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Toxicity of chlorantraniliprole to the Collembola *Folsomia candida*; toxicodynamics and effects of organic matter content

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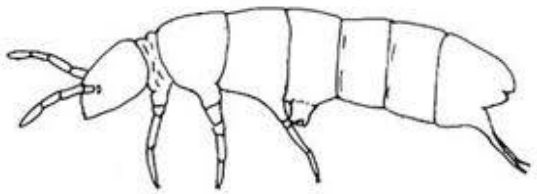

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ABSTRACT

Toxicity of pesticides to non-target organisms determines their impact on natural environments. And to find pesticides with a new, target-specific mode of action, which are safe to farmers and organisms in the surrounding environment, is important when developing new pesticides.

In this study, toxicity of the insecticide chlorantraniliprole to the Collembola *Folsomia candida* was investigated, showing that the test animals were adversely affected by the compound. Toxicity was tested in two experiments; a reproduction test in four soils with different organic matter content, following the OECD Guideline 232, and a toxicodynamic test where mortality, mobility, reproduction and morphological changes were recorded.

The reproduction test showed a lower toxicity of chlorantraniliprole in the high-organic soils compared to the low-organic. When organic matter content increased two times, the difference between the lowest and the highest EC_{50} and EC_{10} values was a factor of 5.3 and 8.4, respectively. pH did not seem to significantly affect toxicity, and organic matter content did not seem to affect the total number of juveniles produced.

The toxicodynamic test showed a fast mode of action on mobility of *F. candida*, but not on mortality. Mobility decreased at the highest treatments of chlorantraniliprole already one day after the animals were introduced to the test vessels, but significant mortality was still not seen after almost three weeks. Reproduction was also adversely affected with a decline in the total number of juveniles produced at the higher treatments. The animals at the higher treatments also showed a possible compound induced reproduction stress, with faster egg laying. Morphological changes, such as affected antennas, increased steadily over time.

Chlorantraniliprole shows high toxicity to some non-target organisms but is, with its new mode of action, still important in the development of more environmentally safe pesticides.

SAMMANFATTNING

Hur toxiskt ett bekämpningsmedel är för icke-målorganismer avgör dess påverkan på naturmiljön. Därför är det viktigt att utveckla bekämpningsmedel med ett nytt målinriktat verkningssätt, vilka är säkrare för jordbrukare och organismer i den omkringliggande omgivningen.

I den här studien undersöktes toxiciteten hos insektsmedlet chlorantraniliprole genom experiment med collembolan *Folsomia candida*, och de visade att preparatet hade en negativ effekt på testdjuren. Toxiciteten testades i två experiment; ett reproduktionstest i fyra jordar med olika innehåll av organiskt material som följde testproceduren i OECDs Guideline 232, och ett toxicodynamiskt test där mortalitet, rörlighet, reproduktion och morfologiska förändringar registrerades.

Reproduktionstestet visade en lägre toxicitet av chlorantraniliprole i jordarna med högre innehåll av organiskt material jämfört med de med lägre. När mängden organiskt material ökades två gånger, var skillnaden mellan det högsta och det lägsta EC_{50} och EC_{10} värdet en faktor av 5.3 och 8.4, respektive. pH verkade inte signifikant påverka toxiciteten och mängden organiskt material verkade inte påverka det totala antalet producerade juveniler.

Det toxicodynamiska testet visade en snabb påverkan på rörligheten hos *F. candida*, men inte på mortaliteten. Rörligheten minskade i de högsta testdoserna av chlorantraniliprole redan en dag efter att djuren introducerats i testbehållarna, men signifikant mortalitet sågs fortfarande inte efter nästan tre veckor. Reproduktionen påverkades också negativt med en

minskning av det totala antalet producerade juveniler i de högre testdoserna. Snabbare äggläggning hos djuren i de högre testdoserna visade även på en möjlig preparatinducerad reproduktionsstress. Morfologiska förändringar, såsom påverkade antenner, ökade stadigt över tiden.

Chlorantraniliprole visar hög toxicitet för vissa icke-målorganismer, men är med sitt nya verkningssätt fortfarande en viktig del av utvecklandet av mer miljömässigt säkra bekämpningsmedel.

INTRODUCTION

Pesticides

Pollution may occur in many forms, and one of them is through the extensive use of biocides, such as pesticides, which can have an adverse effect on natural environments. Pesticides are mainly used on agricultural land and forests, but also to control insect disease vectors like malaria mosquitoes and tsetse flies, pests in water systems, in households as insect repellents, and as sanitizers and bath disinfectants (*16, 18*).

Pesticides applied on the soil directly, e.g. granules, in the soil on dressed seeds or sprayed on crops are the most significant soil pollutants in agricultural landscapes. The use of pesticides poses a problem of contaminating surrounding environments such as land and surface waters in form of spray drift from aerial spraying, and ground water from leaching through the soil (*16*). Heavy rainfalls can wash pesticides into rivers and oceans and transport them long distances from where they were applied. Pesticides transferred to lakes can easily accumulate, which can have a serious adverse impact on aquatic ecosystems (*16, 18*).

The properties of a pesticide, like chemical stability, vapor pressure and solubility, determine how they distribute in the environment. When broken down, pesticides normally, but not always, become less toxic (*16*). Pesticides that are highly target-specific normally do not cause harm to non-target organisms, but many pesticides are not selective for a specific pest and can therefore be highly toxic to non-target organisms such as soil and aquatic organisms (*16, 18*).

Some persistent lipophilic pesticides (e.g. organochlorine insecticides) may also accumulate in food chains, and predators of the highest trophic levels, often birds and mammals, at times carry the highest concentrations (*16*). Nowadays, new types of pesticides, which are more target-specific and therefore have less adverse impact on non-target organisms, are under development and evaluation. One such pesticide is chlorantraniliprole, which belongs to a new class of insecticides with a novel mode of action.

Ecotoxicology and toxicity testing

Pollution has long been a problem, and the concern for public and environmental health has made the management and control of chemicals necessary. Toxicological testing may show how toxic different compounds are to organisms, including humans, and to conduct risk assessment to set up standards for how much of the compound will be allowed in terms of usage, amounts in food and in the environment etc.

Ecotoxicology addresses the impact of toxic chemicals on the environment and their effects on microbial, plant and animal populations, community structure and ecosystems (*16, 17*).

Ecotoxicological research is necessary upon the introduction of new pesticides for evaluating their impact on the natural environment, crops and humans. Within agriculture it is important to keep a profitable production and the use of pesticides normally is an inevitable action to get rid of crop-destroying pests. Toxicological testing is continuously being performed in laboratories and in the field to make sure pesticides do not leave any toxic residues on crops or contaminate drinking water, but also to minimize their potential harm to farmers and the surrounding environment, and to estimate the possible adverse effect on organisms that are essential for the functioning of the soil. This study will evaluate the effect of the new insecticide chlorantraniliprole on a soil-dwelling arthropod, the collembolan *Folsomia candida*.

Use and mode of action of chlorantraniliprole

Chlorantraniliprole (CAP) is a relatively new insecticide (registered in April 2008) which belongs to the chemical class of anthranilic diamide insecticides (*1-3, 5, 6*). It is the active compound in several pesticides such as Coragen and Rynaxypyr, and has been tested in the field extensively since 2002 (*5*). Just like all the anthranilic diamide pesticides, chlorantraniliprole shows no significant effects on the central nervous system and is therefore not neurotoxic (*4, 5, 11*), nor is it genotoxic (*4, 8*). The Environmental Protection Agency (EPA) has classified it as a reduced-risk pesticide (*3*).

Chlorantraniliprole works foremost on chewing pest insects, mainly by consumption but also by contact (*5, 6*), and it is effective against several lepidopteran pests, and some species of Coleoptera, Diptera and Hemiptera (*2, 3*). It has very low toxicity to mammals, birds, bees and earthworms, and only slightly toxic to fish (*1, 4*), but is highly toxic to *F. candida* and *Daphnia magna*, and medium to very highly toxic to several insects and crustaceans. Chlorantraniliprole has a moderate potential for bioconcentration, and metabolites show low toxicity (*4*).

