, the values of "no observed effect concentration (NOEC)" were estimated. In evaluating the effect on algae, the NOEC was estimated as the "effective inhibitory concentration at 10% of algal growth (EC10-7d)" (OECD 1995). For other organisms, the NOEC was estimated by the ratio EC50 or LC50 / 10 as described by various authors (Gherardi-Goldstein 1990, OECD 1995, Elmeggard *et al.* 2000, Rebechi *et al.* 2014). The hypothetical environmental concentration at which the probability of only 5% of species in a community would be affected (HC5) (Aldenberg & Slob 1993, OECD 1995) was determined using the program ETX (ETX 2.0 RIVM; Van Vlaardingen et al. 2004) based on the method of Aldenberg & Jaworska (2000). For this calculation, it was adopted the lower confidence limit 50% of HC5 (HC5-50%) as the risk concentration (Aldenberg & Slob 1993, OECD 1995).

The distribution of normality of the NOEC values was previously analyzed by the Kolmogorov-Smirnov test contained in the Statgraphics Centurion XVII Version 1.17.04 program. (StatPoint Technologies 2014).

Removal of the diflubenzuron toxicity by inert materials: Gravel and expanded clay were used as inert materials. The former was a crushed stone n. 02 measuring 9.5 to 31.5 mm in diameter. The expanded clay was equivalent to the grit n. 02 with a diameter between 22 to 32 mm and apparent density of 450 kg / m^3. Each of these substrates were placed within an external aquarium filter type hang-on (Eheim Liberty 2040, EHEIM GmbH & Co. KG, DE) which was coupled in a glass container. The filters had a volume of 650 cm^3 to deposit the substrate and flow capacity of 380 L/h. Five hundred grams of the material were placed in the filter through which was recirculated 2 L of the insecticide solution containing 1 mg L^{-1} of the active ingredient.

Freshwater from an artesian well with the same characteristics described above in "Evaluation of the acute effect and prolonged exposure in *D. similis*" was used for the preparation of the diflubenzuron solution. Each recirculation system related to each inert material was assessed in duplicate. A system evaluated without the inert material was considered as a control. After 24 h of recirculation, the salinity of the recirculated solution was adjusted by adding 30 g L^{-1} "Sera Premium®" salt (Sera GmbH, Heinsberg) so as to be compatible with the survival of *A. salina*. This solution was diluted with various proportions of 30 g L^{-1} Sera Premium salt medium in order to obtain test-solutions with concentrations of 0.0 (control); 5.3; 9.5; 17.0; 30.0; 55.0 and 100.0% of recirculated solution.

The toxicity evaluation of the diflubenzuron solution passed through each filter system was performed by transferring five *A. salina* nauplii to beakers containing 30 mL of the above test-solutions. Each recirculation system related to each inert material was assessed in duplicate.

The toxicity assays were carried out in quadruplicates, performing a total of 40 organisms for each test dilution. The EC50-48h and its 95% confidence interval was evaluated as described in "Evaluation of the acute effect on *Artemia salina*". These values were compared and considered significantly different from each other ($p<0.05$) when their confidence intervals showed no overlap (Yang *et al.* 2002, Bejgarn *et al.* 2015). This was performed by the module "Probit Analysis" contained in the Statgraphics Plus Version 5.1 software which calculated the confidence intervals at a given significance level (Manugistics 2001).

The EC50 values were transformed into Toxic Units (TU) by the ratio 100/EC50-48h (Araujo *et al.* 2005). TU values were used to calculate the percentage of removal of toxicity by the following equation (Araujo *et al*. 2005, Megateli *et al*. 2009):

Toxicity removal (%) = [(TU control - TU material) / TU control)] x 100

where :
TU material: toxic units of the diflubenzuron solution recirculated (24 h) through the system containing the filter filled with inert material.
TU control: toxic units of the diflubenzuron solution recirculated (24 h) through the system with the filter without the inert material.

Results

Toxicological evaluation: The order of toxicity according to the EC50 or LC50 values for the different organisms was *D. similis* > *A. salina*> *P. subcapitata* > *C. sancticaroli* = *O. niloticus* (Table I). The toxicity of diflubenzuron for *P. subcapitata* and for *A. salina* was approximately 60,720 and 80 times lower than the observed for *D. similis* (Table I).

D. similis was the more sensitive organism to the diflubenzuron effects which presented an EC50-48h value equal to 0.97×10^{-3} (0.65×10^{-3} - 1.62×10^{-3}) mg L^{-1} (Table I). This result allowed outlining a long exposure experiment (7 days) with this organism in order to estimate an NOEC based on the EC50-48h value.

Figure 2 shows the results of the effect of diflubenzuron on microcrustacean growth rate. At the concentrations of 1.1×10^{-3} and 3.3×10^{-4} mg L^{-1}, it was no possible to evaluate growth after the second day because the organisms died after this time. It was found a significant decrease ($p<0.005$) in the growth rate from 1.1×10^{-4} mg L^{-1} to the higher concentrations, compared to the control. So it was estimated an NOEC equivalent to 0.033×10^{-3} mg L^{-1} since this was the highest concentration that not affected ($p>0.5$) the test-organisms.

It was assigned a EC50-48h value >100 mg L^{-1} to *C. sancticaroli* larvae since it was verified the presence of mobility during all the exposure period at 100 mg L^{-1} . Similarly, the diflubenzuron proved to be practically non-toxic to the fish *O. niloticus* with an LC50-96h >100 mg L^{-1} (USEPA 1985b).

