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Combined effects of technical grade fenitrothion, humic acids and particulate matter on cholinesterase activity in freshwater invertebrates

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Abstract
Purpose The relative sensitivity of two freshwater invertebrate organisms to the organophosphorus insecticide fenitrothion was assessed by measuring cholinesterase (ChE) activity, a well-known biomarker of both exposure and effect to organophosphorus pesticides. The influence of different concentrations of humic acids (HAs) and particulate matter on fenitrothion bioavailability was assessed in the more sensitive species.
Materials and methods The selected invertebrates were the dwelling feeding oligochaete *Lumbriculus variegatus* and the pulmonate gastropod *Biomphalaria glabrata*. Acute 48-h bioassays were performed exposing organisms to different fenitrothion concentrations. The concentrations that induced 50 % inhibition of enzyme activity (EC_{50}) were calculated. Fenitrothion bioavailability was investigated using different concentrations of commercial HA or particulate matter. Sand and a diverse selection of chromatographic resins that have been proposed as analogues of natural sediments were selected. For these experiments, animals were exposed to a fenitrothion value similar to the EC_{50}.
Results and discussion The 48-h EC_{50} values were 12±2 and 23±3 $\mu g l^{-1}$ for *L. variegatus* and *B. glabrata*, respectively. Depending on HA concentration and the characteristics of particles, ChE activity was similar or higher than the value recorded for animals exposed only to the pesticide in aqueous solution.
Conclusion The results indicated that *L. variegatus* was the more sensitive species of the two. In this species, fenitrothion bioavailability did not increase due to the presence of either different HA concentrations or particulate matter. The experimental approach may constitute a useful tool to predict the influence of dissolved organic matter and sediment particles on fenitrothion bioavailability and toxicity to non-target aquatic invertebrates.

Keywords Bioavailability · Biomarkers · *Biomphalaria glabrata* · *Lumbriculus variegatus* · Organic matter

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1 Introduction

Most pesticides used to control pests in agricultural settings are applied on terrestrial environments. However, the application of chemicals may constitute an important non-point source of contamination to nearby aquatic environments posing a risk to biological communities. Fenitrothion (*O*, *O*-dimethyl-*O*-[3-methyl-4nitrophenyl] phosphorothiate) is an organophosphorus pesticide (insecticide) that has been in use since 1959 for the control of insects in rice, fruit and cereal crops (WHO 1991). The compound proved to be toxic to many non-target aquatic invertebrates, with LC_{50} values in the order of microgramme per litre (WHO 1991).

Depending on the physicochemical properties of both chemicals and water courses, a proportion of pesticides reaching aquatic systems remains in solution, whilst the rest becomes associated with sediment particles. It has long been established that a large fraction of fenitrothion can be taken up by particulate matter (both suspended material and bottom sediments) (Eidt et al. 1984). Natural particles are composed of a mineral or biological core, which is coated

by organic and inorganic compounds that are able to interact with many contaminants (Verrengia Guerrero 2007). These interactions play a crucial role in controlling the bioavailability of contaminants to aquatic biota. Hydrophobic organic compounds mainly associate to the organic matter fraction, as has been shown for fenitrothion (Eidt et al. 1984). These interactions preclude or slow down the degradation processes by microorganisms present on the particle surface. As a consequence, fenitrothion can persist in water bodies for weeks to months (SEPA 2010).

Few studies have investigated the influence of particulate matter on the bioavailability of fenitrothion to non-target aquatic invertebrates. Assessing the bioavailability of contaminants to aquatic organisms is complicated by the low concentrations that may be present, by their partitioning between the aqueous and particulate phases, and by the variety of uptake routes that may be involved with the biota.

