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Are insect repellents toxic to freshwater insects? A case study using caddisflies exposed to DEET

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HIGHLIGHTS

- Ecotoxicological data on the effects of insect repellents in aquatic systems is needed.
- Effects of DEET were assessed in the caddisfly *Sericostoma vittatum*.
- Deleterious effects of DEET were only observed at concentrations above environmental levels.
- DEET exposure decreased feeding rate and carbohydrates contents in *S. vittatum*.

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ABSTRACT

Stream ecosystems face ever-increasing pressures by the presence of emergent contaminants, such as, personal care products. *N*, *N*-diethyl-3-methylbenzamide (DEET) is a synthetic insect repellent that is being found in surface waters environments in concentrations up to 33.4 μg/L. Information concerning DEET's toxicity in the aquatic environment is still limited and focused only on its acute effects on model species. Our main objective was to assess the effects of DEET exposure to a caddisfly non-target species using sub-lethal endpoints. For that, we chose *Sericostoma vittatum*, an important shredder in Portuguese freshwaters that has been already used in different ecotoxicological assays. Besides acute tests, *S. vittatum* were exposed during 6 days to a gradient of DEET concentrations (8, 18 and 40.5 mg/L) to assess effects on feeding behaviour and biochemical responses, such as, lipid peroxidation levels (LPO), catalase and acetylcholinesterase (AChE) activities, and also assess effects on energy reserves and consumption. Acute tests revealed a 48 h-LC_{50} of 80.12 mg/L and DEET exposure caused feeding inhibition with a LOEC of 36.80 mg/L. Concerning the biochemical responses, DEET caused no effects in LPO nor on catalase activity. A non-significant decrease in AChE activity was observed. Regarding energetic reserves, exposure to DEET caused a significant reduction in *S. vittatum* carbohydrates levels. These results add important information for the risk assessment of insect repellents in the aquatic environment and suggest that reported environmental concentrations of DEET are not toxic to non-target freshwater insects.

1. Introduction

Insect repellents are a class of personal care products that are applied to skin, clothes or other surfaces to prevent arthropod biting and consequently control dissemination of diseases (Costanzo et al., 2007). These compounds can be based on synthetic or natural substances and information about their chronic effects in aquatic environment is still lacking (Pedrouzo et al., 2011). The most widely used substance in commercial insect repellents is *N*, *N*-diethyl-3-methylbenzamide (DEET), an active ingredient that was first synthetized in 1946 by the U. S. Army (Costanzo et al., 2007). DEET has been detected in different matrices of aquatic environments, such as wastewater treatment plants influents and effluents (Costanzo et al., 2007; Glassmeyer et al., 2005), surface water (Calza

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et al., 2011; Costanzo et al., 2007; Yoon et al., 2010), seawater (Weigel et al., 2004) and even drinking water (Stackelberg et al., 2004). DEET has been detected in different regions of the world, such as, Europe (Calza et al., 2011), USA (Glassmeyer et al., 2005), Australia (Costanzo et al., 2007) or South Korea (Yoon et al., 2010) in concentrations ranging from 0.001 to 33.4 μg/L in surface waters worldwide (for more detail see Aronson et al. (2012)).

DEET's mode of action has been the subject of investigations using different insect species, namely *Drosophila melanogaster* (Pellegrino et al., 2011), *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles albimanus* (Leal, 2014). Recent studies have shown that DEET modify insect's behaviour by activation or modulation of olfactory receptors (Ditzen et al., 2008; Pellegrino et al., 2011) and can directly activate gustatory receptors neurons mimicking feeding deterrents (Lee et al., 2010). DEET has also been shown to inhibit the activity of acetylcholinesterase (AChE) in neuronal preparations of mammals and insects (Corbel et al., 2009). Collectively these studies suggest that although not designed to have biocidal properties, exposure to insect repellents such as DEET can affect non-target insects through behaviour impairment (feeding, predator and prey attack-escape performance) and neurotoxicity. Thus, it is important to evaluate their ecological effects in the aquatic environment.

However, only a few studies were conducted using aquatic organisms exposed to DEET and the majority of those investigations are related with its acute toxicity (Aronson et al., 2012). DEET appears to be slightly toxic, but taking into account the frequency of detection in surface waters and its persistence more studies are required to assess the chronic toxicity of DEET for an accurate risk assessment (Brausch and Rand, 2011; Costanzo et al., 2007). Moreover, it is also important that this assessment is conducted with different non-target species.

Caddisflies are used as model species for the assessment of effects of different contaminants in lotic ecosystems (Campos et al., 2014; Damásio et al., 2011; Pestana et al., 2009). The caddisfly *Sericostoma vittatum* Rambur (Trichoptera: Sericostomatidae) is an endemic species present in streams of the Iberian Peninsula during all year with an annual life cycle. They are benthic organisms with an important role in the fragmentation of allochthonous organic matter in streams being efficient shredders (Feio and Graça, 2000).

