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Do insect repellents induce drift behaviour in aquatic non-target organisms?

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ABSTRACT

Synthetic insect repellents are compounds applied to surfaces to discourage insects, mainly mosquitoes, from landing on those surfaces. As some of these repellents have repeatedly been detected in surface waters at significant concentrations, they may also exert repellent effects on aquatic non-target organisms. In running water systems, aquatic invertebrates actively enter downstream drift in order to avoid unfavourable environmental conditions. We thus tested the hypothesis that the widely used insect repellents DEET (N,N-Diethyl-*m*-toluamide), EBAAP (3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester) and Icaridin (1-piperidinecarboxylic acid 2-(2-hydroxyethyl)-1-methylpropyl ester) induce downstream drift behaviour in the aquatic invertebrates *Gammarus pulex* (Crustacea, Amphipoda) and *Cloeon dipterum* (Insecta, Ephemeroptera), using a laboratory-scale drift assay. We found no clear increase in the drift behaviour of both invertebrate species across a concentration gradient of eight orders of magnitude and even beyond maximum environmental concentrations for any of the three repellents. We found no evidence for a direct drift-inducing activity of insect repellents on aquatic non-target organisms.

1. Introduction

Insect repellents are products that are applied to organisms or materials in order to deter insects and ticks from approaching these surfaces. The application of these insect repellents to human skin to discourage the landing of blood-sucking mosquitoes is common, in particular in the tropics and during the summer season in temperate climate zones. In contrast to insecticides, these repellents are designed to alter the behaviour of the target organisms (e.g. host-finding). The most common ingredient of insect repellents is DEET (N,N-diethyl-*m*-toluamide, CAS no. 134-62-3). Despite being the most widely used insect repellent worldwide, it has never been tested what consequences its introduction into aquatic environment may have, as it was originally registered for indoor use (Costanzo et al., 2007). Since DEET can cause dissolution of certain plastics and an irritation of mucous membranes of human and animal users (Briassoulis et al., 2001; Osimitz and Murphy, 1997), the demand for alternative repellents increased in the 1980s. The two most common DEET alternatives in use are EBAAP (IR3535®), (3-[N-butyl-N-acetyl]-aminopropionic acid ethyl ester, CAS no. 52304-36-6) and Icaridin (Picaridin, Bayrepel, Saltidin, 1-piperidinecarboxylic acid 2-(2-hydroxyethyl) 1-methylpropyl ester, CAS no. 119515-38-7).

Such repellents become washed off the skin and fabric upon use and cleaning. Because their microbial degradability appears to be limited, it is not surprising that in both the US and Europe concentrations of up to several μg L^{-1} have frequently been detected in surface waters (Nendza et al., 2013). DEET has been detected in ground- and surface waters in concentrations up to 3 μg L^{-1} in Europe and even 33 μg L^{-1} in the U.S. (reviewed by Nendza et al., 2013). Since European industries have increasingly replaced DEET by EBAAP and Icaridin in the early 2000s, DEET concentrations in surface waters of Europe have declined steadily (Knepper, 2004b). On the other hand, Icaridin is meanwhile found in low μg L^{-1} concentrations in European lakes and rivers (Knepper, 2004a). Although the use of EBAAP has increased as well (Büchel et al., 2015), reports on its concentrations in surface waters are lacking (Nendza et al., 2013). Due to this lack of published data, it is unclear whether EBAAP occurs in similar environmental concentrations as reported for the other two repellents or if its concentrations are typically below the analytical detection limit due to biodegradation

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or adsorption to organic surfaces. Overall, considerable repellent (DEET) concentrations were found in agriculturally dominated areas (Liu et al., 2015) and even in areas distant from potential sources (Costanzo et al., 2007; Kolpin et al., 2002). Compounds with limited biodegradability are not unlikely to be transported downstream in running waters or spread by turbulent flows through lake systems and could thus impact even organisms further away from the point of their introduction into the system. It is thus plausible to hypothesize that in these natural surface waters, the compounds may also exert repellent effects on aquatic non-target organisms (Nendza et al., 2013). This may not only affect aquatic insects (or their aquatic larval stages), but potentially as well crustaceans, which are phylogenetically much more closely related to insects than e.g. ticks, which are major target organisms for repellents (Büchel et al., 2015). Obviously, the validity of this hypothesis depends on the specific mode of action of the repellents on the molecular level. However, this is not yet known in detail despite detailed studies in recent years (Kain et al., 2013). Such potential behavioural effects on aquatic non-target organisms have never been considered in the registration process of those chemicals.

As repellents fall under the EU biocides legislation (ECHA -European Chemicals Agency, 2016), their registration process involves only assays for potential toxicity to non-target organisms. For this reason, the German Federal Environment Agency initiated a research project to screen for possible effects of repellents on aquatic non-target species. As a first step, based on an initial literature survey (Nendza et al., 2013), the most relevant substances with regard to criteria like environmental concentrations, chemical detectability, mode of action, etc. were selected. In the following, laboratory studies were carried out in order to evaluate test systems for their suitability to detect induced behavioural changes (such as infochemical effects, Klaschka, 2008), caused by the most frequently applied repellents. These experiments were conducted at a wide range of concentrations even beyond the level of environmental concentrations in order to evaluate potential hazards. The results of the first experiments on possible changes in vertical migration of freshwater zooplankton under the influence of repellents were recently published by Von Elert et al. (2016). While this first study addressed the pelagic habitat of lakes, we here report the findings of the second experiment focussing on invertebrate downstream drift in running water systems.