In the case of non-degradable chemicals, such as metals or persistent organic pollutants, analyses of bioaccumulation provide direct evidence of their bioavailability. For degradable organic chemicals, such as fenitrothion, chemical analyses limited to the parent compounds are not good indicators of their bioavailability as they do not consider metabolic processes. For instance, it is known that fenitrothion requires metabolic activation before being able to interact with the enzyme acetylcholinesterase (AChE) (Sánchez-Hernández 2007). This enzyme (E.C.3.1.1.7.) catalyzes the hydrolysis of the neurotransmitter acetylcholine. Organophosphorus and carbamate pesticides exert an inhibitory effect on enzyme activity that results in the accumulation of acetylcholine in the synaptic cleft, leading to disruption of neurotransmission. The impact on the nervous system may lead to serious disorders for individuals, such as changes in inter-individual relationships, and can eventually affect biological communities. Acetylcholinesterase activity has been recognized as a useful biomarker of exposure and effect to organophosphorus and carbamate pesticides. Consequently, determination of its activity may be considered a reliable indicator of fenitrothion bioavailability under controlled laboratory conditions, where no other biological or chemical confounding factors can mask the results. The enzyme activity assay generally involves the use of acetylthiocholine (ATCh) as a substrate, allowing comparisons with data from the literature (Whitehead et al. 2005). Since other serine esterases are also capable of catalyzing the hydrolysis of ATCh, the term cholinesterases (ChE) is more appropriate than AChE and will be used from here.

The aims of this work were: (1) to investigate the dose–response relationship between ChE activity in *Lumbriculus variegatus* and different concentrations of fenitrothion in aqueous solutions, and to compare its sensitivity with that of *Biomphalaria glabrata*; (2) to investigate the influence of commercial humic acids (HA) on the bioavailability of this pesticide; and (3) to investigate the influence of different types of particulate matter on the bioavailability of fenitrothion. The most sensitive species was used for the last two studies.

L. variegatus is a freshwater oligochaete that has been widely recommended and used as a standard bioindicator organism for toxicity tests to evaluate both water and sediment quality (USEPA 2000; OECD 2007; ASTM 2010). It is a dwelling feeding organism that inhabits areas throughout the Northern Hemisphere. They serve an important role as prey organisms for other animals in aquatic systems, and can therefore promote transference of contaminants to higher trophic levels (Mount et al. 2006). The pulmonate hermaphroditic freshwater gastropod *B. glabrata* was selected for comparison since it inhabits tropical and subtropical areas of the Southern Hemisphere. In natural habitats, it feeds on bacterial films, algae, diatoms and decaying macrophytes. *B. glabrata* not only has ecological relevance as a prey organism (Giovanelli et al. 2005), but also medical significance as it is the intermediate host for the trematode *Schistosoma mansoni*, the causal agent of schistosomiasis (Pointier et al. 2005).

Humic acids were selected as they constitute the principal source of organic matter in natural particles (Koopal et al. 2001). The particulate matter consisted of sand particles and a diverse selection of chromatographic resins that had been previously proposed as a useful experimental model for natural sediments (Davies et al. 1999; Simkiss et al. 2000, 2001; Verrengia Guerrero 2007). Specifically, an anionic exchanger, a cationic exchanger and a neutral resin devised for investigating hydrophobic interactions were chosen for this study. Although some of the functional groups present in these resins are not likely to occur in natural sediments, the synthetic particles selected may reflect some of the possible interactions that could be occurring in the natural environment, such as charge effects and non-charged interactions.

2 Materials and methods

2.1 Chemicals

Fenitrothion (technical grade, purity ≥85 %) was obtained from Summit Agro Argentina S.A. Acetylthiocholine iodide and 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma (St. Louis, MO). All other chemicals used were of analytical reagent grade. Humic acids were obtained from Fluka Chemie AG, Switzerland, and were used as provided. The selected concentrations (5, 20 and 60 mg HA l^{-1}) were prepared by dissolving the humic material in dechlorinated tap water using a sonicator.

Sand particles were obtained from Merck (Buenos Aires, Argentina). The material consists of acid prewashed marine sand, heated to 900 °C and has a particle size range of 100–

315 μm (Table 1). The following resins were selected as experimental models: Toyopearl Phenyl 650 M (a neutral material); Toyopearl SP650M (a cationic exchanger); and Dowex 1x8-400 (an anionic exchanger). Both Toyopearl resins were obtained from Fisher, Loughborough, U.K. These two resins share the same particle size composition and backbone structure, consisting of polymers of ethylene glycol and methyl methacrylate, and differ only in their functional groups (see Table 1). The Dowex resin (Dowex 1x8-400) was obtained from Sigma-Aldrich (Poole, Dorset, U.K.); it has a backbone structure formed from a cross-linked copolymer of styrene and divinylbenzene and has quaternary ammonium as the functional group (see Table 1).