The biochemical (*8*) mode of action of chlorantraniliprole is new and different from other pesticides (*3, 4-6*). It binds to a novel target site and interrupts the normal contraction of the muscle by activating the ryanodine receptors (RyRs) located in the sarcoplasmic reticulum of the muscle cells and endoplasmic reticulum of non-muscle cells. This result in uncontrolled release of stored intracellular calcium from the sarcoplasmic reticulum, leading to Ca^{2+} depletion, which causes impaired regulation of muscle contraction. This leads to rapid feeding cessation, lethargy, paralysis, since the muscles get locked in a contracted state, and eventually death (normally within 24-72 hours) (*1-3, 5-11*).

Importance of Collembola in the environment and in ecotoxicology

Collembolans are among the most abundant soil arthropods in different environments all over the world (*12, 14-16, 19*). They can be found on every continent, including Antarctica, in soils, moss, lichen, water, snow and in trees (*12, 14, 15, 19*), and population densities can sometimes reach as much as 10^5 m^{-2} (*14, 15*). Collembolans feed on fungal hyphae, yeasts and decaying organic material. They also consume pest fungi, and thus might also play a role in the growth of mycorrhizae and act as plant fungal disease control. Experiments have shown that collembolans contribute to enzyme activity, respiration (calculated through production of carbon dioxide or oxygen consumption) and nutrient release from leaf litter (*12, 19*). They are extremely important to some soils where their faeces is an essential source of nutrients for plant roots. Because of their abundance and function in ecosystems, collembolans are often used as bioindicators for the effects of pollutants and pesticides (*15*).

In environments where earthworms and diplopods are present, the collembolans probably contribute to a very small part of the total decomposition. But where earthworms and diplopods are not present, for example in acid or polluted soils, they are necessary in the decomposition process. They are also important food sources for many invertebrates, mainly other arthropods, and some vertebrates such as birds and amphibians (*12, 14, 15*). Since they are ecologically important organisms (*12, 15*) with their contribution to nutrient release through decomposition of organic matter and respiration in the soil (*12*), they are of high value to preserve.

Collembolans are widely used animals in ecotoxicological testing to predict the effects of chemicals in natural environments and implement risk assessment, but also to test the toxicity of different chemicals to natural populations of Collembola (*13, 15, 16, 19*). Collembolans have a thin exoskeleton easily penetrated by air and water and their rate of exposure is different from other invertebrates such as earthworms and enchytraeids. Hence, they pose an interesting aspect in ecotoxicological testing (*14*).

Folsomia candida in toxicity testing

The most commonly used species of collembolan is *Folsomia candida*, which has been selected as a representative for soil arthropods. It has been assigned an ISO standard to toxicity testing of chemicals and contaminated soils since 1999 (*13*), and an OECD test guideline since 2009 (*14*). *F. candida* lives in the top layer of soils and has been located in almost all parts of the world except for Africa and India, and is widespread in Europe (*13, 15*).

However, they are not present in many types of natural and agricultural habitats, but due to their asexual reproduction (*13-15, 19, 20*) which makes them easy to breed, and short lifespan, they are perfect as test animals (*13, 19, 20*). They are therefore extensively used in laboratories in both ecotoxicological and non-ecotoxicological testing (*13, 19*).

Aim of this study and expected results

As mentioned before, the aim of this study is to evaluate the toxicity of the insecticide chlorantraniliprole by analyzing how it affects the collembolan *Folsomia candida*. This is done through two different experiments.

The first test will be a reproduction test to examine if there is any difference in toxicity between soils with different amounts of organic matter. The parameters EC_{50} and EC_{10} will be calculated to determine the toxicity.

The second test will be a toxicodynamic test, to see how the collembolans react over time when being exposed to the compound, by observing morphological and behavioural changes.

The toxicity of a chemical can vary substantially between environments (*18*) and earlier studies have shown that chemicals tend to be less toxic in soils with higher organic matter content (*13, 18*). Chlorantraniliprole is lipophilic and has a low solubility in water; consequently, it will bind to the organic matter in soils and be less present in pore water, which is the main route of exposure for collembolans with their highly permeable exoskeleton. Therefore, the predicted result of the reproduction test is that chlorantraniliprole will show lower toxicity to *F. candida* in the soils with higher organic matter contents.
It is already known that chlorantraniliprole is toxic to *F. candida* (*1, 4*), and it is also said to have a fast mode of action (*3, 5, 11*). Hence, the toxicodynamic test is expected to show a fast onset of effects on the test animals.

MATERIALS AND METHODS

Test organism and test chemical

Collembola are widespread, ecologically important soil organisms. They are commonly used in ecotoxicological experiments for their susceptibility to many chemicals, and for the easiness to sample them in the field and to cultivate them (*12, 19*).

The test species, *Folsomia candida* Willem (Collembola: Isotomidae) has been shown to tolerate a wide range of soil substrates (*18*). It is parthenogenetic, meaning the offspring develops from unfertilized eggs from females, and has a short reproduction cycle in 20°C, which make them excellent as test animals (*12, 15, 19, 20*). Males occur, but are rare (<1 per 1000) (*13, 14*).

The animals used were taken from a >10 year old laboratory culture from the Department of Animal Ecology, VU University Amsterdam. The animals were cultivated in plastic boxes (12 x 17 cm) with moist plaster of Paris mixed with charcoal, in climate rooms of 20°C, 12:12 h light/dark regime, and fed dry baker's yeast (Dr Oetker).

To obtain test animals of the same age, juveniles were synchronized prior to the start of the test. Cultured adults were left in small plastic boxes (7 x 10 cm) with fresh plaster of Paris/charcoal for 2 days in a climate room for oviposition, and then removed. The boxes were then aerated and moistened every 2-3 days, and after hatching dry baker's yeast was added when needed. The tests started when the synchronized animals were 9-12 days old.

The chemical used was a standard of the insecticide chlorantraniliprole (CAP) (99.5 % purity, Dr. Ehrenstorfer GmbH, Augsbury, Germany). It was chosen since it is a rather new insecticide on the market and not much is known about its effects on *F. candida*. For more detailed information on chlorantraniliprole, see Table 1.

Table 1. Physiochemichal properties of chlorantraniliprole.

Parameter	Value
Water solubility:	Deionized water 1.023 mg/L pH 4 0.972 mg/L pH 7 0.880 mg/L pH 9 0.971 mg/L
Vapour pressure:	6.3×10^{12} Pa
Henry's constant:	3.2×10^{9} Pa m^3/mole
Dissociation constant, pK_a:	10.88 ± 0.71
Octanol-water partition coefficient, log Kow:	pH 4.0 2.77 ± 0.067 pH 7.0 2.86 ± 0.010 pH 9.0 2.80 ± 0.116
Hydrolysis:	pH 4 stable pH 7 stable pH 9 ~10 days (half-life)

Test soils

For the reproduction test, four soils with different amounts of organic matter (OM) were selected, two with a lower OM content and two with a higher. Three of the soils were natural soils sampled from different geographical places in Europe, and one was a standard laboratory soil. The different soils were; a soil from Coimbra in Portugal (soil 1), the standard European soil Lufa 2.2 (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, Germany) (soil 2), a grassland soil excavated from a football field in The Netherlands (soil 3), and a soil from North Wales in UK (soil 4). The soils also had a difference in pH, which ranged from 5.04 to 6.78. See Table 2 for soil properties.

The CAP concentrations used for spiking soil 1 and 2 were 0.0256 – 0.064 – 0.16 – 0.4 – 1.0 – 2.5 µg/g soil. And the concentrations for soil 3 and 4 were 0.064 – 0.16 – 0.4 – 1.0 – 2.5 – 6.25 µg/g soil. These concentrations were determined from an earlier reproduction test on *F. candida* in Lufa 2.2 soil. Soil 3 and 4 were given a concentration range one step higher than soil 1 and 2 since they were expected to be less toxic due to the higher amount of organic matter.

Table 2. Properties of the four test soils Coimbra (soil 1), Lufa 2.2 (soil 2), Dutch grassland (soil 3) and North Wales (soil 4), used to examine the effect of organic matter content on the toxicity of chlorantraniliprole to *Folsomia candida*.

