ACUTE TOXICITY OF METHYL PARATHION ON *DAPHNIA LAEVIS* (BIRGE, 1879) AND ITS IMPACT ON THE ACTIVITY OF FARMED FISH

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ABSTRACT

Keppeler, E.C.; Plese, L. P. M. & Vieira, L. J. S. 2015. AcuteToxicity of Methyl Parathion on Daphnia laevis (Birge, 1879). Braz. J. Aquat. Sci. Technol. 19(1):33-38. eISSN 1983-9057. DOI: 10.14210/bjast.v19n1.p33-38 The present study was carried out to determine the short-term effective concentration 50% toxicity (EC50) of methyl parathion, and to evaluate whether *Daphnia laevis* has the potential to be used as test organism, in medium M4, using potassium dichromate as reference substance. Twenty neonates with aged less than 24h were separated into four groups, exposed to concentrations of 5 x 10-6 mg L-1, 10 x 10-6 mg.L-1, 20 x 10 -6 mg.L-1, 40 x 10-6 mg.L-1, 80 x 10-6 mg.L-1, plus a zero control, for 24h in a static test. Spearman-Karber method was is adoted for estimating of the median effective concentration (EC50). The EC50 of methyl parathion in *Daphnia* was 19.4 x 10-6 mg.L-1. Thus, *Daphnia laevis*, in medium M4, has potential to be used as test organism using as reference substance the potassium dichromate.

Key words: Cladocera, EC50, Potassium dichromate.

Pesticides are chemicals produced for various purposes, such as in the areas of growing, storing and improving agricultural products in the field (Rosenzweig & Liverman, 1992) and represent a serious threat to ecosystems (Howarth, 1983), because are used also in protecting natural and planted forests (Venzon *et al*., 2006), and of other ecosystems, such as water bodies.

The high concentration of fish cultivated always constitutes a factor that favors the emergence of diseases (Ueda *et al*., 2013). This is especially true in the case of intensive fish farms, because under conditions of confinement, fish are subjected to chronic stress, resulting above all in the deterioration of water quality. For example, methyl parathion is a toxic compound utilized to eliminate aquatic insect larvae, and very used in aquaculture (Almeida *et al*., 2005).

Daphnids are cladocerans freshwater microcrustaceans abundant in lakes and pond, because the animals are effective grazers on phytoplankton (major primary producer in lakes) and are favorite food for vertebrate and invertebrate predators (Pennak, 1978; Cooney, 1995). The species of cladocerans of genus *Daphnia* are the most often utilized for toxicity tests because of their high sensitivity to many toxic chemicals. Thus, it is reasonable to use *Daphnia* as a test organism.

The genus *Daphnia* (Cladocera, Daphnidae) is widely used as a test organism for acute and chronic tests (Hanazato, 1998; Kungolos *et al*., 1999) in the field of ecotoxicology. A standardized acute with *Daphnia* test has been proposed by DIN (1981) e ISO (1982), where the test animals are used with ≤ 24h, in test with 24h in photoperiod in dark.

Bioassays with reference substances are used for the evaluation of the sensitivity of the animal utilized in toxicological tests (Buikema Jr. *et al*., 1982). The toxicity of potassium dichromate is well known and your toxicity to invertebrates (Diamantino *et al*., 2000). The potassium dichromate was used as reference substance.

The acute toxicity of this reference substance potassium dichromate has been reported for many species of *Daphnia* (Persoone *et al*., 2009; Gopi *et al*., 2012). However, is not known the toxicity on Daphnia laevis in medium M4, a rich medium in salts and nutrients (Beatrici, 2004), standardized by the Organization for Economic Cooperation and Development (OECD, 2000).

Methyl parathion is a pesticide organophosphorous and is widely utilized in agriculture, it is also applied to eliminate aquatic insect larvae that prey on fish larvae (Silva *et al*., 1993; Fanta *et al*., 2003).

The present study was carried out to determine the short-term effective concentration 50% toxicity (EC50) of methyl parathion, and to evaluate whether *Daphnia laevis* has the potential to be used as test organism, in medium M4, using potassium dichromate as reference substance.

