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Avoidance response of sediment living amphipods to zinc pyrithione as a measure of sediment toxicity

Ann-Kristin Eriksson Wiklund [a,*], Therese Börjesson [a], Stig Johan Wiklund [b]

[a] Department of Applied Environmental Science, Stockholm University, 106 91 Stockholm, Sweden
[b] Fornstigen 7 151 52 Södertälje, Sweden

Abstract

An avoidance test was developed using non-cultured individuals of the sediment dwelling amphipod *Monoporeia affinis*. As test substance we used zinc pyrithione, an antifouling agent and a common shampoo ingredient. The toxicity to Daphnia and fish is well known but sediment toxicity of this very hydrophobic compound is less known. The preference of juvenile *M. affinis* was tested in jars, each including 12 petri dishes. In each replicate, half of the petri dishes contained sediment mixed with six concentrations ranging from 0 to 10 μg zinc pyrithione per L sediment and half of the petri dishes contained the corresponding sediment-substance mixture plus an extra food addition. The amphipods significantly avoided petri dishes with the three highest concentrations of zinc pyrithione and the calculated EC_{50} was 9.65 μg L^{-1} sediment. No difference in mortality was observed between concentrations. Using the avoidance behaviour in sediment toxicity testing is a simple and cost-effective screening for toxicants.

Keywords: Avoidance behaviour; Sediment test; Amphipod; Zinc pyrithione

1. Introduction

The concentration causing avoidance behaviour is often lower than the lethal concentration of a contaminant, but if populations' demonstrate avoidance behaviour it is equivalent to the populations extinction in that area (Lopes et al., 2004). The capability of avoiding metals and organic contaminants is well known in fish (Giattina and Garton, 1983) but the ability to avoid contaminants can be impaired at high contaminant concentrations (Hansen et al., 1999). Invertebrate behavioural studies are most commonly developed for terrestrial species in pesticide experiments, earthworms and Collembola being the most commonly studied (i.e. Heupel, 2002 and references herein, Da Luz et al., 2004). Amphipods are normally considered as sensitive to contaminants (Swartz et al., 1982) and avoidance behaviour to metals was reported in amphipods in the mid eighties (Swartz et al., 1986) and to organic contaminants in the late nineties (Kravitz et al., 1999).

Studies on avoidance response to toxicity are normally performed in two-compartment chamber systems with control and contaminated as the options but there are few studies where the tested organisms have a multiple choice situation. The aim of this study was to develop a small and cheap multiple test system studying avoidance behaviour using a field population of an ecologically relevant lipid rich and common benthic species in the Baltic Sea, *Monoporeia affinis*. We also wanted to examine the effects of the increasingly used substance zinc pyrithione. Since sediment toxicity tests in Europe are not standardised, toxicity studies of zinc pyrithione are mainly performed in water only tests, despite its hydrophobicity and particle binding ability. In view of the fact that there are diverging opinions regarding the effects of food quality and quantity when combined with contaminants, we decided to combine food addition and contaminant exposure.

* Corresponding author. Tel.: +46 8 674 7251; fax: +46 8 674 7638.
E-mail address: annkristin.eriksson@itm.su.se (A.-K. Eriksson Wiklund).

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2. Materials and methods

2.1. Test substance and test species

Zinc pyrithione is used as an industrial preservative to prevent microbial degradation. It has replaced tributyltin (TBT) as the main antifouling substance (Doose et al., 2004). Zinc pyrithione is used extensively as an active ingredient in medical and hygienic products such as antidandruff shampoo. It is not considered to pose considerable toxicity to vertebrates but is found severely toxic in low concentrations to fish (EC_{50} 5–9 μg L^{-1}) and invertebrates (EC_{50} 3–20 μg L^{-1}) and NOEC to sea urchin development was 0.01 fg/L (Kobayashi and Okamura, 2002). The usage of the substance as an antifouling agent is environmentally regulated in both Europe and USA, while the usage in medical and hygienic products is governed by health authorities. Environmental guidelines regarding medical products are currently being developed (www.emea.eu.int/pdfs/human/swp.444700en.pdf).

M. affinis is a glacial relict occurring in both brackish-water and freshwater environments. It is the most productive macrofauna species on soft bottoms in greater Swedish lakes such as Lake Mälaren and the Baltic Sea, showing extremely low species richness (Hill and Elmgren, 1987). The high abundance, ecological importance and high lipid content (Eriksson Wiklund et al., 2003), make it a relevant test species. As many other amphipods it is proven as sensitive to contaminants (Sundelin, 1983; Sundelin and Eriksson, 1998).