Soil	OM (%)	pH	CEC (mval/100g)	DOC (mg/l)	WHC (g/100g)
1	2.37 ± 0.06	5.85	5.17 ± 2.47	64.9 ± 4.37	32
2	3.09 ± 0.04	5.67	6.34 ± 0.81	64.1 ± 4.93	45
3	10.6 ± 0.31	6.78	20.0 ± 0.8	265 ± 1.64	73
4	14.7 ± 0.18	5.04	11.8 ± 0.2	3605 ± 279	96

OM = organic matter (± SD; n=2); CEC = cation exchange capacity (± SD; n=2); DOC = dissolved organic carbon, measured in pore water (± SD; n=2); WHC = water holding capacity.

For the toxicodynamic test the standard soil Lufa 2.2 was used. The concentration range 0.1 – 0.33 – 1.0 – 3.3 – 10 – 33 – 100 – 330 µg/g soil was chosen to examine the mortality of *F. candida* with time, when exposed to chlorantraniliprole. This concentration range was determined from an earlier 28 days-reproduction test in Lufa 2.2 soil.

Prior to use in the tests, all soils were dried for 24 hours in an oven at 60°C, to remove any traces of moist and any organisms, such as Collembola.

Experimental set up of the collembolan reproduction test

The first experiment followed the OECD Guideline 232 on Testing Chemicals for the Collembolan Reproduction Test in Soil (OECD 2009) (*14*), to test the toxicity on *Folsomia candida* of the insecticide chlorantraniliprole in four soils with different content of organic matter. Each soil was tested separately.

The four soils were spiked with 6 concentrations of the insecticide. The test also included a control and a solvent control. Since CAP has low solubility in water, before spiking, it was diluted in acetone. Only 10 % of the total amount of soil for each treatment was used during

the spiking. This was for two reasons; to avoid using a huge amount of acetone and to avoid damaging the organic matter in the soil, which is sensitive to acetone. The soils were spiked in big glass jars and the soil saturated with the acetone/CAP solution. After spiking, the jars were left in a fume hood with their lids on for 24 hours and without lids for 24 hours. When the soils had dried they were merged with the rest of the soil measured for each treatment, and then moistened with deionized water to 50 % of their water holding capacity (WHC), and mixed thoroughly with a spoon.

For the test, glass jars of 100 ml were filled with 20-30 g of moist soil, the exact weights noted. Five replicates were made for each treatment, and two extra jars for measuring the concentration and the pH at the end of the test. Soil for measuring the initial concentration was divided into falcon tubes and directly put in a freezer. The initial pH was measured by shaking 6 g of soil with 25 ml of 0.01 M $CaCl_2$ in small plastic bottles (2/treatment) at 200 rpm for 2 hours. When the soil had settled, pH was measured using a WTW pH 7110 (inoLab) meter. Two blanks containing only $CaCl_2$ were also measured. A Lufa 2.2 control was set up for soil 1, 3 and 4 to secure the outcome was due to the properties of the soils and not to a poor batch of animals. These controls were treated in the same way as the controls of the test soils.

Ten 9-12 days old synchronized *F. candida* were put in each test jar randomly at the start of the test, and a few granules of baker's yeast were added. The animals were checked under a microscope to make sure they looked healthy. Lids were put on loosely to let air in. The test vessels were put in a climate room of 20°C, 12:12 h light/dark regime, and the test organisms exposed for 28 days to the insecticide. During the test the jars were aerated twice a week, moistened to their initial weight with deionized water once a week, and more food was added after half of the test period.

At the end of the test, deionized water was added to the test jars and the content emptied into 250 ml beakers. The solution was stirred gently with a small spatula to make the animals float to the surface. Surviving adults were counted manually and the surface of the solution was photographed using a digital camera (Nikon Coolpix P510). Two pictures per sample were taken. The end pH soil was measured by following the same procedure as at the start of the test. To determine the reproduction, the juveniles on all the pictures were counted using a counting tool in Photoshop, and a mean value of every sample was calculated.

Experimental set up of the toxicodynamic test

The second experiment was a toxicodynamic test, set up to test the effect of the insecticide chlorantraniliprole on *Folsomia candida* over time. The soil used for this experiment was the standard soil Lufa 2.2. The spiking process of the soil followed the same procedure as for the reproduction test, and it was spiked with 8 different concentrations of the insecticide. The test also included a control and a solvent control of acetone.

After the spiking process was finished, the soil was mixed and moistened to 40 % of the WHC and divided into small, round, plastic test vessels (5 cm in diameter and 3 cm in height). In every test vessel 20 g of soil was added. To facilitate the monitoring of the normally soil-dwelling animals, the soil was flattened to force them to stay on the soil surface. 4 replicates were made for each treatment and also two test vessels per treatment with only soil, for measuring the concentration and pH at the end of the test.

At the start of the test, 10 healthy adult animals from the same culture were put in each test vessel, a few granules of baker's yeast added and lids put on. For this test a synchronization of the animals was not necessary since adult animals were used. The test vessels were put in a climate room of 20°C, 12:12 h light/dark regime. The initial pH was measured at the start of

the test in the same way as for the reproduction test, and soil for measuring the initial concentration was divided into falcon tubes and directly put in a freezer.

During the exposure time, the animals were observed every day for 4 weeks and parameters such as mortality, mobility, behavioural changes, reproduction (eggs and juveniles) and affected antennas was recorded. New food was added twice a week and fungus removed when needed. The soil was moistened 2 times every day to prevent the soil from becoming drenched from too much water added at the same time.

Calculations and statistical analysis

A two-tailed T-test with equal variances was done to each soil to see if the control and the solvent control showed any significant differences in reproduction. If this was not the case, the control data were pooled for further analysis. Performance of the controls was calculated using adult survival and reproduction.

The number of juveniles for each soil was plotted against the concentrations. A logistic model following Haanstra *et al.* (1985) was fit to the data to determine the sublethal endpoint of reproduction, the concentrations at which 50 and 10 % of the test animals showed an effect in form of reduction, EC_{50} and EC_{10}. The statistical program IBM SPSS software was used to calculate the 95 % confidence intervals for the EC_{50} and EC_{10} values.

No mean lethal concentration LC_{50} could be calculated because less than 50% mortality occurred at the highest test concentrations.

RESULTS

Collembola reproduction test

T-tests

None of the T-tests calculated for the control and the solvent control of the four soils showed any differences between them. The lowest p-value calculated was for soil 3 ($p = 0.31$). Hence, they were not significantly different from each other in any of the soils since $p > 0.05$, the threshold value. Therefore the two controls for each soil were pooled.

Control performance

The validity criteria for the untreated controls was set according to the OECD Guideline 232 (OECD 1999) (*14*) and the ISO Standard 11267 (1999) (*21*); adult survival > 80 %, > 100 juveniles per test vessel and coefficient of variance (CV) < 30 %.

Control performance (Table 3) of the two pooled controls in the different soils was in general high. After 28 days of exposure the adult survival for soil 1, 2, 3 and 4 was 91, 99, 99 and 79 %, respectively. Only soil 4, which has the highest OM content, but also the lowest pH, was slightly below 80 % survival, but it was considered negligible. The average number of juveniles in soil 1, 2, 3 and 4 was 326 (CV 32 %), 184 (CV 27 %), 156 (CV 33 %) and 242 (CV 31 %), respectively, which means all soils had a control reproduction of >100 juveniles.

The highest control reproduction was seen in soil 1, which has the lowest OM content. The lowest reproduction was in soil 3, which has the highest pH. In soil 1, 3 and 4 the CVs were slightly above the validity criteria of 30 %. This is due to one replicate in soil 1, two in soil 3 and one in soil 4 which had deviant values compared to the rest of the replicates.

The Lufa 2.2 controls for soil 1, 3 and 4 also showed good performance (Table 3), with an adult survival of 96 % for soil 1 and 84 % for soil 3 and 4. Since soil 3 and 4 were run practically simultaneously, they had the same Lufa 2.2 control. The average number of juveniles was 325 (CV 29 %) for soil 1 and 248 (CV 21 %) for soil 3 and 4. In the Lufa 2.2 controls, all the criteria for a valid test were met.