This work was performed at the Laboratory of Ecotoxicology of Agrochemicals & Health, Department

of Occupational Plant Protection, Faculty of Agricultural Sciences and Veterinary/UNESP, Jaboticabal associated with the Aquaculture Center of UNESP (CAUNESP).

The product chemicals used in this work were Potassium dichromate, as reference substance, and methyl parathion, as chemical compound, with active ingedient on the concentration of 600 g.L^{-1}, belonging to toxicity class I, considered highly toxic.

The cultures consisted of 3 glass flasks containing about of 3 liters. *Daphnia laevis* was cultured in a fully defined medium M4 (OECD, 2000). Medium M4 was renew weekly. Cultures were maintained at 20ºC under 12h light: 12h dark photoperiod. The food organism for the cladocerans was *Scenedesmus subspicatus* cultured in Chu medium (Chu, 1942).

Twenty neonates with aged less than 24h (OECD, 1981; DIN, 1981; ISO, 1982), divided into four groups were exposed to each concentration for 24h in a static test (US EPA, 1985), along with a control (Kungolos *et al*., 1999) in dark photoperiod (AFNOR, 1974, DIN, 1981, ABNT, 1987). The number of mobile and immobile specimens were recorded after 24h (AFNOR, 1974; DIN, 1981; ISO, 1982, ABNT, 1987) and were calculated by the EC- values.

In order to evaluate *Daphnia laevis* as a potential test organism, two sensitivity tests were conducted (ISO, 1982; DIN, 1989), using 4 and 5 treatments . Test solutions of potassium dichromate were prepared by diluting a stock solution of 600 mg.L^{-1}. The tests were carried out in a acclimatized room at 20ºC in the dark. Six logarithmically scaled concentrations were used, 0.05 mg.L^{-1}, 0.10 mg.L^{-1}, 0.20 mg.L^{-1}, 0.40 mg.L^{-1}, 0.80 mg.L^{-1}, 1.60 mg.L^{-1} plus a zero control.

Test solutions of methyl parathion, were prepared by diluting a stock solution of 600 mg.L^{-1}. The tests were carried out in a acclimatized room at 20ºC in the dark. Five logarithmically scaled concentrations were used, 5 x 10-6 mg L^{-1}, 10 x 10-6 mg.L^{-1}, 20 x 10 -6 mg.L^{-1}, 40 x 10-6 mg.L^{-1}, 80 x 10-6 mg.L^{-1}, plus a zero control.

EPA's DOS program was used to perform the Spearman-Karber method (U.S. E.P.A., 1999), that is a program integrated with a plotting routine for obtaining plots of toxicity curves, which method is adoted for estimating of the median effective concentration (EC50) (Hamilton et al., 1977).

This program was employed to calculate the EC50 in Daphnia laevis for the 24-h acute toxicity test, at effective concentrations. In this test proportions no shows monotonically increasing, being P(1)<P(2) (Hamilton et al., 1977, Hamilton et al., 1978). Descriptive statistics were also used, aiming to calculate the Pearson correlation coefficient (Zar, 1999).

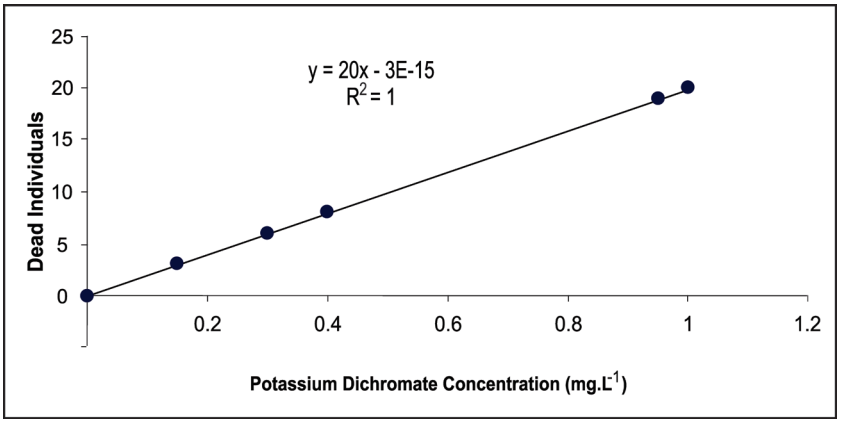

Figure 1. The correlation coefficient between dead individuals values for the potassium dichromate in 24h (Test 1).

