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Molluscicide baits impair the life traits of *Folsomia candida* (Collembola): Possible hazard to the population level and soil function

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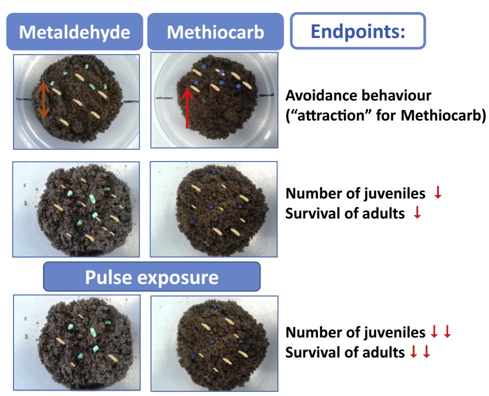
HIGHLIGHTS

- Molluscicide baits attracted collembolans.
- Collembolans reproduction and survival were adversely affected by molluscicide baits.
- One bait pulse exposure increased toxicity when compared to a single bait exposure.
- Molluscicidal baits can harm collembolans in realistic exposure scenarios.

GRAPHICAL ABSTRACT



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ABSTRACT

The application of molluscicides baits on the soil surface is the most common practice to control terrestrial gastropods. There seems to be a gap in the accurate evaluation of molluscicidal baits effects to soil arthropods, since their hazard to non-target organisms has been considered low after mixing baits into soil. In this work the ecotoxicological effects of two molluscicide baits (metaldehyde and methiocarb) to the collembolan *Folsomia candida* were evaluated using two different approaches: (1) molluscicidal baits were applied to the top soil once and only at the beginning of the exposure and avoidance behaviour and reproduction were evaluated; and (2) baits were replaced by new ones after 14-d of exposure, simulating the recommended application rate recommended by the manufacturer and reproduction was assessed (repeated/pulse exposure). A preference for the side contaminated for methiocarb was observed but the distribution of collembolans in the avoidance test with metaldehyde was random. Exposure to metaldehyde resulted in a significant increase in mortality. For methiocarb, a reduction in the juveniles produced but no acute effects were observed. In the bait pulse test, the toxic effects of each chemical was significantly increased compared with the single exposure test, for all treatments used (both reproduction and mortality). In summary, molluscicides have an adverse effect on *F. candida*, with severe effects on their behaviour (only for methiocarb), reproduction and survival (for both), which can lead to population collapse with time.

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1. Introduction

Currently the chemical control of slugs and snails is mainly based on the use of metaldehyde and methiocarb formulated as flour-based pellets, or to a less extent, granular and liquid formulations. Both compounds represent more than 90% of all European sales for molluscicides (Henderson and Triebskornz, 2002). Metaldehyde baits are toxic to snails and slugs upon ingestion, causing increased slime secretion and inducing ultrastructural destruction of mucocytes, leading to dehydration and subsequent death of the organisms (Bailey and Barker, 2002). As a carbamate, methiocarb acts upon the central nervous system inhibiting acetylcholinesterase (AChE), which leads to an overstimulation of the nervous system and to the death of the animal (Santos et al., 2010).

The application of molluscicides in agricultural fields is conducted by randomly distributing baits on the soil surface (by manual or mechanical dispersion). On that way, the number of baits that are present in the field is normally higher than the recommended dose, per area. The application of molluscicides in some cultures (e.g. vineyards and orchards) implies several applications within a safety interval of 14 d, as recommended by the manufacturer. Moreover, in some horticultural crops baits are applied near the stem as agglomerates, to prevent snails and slugs from attacking the aerial part of plants. Due to this method of application there will be some hotspots where the number of baits will be higher than the recommended application dose. However, although near plant stems they can be more concentrated, baits are usually randomly distributed by the way they are hand propel using forward motion (thrown) and therefore several baits can be found simultaneously in agricultural fields within a small area.

The half-life of metaldehyde in soil in laboratory conditions (DT_{50lab}) has been reported to be between 8.5 and 22 d (20 °C), and for methiocarb between 0.7 and 1.5 d (EFSA, 2006; EFSA, 2010); therefore, both active substances are considered to have low to very low persistence in soil. This is important when looking at the DT_{50lab} for both compounds and the exposure time used in standardized protocols with collembolan; here it is clear that a complete dissipation of the two active ingredients in the soil may occur in chronic tests (e.g. reproduction test) (ISO, 1999; OECD, 2009). However, in short-term exposures like those in avoidance tests (ISO, 2007) the dissipation will not be completed at the end of the 48 h exposure period. In addition, one should also consider the common agriculture practice of molluscicide baits, as previously stated, where multiple application are used.

