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Comparative ecotoxicity of chlorantraniliprole to non-target soil invertebrates

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HIGHLIGHTS

- CAP was severely toxic to *Folsomia candida* in a chronic reproduction toxicity test.
- Toxicity of CAP to *F. candida* was reduced by higher soil organic matter contents.
- CAP appears to act promptly, affecting locomotor abilities of *F. candida*.
- CAP was not toxic to isopods, oribatid mites and enchytraeids in chronic toxicity tests.
- CAP may especially pose a risk to non-target soil arthropods closely related to insects.

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ABSTRACT

The insecticide chlorantraniliprole (CAP) is gaining importance in agricultural practice, but data on its possible negative effects on non-target organisms is severely deficient. This study therefore determined CAP toxicity to non-target soil invertebrates playing a crucial role in ecosystem functioning, including springtails (*Folsomia candida*), isopods (*Porcellio scaber*), enchytraeids (*Enchytraeus crypticus*) and oribatid mites (*Oppia nitens*). In sublethal toxicity tests in Lufa 2.2 soil, chronic exposure to CAP concentrations up to 1000 mg/kg_{dw} did not affect the survival and reproduction of *E. crypticus* and *O. nitens* nor the survival, body weight and consumption of *P. scaber*. In contrast, the survival and reproduction of *F. candida* was severely affected, with an EC_{50} for effects on reproduction of 0.14 mg CAP/kg_{dw}. The toxicity of CAP to the reproduction of *F. candida* was tested in four different soils following OECD guideline 232, and additionally in an avoidance test according to ISO guideline 17512-2. A significantly lower toxicity in soils rich in organic matter was observed, compared to low organic soils. Observations in the avoidance test with *F. candida* suggest that CAP acted in a prompt way, by affecting collembolan locomotor abilities thus preventing them from escaping contaminated soil. This study shows that CAP may especially pose a risk to non-target soil arthropods closely related to insects, while other soil invertebrates seem rather insensitive.

1. Introduction

Modern agricultural practice continues to apply high amounts of insecticides in order to protect crop yields. The tendency is, however, to develop new, less harmful and more effective active compounds for crop protection. Likewise, DuPont synthesized chlorantraniliprole (CAP), an insecticide acting as a ryanodine receptor activator. Following uptake by an insect, CAP binds to this receptor, causing a depletion of internal calcium stores in the sarcoendoplasmatic reticulum (Cordova et al., 2006). The unregulated release of internal calcium stores subsequently causes an impaired regulation of muscle contraction, resulting in feeding cessation, lethargy and partial paralysis, leading to death of the insect (Cordova et al., 2006, Lahm et al., 2007). Due to the differential

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receptor selectivity of CAP between mammalian and insect ryanodine receptors, CAP features high insecticidal activity but low mammalian toxicity, for which it also holds promises for an effective pest control in integrated pest management practices (Lahm et al., 2007).

Apart from being relatively safe to mammals, CAP also appears to be safe to birds and fishes, at environmentally relevant concentrations (USEPA, 2008), but very high toxicity was reported for several aquatic invertebrates. Very high acute toxicity has been reported for the water flea *Daphnia magna* (Lavtižar et al., 2015), the crayfish *Procambarus clarkii* (Barbee et al., 2010), mayflies, caddisflies, midges, amphipods, estuarine and marine oysters, and moderate toxicity was observed for the crayfish *Oronectes virilis* (USEPA, 2008; EFSA, 2008).

In contrast to aquatic organisms, CAP appears to be relatively safe to most non-target terrestrial arthropods, such as parasitic wasps (Brugger et al., 2010), predatory insects (Gontijo et al., 2015), honeybees and bumblebees (Dinter et al., 2009), predatory mites, hoverflies and earthworms (USEPA, 2008; EFSA, 2008). CAP also had little or no effect on the abundance of several non-target soil invertebrate species and on predation or decomposition tested in a turfgrass field experiment (Larson et al., 2014). A very high toxicity, however, has been reported for the springtail *Folsomia candida*, with an EC_{50} of 0.48 mg/kg_{dw} for effects on reproduction (USEPA, 2008), but no information was provided on the type of test soil and its properties. EFSA (2008) reported a similar low EC_{50} of 0.85 mg/kg_{dw} for the effect of CAP on *F. candida* reproduction in an artificial soil with 5% organic matter (OM) and a pH of approximately 5.5. However, no ecotoxicity data are available for other soil invertebrate species, while CAP residues are expected to accumulate in soil due to its persistence in soil (half-life up to 1130 days in dissipation studies on bare ground, USEPA, 2008). Sharma et al. (2014) reported an average initial CAP concentration in a sugarcane field soil of 0.88 mg/kg_{dw} after the application of CAP at the recommended dose of 100 g a.i./ha. This indicates that exposure of soil invertebrates is likely to occur. Hence, there is an urgent need to generate toxicity data for non-target soil organisms playing an important ecological role in decomposition processes. The aim of the present study was therefore to investigate the effects of chronic exposure to CAP of the isopod *Porcellio scaber*, the oribatid mite *Oppia nitens*, the potworm *Enchytraeus crypticus* and the springtail *F. candida*. To determine whether the springtails are able to avoid soil contaminated with CAP and whether this avoidance behaviour is dose-related, *F. candida* was also subjected to an avoidance test. Since soil characteristics influence the bioavailability and toxicity of xenobiotics (see for example Johnson and Sims, 1993), we also determined the influence of soil properties, especially organic matter content, on CAP toxicity to the springtails. The concentrations used in the springtail reproduction test were based on literature data (USEPA, 2008), while for the other organisms a range-finding test with wider concentration range was performed to enable proper quantification of CAP toxicity.