Table 3. Control performance of *Folsomia candida* in the Lufa 2.2 controls for each test (left), and the pooled controls (the control and the solvent control) of the four test soils (right).

	Lufa 2.2 controls			Pooled controls		
Soil	Survival (%)	Reproduction	CV reproduction (%)	Survival (%)	Reproduction	CV reproduction (%)
1	96 ± 5	325 ± 94	29	91 ± 19	326 ± 105	32
2	100 ± 0	182 ± 36	20	99 ± 26	184 ± 49	27
3	84 ± 9	248 ± 51	21	99 ± 3	156 ± 51	33
4				79 ± 17	242 ± 76	31

Soil 3 and 4 were run approximately simultaneously, therefore, their Lufa 2.2 control values are the same. Survival and reproduction are mean values of five replicates (± SD) of the Lufa 2.2 control, and of 10 replicates (± SD) in the two pooled controls, except for reproduction of the pooled controls of soil 2 which had one outlier removed (n=9). CV = coefficient of variance. For soil abbreviations, see Table 2.

Influence of organic matter on toxicity

As seen in Figure 1, the total number of juveniles for each treatment varied between the different soils, but decrease in reproduction was similar between soil 1 and 2 and between soil 3 and 4.

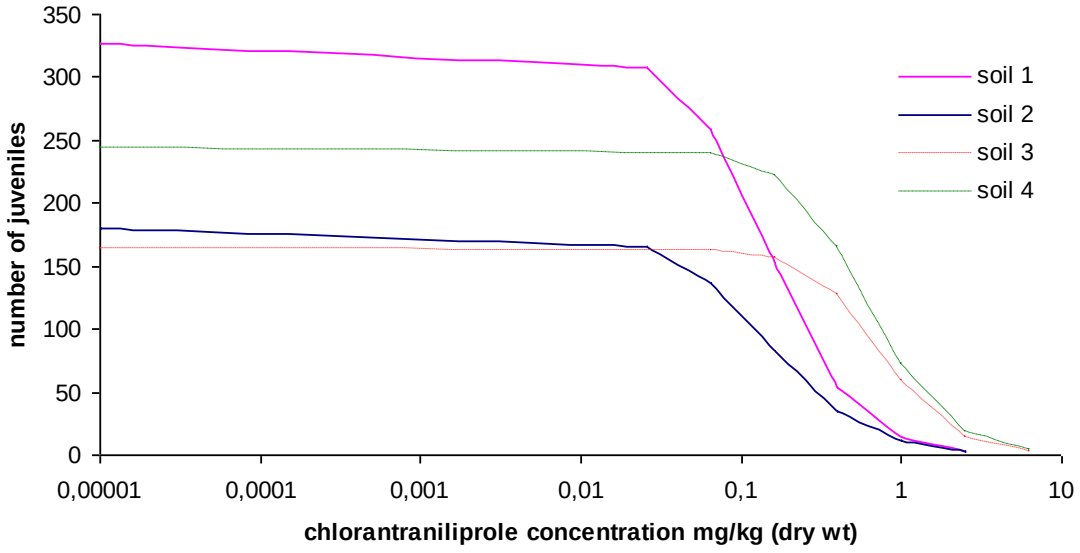

Figure 1. Reproduction of *Folsomia candida* after 28 days of exposure to different concentrations of chlorantraniliprole in the four test soils, presented as mean values deriving from five replicates. For abbreviations, see Table 2.

The effect of CAP on the sublethal endpoint of reproduction (EC_{50} and EC_{10}) decreased with increasing OM content (Table 4), and there was a significant difference in toxicity between the two low-organic soils (soil 1 and 2) than in the two high-organic soils (soil 3 and 4). However, soil 1 showed a lower toxicity than soil 2, and soil 3 a lower toxicity than soil 4, but their differences were not significant. The highest toxicity (EC_{50} 0.14 mg CAP/kg) was observed in the Lufa 2.2 soil (soil 2), with an OM content of 3.09 %, and the lowest in soil 3 (0.76 mg/kg), with an OM content of 10.6 %.

The difference between the lowest and the highest EC_{50} and EC_{10} values, when OM content was increased more than two times, was a factor of 5.3 and 8.4, respectively.

The soil with the highest pH (soil 3) showed the lowest toxicity, and the soil with the lowest pH (soil 4) showed the second lowest toxicity.

Table 4. Toxicity of chlorantraniliprole to *Folsomia candida* after 28 days of exposure to different concentrations of CAP in the four test soils.

Soil	OM (%)	pH	EC_{50} (mg/kg)	EC_{10} (mg/kg)
1	2.37	5.85	0.16 (0.085-0.209)	0.04 (0.002-0.074)
2	3.09	5.67	0.14 (0.088-0.199)	0.03 (0.004-0.056)
3	10.6	6.78	0.76 (0.433-1.092)	0.25 (0.003-0.501)
4	14.7	5.04	0.62 (0.347-0.884)	0.17 (0.002-0.346)

EC_{50} and EC_{10} values for reproduction presented as mg CAP/kg dry soil with 95 % confidence intervals in parenthesis. For abbreviations see Table 2.

Validity of the tests

The 95 % confidence intervals for EC_{50} and EC_{10} values are presented in Table 4. In case of EC_{10} values, the 95 % confidence intervals overlap for all values, meaning that the EC_{10} values doe not differ significantly between the different soils. For the EC_{50}s, situation is different. From the overlap of the confidence intervals, it may be concluded that EC_{50}s for the two low-organic soils are the same, while they do differ from those for the two high-organic soils, which in turn also are similar.

In one of the control replicates for soil 2, the number of juveniles was so much higher compared to the rest of the replicates it was considered an outlier and removed from the analysis. When the outlier was included in the analysis, EC_{50} for soil 2 was 0.10 mg CAP/kg, instead of 0.14 mg/kg when removed.

In one replicate of the control and one replicate of the concentration of 0.0256 µg/g for soil 1, and in two replicates of the solvent control and one replicate of 0.4 µg/g for soil 2, the number of juveniles at the end of the test were corrected according to the number of adults present in the test vessel, since either too many or too few adults had been added by mistake at the start of the test.

Influence of chlorantraniliprole on pH in the soils

The pH measured at the start and end of the tests for each soil showed a slight decrease during the test for soil 1, 2 and 4, but was quite steady between the different concentrations of CAP. The pH for soil 3, which was the soil with the highest pH, increased slightly during the test.

The pH shown in Table 2 and 4 was, together with the rest of the soil properties, derived from an earlier study of the same soils. The pH measured in this test showed equivalent values with the values presented in Table 2 and 4 and is presented below (Table 5) as mean values from start and end measurements.

Table 5. pH of the four different soils at the start and end of the 28-day toxicity tests with *Folsomia candida*.

Soil	pH start	pH end
1	5.97	5.81
2	5.71	5.39
3	6.74	6.98
4	5.15	4.68

All values presented are mean values of all the treatments for each soil, including 2 replicates per treatment. For abbreviations, see Table 2.

The soil put in the freezer for measuring start and end concentrations were never measured during the test period, since there was not enough time. They are kept at the University of Amsterdam (UvA) where they will be measured later on.

Toxicodynamic test

Effect on mobility and mortality

Mobility and mortality was observed and recorded into 6 different classes; [1] fine (not affected), [2] moving a bit slow, [3] moving slow, [4] moving very slow, [5] paralyzed, and [6] dead. For twelve of the days observed, these parameters were plotted into 10 graphs, one for each treatment (Figure 2-4).

The observations show that the control and solvent control stayed fine for 3 weeks, but at day 20 they started to die, probably due to excess water applied to the soil. Within a week the two controls went from 96 % survival (day 17) and no paralyzed, to 71 % survival and 10 % paralyzed (day 24) due to changes in the soil moistening regime from day 20 and forward. At day 27, survival was down to 60 % in the controls and the test was terminated.