2.2 Test organisms

Both invertebrate species were obtained from laboratory cultures. *L. variegatus* were maintained under static conditions in a 12-l aquaria, at 22±2 °C, and a 14–10-h artificial light–dark photoperiod regime. Aquaria were filled with shredded non-bleached paper towels (depth of 3 to 6 cm) and contained approximately 8 l of dechlorinated tap water and constant aeration. The overlying water was changed every 7 days. Animals were fed three times a week with suspensions consisting of 0.5 g of finely ground TetraFin® (TetraWerke, Melle, Germany) in 25 ml of dechlorinated water. Adult organisms of 3.5±0.5-cm length were used for the assays.

B. glabrata snails were cultured under standard conditions in aerated glass aquaria (17–20 l), at a temperature of 22±2 °C, and a 14–10-h artificial light–dark photoperiod regime. Animals were fed with lettuce leaves ad libitum (Fried et al. 1992). For all experiments, adult snails of similar size (18±2 mm) were used, weighing on average 0.755±0.100 g total whole body wet weight.

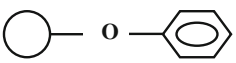
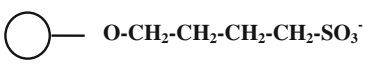
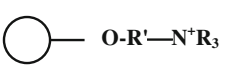
2.3 Bioassays

Acute 48-h bioassays were performed with both species. Test solutions were renewed every 24 h. In the case of worms, 10 organisms were placed in glass vials containing 20 ml of the test solutions. Five or 10 replicates were used for each exposure concentration. Three snails were placed in a glass vessel (500 ml) containing 300 ml of each test solution. Each exposure system was assayed in triplicate (n=9). Animals were not fed during bioassays. Tap water was dechlorinated by letting it sit at least 24 h and by filtering through a carbon column prior to use. The following physicochemical parameters were recorded: total hardness=48±3 mg $CaCO_3$ l^{-1}; alkalinity=29±2 mg $CaCO_3$ l^{-1}; pH=7.0±0.2; and conductivity=250±17 μS cm^{-1} (APHA-AWWA-WPCF 2005).

Aqueous solutions of fenitrothion were prepared by dissolving the insecticide in acetone and diluting with an appropriate amount of dechlorinated water. Control organisms were exposed to aqueous solutions containing only the acetone dilution. The concentration of acetone was 0.05 % for all insecticide and control solutions. Fenitrothion concentrations in the exposure containers were quantified after solvent extraction with hexane using a Hewlett-Packard Model 5840A gas chromatograph equipped with ^{63}Ni electron capture detector and a glass column (2-m length×2-mm I.D.) packed with 3 % SE-30 on Chromosorb WAW/DMCS, 80–100 mesh. The operating conditions of the gas chromatograph were: column temperature, 160 °C; injector temperature, 220 °C; detector temperature, 300 °C and carrier gas (nitrogen), 40 ml min^{-1}. Fenitrothion solutions were stable for at least 24 h.

To investigate the differential sensitivity of ChE in both species, animals were exposed to varying levels of fenitrothion (0, 1, 10, 50, 100, 250, 500 and 1,000 μg l^{1}) for 48 h. To investigate the influence of HAs, *L. variegatus* were exposed to 10 μg l^{-1} of fenitrothion in the presence of 0, 5,

Table 1 Type, functional group and particle size of the selected particulate matters

Particle	Type	Functional group	Particle size
Sand	Neutral material	SiO_2	100 - 315 μm
T-Phenyl	Hydrophobic interactions resin		40 μm
T-SP	Cationic exchanged resin		40 μm
Dowex	Anionic exchanged resin		60 μm

20 or 60 mg HA l^{-1}. The pesticide concentration was selected as it induced approximately a 50 % enzyme inhibition, allowing the determination of both positive and negative changes on fenitrothion bioavailability. To investigate the influence of particulate matter, oligochaetes were exposed to 10 $\mu g l^{-1}$ of pesticide in systems containing either 2 g of sand particles or a mixture consisting of 1.5 g sand plus 0.5 g of each resin. Particle systems were equilibrated by shaking for 4 h before performing the bioassays. Previous experiments where samples were taken every 30 min showed that this period was enough to reach equilibrium for all particle systems. Dissolved oxygen and pH values were recorded immediately at the beginning of each bioassay, and after 24 and 48 h (Water Quality Meter, Sper Scientific Ltd, Arizona, USA). Temporal changes in pH values were ≤0.02; dissolved oxygen concentration did not fall below 75 % saturation in any case.