HC5-50% determination: The values of the estimated NOECs for each test-organism, are shown in Table I. These values were calculated by different criteria according described in Materials and Methods and were helpful in determining the HC5-50% value.

Table I. Acute toxicity, not observed effect concentration (NOEC) and hazardous concentration of diflubenzuron for 5% of species (HC5-50%).

Test-organism	EC50 or LC50 (mg L^{-1})	Calculation criteria of NOEC	NOEC (mg L^{-1})
Pseudokirchneriella subcapitata	58.90 (44.00 – 85.50)	EC10 -7d	6.27
Daphnia similis	0.97×10^{-3} (0.65×10^{-3} – 1.60×10^{-3})	Length growth (7 d)	0.033×10^{-3}
Artemia salina	0.08 (0.03-0.12)	EC50-48h/10	8.0×10^{-3}
Chironomus sancticaroli	>100	EC50-48h /10	10
Oreochromis niloticus	>100	LC50-96h/10	10

HC5-50% = 7.3×10^{-6} mg L^{-1}

According to the OECD (1995) methodology, the NOEC values distribution must follow a log-logistic function (Figure 3) that can be evaluated by the Kolmogorov-Smirnov test. As such, it was determined a value of p = 0.2464 by this test, thus it was not rejected the hypothesis that the NOEC values are from a log-logistic distribution to a certain level of 95%.

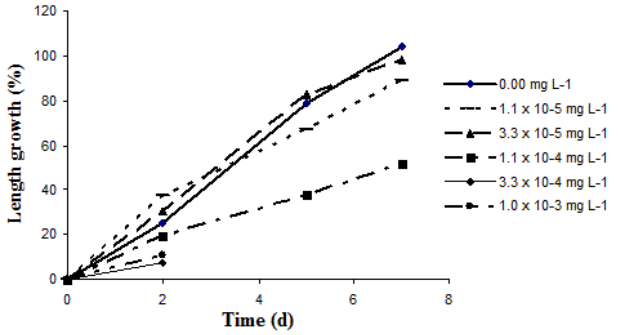

Figure 2. Change of *Daphnia similis* size as a function of exposure time to diflubenzuron at different concentrations (mg L^{-1}). Results are expressed as % of body growth from the initial time. Each point is the mean of 10 observations for each treatment.

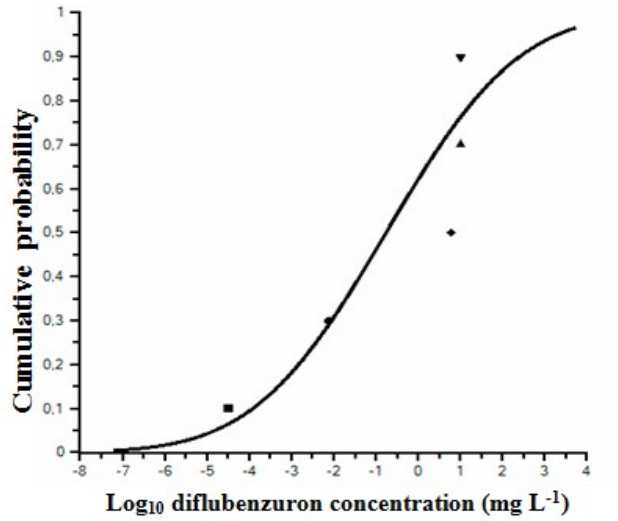

Figure 3. Log-logistic distribution of cumulative sensibility according of the NOEC values of diflubenzuron for the test-organisms: ■ *Daphnia similis;* ● *Artemia salina*; ♦ *Pseudokirchneriella subcapitata*; ▲ *Chironomus sancticaroli*; ▼ *Oreochromis niloticus* .

Data showed a high variability of susceptibility of the test-organisms to diflubenzuron. This difference was approximately 300,000 times between the more susceptible and the less sensitive specie. This fact, together with the extremely low NOEC value for *D. similis*, given a HC5-50% equivalent to approximately 7 ng L^{-1}. So, this is the maximum concentration in the environmental compartment that theoretically not would cause adverse effect to 95% of community species, and is associated with an uncertainty factor that is the lower confidence interval limit at 50% of confidence (Aldenberg & Slob 1993) .

Reduction of diflubenzuron toxicity by inert materials: Table II indicates that the removal rate of diflubenzuron toxicity by expanded clay and gravel was approximately 50% and 30%, respectively.

There was an increase on the EC50-48h value for *A. salina* equivalent to 51.36%. This was observed after recirculating the diflubenzuron solution through the filter containing expanded clay in comparison to the solution recirculated through the filter free of this inert material. Such increase was significant at the 95% level ($p<0.05$), demonstrating that there is strong evidence on the propriety of expanded clay in reducing the toxicity of effluents containing diflubenzuron.

For gravel, the increase on the EC50-48h value was 44.29% which was not significant ($p>0.05$). However, it was significant at the 93.5% level ($p <0.065$) because the 93.5% confidence intervals did not overlap (Yang *et al.* 2002, Bejgarn *et al.* 2015). This fact also states that the presence of this other material in the filter systems can reduce the adverse effects of the insecticide in organisms.

Discussion

Our results showed slightly toxicity effect of diflubenzuron on *P. subcapitata* and are partially in agreement with the data published by WHO (2005) that reported EC50 values (growth inhibition) higher than 80 mg L^{-1} for such compound in microalgae.