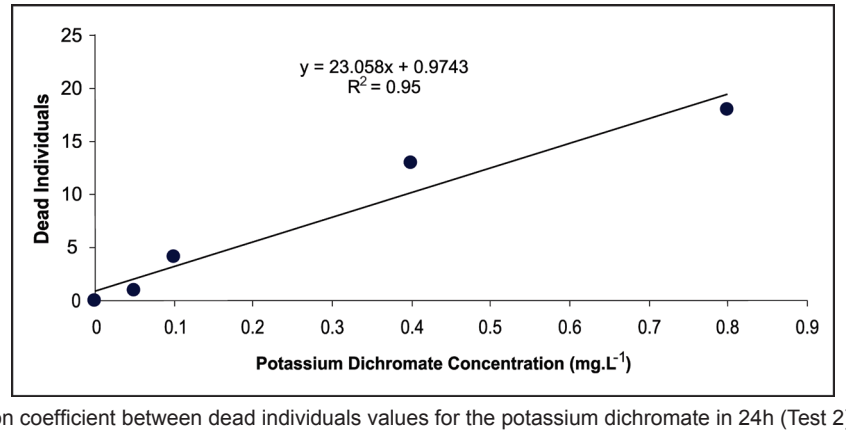

Figure 2. The correlation coefficient between dead individuals values for the potassium dichromate in 24h (Test 2).

In the results, the sensitivity of *Daphnia laevis* to potassium dichromate shows an EC50 of 0.332 mg.L^{-1}. Figures 1 and 2 also demonstrates a high pearson correlation coefficient of the effect of potassium dichromate on the dead individuals. The results with *Daphnia laevis* to potassium dichromate no did show notable difference in our study in regard to sensitivity to control and 0.05 mg.L^{-1}. With regard to others concentrations, only the results for concentration of 1.60 were more different.

To the methyl parathion there was a positive correlation between concentration and mortality of individuals for methyl parathion and *Daphnia laevis* (Figure 3). The concentrations that caused significant effects, with respect to control, ranged from 5 x 10-6 to 80 x 10-6 mg.L^{-1} for methyl parathion. The toxicity of Daphnia to the Methyl parathion shows and EC50 of 19.4 x 10-6 mg.L^{-1}.

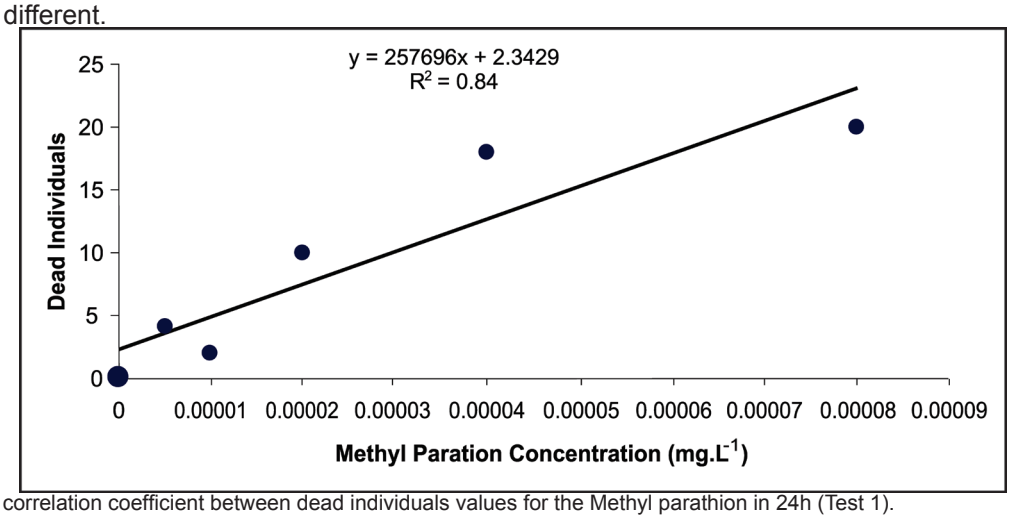

Figure 3. The correlation coefficient between dead individuals values for the Methyl parathion in 24h (Test 1).