2.2. Experimental set up

Natural sediment from a reference site in the Baltic proper was mixed with zinc pyrithione. A stock solution was prepared by dissolving 1 mg of zinc pyrithione in 50 ml acetone and sonicated for 25 min. Six batches of sediment were prepared by thoroughly mixing 530 mL sediment (25% dry weight) with a specified volume of the stock solution giving the nominal zinc pyrithione concentrations of: 0, 0.1, 0.5, 2.5, 5.0 and 10.0 μg L^{-1} sediment. To avoid differences in acetone concentration, a corresponding amount of acetone–water mixture was added to all batches. The acetone concentration in each batch was less than 1‰. To avoid zinc pyrithione degradation by sunlight, sediment beakers and stock solution were covered in aluminium foil. The six sediment batches were allowed to settle for 2 h before administrating into petri dishes. Ten aquaria with 12 petri dishes (∅ = 5.3 cm, height = 1.2 cm, V = 26.5 mL), two per concentration of zinc pyrithione in each aquarium were prepared. In six petri dishes per aquarium, one from each concentration, additional food in the form of Tetraphyll® (corresponding to 2.33 g C/m^2 week) was added. The petri dishes, two of each concentration, with and without additional food addition were randomly put in each aquarium and allowed to settle for 2 h. The aquaria were gently filled with water before the flow through system was connected. To avoid potential effects of acetone and to achieve similar substance concentrations during the incubation period (there is a fast initial degradation followed by a slower process), the aquaria were connected to the flow through system for three days before addition of 100 juvenile *M. affinis*. The temperature was (4.5 °C) and light was synchronised after natural seasonal regime and at a low intensity (0.1 μmol/m^2 s^1) since *M. affinis* is a bottom dwelling species. *M. affinis* is sensitive to hypoxia; thus the oxygen concentration was monitored twice during the experiment. The oxygen concentrations were >9.0 mg L^{-1} in all aquaria. The toxicity test was run for 10 days. At experiment termination, individuals were counted in each aquarium by screening the overlying water in the aquarium and sediment in each petri dish. Throughout the paper the initial nominal concentration of zinc pyrithione will be used.

2.3. Statistical analyses

The statistical analyses of the number of animals were performed using a generalized linear model (e.g. Agresti, 1990) assuming a Poisson distribution and using a logarithmic link function. The model included a factor allowing for over-dispersion and allowed for correlation between Petri dishes from the same aquarium. The model included factors for concentration and food addition and the interaction between these two factors. The proportion of living animals was analysed with a similar model, but assuming a binomial distribution and using a logistic link function. The calculations were performed with the GENMOD procedure of the SAS software ver. 8.2.

The EC50 for the number of living animals was estimated using a non-linear mixed effects regression model, with Emax and EC50 modelled as random effects in a sigmoid concentration-response function. The calculations were performed with the NLMIXED procedure of the SAS software ver. 8.2.

3. Results

The spill of sediment from petri-dishes was small and the overlying water was clear. Oxygen concentrations were more than 9 mg L^{-1} in all aquaria. The mean survival was more than 90% in all but one aquarium in which it was 66%. More than 80% of the individuals were found buried in the sediment. We found significantly fewer living amphipods in the three highest concentrations of zinc pyrithione compared to the control and a significant decreasing trend of live amphipods with increasing contaminant concentration (Fig. 1, Table 1). We observed no trend with increasing contaminant concentration if we analyse the number of dead amphipods (Table 1). Neither did we observe any differences between the number of dead amphipods in control and treatments. If we analyse the proportion live amphipods, there is a significant decreasing trend with increasing contaminant concentration but only

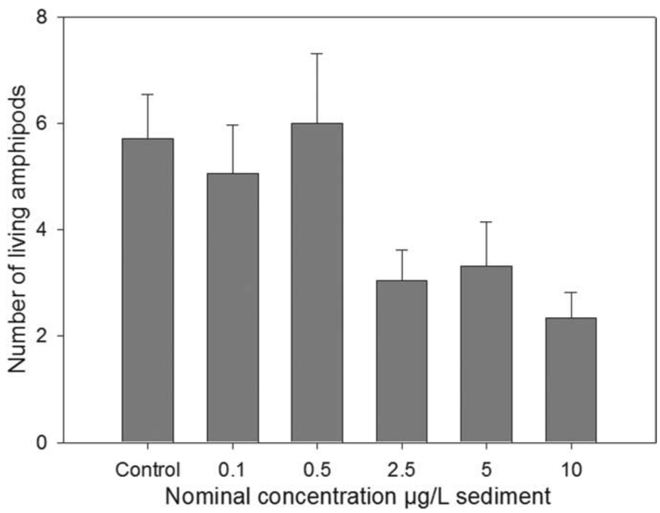

Fig. 1. Number of living amphipods per petri dish simultaneously exposed to a control and five concentrations of zinc pyrithione. Data is given as average of 10 replicates + SE.

the highest concentration was significantly separated from control (Table 1). We observed no effect of the food addition analysed separately or in combination with contaminant concentration. An EC_{50} value was calculated for the number of living amphipods and was estimated to be 9.6 µg L^{-1} (confidence interval 2.8–17).