2. Materials and methods

2.1. Test organisms

Springtails, enchytraeids and oribatid mites originated from cultures present at the Department of Ecological Science of the Vrije Universiteit in Amsterdam. Springtails and mites were cultured on charcoal-amended plaster of Paris and fed *ad libitum* with Dr. Oetker dry baker's yeast. The enchytraeids were cultured on an agar substrate, prepared with aqueous Lufa 2.2 soil extract and fed with a mixture of oat meal, dried yeast, yolk and milk powder, and fish oil.

Adult isopods *Porcellio scaber* were collected from an uncontaminated area in Bilthoven, the Netherlands, and kept in a glass terrarium with a thick layer of Lufa 2.2 soil covered with leaves (mainly maple and poplar) obtained from the same site. Isopods were kept in a climate room at 16 °C with 16/8 h light/dark for approximately one month to acclimatize. Alder leaves were provided as a food source and the substrate was moistened regularly.

Cultures were maintained at 16 ± 1 °C (20 ± 1 °C for the oribatid mites), 75% relative humidity, 16/8 h light/dark and 400–800 lux illumination.

Prior to the toxicity tests, the springtails were synchronized to obtain animals of the same age: for the reproduction toxicity tests, animals of 10–12 days old were selected, while in the avoidance test animals were around 32 days old. For age synchronization, 30 visually healthy adults from the cultures were randomly introduced into boxes with moistened black-coloured plaster of Paris, and fed with dry baker's yeast. After 2 days, all adults were removed, leaving only the freshly laid eggs. The boxes were regularly moistened and aerated. The springtails hatching after approximately 8–10 days were fed dry baker's yeast twice a week until they were used in the toxicity tests. The synchronizations were incubated at 20 ± 1 °C under the same conditions of humidity and illumination as the cultures.

2.2. Test soils

The toxicity tests with isopods, mites, enchytraeids, springtails and the springtail avoidance test were performed in natural Lufa 2.2 soil (Lufa Speyer, Germany; LF). In addition to the Lufa 2.2 soil, in the reproduction toxicity tests with springtails, three other non-contaminated natural soils from Portugal (Coimbra; CO), the Netherlands (Dutch grassland; DG) and the United Kingdom (North Wales; NW) were included. These soils were chosen to have different properties, in particular, soil organic matter (OM) content. Prior to the toxicity experiments, the soils were homogenized, air dried, 5 mm sieved and characterized as described by Waalewijn-Kool et al. (2014). Soil pH was measured at the beginning and at the end of the tests following ISO guideline 10390 (ISO, 2005), except where noted. For the pH measurement, moist soil was shaken with 0.01 M analytical grade $CaCl_2$ solution (1:5) for 2 h at 200 rpm. After settling, the pH of the overlying solution was measured in duplicates with a pH meter (Consort P907).

2.3. Toxicity tests

Soil spiking followed the same procedure for all toxicity tests. The soil was dried at 50 °C overnight. A portion (10–25%) of the specified amount of soil needed for each treatment was used for spiking. Since CAP has a low water solubility, acetone was used as a carrier solvent. All treatments received the same amount of solvent and a solvent control was included as part of the tests. The glass beakers with the spiked soil were closed and placed in the fume hood overnight. The next day, the jars were opened to allow for evaporation of the solvent (for about 18 h) and then the remaining portion of the soil was added and mixed thoroughly. Next, demineralized water was added, while constantly stirring, to obtain a soil moisture content equivalent to 50% of the respective soil water holding capacity (WHC). The prepared soil was then distributed into test containers. All toxicity tests were incubated at 20 °C, 75% relative humidity and a 16/8 h light/dark photoperiod. The reported toxicity effects were based on nominal CAP concentrations.

2.3.1. Toxicity tests with Porcellio scaber, Enchytraeus crypticus and Oppia nitens

As no information on the toxicity of CAP to isopods, oribatid