The body growth rate measure of daphnids is a parameter that has been suggested by several authors because the good correlation with the reproduction effect studies (Johnson & Delaney 1998, Santojanni *et al.* 1998). In our study, NOEC value derived of the body growth rate of *D. similis* was 0.033 x 10^{-3} mg L^{-1}.

In consonance with our findings, the literature cited very low NOEC values of diflubenzuron for daphnids. As reported by WHO (2005), the NOECs values calculated for *Daphnia magna* were equivalent to 0.45 x 10^{-3} mg L^{-1} and 0.38 x 10^{-3} mg L^{-1}. However these reported values were from 48 h exposures. According an USEPA (1997) report, the NOEC values <0.06 x 10^{-3} mg L^{-1} and <0.09 x 10^{-3} mg L^{-1} were obtained from life cycle studies with *D. magna*.

Table II. Toxicity to *Artemia salina* of the recirculated diflubenzuron solution (1 mg L^{-1}) through the inert materials.

	EC50-48h (fiducial limit 95%) (% test-solution)	Toxic Units (TU)	Toxicity removal (%)
Expanded clay test			
Without inert material (control)	30.67 (26.18-36.28)	3.26	-
With inert material	63.06 (55.60-72.56)	1.58	51.60
Gravel test			
Without inert material (control)	47.75 (37.67-61.46)	2.09	-
With inert material	68.90 (60.42-80.01)	1.45	30.62

Also in analogy to the present work, 0.04 x 10^{-3} mg L^{-1} was the NOEC of diflubenzuron reported for *D. magna* derived from a 21 days chronic test (Swedish Chemicals Agency 2007).

For *A. salina*, the EC50-48h value calculated in our work is much higher to that reported by Gartenstein et al. (2006) who determined the EC50-48h of 3.7 x 10^{-4} mg L^{-1}. However, this author reports that the LC50 of diflubenzuron to brine shrimp is not well established. Thus, for example, nauplii exposed to 0.1; 1.0 and 10.0 mg L^{-1} had the survival affected, while those exposed to 0.01 mg L-1 behaved like the control (Cunningham 1976).

The value of NOEC assigned to *A. salina* and estimated from our acute toxicity study (Table I) is close to that described in the report of the USEPA (1997). In this case, it was assessed the sensitivity of *A. salina* to diflubenzuron during the life cycle.

An EC50-48h >100 mg L^{-1} rates diflubenzuron as "practically non-toxic" for *C. sancticaroli* in relation to its short term toxicity. This pattern of Chironomus larvae sensitivity to diflubenzuron is very different when compared to other insecticides, although using *Chironomus dilutis* larvae as test-organism. For the later organism, the insecticides chlorpyrifos, dimethoate and imidacloprid showed LC50-96h values equivalent to 0.63 x 10^{-3}; 2.65 x 10^{-3} and 1.29 x 10^{-3} mg L^{-1}, respectively (Le Blanc *et al*. 2012). Karnak and Collins (1974) evaluated the toxicity of 15 insecticides belonging to different chemical groups in *Chironomus tentans* and found that the less toxic had an LC50-24h value equal to 9.5 x 10^{-3} mg L^{-1}. Perhaps, the reason we found a high LC50-48h value for *C. sancticaroli* should be that the diflubenzuron do not affect the biochemical mechanisms which directly affect the survival within a short period of exposure. Such property would be associated to acetylcholinesterase inhibitors but not to insecticides that interfere on chitin biosynthesis. In this context, it was demonstrated that microssomal preparations from arthropods extracts catalyze the transfer of groups N-acetyl-D-glucosamine from UDP-N-acetylglucosamine to an endogenous acceptor. The formed product was identified as chitin and the reaction is inhibited by diflubenzuron (Horst 1981).

Similarly to this work, others works also noted the absence of toxicity of diflubenzuron for fish. Thus, the LC50-96h values for *P. mesopotamicus* (Lopes 2005), rainbow trout (Fischer & Hall 1992) and catfish (Fischer & Hall 1992) were >2000; 240 and >100 mg L^{-1}, respectively. However, a slight toxicity was observed for some species (EPA 1985), such as for *Fundulus heteroclitus* (LC50-96h = 33 mg L^{-1}; Lee & Scott, 1989) and trout (LC50 = 57 mg L^{-1}; USEPA 1997).

According to Table I, the EC50-48h or the LC50-96h values for Chironomidae and fish were not effectively set due to the lack of signs of toxicity at the highest concentration tested. Thus, it was established an EC50-48h or LC50-96h >100 mg L^{-1}. However, to enable the NOEC estimation for these organisms, it was adopted the LC50 and EC50 values as being equivalent to 100 mg L^{-1}. This value is given as "test limit value" in the acute toxicity assessment for fish and *Chironomus* sp. (OECD 1992a, OECD 2010). So compounds with LC50 or EC50 greater than 100 mg L^{-1} are considered practically non-toxic (USEPA 1985a) and the procedures consider that is unnecessary to test greater concentrations.

The HC5-50% value calculated by the program ETX in the present work is very close to the HC5 estimated by applying the uncertainty factor equal to 10. In this way, HC5 can also be obtained by dividing by 10 the lower NOEC value (0.033 x 10^{-3} / 10) from a study with at least one algae (primary producer), an microcrustacean (primary consumer) and a fish (secondary consumer) (OECD 1992b, OECD 1995, Pennington 2003). It is also

closely to the determined by the formulae described in OECD (1995) based in Aldenberg & Slob (1993), that is, 4.9 ng L^{-1}.