An effect of the compound was not seen at the two lowest concentrations during the test. Effects were first seen at 1.0 µg CAP/g soil at day 6 and at 3.3 µg/g at day 2. At the concentration of 10 µg/g and higher an effect was seen already one day after the animals were introduced into the test vessels. The ability to move decreased radically over time at the concentration of 1.0 µg/g and higher.

Significant mortality was seen first at day 20, after changes in the soil moistening regime. Paralyzed animals were found already after one day at the two highest concentrations, but were not seen at the two lowest concentrations until day 24 and 22, respectively.
Mobility and mortality, divided into 6 different classes, are displayed below in Figure 2-4.

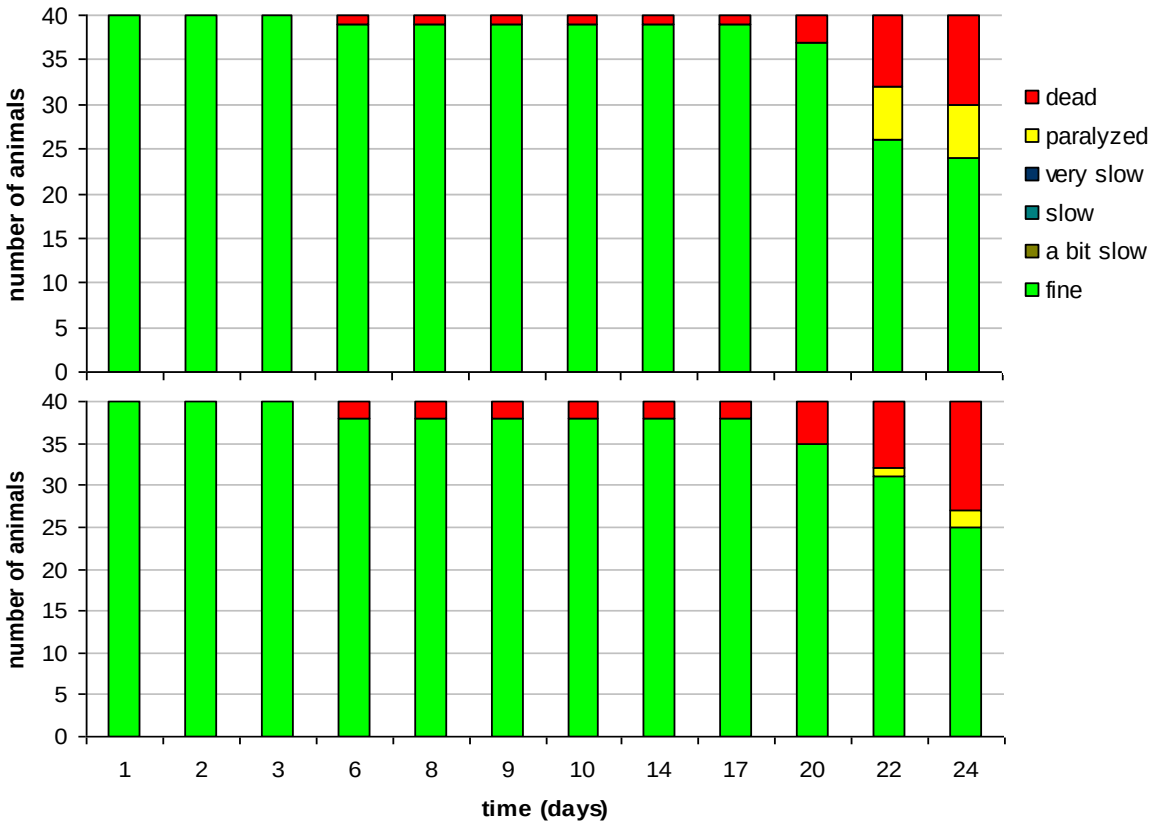

Figure 2. Development of mobility/mortality over time of control (top) and solvent control (bottom) for *Folsomia candida* kept on Lufa 2.2 soil in a toxicodynamic test.

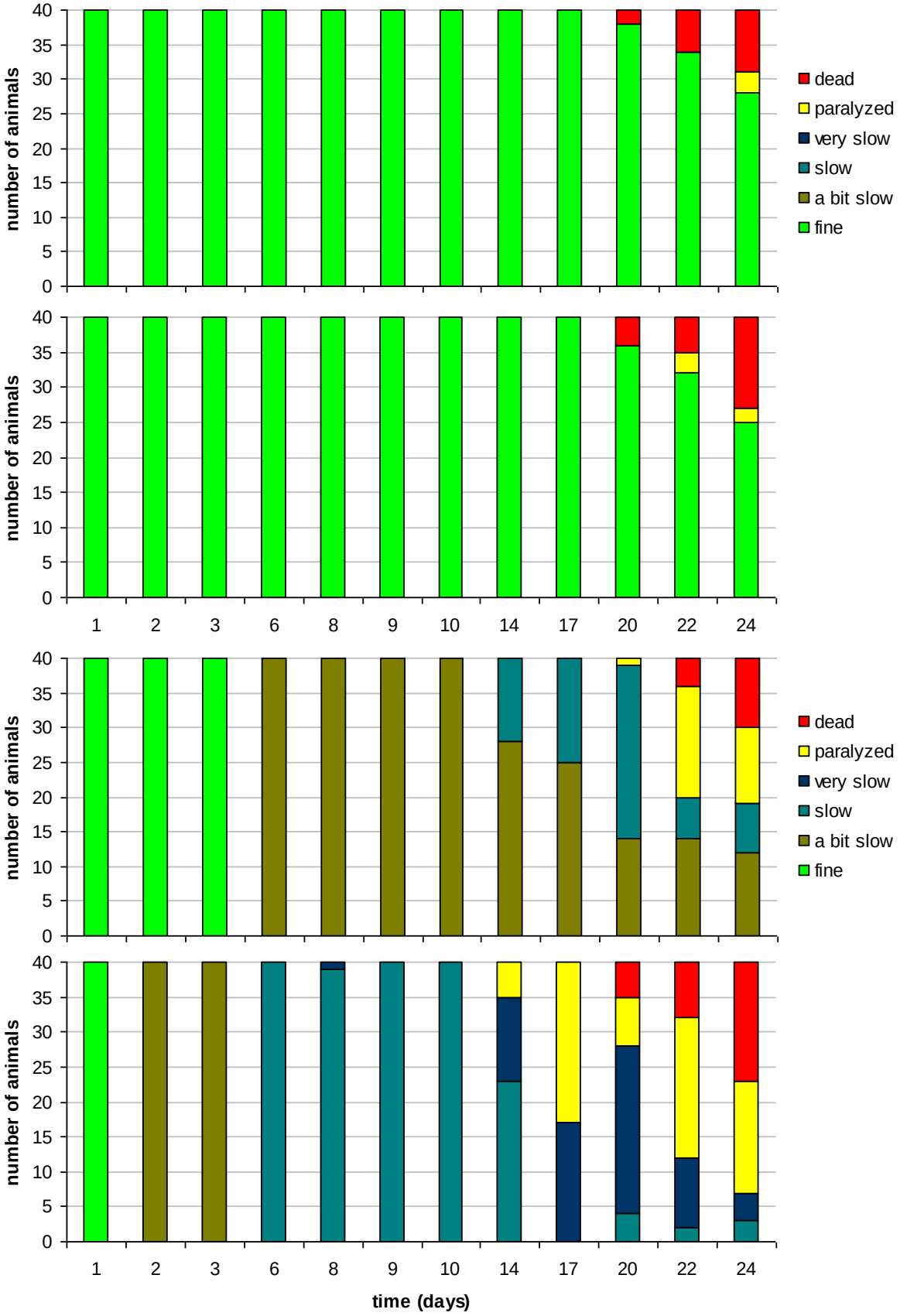

Figure 3. Development of mobility/mortality over time of 0.1 (top), 0.33 (top middle), 1.0 (lower middle) and 3.3 µg CAP/g soil (bottom) for *Folsomia candida* kept on Lufa 2.2 soil in a toxicodynamic test.

