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Sensitivity of Costa Rica´s native cladoceran *Daphnia ambigua* and *Simocephalus serrulatus* to the organophosphate pesticide ethoprophos

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Abstract

The study of pesticide toxicity in aquatic environments is assessed with ecotoxicological tests and most research has been performed using species from temperate regions. In the present study, series of acute (48 hrs) toxicity tests to compare the sensibility of two indigenous cladocera of Costa Rica and two reference species were used in temperate regions to the organophosphate pesticide, Ethoprophos. Additionally, reproduction tests using *S. serrulatus* with sub lethal concentrations of ethoprophos and a control were assayed to check its sensitivity over a longer period exposure. The sensitivity of Costa Rica´s native species *Daphnia ambigua* (EC_{50} 48 hr : 12.9 ± 3.0 $\mu g l^{-1}$) and *Simocephalus serrulatus* (10.6 ± 2.1 $\mu g l^{-1}$) to ethoprophos were higher ($p<0.05$) when compared to the exotic species *Daphnia magna* (289.8 ± 77.4 $\mu g l^{-1}$), and were comparable to that of the more widely distributed species, *Ceriodaphnia dubia* (18.2 ± 5.2 $\mu g l^{-1}$). No effect on *S. serrulatus* reproduction was observed at concentrations between 1 and 4 $\mu g l^{-1}$. This study provides information that can be considered in the selection of species for ecosystem studies of pesticide toxicity in neotropical regions.

Key words

Ecotoxicology, Native cladocera, Pesticides, Tropics

Introduction

The environmental risk assessment of pesticides in tropical regions has been discussed frequently over recent years, since they encompass highly valuable ecosystems and often demonstrate deficiencies in pollution control (Rico *et al.,* 2011; Daam *et al.,* 2010; Kwok *et al.,* 2007). Thereby, efforts have been made in tropical countries to develop technical capabilities needed for the ecotoxicological assessment of agricultural chemicals (Castillo *et al.,* 2000a). This includes the sensitivity evaluation of aquatic tropical organisms such as fish(Mena-Torres *et al.,* 2012), Cladocera (Freitas and Rocha, 2011; Lopes *et al.,* 2011) and microalgae (Magnusson *et al*., 2008), as well as changes in the assemblage of macroinvertebrate communities (Castillo *et al.,* 2006).

In Costa Rica, pesticide use has been steadily increasing (Programa Estado de la Nación, 2004) and residues of different organophosphate (OP) pesticides are found frequently in surface waters near locations with intensive permanent agriculture activities (Castillo *et al.,* 2000a; Castillo *et al.,* 1997; Castillo *et al.,* 2000b; Shunthirasingham *et al.,* 2011).Among these substances, the insecticide Ethoprophos remains among the most imported (3,081 t a.i.)OP active ingredient in Costa Rica (Ramírez *et al.,* 2009), and some estimates indicate it belongs to a group of pesticides accounting for a high percentage of the aquatic ecotoxicity in the country (Humbert *et al.,* 2007).

Acute toxicity is still an important tool in risk assessment for regulatory purposes (Hayasaka *et al.,* 2011) and the evaluation of species that can be obtained locally for regular toxicity testing of these organic pollutants is useful. The present aimed to evaluate the sensitivity ofcladocera that can be obtained in Costa Rica´s aquatic ecosystems, *D. ambigua* and *S. serrulatus*, for ecotoxicity assessment of a pesticide frequently found in local monitoring of surface waters.

Materials and Methods

Chemical reagents : Pesticide standards were prepared at the Laboratory of Pesticide Residue Analysis (LAREP) of the Regional Institute for Studies on Toxic Substances (IRET): Ethoprophos (Sigma-Aldrich, 98.1% purity; Dr. Ehrenstorfer, 90.0% purity). Stock solutions were prepared in volumetric flasks with purified water (Milli- Q Plus System, Millipore Co.) and kept at 4ºC for no more than 4 weeks. Working solutions were also prepared in volumetric flasks with MilliQ+ purified water (Millipore Inc., Bedford, MA, USA). The concentrations of pesticide analyzed by GC/MS in the dilutions used for chronic toxicity testing(1, 2 and 4 μgl^{-1}) were between 78 and 100 % of the nominal concentrations.

Culture of organisms : Cladocera were cultured in IRET's Ecotoxicological Studies laboratory (ECOTOX) under controlled temperature (20±2 °C) and light period (16:8 Light/Darkness). Culture media contained microalgae *Pseudokirchneriella subcapitata* and *Chlorella* sp. (1.05x10^{8} cells l^{-1}), YCT (Yeast, Cereal Leaves, Tetramin; 0.5 ml l^{-1}), B12 vitamin (2 μg l^{-1}) and Selenium (2 μg l^{-1}) (US EPA, 1982; US EPA, 1989, WDNR, 1991; Mount and Mount 1992).Hard Reconstituted Water (HRW) was used as dilution media for *Daphnia magna* and Moderately HRW (MHRW) was used for the rest of the species. MHRW was prepared by diluting HRW 1:1 with filtered (MILLIPORE) and UV-treated (PURA) water.