Toxicity tests with *A. salina* and others microcrustaceans constitute a promising tool of relatively low cost and simplicity in evaluating the efficiency of materials for removal water pollutants. For example, Pimentel *et al.* (2009) analyzed the toxicity to *A. salina* of a raw and treated wastewater from a processing fruit industry. The authors concluded that treatment strategies employed to minimize the toxicity of cardol and cardanol present in the effluent should be reviewed. Bortolotto *et al.* (2009) observed that the leachate from landfills had their EC50-24h values altered from 71.63 to 97.99% in *A. salina* . This fact occurred before and after treatment by microbiological degradation, respectively. Degradation of the ethion insecticide by photocatalysis with TiO_2 was evaluated using the same test-organism (Hassarangsee *et al.* 2015).

Results of this work corroborate with literature data about the use of clay for the withdrawal of xenobiotics toxicity. Thus for example, in a wastewater treatment system in which was found 2 herbicides and 8 pharmaceuticals, the removal efficiency by the expanded clay was 54,8% to 98,4%. This was performed by the contact of this material with the contaminated effluent during 20 hours in the rate 35:1 (g : L) (Tahar *et al.* 2014). Mesocosms experiments containing an expanded clay layer (35cm depth) flooded until its surface, removed about 52% of the initial concentration (1 mg L^{-1}) of the herbicide MCPA (2-methyl-4-chlorophenoxyacetic acid) after the contact of the material with the pesticide solution during 3, in the absence of flow (Dordio *et al.* 2013). According with Thiebault *et al*. (2015), who studied the adsorption mechanisms of pharmaceutical products (emerging micro-pollutants) with clay, the adsorption of several pollutants onto clay materials is mainly driven by electrostatic interaction through a cation exchange. These authors reported that these layered materials show non-singular hydration properties with a macroscopic swelling, a cation exchange capacity allowing the intercalation of micro-pollutants within the interlayer space. Nkansah et al. (2012) reported that the main sorption mechanism of expanded clay aggregates seem to be based on a fast attraction of hydrophobicity or low polarity compounds towards the hydrophobic sites of the material surface immediately on exposure. This mechanism was enhanced with contact time. It is possible that this pollutant removal mechanism may also be assigned to diflubenzuron due its hydrophobicity properties (log Kow = 3.88, Sangster 1997).

The gravel is another material of relatively low cost that has been used in wastewater decontamination processes in constructed wetland systems. The use of gravel as a support medium for effluent treatment systems enables the installation of biofilms composed of various microorganisms . These will settle and develop on the empty spaces of the material to degrade the organic matter (Colombo *et al*. 2010). Although, there is a lack of works about the yield of removal of aquatic organic pollutants using this material.

In this study, the percentage of removal of the toxic effects by the gravel was lower than that calculated for the expanded clay, nevertheless it is also a promising material for the adsorption and consequent removal of agrochemicals from effluents. Stearman *et al.* (2012) used gravel in removal systems by wetlands during 1.2 days of hydraulic retention time. It was found 48.9 % removal of the herbicide prodamine contained in the wastewater. Removal of herbicides metolachlor and simazine were also reported by the same author, also assigning high efficiency to the gravel use (Stearman *et al.* 2003).

However, despite the gravel be recommended for water remediation systems , it belongs to the very low sorption capacity materials (Ahmedi & Pelivanoski 2011) . On the other hand the clay was qualified as the better material for the pollutant removal due the higher potential to trap and hold the contaminant comparing to gravel materials (Ahmedi & Pelivanoski 2011). These findings are in agreement with our results.

In conclusion, it is expected that the results of this work contribute to support public policies in Brazil for the establishment of maximum permissible limits of xenobiotics in the aquatic compartments, as is the absence of these levels in the current legislation. It is also considered that data presented here are an advance in the knowledge about the use of materials to reduce pollutant toxicity . Such materials could be used in effluents output of aquaculture or agriculture systems.

Therefore, this study represents a contribution to the risk assessment of the insecticide diflubenzuron in the aquatic compartment. Organisms of different taxonomic groups showed large differences in sensitivity to the test-compound. Both microcrustaceans species were the more susceptible, while fish and dipterans were the more

tolerant. With respect to the dipterans, the resistance to the adverse effects would be associated with the mode of action on the chitin biosynthesis. In these organisms and in fishes, this lack of effect was not evidenced in the short term exposures even at very high doses. An extremely low concentration value for diflubenzuron (~7 ng L^{-1}) was assigned for the protection of aquatic communities based on the species sensitivity distribution. The extremely high toxicity of diflubenzuron to cladocerans found in this work, as was reported in the literature, contributed to for determining the low HC5-50% value.

Both filter systems containing inert materials led to a decrease of the toxic effects to *A. salina*. The filter filled with expanded clay was more effective in reducing the toxicity of diflubenzuron when compared with the gravel filter. Although, this last material as well as the clay, showed capacity to remove the solution toxicity and could be used in successive filtrations to increase the removal efficiency.