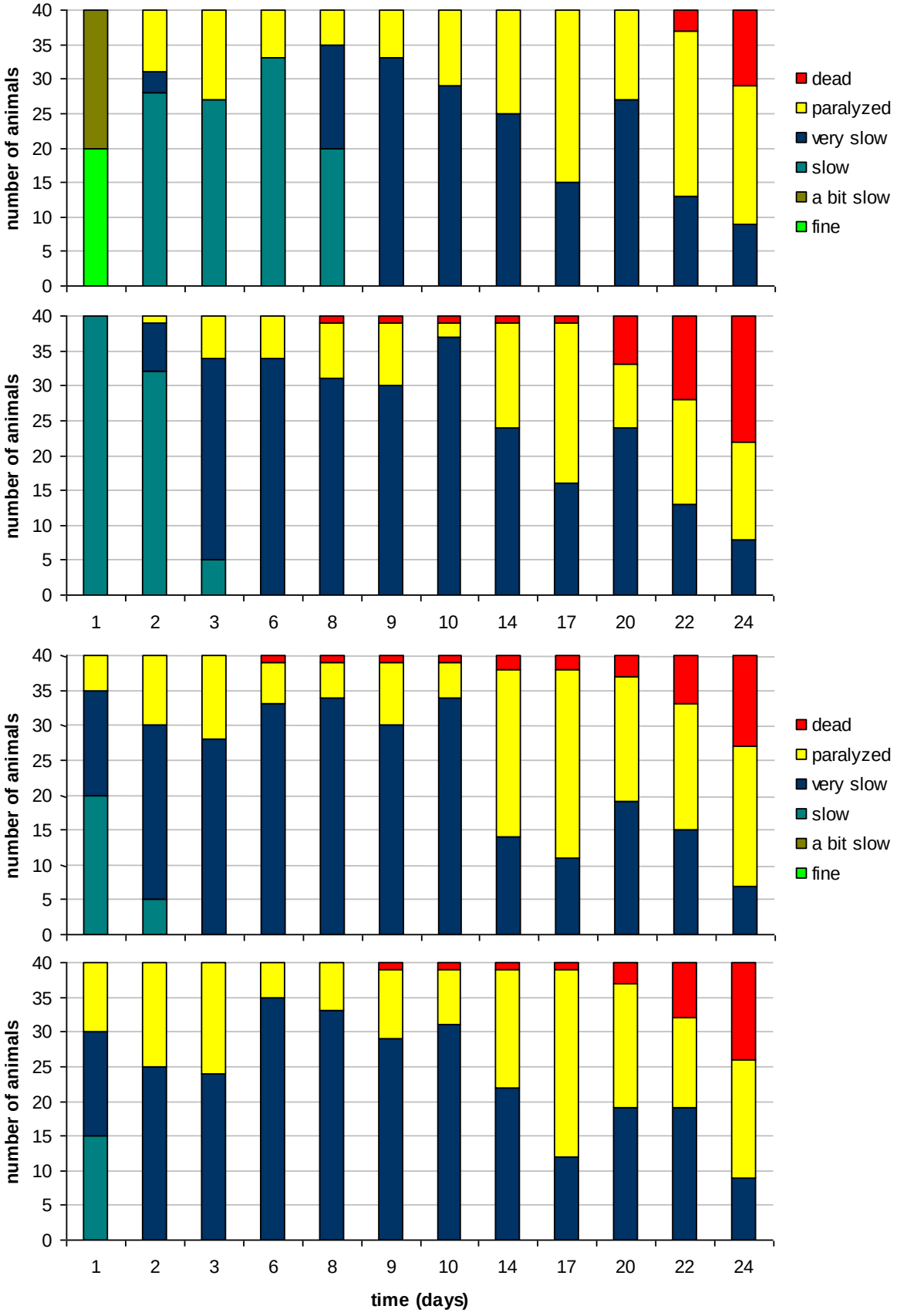

Figure 4. Development of mobility/mortality over time of 10 (top), 33 (top middle), 100 (lower middle) and 330 µg CAP/g soil (bottom) for *Folsomia candida* kept on Lufa 2.2 soil in a toxicodynamic test.

Effect on reproduction

Figure 5 shows when eggs were laid, the estimated number of juveniles hatched (in four different estimations), and mortality of the juveniles. Reproduction was observed and recorded into 7 classes; [1] no eggs, [2] eggs, [3] > 80 juveniles, [4] 40-80 juveniles, [5] 10-39 juveniles, [6] < 10 juveniles and [7] juveniles dead.

Eggs were laid at the four highest concentrations (10, 33, 100 and 330 µg CAP/g soil) already after one day, but not at the rest until day 2. At day 14, juveniles had hatched. At the concentration of 3.3 µg/g and higher, there was a slight decrease in the number of juveniles observed, compared to the lower concentrations. The number of juveniles observed at 3.3 µg/g increased from day 14 till day 15. At day 20, the first dead juveniles were observed at the 4 highest concentrations, and at the 3 highest concentrations, mortality was so high the number of juveniles decline significantly. From day 22, a few dead juveniles were found at the concentrations of 1.0 and 3.3 µg/g, and at day 27, all juveniles at the 5 highest concentrations were dead. In the two controls and the lowest concentration there was no decline in the number of juveniles recorded, not even on the last day of the test.

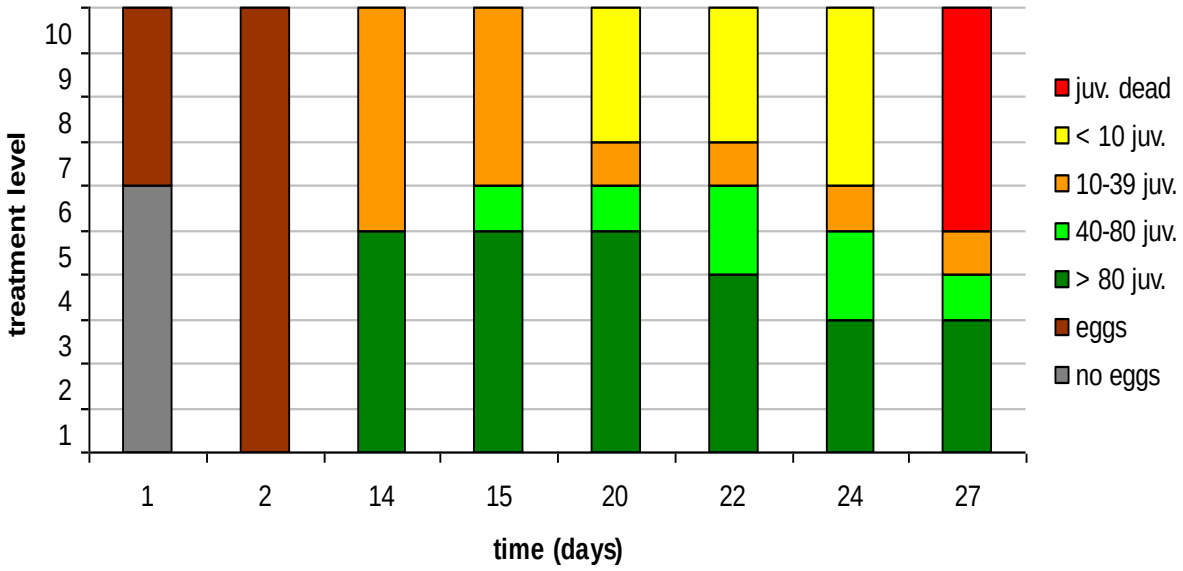

Figure 5. Reproduction of *Folsomia candida* upon exposure to chloranthraniliprole (CAP) in a toxicodynamic study on Lufa 2.2 soil. Reproduction is shown per treatment over time divided into 7 classes shown in the legend. The different treatments are presented on the y-axis as numbers ranging from 1 to 10; 1 being the control, 2 the solvent control, 3 is 0.1 µg/g, 4 is 0.33 µg/g, 5 is 1.0 µg/g, 6 is 3.3 µg/g, 7 is 10 µg/g, 8 is 33 µg/g, 9 is 100 µg/g, and 10 is 330 µg/g soil.