2.4 Cholinesterase (ChE) activity

Following exposure, animals were cooled on ice for 6–8 min. Pools of 10 oligochaetes were washed twice in distilled water (T=4 °C), placed on filter papers to drain extra fluids and weighed. In the case of snails, shells were carefully removed and the soft tissue isolated on ice and treated similarly. Each pool of 10 worms or each separate snail was homogenized in 20 mM Tris/HCl buffer at pH 7.5. Homogenates were centrifuged at 11,000×g for 20 min at 4 °C. ChE activity was measured in 100 mM phosphate buffer at pH=8 (0.2 mM DTNB), according to the method of Ellman et al. (1961). Acetylthiocholine iodide was used as a substrate. Previous studies had shown that both organisms differed in ChE activity, type and subcellular location of enzymes (Kristoff et al. 2006). Nevertheless, for both invertebrates, the higher enzyme activity was observed when using acetylthiocoline as a substrate (Kristoff et al. 2006). Activity was recorded continuously at 412 nm. All measurements were conducted at least in duplicate. Rates were corrected for spontaneous hydrolysis of the substrate and for non-specific reduction of the chromogen by tissue extracts. The specific activity was expressed as micromole per minute per milligram of protein (extinction coefficient= 13,600 M^{-1} cm^{-1}). Protein content was determined according to the method of Lowry et al. (1951), using bovine serum albumin as a standard. The percentage of enzyme activity was defined as: (specific activity in treated organisms/specific activity in controls)×100.

2.5 Data analyses

All results were expressed as mean ± standard deviation (SD). The concentrations that caused 50 % of enzyme inhibition (EC_{50}) were calculated using the non-linear log-logistic regression analysis (Microcal Origin 8.0 Professional, OriginLab, Northampton, MA, USA). To investigate the influence of humic acids and particulate matter on the enzyme activity, data were first tested for normality (Kolmogorov–Smirnov's test) and homogeneity of variances (Bartlett's test). Means were compared by one-way ANOVA (parametric) or non-parametric Kruskal–Wallis tests. Tukey–Kramer or the non-parametric Dunn's tests were applied for post hoc comparison of means. All tests were performed using InfoStat statistical software (Di Rienzo et al. 2008). In all cases, results were considered significant at $p<0.05$.

3 Results

3.1 ChE inhibition by fenitrothion in *L. variegatus* and *B. glabrata*

Figure 1 shows ChE activity as a function of the different fenitrothion concentrations for the two invertebrate species. No mortality was registered at any exposure level. The calculated EC_{50} were 12±2 $\mu g l^{-1}$ for *L. variegatus* and 23±3 $\mu g l^{-1}$ for *B. glabrata* and were statistically different ($p<0.05$).

3.2 Influence of humic acids and particulate matter on ChE activity in control *L. variegatus*

Enzyme activity values in *L. variegatus* exposed to the different HA concentrations or to the selected particles in the absence of the insecticide are presented in Table 2. No significant differences in ChE activity were found in any

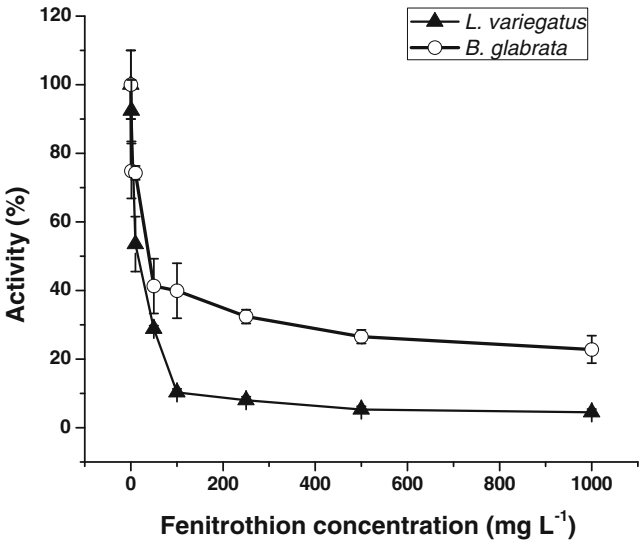


Fig. 1 Percentage of cholinesterase activity (mean values ± SDs) in whole-body soft tissue of *L. variegatus* and *B. glabrata* exposed to different concentrations of fenitrothion for 48 h. For *L. variegatus*, each point represents the mean value of 10 pools of 10 organisms. For *B. glabrata*, nine organisms were analyzed for each concentration