Due to constant detection of DEET in freshwaters and also due to their mode of action is expectable that DEET exposure can cause effects in non-target aquatic insects through feeding inhibition and/or neurotoxicity. Although the concentrations tested in our study (in order of mg/L) are above environmental relevant concentration (in order to μg/L), understanding ecological effects of DEET in aquatic ecosystems, its biochemical effects and tolerance of non-target organisms is a pertinent issue. So the aim of this study was to evaluate the ecotoxicological responses of *S. vittatum*, a freshwater caddisfly, to DEET exposure at different levels of biological organization. The endpoints chosen included feeding rate as organismal endpoint and oxidative stress (lipid peroxidation; LPO), antioxidant enzymes (catalase; CAT), and neurophysiological activity (AChE) as biochemical endpoints. We also wanted to evaluate the energy available (E_a) (measuring levels of carbohydrates, lipids and proteins contents) and energy consumption (E_c) (measuring electron transport system- ETS - activity).

2. Methods

2.1. Animals

S. vittatum were collected from Ribeira de São João, Lousã, Portugal (40°06′N, 8°14′W) using an hand net. Organisms were acclimated to laboratory conditions (20 ± 1 °C, light–dark cycle of 16:8 h) for one week in plastic containers with inorganic fine sediment (<1 mm) previously burnt (500 °C for 4 h), and filled with American Society for Testing Materials ASTM (1980) hard water. Following the protocol described in Pestana et al. (2009), organisms were fed *ad libitum* with unconditioned alder leaves (*Alnus glutinosa*), which provide adequate nutrition for maintenance and reproduction of this species under laboratory conditions.

2.2. S. vittatum acute experiments

S. vittatum were exposed to a range of DEET concentrations (39.05, 50.77, 66.00, 85.80, 111.54 and 145 mg/L) during 48 h plus control treatment (ASTM hard water only). The experimental setup consisted in five replicates with five organisms each, for each treatment. The organisms were exposed in glass vials with 150 mL of respective medium at 20 ± 1 °C and 16:8 h light: dark photoperiod. No food or sediment was added during the exposure period. In the end of 48 h all organisms in control treatment were alive.

2.3. S. vittatum feeding experiments

Based on preliminary experiments *S. vittatum*, were exposed to a gradient of three concentrations (8, 18 and 40.5 mg/L) of DEET (CAS number: 134-62-3; molecular weight: 191.27; Sigma–Aldrich, Germany) plus a control treatment (ASTM hard water only). Feeding trials were based on previous laboratory toxicity assays conducted with *S. vittatum* (Campos et al., 2014; Pestana et al., 2009). Briefly, we used ten replicates with one animal per replicate. In each replicate *S. vittatum* were allocated to glass vials containing 1 cm layer of inorganic fine sediment (<1 mm), 150 mL of respective solution and 6 conditioned alder leaf discs as food. Alder leaves used in these assays were collected from riparian vegetation of Alfusqueiro river near Destriz (40°38′N, 8°16′W). The leaves were air dried and stored in the darkness. Before use in feeding trials, the leaves were soaked in distilled water and leaf discs (Ø 10 mm) were prepared with a cork borer. Alder leaf discs were then autoclaved and conditioned during one week in 1500 mL of local river water, at 20 ± 1 °C, 16:8 h light: dark photoperiod and with aeration. After conditioning, alder leaf discs were dried at 50 °C during 96 h and weighed.

Alder leaf discs used in each replicate are soaked in the respective DEET solutions during 96 h before use. The test were conducted at 20 ± 1 °C with a photoperiod of 16 h light: 8 h dark. After 6 days of exposure, *S. vittatum* were collected, removed from their case, quickly dried on filter paper, immediately weighted, frozen in liquid nitrogen and stored at −80 °C. In the end of the test no mortality was observed in the control treatment. In this control treatment one of the caddisfly was in the pupal stage and thus this replicate was removed from the feeding calculations.

Alder leaf discs were also collected and dried at 50 °C during 96 h. Feeding rate was calculated as the difference between the initial and final leaf disc dry mass (mg) and divided by the wet mass of organism (mg) and elapsed time (days). Three replicates in control and highest concentration of DEET were performed with leaves discs in the absence of organisms in order to correct weight change of leaf discs due to other factors rather than feeding. Since no difference was found between leaf discs weight loss between these two treatments, the combined average of loss of weight of leaf discs of control and highest concentration of DEET was used as a correction factor in all experimental treatments.

2.4. S. vittatum biochemical experiments

After six days organisms used for feeding experiments were frozen at −80 °C and were used to assess effects of DEET on biochemical parameters. Each organism was homogenized in 1600 μL of Milli-Q water by sonication. After homogenization three