Acute toxicity tests : Dilutions from a stock pesticide solution were prepared with reconstituted water on the same day as the tests were started with the native species *D. ambigua* and *S.serrulatus* as well as the reference species *D. magna* and *C. dubia* were used for toxicity testing. A series of preliminary tests were carried out in order to achieve a range of pesticide concentrations that caused mortalities between 0 and 100% in 48 hours. Nominal concentrations of 150 to 1000 μgl^{-1} for *D. magna* (aprox. factor of 0.8) and 4 to 30 μg l^{-1} (aprox. factor of 0.5) for the other cladoceran species were determined for final testing.The method for acute toxicity testing was based on standard procedures (OECD, 2004). Neonates less than 24 hours old of each species were exposed in triplicate to 30 ml of each dilution of pesticide solution placed on glass recipients at 20±2°C. Negative controls included only dilution media. Observations of organisms' mobility were made at 24 and 48 hours of exposure. The tests were repeated three different times.

21 day reprodution tests with *S. serrulatus*: Chronic exposure to the pesticide was evaluated by assays that measured the reproduction rate of *S. serrulatus* (*D. ambigua* was not tested because of difficulties in handling reproduction tests over prolonged periods, since organisms showed a strong tendency to float). Less than 24 hours old neonates of *S. serrulatus* were exposed to culture media prepared as mentioned before and amended with sub lethal concentrations of Ethoprophos. Pesticide concentrations of 0, 1, 2 and 4 μgl^{-1} were established for tests based on acute toxicity results. Ten replicates of one organism in 50 ml of test solution were prepared for each treatment, including a control of reconstituted water supplemented as mentioned in culture section. The medium was renewed three times per week during 21 days of exposure, and the offspring produced by each parent animal was removed and counted daily (OECD, 2012).

Statistical analysis : Acute toxicity results were analyzed with the probit method (using SPSS software) in order to obtain EC10, 50 and 90 values at 48 hours. These values, as well as the mean neonate production per treatment in reproduction assays were compared by analysis of variance and *post Hoc* tests (Tukey for acute tests and Dunnett´s for reproduction tests).

Results and Discussion

The EC_{10}, EC_{50} and EC_{90} values obtained for *D. magna* were higher than their respective values for the native species, *D. ambigua* and *S. serrulatus* ($F=32.9$, $p<0.05$, Table 1). *C. dubia*, another well-known cladoceran used for ecotoxicological tests (OECD, 2004), showed similar acute sensitivity to the native species. Survival of the organisms in reconstituted water controls was ≥ al 90% in all tests. The sensitivity shown by both species was higher when compared to EC_{50}s reported for this pesticide and *D. magna* in different databases, ranging from 43.9 to 200 μgl^{-1} (PAN, 2011; Plaguicidas de Centroamerica, 2011).

Measured environmental concentrations (MEC) of ethoprophos analyzed by Suarez *et al*. (2008) in surface waters near intensive banana plantations reached 4 μgl^{-1}. The predicted no-effect concentration for aquatic biota (PNEC orthe concentration of the toxicant below which adverse effects are unlikely to occur to the potentially exposed ecological assemblage) obtained by dividing the lowest mean EC_{50} from the native species (10.6 μgl^{-1}) by an assessment factor of 100 (EU, 1996) was 0.1 μgl^{-1}. A first tier value obtained by comparing MEC / PNEC indicates that the amount of pesticide that was found in surface water near banana plantations, were at least 40 times higher than the concentration estimated to be protective for aquatic biota. However, it must be considered multiple active ingredients including other OPs are commonly found in this zone. For instance, Ethoprophos and other organophosphate insecticides used in pineapple and banana, such as Diazinon and Terbufos, were detected simultaneously in surface waters near plantations in the Caribbean coast of Costa Rica in four sampling periods during 2009-2010 (Echeverría-Sáenz *et al*., 2012).

Table 1 : Acute toxicity values (μgl^{-1}) of ethoprohos in 48 hrs toxicity tests with cladocerans (mean ± SD)

Species	EC10	EC50	EC90
Daphnia magna	170.1 ± 44.5	289.8 ± 77.4	506.7 ± 179.8
Daphnia ambigua	6.3± 4.2	12.9 ± 3.0	29.8 ± 6.4
Simocephalus serrulatus	4.8± 0.4	10.6± 2.1	24.5± 10.5
Ceriodaphnia dubia	14.2± 3.8	18.2 ± 5.2	23.5 ± 6.9