Acknowledgments

The authors are grateful for financial support provided by the "Fundação de Apoio à Pesquisa do Estado de São Paulo – FAPESP" (processes 2010/08097-5; 2011/19204-0 and 2011/04788-6) and to the "Programa Institucional de Bolsas de Iniciação Científica - PIBIC)" of the "Conselho Nacional de Desenvolvimento Cientifico e Tecnológico – CNPq". We are also grateful to Prof. Susana Trivinho-Strixino (UFSCar – São Carlos, SP), for the donation of egg masses of *Chironomus sancticaroli*.

References

Ahmedi, F. & Pelivanoski, P. 2011. Sand, gravel, clay, and coal combustion byproducts used as a filter material for phosphorus removal in small scale on-site wastewater. Pp. 98-101. *In*: Mastorakis, N., Mladenov, V., Lepadatescu, B., Karimi, H. R. & Helmis, C.G. (Ed**.). Recent advances in environment, energy systems and naval science** . **Proceedings of the 4th International Conference on Environmental and Geological Science and Engineering (EG '11)** . WSEAS Press, Barcelona, Spain, 297 p.

Al Hattab, M. T. & Ghaly, A. E. 2012. Disposal and treatment methods for pesticide containing wastewaters: Critical Review and Comparative Analysis. **Journal of Environmental Protection**, 3: 431-453.

Aldenberg, T. & Jaworska, J. S. 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. **Ecotoxicology and Environmental Safety**, 46: 1–18.

Aldenberg, T. & Slob, W. 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. **Ecotoxicology and Environmental Safety**, 25: 48-63.

Araujo, C. V. M., Nascimento, R. B.; Oliveira, C. A. & Strotmann, U. J. & Silva, E. M. 2005.The use of Microtox_to assess toxicity removal of industrial effluents from the industrial district of Camaçari (BA, Brazil). **Chemosphere**, 58: 1277–1281.

Assis, C. R., Linhares, A. G., Oliveira, V. M., França, R. C., Carvalho, E. V., Bezerra, R. S. & Carvalho Jr., L. B. 2012. Comparative effect of pesticides on brain acetylcholinesterase in tropical fish. **Science of the Total Environment**, 441: 141-150.

Augusto, K.V. Z. 2011. Tratamento e reuso do efluente de biodigestores no processo de biodigestão anaeróbia da cama de frango. **PhD. Thesis.** Unicamp, Campinas, Brazil, 72 p.

Becaro, A. A., Jonsson, C. M., Puti, F. C.,Siqueira, M . C., Mattoso, H. C., Correa, D. S. & Ferreira, M. D. 2015. Toxicity of PVA-stabilized silver nanoparticles to algae and microcrustaceans. **Environmental Nanotechnology, Monitoring & Management**, 3: 22–29.

Bejgarn, S., Mac Leod, M., Bogdal, C. & Breitholtz, M. 2015. Toxicity of leachate from weathering plastics: An exploratory screening study with *Nitocra spinipes*. **Chemosphere** 132: 114–119.

Bortolotto, T., Bertoldo, J.B., Silveira, Z. F., Defaveri, T.M., Silvano, J. & Pich, C.T. 2009. Evaluation of the toxic and genotoxic potential of landfill leachates using bioassays. **Environmental Toxicology and Pharmacology**, 28: 288–293.

BRASIL. 1981. **Lei Federal Nº 6.938, de 31 de agosto de 1981.** Diário Oficial da União, Brasília. World Wide Web electronic publication accessible at http://www.planalto.gov.br/ccivil_03/Leis/L6938.htm (Accessed 12/21/2015).

BRASIL. 2005. **Resolução CONAMA Nº 357, de 17 de março de 2005.** Diário Oficial da União, Brasília. World Wide Web electronic publication accessible at http://www.mma.gov.br/port/conama/res/res05/res35705.pdf (Accessed 09/16/2015).

Clemente, Z., Castro, V. L., Jonsson**,** C. M. & Fraceto, L. F. 2014. Minimal levels of ultraviolet light enhance the toxicity of TiO2 nanoparticles to two representative organisms of aquatic systems**. Journal of Nanoparticle Research**, 16: 1-16.

Colombo, L. G. E., Bordin, F., Rocha, D. C. & Gomes, L. P. 2010. Emprego de brita, cerâmica vermelha e concretos como meios suporte de reator anaeróbio no tratamento de lixiviado de aterro sanitário. **VII Simpósio Internacional de Qualidade Ambiental,** Porto Alegre, RS, Brazil, 1-11. World Wide Web electronic publication accessible at https://www.researchgate.net/publication/261052934_Emprego_de_brita_ceramica_vermelha_e_concretos_como_meios_suporte_de_reator_anaerobio_no_tratamento_de_lixiviado_de_aterro_sanitario (Accessed 12/21/2015).

Cunningham, P. A. 1976. Effects of Dimilin (TH-6040) on reproduction in the brine shrimp, *Artemia salina*. **Environmental Entomology**, 5:701-706.

Dordio, A. & Carvalho, A. J. P. 2013. Constructed wetlands with light expanded clay aggregates for agricultural wastewater treatment. **Science of the Total Environment**, 463–464: 454–461.

Elmeggard, N., & Jagers op Akkerhuis, G. A .J. M. 2000. **Safety factors in pesticide risk assessment. Differences in species sensitivity and acute-chronic relations**. National Environmental Research Institute. NERI Technical Report 325. 57 p. World Wide Web electronic publication accessible at http://www2.dmu.dk/1_viden/2_Publikationer/3_fagrapporter/rapporter/fr325.pdf (Accessed 09/11/2015).