Table 2 Cholinesterase (ChE) activity (mean value ± SD) in control *Lumbriculus variegatus* exposed to different humic acid concentrations and different types of particulate matter

Sample	ChE activity[a] (μmol min^{-1} mg^{-1} protein)
Control	0.52±0.04
5 mg HA l^{-1}	0.50±0.10
20 mg HA l^{-1}	0.54±0.08
60 mg HA l^{-1}	0.60±0.10
Toyopearl SP	0.49±0.06
Toyopearl Phenyl	0.48±0.06
Dowex	0.50±0.10
Sand	0.54±0.10

[a] Mean value of five pools each of 10 organisms

case ($p>0.05$), ensuring that ChE activity was not modified by either the humic material or the particulate matter.

3.3 Influence of humic acids on ChE activity in *L. variegatus* exposed to fenitrothion

The addition of 5 mg HA l^{-1} to the medium containing fenitrothion did not modify ChE inhibition ($p>0.05$) (Fig. 2). In the presence of 20 or 60 mg HA l^{-1}, however, the activity was significantly higher ($p<0.05$), reaching control values (0.52±0.04 μmol min^{-1} mg^{-1} protein).

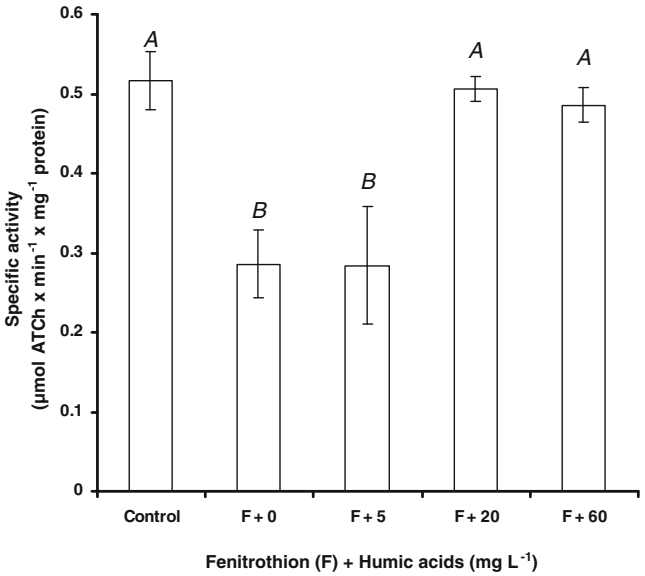


Fig. 2 Cholinesterase activity (mean value ± SD) in *L. variegatus* exposed to 10 μg fenitrothion (F) l^{-1} and different concentrations of commercial humic acids for 48 h. *Control* refers to animals exposed to the aqueous phase and 0.05 % acetone without addition of pesticide. Each system was assayed using 10 pools of 10 organisms. *Different letters* indicate significant differences between treatments ($p<0.05$)

3.4 Influence of particulate matter on ChE activity in *L. variegatus* exposed to fenitrothion

L. variegatus oligochaetes exposed to fenitrothion in systems containing either sand or both Toyopearl particles showed similar ChE activity values, compared to those exposed to fenitrothion only ($p>0.05$) (Fig. 3). In contrast, animals exposed to the pesticide in the presence of Dowex particles had higher values of ChE activity, similar to those of control organisms ($p>0.05$).

4 Discussion

The 48 h-EC_{50} value obtained for *L. variegatus* was less than half the value for *B. glabrata*, indicating that the oligochaetes were more sensitive to the inhibition induced by fenitrothion. These values, in the order of microgramme per litre, are in good agreement with data reported for other invertebrate aquatic species and are generally lower than values obtained for fish (WHO 1991; Sancho et al. 1998).

As was observed for fenitrothion, *L. variegatus* showed higher sensitivity than *B. glabrata* to the anticholinesterase effect induced by the organophosphorus azinphos-methyl (993 times) (Kristoff et al. 2006) and the carbamate carbaryl (20 times) (Kristoff et al. 2010). On the contrary, *B. glabrata* snails were more sensitive than *L. variegatus* (410 times) to the inhibitory effect of chlorpyrifos, another organophosphorus pesticide (Cochón, personal communication). These differences in the susceptibility of both organisms to the organophosphorus