Values are mean of replicates ± SD

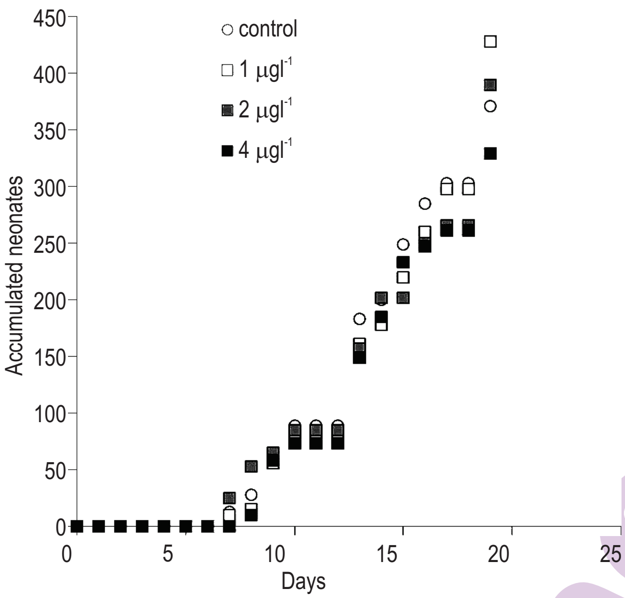

Fig. 1 : Reproduction of *S. serrulatus* over 21 days in control and ethoprophos treatments (μ gl^{-1})

In the 21 day test with S. *serrulatus,*reproduction was first recorded around day 7-8, and three reproductions per organism were achieved in the whole test. There was a 100% survival in all treatments. The control had an average reproduction of 37.1 ± 10.5 neonates. In 1, 2 and 4 ugl^{-1} treatments, average reproduction was similar to control with values of 42.8 ± 15.5, 39.0. ±12.1 and 32.9 ± 10.8 neonates produced per parent respectively (F=0.097, p=0.96, Dunnett; Fig. 1). There were no differences observed between treatments and control on the first day of neonate production. The highest concentration of Ethoprophos that caused no acute effects in *S. serrulatus* was 4 μgl^{-1}.

As established in protocols with *D. magna*, *S. serrulatus* achieved at least three broods in the 21 days test, with the first brood about a similar time, but with fewer neonates per parent (reproduction rate should be ≥ 60 for *D. magna* according to OECD guidelines). Therefore, the use of *S. serrulatus* in ecotoxicity testing for environmental research allows some similarity in data management as in protocols for *D. magna*. More ecotoxicological studies concentrate on planktonic genus like *Daphnia*, *Ceriodaphnia* and *Moina* (Sarma and Nandini, 2006).Thus, in addition to the practical reason of having a source of organisms in local environments to start a culture, our study also provides more data on a benthic genus, *Simocephalus*.

In this study, two species of cladocera collected from tropical aquatic ecosystems of Costa Rica demonstrated an elevated acute sensitivity to ethoprophos when compared to *D. magna*, a standard hard water test organism currently used for freshwater ecotoxicity tests (OECD, 2004).The importance of using autochthonous species to assess the effects of contaminants on tropical ecosystems has been highlighted by others, particularly for *in situ* testing (Moreira *et al*., 2010).

However, difference in chemical sensitivity distributions between species based on geographic distribution are not always to be expected. Also, there is a trade-off to be considered during species selection between a native species that may render more ecological relevance to a site, and choosing the most sensitive species available (Krull and Barros, 2012).

Daam *et al*. (2008, 2009a and 2009b) assessed the ecological effect of the insecticide chlorpyrifos, the herbicide linuron and the fungicide carbendazim using tropical freshwater microcosms in Thailand. The results showed no difference in sensitivity distributions and threshold values between their results and data generated from temperate regions, supporting the use of toxicity data from temperate countries in the risk assessment for the tropics. Still, they conclude variances may indeed be found on ecosystem function level and recovery potentials for these substances.

The authors also conclude more ecosystem tropical models with a broader variety of substances and geographical settings within tropical zones, and representing sensitive endpoints should be developed in order to improve the risk assessment of pesticides in tropical areas (Daam *et al.*, 2009c; Daam and Van den Brink, 2011). This implies further development of single species toxicity tests with indigenous species that can be used for the validation of local model ecosystems (Daam and Van den Brink, 2011) and in doing so, using the most sensitive groups for the substances of interest, such as freshwater arthropods and insecticides (Maltby *et al*., 2005).

Even though comparison of the sensitivities towards pesticides of tropical and temperate freshwater organisms has increased over the past years, still less information is available on tolerance of taxa mainly distributed in the tropics, where much of the world's species diversity is located (Nagendra *et al.,* 2008), and where there is lack of extensive and intensive field collections for certain groups such as Cladocera (Sarma *et al.,* 2005). Other factors such as fate, transport and degradation studies of organophosphate insecticides in formulation should also be considered when posing laboratory toxicity assessments. Although ethoprophos reaching rivers by runoff (up to 30% of what is being applied) may undergo biotic and abiotic degradation, granule formulation may increase persistence and thus toxic risk (Robinson *et al*., 1999)

More research must be done to validate tropical species ecotoxicological information for environmental risk assessment of selected substances relevant to tropical ecotoxicology. Meanwhile, more sensitive taxa with broad geographic distribution such as *C. dubia* are a better choice for toxicity assessment than *D. magna*.

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