FAO 2014. Food and Agriculture Organization of the United Nations. **The State of World Fisheries and Aquaculture. Opportunities and Challenges.** World Wide Web electronic publication accessible at http://www.fao.org/3/a-i3720e.pdf (Accessed 09/10/2015).

Fischer, S. A. & Hall, L. W. 1992. Environmental concentrations and aquatic toxicity data on diflubenzuron (Dimilin). **Critical Reviews in Toxicology**, 22: 45-79.

Fonseca, A. L. & Rocha, O. 2004. Laboratory cultures of the native species *Chironomus xanthus* Rempel, 1939 (Diptera – Chironomidae). **Acta Limnologica Brasiliensia**, 16(2): 153-161.

Gartenstein, S., Quinnell, R. G., Larkum, W. D. 2006. Toxicity effects of diflubenzuron, cypermethrin and diazinon on the development of *Artemia salina* and *Heliocidaris tuberculata*. **Australasian Journal of Ecotoxicology**, 12: 83-90.

Gherardi-Goldstein, E., Bertoletti, E., Zagatto, P. A., Araujo, R. P .A. & Ramos, M. L. L. C. (Eds.). 1990. **Procedimentos para a utilização de testes de toxicidade no controle de efluentes líquidos**. Cetesb, São Paulo, 17 p.

Grillo, R., Clemente, Z., Oliveira, J. L., Campos, E. V. R., Chalupe, V. C., Jonsson, C. M., Lima, R. , Sanches, G., Nishisaka, C. S., Rosa, A. H., Oehlke, K., Greiner, R. & Fraceto, L. F. 2015. Chitosan nanoparticles loaded the herbicide paraquat: The influence of the aquatic humic substances on the colloidal stability and toxicity. **Journal of Hazardous Materials** 286: 562-572.

Hassarangsee, S., Uthaibutra, J., Nomura, N. & Whangchai, K. 2015. Degradability of treated ethion insecticide by TiO_2 photocatalysis. **Pakistan Journal of Biological Sciences** 18(1): 27-31.

Heijerick, D. G., Regoli, L. & Carey, S. 2012. The toxicity of molybdate to freshwater and marine organisms. II. Effects assessment of molybdate in the aquatic environment under REACH. **Science of the Total Environment** 435–436: 179–187

Horst, M. N. 1981.The biosynthesis of crustacean chitin by a microsomal enzyme from larval brine shrimp. **The Journal of Biological Chemistry**, 256(3): 1412-1419.

IBAMA (Ed.). 1988. **Manual de testes para a avaliação da ecotoxicidade de agentes químicos**. Secretaria do Meio Ambiente, Brasília, 351 p.

Ibrahim, T. G., Goutelle, A., Healy, M. G., Brennan, R., Tuohy, P., Humphreys, J., Lanigan, G., Brechignac, J. & Fenton, O. 2015. Mixed agricultural pollutant mitigation using woodchip/pea gravel and woodchip/zeolite permeable reactive interceptors. **Water, Air and Soil Pollution**, 226: 51. DOI

10.1007/s11270-015-2335-4

Johnson, I. & Delaney, P. 1998. Development of a 7-Day *Daphnia magna* growth test using image analysis. **Bulletin of Environmental Contamination and Toxicology**, 61: 355-362.

Jordaan, M. S., Reinecke, S. A. & Reinecke, A. J. 2013. Biomarker responses and morphological effects in juvenile tilapia *Oreochromis mossambicus* following sequential exposure to the organophosphate azinphos-methyl. **Aquatic Toxicology**. 144-145, 133-140.

Karnak, R. E. & Collins, W. J. 1974. The susceptibility to selected insecticides and acetylcholinesterase activity in a laboratory colony of midge larvae, *Chironomus tentans* (Diptera:Chironomidae). **Bulletin of Environmental Contamination and Toxicology**, 12(1): 62-69.

Le Blanc, H. M. K., Culp, J. M., Baird, D. J., Alexander, A. C. & Cessna, A. J. 2012. Single versus combined lethal effects of three agricultural insecticides on larvae of the freshwater insect Chironomus dilutus. **Archives of Environmental Contamination Toxicology**, 63: 378–390.

Lee, B. M. & Scott, G. I. 1989. Acute toxicity of temephos, fenoxycarb, diflubenzuron, and methoprene and *Bacillus thuringiensis* var. israelensis to the mummichog (*Fundulus heteroclitus*). **Bulletin of Environmental Contamination and Toxicology**, 43: 827-832.

Lee, S. B. & Choi, J. 2006. Multilevel evaluation of nonylphenol toxicity in fourth-instar larvae of *Chironomus riparius* (Diptera, Chironomidae). **Environmental Toxicology and Chemistry**, 25(11): 3006–3014.

Lopes, R. B. 2005. Análise ecotoxicológica dos xenobióticos triclorfon e diflubenzuron empregados na aqüicultura continental. **PhD. Thesis.** University of São Paulo, Piracicaba, Brazil, 104 p.

Maduenho, L. P. & Martinez, C. B. R. 2008. Acute effects of diflubenzuron on the freshwater fish *Prochilodus lineatus***. Comparative Biochemistry and Physiology, Part C**, 148: 265–272.

MANUGISTICS. Statgraphics Plus: version 5.1 for Windows. Rockville, 2001.

Megateli, S., Semsari, S. & Couderchet, M. 2009. Toxicity and removal of heavy metals (cadmium,copper, and zinc) by *Lemna gibba*. **Ecotoxicology and Environmental Safety**, 72, 1774–1780.