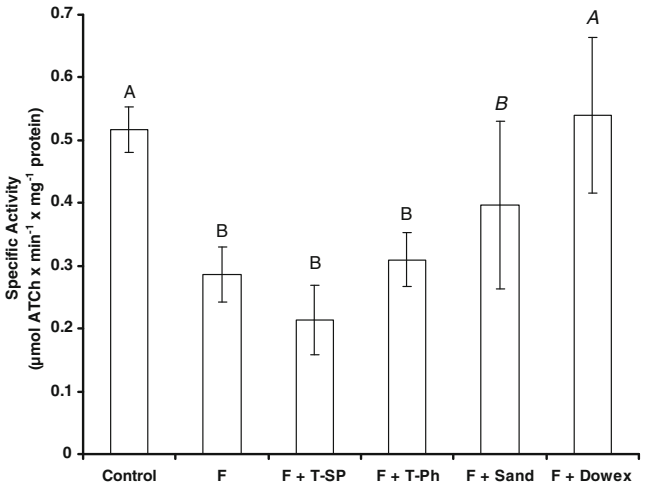


Fig. 3 Cholinesterase activity (mean value ± SD) in *L. variegatus* exposed to 10 μg fenitrothion (F) l^{-1} in aqueous phase and different types of particulate matter for 48 h. *Control* refers to animals exposed to the aqueous phase and 0.05 % acetone without addition of pesticide. Each system was assayed using 10 pools of 10 organisms. *T-SP*, Toyopearl SP; *T-Ph*, Toyopearl Phenyl. *Different letters* indicate significant differences between treatments ($p<0.05$)

and carbamate pesticides may be due to many possible explanations. Toxicokinetic processes may be responsible for different absorption, distribution, metabolism and/or excretion of the parent compounds and their metabolites. Toxicodynamic processes may also vary, since *L. variegatus* and *B. glabrata* present different ChE enzymes (Kristoff et al. 2006), as mentioned above (Section 2.4). In addition, both toxicokinetic and toxicodynamic factors could combine to cause the observed differences.

After a 48-h exposure, no mortality was observed in either species even at the highest level of fenitrothion tested (1,000 μg l^{-1}), whereas the ChE inhibition reached values of about 80 and 90 % for *B. glabrata* and *L. variegatus*, respectively. However, the oligochaetes exhibited visible signs of locomotive alterations. At the lower fenitrothion concentrations tested, animals showed uncoordinated movements and curling of the tail. At the EC_{50} level, only the anterior segments of the oligochaetes conserved mobility, whilst at higher concentrations individuals showed total lack of movement, contraction and curling of the whole body. In contrast, gastropods did not show any visible locomotive alteration. A correlation between ChE inhibition and mortality has not been fully established, especially for aquatic organisms (Sánchez-Hernández 2007). Whilst some species tolerate high degrees of ChE inhibition, others exhibit high mortality (Chambers and Carr 1995; Fulton and Key 2001). In agreement with the present results, Ferrari et al. (2004) described that exposure of goldfish for 96 h to 0.05 mg azinphos-methyl l^{-1} induced a ChE inhibition of 90 % without lethality. Similar results have been reported for other fish and crustacean species, where sublethal concentrations of organophosphates induced more than 70–90 % ChE inhibition (Gruber and Munn 1998). Moreover, the correlation between ChE inhibition and the appearance of signs of intoxication is complex and variable (Sánchez-Hernández 2007).

It is well-known that ChE activity can be inhibited by substances other than organophosphorus and carbamate pesticides in several species. Organic compounds, like surfactant agents and the bipyridylium herbicide paraquat, were found to be non-specific anti-cholinesterase agents (Szabó et al. 1992; Payne et al. 1996; Láng et al. 1997; Guilhermino et al. 2000). Several metals such as Hg(II), Cu(II), Zn(II), Cd(II) and Pb(II) may also decrease ChE activity in fish and invertebrates either in vivo or in vitro (Schmidt and Ibrahim 1994; Devi and Fingerman 1995; Martinez-Tabche et al. 2001; Alves Costa et al. 2007). Therefore, before attempting to analyze enzyme activity variations when animals were exposed to a given level of fenitrothion in the simultaneous presence of humic material or particulate matter, their individual potential inhibitory effect on ChE was studied. However, commercial HAs, sand and the selected chromatographic resins alone had no effect on ChE activity of *L. variegatus*.

All the bioassays that involved humic materials and particles were conducted using the same concentration of fenitrothion. Therefore, there was a fixed mass balance that allowed direct comparisons of ChE activity among the different conditions.