Effect on mobility of the juveniles

The mobility of the juveniles was also observed, but not put into a graph. It showed that the juveniles at the three highest concentrations (33, 100 and 330 µg/g) were moving slow already from the first day they were observed (day 14). At day 15, the juveniles at the highest concentration were moving very slow and some were paralyzed. At day 17, the juveniles at 10 µg/g were also moving slow, which means the juveniles at the four highest concentrations were now affected in their mobility. At day 24, the juveniles at the concentrations of 1.0 and 3.3 µg/g were moving a bit slow, at the higher concentrations they showed the same mobility as on day 17.

Condition of the antennas

The last parameter observed was the condition of the antennas on the adult animals, and any form of affected antennas (such as weak or broken antennas, or antennas stuck together or to the head of the animal) was recorded. The number of animals with affected antennas, regardless of the type of disorder, was plotted in a graph over time (Figure 6).

Affected antennas was not observed in the two controls or the two lowest treatments of CAP until around day 22, and the effect is considered to be due to the excess water applied to the soil. At the higher concentrations (the 6 highest), animals with affected antennas were observed from day 9, and were forward on fluctuating over the different treatments over time. But an effect of the compound still increased over time of the total number of animals affected. At day 22, the highest number of affected animals was recorded. Two days later (day 24) the number was decreasing, but also a lot of animals were dead by then.

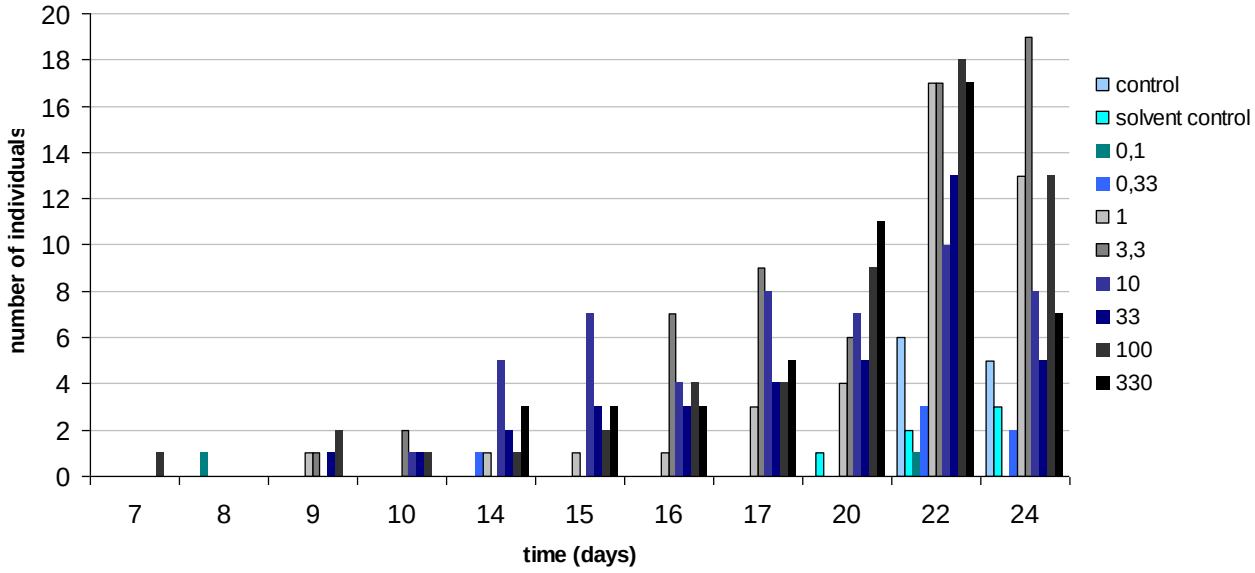

Figure 6. Number of adults of *Folsomia candida* with affected antennas per treatment over time upon exposure to chloranthraniliprole (CAP) in a toxicodynamic test on Lufa 2.2 soil.

DISCUSSION

Earlier studies have shown that chlorantraniliprole has a low impact on non-target organisms, such as pollinators, beneficial insects, predatory mites, earthworms and many other soil organisms. Experiments have been performed on foraging honey bees, showing no negative effect, which is an important feature not shared with many other pesticides (*5, 6*). However, CAP has also shown to be highly toxic to some soil and water organisms, e.g. the collembolan *Folsomia candida* and the cladoceran *Daphnia magna*. This study determined the toxicity to *F. candida*, and showed that it both affects behaviour and reproduction. Effects of CAP on the reproduction of *F. candida* have been determined in other studies, giving the endpoint values EC_{50}: 0.48 mg/kg d.w. soil and NOEC: 0.39 mg/kg d.w. soil (*1, 4*).

Fate of chlorantraniliprole in the environment

Distribution, availability and biodegradation

One important feature for a pesticide to be environmentally safe is how it distributes in the natural environment. The octanol-water partition coefficient (K_{ow}) defines the hydrophobicity (water solubility) of a compound and therefore the distribution and bioconcentration of the compound in the environment (*16, 18*). Since CAP is lipophilic (has a high K_{ow}) and binds to organic matter and other fatty tissues, it should not be distributed that easily. As mentioned earlier, it has a moderate potential for bioconcentration, and the bioconcentration factor (BCF) in fish is 13-15 (*4*). Hence, it should have a low impact on the surrounding environment of the application site. Hartnik (2008) suggests that when lipophilic chemicals bind to OM they become less available for uptake by organisms and can not cause any harm, neither can they be degraded. But are they still toxic for beneficial soil organisms?

Since organic matter is never the sole property to interact with chemicals upon introduction to different environments, the conditions have to be perfect, and a compound has to entirely bind to the organic matter to become absolutely non-toxic to organisms. Furthermore, different organisms differ in sensitivity and fractions of the compound will always be available to organisms. So if the compound is toxic to non-target organisms it will always show an effect. But depending on the amount available for uptake by the organisms, it will show different levels of effect. Therefore, it is important to develop pesticides that have an as low adverse impact on beneficial organisms and the environment as possible. Chlorantraniliprole, with its low impact on non-target organisms, is hopefully one step in the right direction.

Persistence in soil and sediment

But what about persistency? If the compound binds strongly to organic matter, how long will it stay in the soil? The potential of CAP to bind to the organic matter in soil might render a problem when applied to the same site for a longer time period. In terrestrial and aquatic environments, CAP may be qualified as persistent, and mineralization goes slowly (*4, 8*). Degradation is abiotic and slow (DT_{50soil}: 233-886 days (aerobic) and 208 days (anaerobic) at 25°C), and the rate depends on temperature (*4*). Higher temperature generates faster degradation. Photolysis contributes to the overall degradation in both soil and water (DT_{50soil}: 43 days, compared to dark control = 416 days, and $DT_{50water}$: 125-231 days) (*4, 8*).

Degradation through field dissipation is moderate to low (DT50: 82-611 days). Half-life for degradation in soil is estimated to be >100 days and sometimes >1000 days, and is sometimes limited by aging (*8*). Sorption in soil is medium to high (Koc: 244-464) and time dependent. Higher sorption gives less bioavailability of compounds in the pore water, and therefore less toxicity (*22, 23*). CAP is most mobile in freshly spiked soil (*4*), but upon aging mobility decreases and it becomes gradually more difficult to extract the compound from soil (*4, 8*). This also protects it from degradation (*8*). Degradation of metabolites is also slow and some are found persistent but with low potential for bioconcentration (*4*). Metabolites are normally of lower toxic potency than the parent compound (*1*).

Hydrolysis of CAP in water is pH dependent and it is stable at pH 4 and 7 (*4, 8*) but hydrolysed at pH 9 (half-life < 10 days) (*8*). So only if pH is high, degradation of CAP residues through hydrolysis in water will be significant. Volatilisation from soil is low, so in essence, pollution will only be in soil and water. This indicates that the major dissipation routes of CAP are through photodegradation, leaching and runoff, and hydrolysis in alkaline water. Therefore, organisms adjacent to application sites may be exposed via drift and runoff, e.g. to drainage channels (*1*).

Accumulation and residues

Because of its persistency, CAP is expected to accumulate in soil and sediment when being used year after year, and since it is lipophilic, it also poses a problem of being translocated in plants. Residues of CAP has been found in leafy and root vegetables and in cereal grain in confined and field rotational crop studies. Residues and metabolites have also been found in plants used for cattle feed (*8*). This means significant residues of CAP might become present in crops on fields where CAP is continuously applied.