Downstream drift is a commonly observed behaviour of invertebrates in running waters as a response to locally changing or deteriorating conditions, including the presence of competitors or predators (Allan and Castillo, 2007; McIntosh and Peckarsky, 1999). The quantification of this avoidance reaction in response to anthropogenic noxae has been an important research focus in both experiments and the field in recent decades (Gunkel, 1994). When entering the drift, animals leave the area of low current velocity near the stream bottom and let themselves be transported downstream passively. Apart from changes in the current velocity, anthropogenic chemicals may cause drastic increases in downstream drift of stream invertebrates (Berghahn et al., 2012; Rasmussen et al., 2008), which can subsequently result in massive ecological changes (Anderson and Lehmkuhl, 1968; Flannagan et al., 1979). Taken together, the ability of insect repellents to deter many arthropods across the whole phylum and the common behavioural response of aquatic invertebrates to escape unsuitable conditions through drift, it is a plausible assumption that repellents (designed to deter invertebrates), induce downstream drift behaviour in stream invertebrates. Here, we test the hypothesis that the insect repellents DEET, EBAAP and Icaridin cause increased drift behaviour in two model invertebrates, the amphipod *Gammarus pulex* (L.) and larvae of the mayfly *Cloeon dipterum* (L.). We addressed this hypothesis by using a new simple laboratory-scale drift assay that allows the quantitative assessment of chemically induced drift behaviour of aquatic invertebrates at a small scale (Berghahn et al., 2012; Werth and Marten, 2007).

2. Materials and methods

2.1. Test organisms

We chose two model invertebrates for our experiments: The amphipod *Gammarus pulex* (L., Crustacea, Amphipoda, Gammaridae) and the larvae of the ovoviviparous mayfly *Cloeon dipterum* (L., Insecta, Ephemeroptera, Baetidae) were chosen as similarly-sized models for two main groups of running water invertebrates. Both species can be found in lentic and lotic inland waters. *G. pulex* has been observed in the field to react to sublethal concentrations of the insecticide fenvalerate with active downstream drift behaviour (Liess, 1994), and gammarids in general have previously been used to assess effects of anthropogenic chemicals on invertebrate drift behaviour (Berghahn et al., 2012; Rasmussen et al., 2008). *C. dipteru*m was chosen as a second study organism because a recent study has indicated higher sensitivity of larvae of this mayfly species to the insecticide imidacloprid compared to amphipods (such as *G. pulex*) and other arthropods (Roessink et al., 2013). *C. dipterum* can be found in a variety of freshwater ecosystems from ponds to slow-running brooks and small rivers (Craig, 1990).

For our experiments, we obtained live *G. pulex* ($\geq$7 mm body length) from a fish food supplier (Fischfutter Etzbach FEE, Schleiden, Germany), where they had been kept in a natural and fishless stream according to the supplier. 8th–9th instar larvae of *C. dipterum* ($\geq$7 mm body length) were collected from a pond on the University of Cologne campus. 10th instar larvae of *C. dipterum* were excluded from the experiments to avoid emergence during the acclimation or the experiment. Both organisms were kept in aquaria in a climatized chamber at 18 $\pm$ 1 °C and dim light (16:8 h L:D cycle). Tap water was passed through an activated charcoal filter and preconditioned by aging for >3 d under continuous aeration, followed by pressure filtration (<0.45 μm) and another storage for > 6 h under aeration to remove supersaturation of gases resulting from the pressure filtration. This pretreatment yields a reliable and suitable medium for the long term culture of various aquatic invertebrates (P. Fink, pers. observation). Maximum densities were 7 individuals L^{-1}, and the animals were fed commercial fish feed (TetraMin and PlecoMin, Tetra GmbH, Melle, Germany) every other day before and between drift assays.

2.2. Test system

The drift assays were conducted in a considerably improved version of the 'drift carousel' developed by Werth and Marten (2007), which had previously been successfully applied for the testing of anthropogenic chemicals on the drift behaviour of aquatic invertebrates (Berghahn et al., 2012). This new test system consists of two circular, flat-bottomed borosilicate glass bowls (140 and 230 mm diameter, respectively). Placing the smaller bowl into the centre of the larger bowl created a channel of 45 mm width, 75 mm depth and 575 mm circular length (in the centre of the channel, see Fig. 1). This channel was subsequently filled with 1700 ml of aged tap water to yield a water depth of 65 mm. To study possible repellent effects on invertebrate drift, the setup was further modified substantially in order to minimize carryover between assays and memory effects of the test system: The pump for the creation of the water current was replaced by a motor-driven glass paddle (a standard microscope slide cut to 26 $\times$ 55 mm and immersed for 30 mm into the water body), which caused a constant current with a speed of 6.1 $\pm$ 0.3 cm s^{-1} (determined with dye, Fig. 1). At this flow