Molluscicidal baits were developed to be effective at low concentrations and have minimal adverse effect on non-target species that shares the same habitat with snails. Effects of molluscicides containing metaldehyde or methiocarb on non-target soil organisms have been assessed in several studies using earthworms, molluscs, nematodes, acarids, isopods and collembolans (Bieri et al., 1989; Wellmann and Heimbach, 1996; Bieri, 2003; Iglesias et al., 2003; Langan and Shaw, 2006; Santos et al., 2010; Calisi et al., 2011; Gavin et al., 2012). Metaldehyde is considered highly specific and non-toxic to beneficial organisms such as earthworms, spiders, ants, millipedes, staphylinid and carabid beetles, when applied at the recommended application dose (RAD) (Bieri, 2003). However, the use of these pellets can represent a hazard to non-target soil invertebrates that can feed directly or indirectly on the pellets (Bailey and Barker, 2002; Bieri, 2003; Santos et al., 2010).

Previous studies observed a reduction in *Lumbricus terrestris* (L.) activity and increased mortality after exposure to molluscicidal iron phosphate pellets in laboratory but no negative effects of metaldehyde pellets were detected (Langan and Shaw, 2006). Another study observed severe lethal effects caused by both molluscicides to the terrestrial isopod *Porcellionides pruinosus* in short-term exposures (Santos et al., 2010). In addition, effects on the isopods' nervous system and oxidative stress were also reported (Santos et al., 2010).

According to the Draft Assessment Report (DAR) for metaldehyde and methiocarb, tests with microarthropods such as collembolans are exempted because no effects on earthworms were observed at higher doses than the predicted environmental concentrations (PEC) of the compounds (EFSA, 2006; EFSA, 2010). Therefore, the risk for earthworms was considered as low and read across for other edaphic species. But recently, the EU regulation 283/2013 set out the data requirements for active substances, concerning plant protection products on the market, and highlighted again the need for ecotoxicity testing using non-target arthropods like collembolan (*F. candida*) and mites (*Hypoaspis aculeifer*) (European Commission, 2013). In addition collembolans have been recommended as bioindicators of changes in soil quality and soil health (Pankhurst et al., 1995; van Bruggen and Semenov, 2000). These organisms are associated to key processes such as decomposition and nutrient cycling and they influence the microbial activity and biomass of the edaphic ecosystem (Hopkin, 1997; Fountain and Hopkin, 2005; OECD, 2009). The collembolan species *F. candida* has a ubiquitous distribution in the world and it is commonly found inhabiting the upper soil layer (Chapman, 1998). This organism has a short life cycle, reproducing through parthenogenesis (ISO, 1999; Fountain and Hopkin, 2005; OECD, 2009). Changes in this species distribution and density can be considered as an early warning indicator of ecosystem changes (Carter and Noronha, 2007).

Considering the above-mentioned reasons, this study aimed at studying the potential toxicity effects of two molluscicidal pesticides, metaldehyde and methiocarb, in the blind and non-pigmented soil dwelling arthropod *F. candida*.

To accomplish this objective two experimental set-ups were used: (1) a single molluscicide application, where metaldehyde or methiocarb baits were applied on the soil surface and avoidance behaviour tests and reproduction tests were carried out; and (2) a pulse exposure test, where two applications of baits within a safety interval of 14 d were carried out and the effects on the reproduction and survival of adults were assessed.

2. Materials and methods

2.1. Test species and test soil

Tests were carried out using the natural standard soil LUFA 2.2 (Speyer, Germany), a sandy-loam soil with the following physical and chemical characteristics: silt (17%), clay (6%) and sand (77%), 2.3% Corg: *C*/*N* of 14, pH (0.01 M, $CaCl_2$) 5.8 ± 0.1, water holding capacity (55% weight per volume), density of 1.13 ± 0.045 g ml^{-1} and cation exchange capacity of 11.2 cmol^{+} kg^{-1}. The collembolan *F. candida* were obtained from synchronised laboratory cultures, maintained in plastic boxes lined with a mixture of plaster of Paris and activated charcoal in a ratio of 9:1 (ISO, 1999), in the dark under a constant temperature regime (20 ± 2 °C). Once a week, granulated dry yeast was added in small amounts as food source.

2.2. Test chemicals and test concentrations

Two molluscicides were used in the experimental set-ups as commercial formulations: HELITOX® (Sipcam™) containing 5% *w*/*w* of metaldehyde and MESUROL® (Bayer™) with 4% *w*/*w* of methiocarb. These formulations were presented to isopods as baits, with approximately concentrations of 9.5 mg metaldehyde kg^{-1} and 3.8 mg methiocarb kg^{-1} per bait.

In all tests, 1, 3, 5, 7 and 10 baits were used to assess the effects of molluscicide baits of metaldehyde and methiocarb. Moreover,