A comparison of the acute toxicity of the values among species indicated that the potassium dichromate, in EC50 is different when compared with others species of *Daphnia* (Table 1). The different values of toxicities determined for test in different species of *Daphnia* may be related to several factors, considering, in this study, as the M4 medium and the species.

The methyl parathion EC50 for *Daphnia laevis* was much lower (19.4 x 10-6 mg.L^{-1}) than the EC50-24h for *Daphnia magna* at 9 x 10-3 mg. L^{-1}. (Kungolos et al., 1999). It is known that small cladocerans like Ceriodaphnia are much more sensitive, for example to chlorpyrifos, than large *Daphnia* species probably due to their smaller size.

A comparison of the acute toxicity values among species indicated that the potassium dichromate in medium M4, showed an EC50 is approximately reduced by half when compared with others species of *Daphnia.*

On the other hand, the sensitivity of daphnids is also influenced by the used culture medium. Beatrici

Tabela 1: EC50 with potassium dichromate for species of Daphnia.

Species	Medium	Exposure time (hr and min)	EC50 (mg.L^{-1})	
D. similis	M4	48h	0.21 - 0.29	Beatrici *et al.* (2006)
D. similis	ISO	48h	0.04 - 0.08	Beatrici *et al.* (2006)
D. magna	M4	48h	1.50 - 1.78	Beatrici *et al.* (2006)
D. magna	ISO	48h	0.81 - 0.97	Beatrici *et al.* (2006)
D. magna	-	1 h	0.72	Janssen e Persoone (1993)
D. magna	-	48 h	0.032	De Coen e Jansen (1997)
D. laevis	**M4**	**24h**	0.332	Present study

(2004) and Beatrici et al. (2006) concluded that D. magna and *D. similis* show more resistence to potassium dichromate in culture medium M4 than in ISO medium, which is a basic medium.

Senhorini et al. (1991) report that methyl parathion, with 60% active ingredient at concentrations between 0.25 $mg.L^{-1}$ and 0.50 $mg.L^{-1}$, decimated populations of Odonata and cladocerans, but copepods remained alive. Moreover, Gáradi *et al*. (1988) reports that Folidol eliminates Odonata at 0.5 $mg.L^{-1}$, but does not select the zooplankton.

Cruz et al. (2004) studied the effect of methyl parathion in Pacu (*Piaractus mesopotamicus*) recommended concentrations of 1.0 $mg.L^{-1}$ for alevinos and 7.5 $mg.L^{-1}$ for juveniles exposed to methyl parathion. This means that the quantities that would cause mortality to fish, are quite high, causing a much higher mortality in the plankton in the tank. Consequently, for this also lead problems toxics in wastes released in effluents in these doses, although recommended for fish farming would be lethal for the plankton present in dams, reservoirs, rivers and lakes. On the other hand, is well known that most of the applied pesticides are subject to many transport and conversion processes. Rovedatti *et al*. (2001) argues that organophosphates are of low persistence because of their short half-lives in aquatic environment.

Daphnia laevis, in medium M4, has potential to be used as test organism using as reference substance the potassium dichromate

In synthesis, the values utilized in baths of methyl parathion for farmed fish are highs for plankton, when the treatment of the fishes is carry out directly in water. The EC50 of methyl parathion to *Daphnia laevis* was much smaller than to other larger *Daphnia* species, when toxicity values were compared to other reported values from the literature, indicating that this species is more sensitive to the toxicants.

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