4. Discussion

There is a growing awareness that the avoidance response is a simple and useful tool and sensitive early warning toxicity test (Da Luz et al., 2004; Lopes et al., 2004) but most avoidance tests evaluate only the difference between a control and a single contaminant concentration in two-chamber systems (Kravitz et al., 1999; Heupel, 2002; Van Zwieten et al., 2004). Our avoidance test simultaneously evaluated multiple factors using a natural population of the amphipod (*M. affinis*). The amphipods demonstrated a significant avoidance behaviour at non-acute toxic concentrations, by decreasing in numbers at increasing contaminant concentrations. Additionally there was a low overall mortality and no difference in mortality between concentrations. The decreasing number of individuals in increasing concentrations of toxicant indicated that the avoidance response was related to the concentration of the toxicant. The EC_{50} concentration of 9.6 µg L^{-1} sediment is more than a magnitude lower than the LC_{50} of the crustacean *Nitocra spinipes* (Karlsson and Eklund, 2004) and demonstrates the sensitivity of the method. We can conclude that examining sediment bound substances using behavioural tests with deposit feeders as test species is fast, cheap and give ecologically relevant results as well as the possibility of testing several variables simultaneously.

We found no response of food addition, solely or in combination with contaminants. There could be several reasons for the lack of interaction between contaminant concentration and food addition. In order to achieve a high ecological relevance we decided to use natural sediment from a reference area in the experiment. The organic content of this sediment was 4%. A carbon addition to this relatively rich sediment might not affect the behaviour and the artificial food addition might attract the amphipods less than the natural sediment.

Until now the risk of zinc pyrithione to the aquatic environment has been considered relatively low due to the fast degradation of the substance by UV irradiation or absorbed to the sediment as an unavailable trace metal

Table 1
Parameters from the statistical analyses

Variable	Concentration comparison	Estimated ratio	95% Confidence interval for the estimated ratio	*p*
Number of live amphipods	0.1 µg L^{-1} vs control	0.87	0.60–1.25	ns
	0.5 µg L^{-1} vs control	1.02	0.71–1.47	ns
	2.5 µg L^{-1} vs control	0.45	0.36–0.57	<0.001
	5 µg L^{-1} vs control	0.55	0.35–0.85	<0.001
	10 µg L^{-1} vs control	0.39	0.24–0.64	<0.001
	Linear trend	0.001		<0.001
Number of dead amphipods	0.1 µg L^{-1} vs control	1.08	0.61–1.89	ns
	0.5 µg L^{-1} vs control	1.46	0.83–2.59	ns
	2.5 µg L^{-1} vs control	0.72	0.36–1.41	ns
	5 µg L^{-1} vs control	1.15	0.49–2.68	ns
	10 µg L^{-1} vs control	1.11	0.63–1.95	ns
	Linear trend	0.99		ns
Percent living amphipods	0.1 µg L^{-1} vs control	0.81	0.39–1.68	ns
	0.5 µg L^{-1} vs control	0.69	0.35–1.36	ns
	2.5 µg L^{-1} vs control	0.62	0.35–1.11	ns
	5 µg L^{-1} vs control	0.48	0.20–1.17	ns
	10 µg L^{-1} vs control	0.35	0.16–0.78	<0.01
	Linear trend	0.001		<0.05

A generalized linear model was used examining food addition (no effect, data not shown) and nominal contaminant concentration in the sediment additionally the occurrence of a linear trend between concentrations was examined.

complex (Comber et al., 2002). Recent results by Mackie et al. (2004) and by Maraldo and Dahlöf (2004) suggest that the photolytic break down process in natural waters could be much lower and that the persistence of zinc pyrithione in the environment must be further examined. Even if zinc pyrithione is bound to the sediment as a trace metal complex it is certainly available to benthic life. Our experiment demonstrates that sediment bound zinc pyrithione affects the behaviour of sediment dwellers at low concentrations. Studies by Petersen et al. (2004) on the bacterial community clearly show that significant effects occur even at low contaminant concentrations. In addition several trans-metallisation and oxidation products of zinc pyrithione are as toxic to rat cells as the mother compound (Doose et al., 2004). Conclusively zinc pyrithione might affect benthos even at low concentrations indicating that adverse effects on biota from the increasing use as an antifouling agent should be carefully monitored.

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