Minguez, L., Di Poi, C., Farcy, E., Ballandonne, C., Benchouala, A., Bojic, C., Cossu-Leguille, C., Costil, K., Serpentini, A., Lebel, J. M. & Halm-Lemeille, M. P. 2014. Comparison of the sensitivity of seven marine and freshwater bioassays as regards antidepressant toxicity assessment. **Ecotoxicology** 23: 1744–1754.

Miranda, J. C., Mazzoni, R. & Silva, C. E. A. 2010. Ocorrência da tilápia do Nilo *Oreochromis niloticus* Linnaeus, 1758 na microbacia do rio Mato Grosso, Saquarema, estado do Rio de Janeiro. **SaBios: Revista de Saúde e Biologia**, 5(2): 47-50.

Morais, G. S., Pesenti, E. C. , Cestari, M. M. & Navarro-Silva, M. A . 2014. Genotoxic effect of phenanthrene on *Chironomus sancticaroli* (Diptera: Chironomidae). **Zoologia (Curitiba): An International Journal for Zoology**, 31(4): 323-328.

Nkansah, M. A., Christy, A. A., Barth, T. & Francis, G. W. 2012. The use of lightweight expanded clay aggregate (LECA) as sorbent for PAHs removal from water. **Journal of Hazardous Materials**, 217-218: 360-365.

Nunes, B., Antunes, S. C., Santos, J., Martins, L. & Castro, B. B. 2014. Toxic potential of paracetamol to freshwater organisms: A headache to environmental regulators? **Ecotoxicology and Environmental Safety,** 107: 178–185.

OECD. 1984. **Guidelines for testing of chemicals. *Daphnia sp.*, acute immobilization test and reproduction test, n. 202.** Organization for Economic Co-operation and Development, Paris, 16 p.

OECD . 1992a . **Guideline for Testing of Chemicals. Fish, Acute Toxicity Test, n. 203.** Organization for Economic Co-operation and Development, Paris, 9 p. World Wide Web electronic publication accessible at http://www.oecd-ilibrary.org/environment/test-no-203-fish-acute-toxicity-test_9789264069961-en;jsessionid=bidue0t0f27au.x-oecd-live-02. (Accessed 09/16/2015)

OECD. 1992b. **Report of the OECD workshop on the extrapolation of laboratory aquatic toxicity data to the real environment. Environment Monograph N. 59.** Organisation for the Economic Cooperation and Development, Paris, 43 p. World Wide

Web electronic publication accessible at http://www.oecd.org/chemicalsafety/testing/34528236.pdf (Accessed 09/10/2015).

OECD. 1995. **Guidence document for aquatic effects assessment . OECD Environment Monographs N. 92.** Organization for the Economic Cooperation and Development, Paris, 1995. p 37, 34 and 28. World Wide Web electronic publication accessible at http://www.oecd.org/chemicalsafety/testing/34290206.pdf (Accessed 09/10/2015).

OECD. 2010. **Draft Guideline for Testing of Chemicals. *Chironomus* sp., Acute Immobilisation Test.** Organization for Economic Co-operation and Development, Paris, 15 p.

Pennington, D.W. 2003. Extrapolating ecotoxicological measures from small data sets**. Ecotoxicology and Environmental Safety**, 56: 238–250.

Pimentel, M. F., Pires de Lima, D., Martins, L. R., Beatriz, A., Santaella, S. T. & Lotufo, L. V. C. 2009. Ecotoxicological analysis of cashew nut industry effluents, specifically two of its major phenolic components, cardol and cardanol. **Pan-American Journal of Aquatic Sciences**, 4 (3): 363-368.

Rebechi, D., Richardi, V. S., Vicentini, M., Guilosky, I.. C., de Assis, H. C. S., Navarro-Silva, M. A. 2014. Low malathion concentrations influence metabolism in *Chironomus sancticaroli* (Diptera, Chironomidae) in acute and chronic toxicity test. **Revista Brasileira de Entomologia**, 58(3): 296–301.

Rodrigues, A. C. M., Jesus, F. T., Fernandes, M. A. F., Morgado, F., Soares, A. M. V. M. & Abreu, S. N. 2013. Mercury toxicity to freshwater organisms: Extrapolation using species sensitivity distribution. **Bulletin of Environmental Contaminantion and Toxicology**, 91: 191-196.

Ruiz-Zarzuela, I., Halaihel, N., Balcázar, J. L., Ortega, C, Vendrell, D, Pérez, T, Alonso, J. L. & de Blas I. 2009. Effect of fish farming on the water quality of rivers in northeast Spain. **Water Science and Technology**, 60(3): 663-671.

Sangster, J. (Ed.) 1997. **Octanol-water partition coefficients: Fundamentals and physical chemistry**. John Wiley & Sons Ltd., Baffins Lane - Chichester, England, p. 165.

Santojanni, A., Gorbi, G. & Sartore, F. 1998. Prediction of fecundity in chronic toxicity tests on *Daphnia magna.* **Water Research**, 32: 3146-3156.

Santos, V. B., de Freitas, T. F. R., Logato, P. V. R., Freato, T. A., Órfão, L. H. & Millioti, L. C. 2007. Rendimento do processamento de linhagens de tilapias (*Oreochromis niloticus*) em função do peso corporal. **Ciência e Agrotecnologia**, 31(2): 554-562.