Humic acids are known to influence the bioavailability and bioaccumulation of organic compounds (Haitzer et al. 1998). However, to the best of our knowledge, specific information about the interactions between fenitrothion and natural organic matter is lacking. The results presented in this study indicate that at the lowest HA concentration tested (5 mgl^{-1}), the commercial material had no affect on the bioavailability of fenitrothion, as reflected by enzyme inhibition values. On the contrary, at levels ≥20 mgl^{-1}, the humic material exhibited a protective role, decreasing fenitrothion bioavailability. Concentrations of humic substances between 1 and 6 mgl^{-1} are generally found in pristine waters whilst values up to 70–80 mgl^{-1} may be found in waters such as bogs, swamps and marshes (Koopal et al. 2001).

Humic acids are natural substances that can derive from vegetable or animal tissues. Their chemical structure is still not well defined (Rand et al. 1995), but consists basically of a complex mixture of hydrocarbon polymers presenting a great number of carboxylic acids, phenols and alcoholic hydroxyls, and carbonyls as main functional groups. Taking into account the structures of HA and fenitrothion, the occurrence of electrostatic interactions between the insecticide and the negative charged functional groups of the humic material would be unlikely. Alternatively, hydrophobic interactions could be established through the hydrocarbon backbone, promoting a decrease in bioavailability.

Similar values of ChE inhibition were found in *L. variegatus* exposed to fenitrothion in the presence of either sand or the two Toyopearl resins. In addition, these values were not significantly different to those observed in animals exposed to the pesticide in the aqueous solution alone. The size of the sand particles was too large to be ingested by the animals (Conrad et al. 2000). Consequently, uptake of contaminants could only occur from the dissolved aqueous phase. This system better reflects a natural environment where the animals can dig holes and burrow in the sediment. In addition, sand particles constitute a neutral material free of organic matter and no significant interactions with the pesticide are to be expected. The Toyopearl Phenyl resin is also a neutral material that allows hydrophobic interactions to be studied. Even though according to its particle size composition that this resin could be ingested by the oligochaetes, it did not modify the bioavailability of fenitrothion.

Similar results were obtained with the cationic exchanger resin Toyopearl SP, which also did not induce changes in ChE activity. As is the case with natural sediment particles, this resin has a negative net charge (Verrengia Guerrero 2007); therefore, electrostatic interactions with the non-charged insecticide would not occur. In fact, the enzyme activity for this group was similar to the value recorded for the neutral Toyopearl Phenyl, suggesting that the functional groups of these particles

have a negligible influence over their interactions with fenitrothion. Only weak hydrophobic interactions would be established through the backbone for both Toyopearl resins, and these interactions would have little to no effect on fenitrothion bioavailability.

Both Toyopearl Phenyl and Toyopearl SP resins have been proposed as useful analogues for natural sediments (Simkiss et al. 2000, 2001). In previous studies, these resins increased the bioavailability and bioaccumulation of several organic compounds in different freshwater species, such as *Chironumus riparius* (Simkiss et al. 2001), the fingernail clam *Sphaerium corneum* (Verrengia Guerrero 2007) and *L. variegatus* (Simkiss et al. 2000). However, no increase in toxicity was observed when combined with fenitrothion.

The presence of Dowex particles induced a significant increase of ChE activity on organisms exposed to fenitrothion, resulting in values similar to those observed for unexposed control animals. This anionic exchanger would thus act to decrease the bioavailability of the pesticide. From its chemical structure, any type of electrostatic interaction between fenitrothion and the positive functional groups present in the particles is improbable. More likely, strong hydrophobic interactions could be occurring with the backbone structure of the Dowex resin, which has a different composition from that of the Toyopearl particles. Hence, even if the particles are ingested by oligochaetes, the pesticide could not be desorbed by the animals.

5 Conclusions

The oligochaete *L. variegatus* was a more sensitive species to fenitrothion than the gastropod *B. glabrata*. Depending on the concentration, dissolved humic acids may exhibit an important role decreasing the bioavailability of this pesticide for *L. variegatus*. This effect may be due to hydrophobic interactions with the hydrocarbon backbone structure. Surface charges present in the particulate matter showed little influence on pesticide bioavailability, whereas when strong hydrophobic interactions could be established between the pesticide and the backbone structure of the particle, a marked decrease in its bioavailability could be observed.

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