Sarmento-Soares, L. M., Mazzoni, R. & Martins-Pinheiro, R. F. 2008. A fauna de peixes dos Rios dos Portos Seguros, extremo sul da Bahia, Brasil. **Boletim do Museu de Biologia Mello Leitão, Nova Série**, 24: 119-142.

Sipaúba-Tavares, L. H. & Pereira, A. M. L. 2008. Large scale laboratory cultures of *Ankistrodesmus gracilis* (Reisch) Korsikov (Chlorophyta) and *Diaphanosoma biergei* Korinek, 1981 (Cladocera). **Brazilian Journal of Biology**, 68(4): 875-883.

StatPoint Technologies. Statgraphics Centurion XVII (Version 17.1.04), StatPoint Technologies, Inc., Herndon, VA, 2014.

Stearman, G. K., George, D. B., Carlson, K. & Lansford, S. 2003. Pesticide removal from container nursery runoff in constructed wetland cells. **Journal of Environmental Quality**, 32(4): 1548-1556.

Stearman, G. K., George, D. B. & Hutchings, L. D. 2012. Removal of nitrogen, phosphorus and prodiamine from a container nursery by a subsurface flow constructed wetland. **Journal of Bioremediation and Biodegradation,** S7-002 World Wide Web electronic publication accessible at http://dx.doi.org/10.4172/2155-6199.S7-002. (Accessed 09/16/2015).

Suciu, N. A., Ferrari, T., Ferrari, F., Trevisan, M. & Capri, E. 2011. Pesticide removal from waste spray-tank water by organoclay adsorption after field application to vineyards. **Environmental Science and Pollution Research** 18: 1374–1383.

Swedish Chemicals Agency. 2007. **Work Programme for Review of Active Substances in Biocidal Products Pursuant to Council Directive 98/8/EC**. Document 1. Diflubenzuron. Swedish Chemicals Agency, Sundbyberg. 46 p. World Wide Web electronic publication accessible at https://www.yumpu.com/en/document/view/36885972/diflubenzuron-doc-i. (Accessed 09/11/2015).

Tahar, A., Choubert, J. M., Miège, C., Esperanza, M., Le Menach, L., Budzinski, H., Wisniewski, C. & Coquery, M. 2014. Removal of xenobiotics from effluent discharge by adsorption on zeolite and expanded clay: an alternative to activated carbon?. **Environmental Science and Pollution Research**, 21: 5660–5668.

Thiebault, T., Guégan, R. & Boussafir, M. 2015. Adsorption mechanisms of emerging micro-pollutants with a clay mineral: Case of tramadol and doxepine pharmaceutical products. **Journal of Colloid Interface Science** 453:1-8.

Tomasella, R. C. 2013. Avaliação do potencial de compostos naturais (argila, turfa e carvão) na remoção do chumbo (Pb) e da toxicidade de um efluente industrial. **MSc. Thesis**. Unesp, Rio Claro, Brazil, 99 p.

USEPA (Ed.). 1985a. **Hazard Evaluation Division. Standard evaluation procedure: acute toxicity test for freshwater invertebrates.** United States Environmental Protection Agency**,** Washington, 12 p.

USEPA (Ed.). 1985b. **Hazard Evaluation Division. Standard evaluation procedure: acute toxicity test for estuarine and marine organisms (estuarine fish 96-hour acute toxicity test)**. United States Environmental Protection Agency**,** Washington, 11 p.

USEPA (Ed.). 1991. **Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth edition. EPA/600-/4-90/027**. United States Environmental Protection Agency, Washington, 293 p.

USEPA (Ed.) 1997. **Reregistration eligibility decision (RED). Diflubenzuron. EPA 738-R-97-008**. United States Environmental Protection Agency, Washington. 199 p. World Wide Web electronic publication accessible at http://www.epa.gov/oppsrrd1/reregistration/REDs/0144red.pdf (Accessed 09/10/2015).

Van Dam, R. A., Barry, M. J., Ahokas, J. T. & Holdway, D. A. 1995. Toxicity of DTPA to *Daphnia carnitata* as modified by oxygen stress and food limitation**. Ecotoxicology and Environmental Safety**, 31: 117-126.

Van Vlaardingen, P. L. A., Traas, T. P., Wintersen, A. M. & Aldenberg, T. 2004. ***ETX* 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. RIVM report 601501028/2004**. National Institute for Public Health and the Environment, Bilthoven, The Netherlands, 68 p.

WHO. 2005. **Specifications and evaluations for public health pesticides. Diflubenzuron 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea**. World Health Organization, Geneva. 29 p. World Wide Web electronic publication accessible at http://www.who.int/whopes/quality/diflubenzuron_eval_march_2006.pdf (Accessed 09/10/2015).

Yang, X., Buschman, L. L., Zhu, K. Y. 6 Margolies, D. C. 2002. Susceptibility and detoxifying enzyme activity in two spider mite species (Acari: Tetrany-chidae) after selection with three insecticides. **Journal of Economic Entomology**, 95: 399–406.

Zago, A. C., Franceschini, L., Garcia, F. & Schalch, S. H. C. & Gozi, K. S. & Silva, R. J. 2014. Ectoparasites of Nile tilapia (*Oreochromis niloticus*) in cage farming in a hydroelectric reservoir in Brazil. **Brazilian Journal of Veterinary Parasitology**, 23: 171-178.

Received: October 2015
Accepted: December 2015
Published: January 2016