The European Food Safety Authority (EFSA) has concluded that the permitted use of chlorantraniliprole on crops will not exceed the authorized toxicological reference values for consumption, and therefore it will not constitute any risks for the public health (*2*). But this does not mean the natural environment and other organisms are out of risk.

Accumulation in higher levels of food chains is not expected since the compound has a low bioconcentration in fish (<21) (*1*). But at lower levels, for example in soil where collembolans eat fungi containing CAP, and predators eat collembolans, the insecticide may accumulate in terrestrial ecosystems, which eventually might increase the persistence of chemicals in the ecosystem (*12*).

To know the fate of chemicals in the environment is very important when evaluating the toxicity of compounds to organisms. It is also important when determining safe amounts and when setting up standards for use. Because of the slow degradation of chlorantraniliprole in soil, toxicity testing on beneficial soil organisms like *F. candida* is also important from an ecological point of view.

Collembola reproduction test

Control performance

In this test, the controls showed a good performance. Only soil 4 had a slightly lower adult survival, but no difference was seen in reproduction where soil 4 had the second highest number of juveniles of all soils. If the lower survival was because of a weaker batch of animals or physical properties of the soil is hard to tell. The Lufa 2.2 control run along the test with soil 4 also showed a slightly lower adult survival, which might indicate weaker animals. But just like soil 4, the Lufa 2.2 control did not show a lower reproduction. Adult mortality in the Lufa 2.2 control was slightly lower than in soil 4, which indicates that other factors might have been involved.

Soil 4 had the highest OM content, and since *F. candida* prefers soils with a high amount of organic matter (*13-15*), the OM content might have influenced the result on reproduction in soil 4. Soil 4 also had the lowest pH (5.04), but the low pH did not seem to have any significant impact on the reproduction, but might have influenced survival.

According to Fountain and Hopkin (2005) *F. candida* seems to have a slight preference for a soil with pH 5.6, and such conditions appear to generate the highest level of reproduction. In this test, soil 2 was the closest to that value (pH 5.67), but had the second lowest reproduction. Nonetheless, the highest reproduction was found in soil 1 with a pH of 5.85, which was the second closest to the preferred value. But instead, soil 1 had the lowest OM content. Also for soil 1, adult survival and reproduction of the pooled controls compared to the Lufa 2.2 control were very similar, which indicates the results were likely to be due to the condition of the animals than physical properties of the soil.

In the end, whether it was quality of the test animals, OM content, microbial factors in the soils, pH or another parameter that influenced the control results in this test is therefore hard to tell. But differences were rather small and animal performances were generally good. So, it

is not very likely that the small variations in control performance will have had any influence on the outcome of the toxicity test with this soil.

Influence of chlorantraniliprole on pH in the soils

The concentration of CAP did not seem to have any impact on the pH of the soil since the pH values from all the measurements did not show any significant difference over the concentration range. The only difference was from the start and end of each test, but a decrease in pH is normal when the soil is repeatedly moistened over time.

Influence of organic matter on toxicity

This test was done to detect an effect of soil organic matter (OM) as an important factor when evaluating the toxicity of different compounds. As predicted, the result showed that increasing OM content decreased the toxicity of chlorantraniliprole to the test species *F. candida*.

An effect of OM content on toxicity has also been shown in other studies. With increasing OM content, Martikainen and Krogh (1999) showed a decrease in toxicity of the insecticide dimethoate to the collembolan *Folsomia fimetaria*. Son *et al.* (2007) showed a decrease in toxicity of cadmium to the collembolan *Paronychiurus kimi* (Lee), and Martikainen (1996) showed a decrease in toxicity of dimethoate with increasing OM content for the earthworm *Aporrectodea caliginosa tuberculata*, the collembolan *Folsomia candida* and the enchytraeid worm *Enchytraeus crypticus*.

In this test, the four soils showed a clear difference in toxicity between the two low-organic soils and the two high-organic soils (see Figure 1). Table 4 shows a slightly higher toxicity for soil 2 than for soil 1, but the EC_{10} and EC_{50} values were practically the same. For soil 3 and 4 the result was also in a reverse order than expected, but again EC_{10} and EC_{50} values did not differ. Yet, this indicates that other factors than the OM content were influencing toxicity of chlorantraniliprole. For example, higher pH also tends to give less toxic soils (*13, 23*). With higher pH, sorption strength and interactions with hydrophobic chemicals decreases (*18*).

The test results show that pH might have influenced the EC_x values for soil 4, but does not seem to have any major impact on the toxicity of CAP in the soil. However, as mentioned before, it might influence the total number of offspring, but that is irrelevant when evaluating the EC_x values.

Influence of other parameters on toxicity

Cation exchange capacity (CEC) and dissolved organic carbon (DOC) can also influence the toxicity of chemicals. But CEC generally only affects the toxicity of ionic compounds. The dissociation constant (pK_a) is the equilibrium constant of the dissociation reaction of an acid (acid-base reactions), and it measures how strong an acid is in a solution. Since CAP has a very high pK_a (around 11), such an effect of CEC was not to be expected as no soil had a pH higher than 7. DOC might affect availability of chemicals in the pore water. The only soil with a very high DOC content in the pore water was soil 4 (DOC: 3605 mg/l). But compared to the total amount of OM in the soil the amount of DOC in the pore water is so small that its influence will be insignificant.

On the other hand, OM content is supposed to influence the total number of offspring, but the results do not show any clear indication of that. However, when looking at the EC_x values, it clearly affects the toxicity of CAP in the soil. This should mean that the influence of soil organic matter is stronger than the influence of pH or any other parameter shown in this test.

Validity of the test
The non-overlapping 95 % confidence intervals around the EC_{50} values demonstrate a significant difference in toxicity between the low-organic soils and the high-organic. The confidence intervals for the EC_{10}s do overlap, but still they indicate a clear difference in toxicity and therefore are worth displaying. Because of the flat slope of the dose-response curve, EC_{10}s are prone to larger variation than the EC_{50}s.

However, the exact concentration calculations should not be done until the soil samples with the initial and end concentrations are measured, to make sure the given concentration range is identical with the one tested.

Toxicodynamic test

Effect on mobility and mortality
The fast mode of action of chlorantraniliprole provides a high feeding cessation efficiency on pest larvae (mainly Lepidoptera) (*3, 5, 11*), and systematically reduces the damage of feeding (*3*). Cessation normally occurs within minutes to a few hours after consumption (*3, 5, 11*), and death 1-3 days after exposure (*11*).

Just as expected, the compound showed a fast effect on the animals in the toxicodynamic test. An effect was seen at the highest exposure concentrations already after 1 day, with paralyzed animals at the two highest concentrations, but after almost 3 weeks they still did not show any significant mortality. This means the compound had a high speed of action on muscle contraction, which induced a fast decrease in mobility, making the animals slow or paralyzed, but the test concentrations were not high enough to cause a fast rate of mortality. This indicates that the compound is not neurotoxic. However, the animals showed signs of weakness at this point and were expected to die within 2 weeks.

Influence of excess water on the flattened soil
The collembolans proved to be very sensitive to too much water on the flattened soil; therefore the soil was only moistened to 40 % of its WHC. In earlier attempts the soil was moistened to 50 % of its WHC but the tests failed within two-three weeks, when the animals seemed to be drowning even in the controls. But even with less moist they started to die in the controls when there was an accidental slight change in the soil moistening regime.

Since there was no significant mortality seen until day 20, after the changes in the soil moistening regime, their death will not be considered to be due to the test compound. However, the number of animals which showed a decrease in their movement pattern increased radically over time, and with increasing concentration.

In general, the effect shown at day 22 and 24 should be considered with some caution, since all the test animals experienced the same change in the moistening regime. Nevertheless, that does not exclude the possibility that the animals exposed to the compound at concentrations from 1.0 µg/g and higher, where an obvious effect is shown already at the beginning of the test, were affected by the compound and therefore more sensitive to changes in the environment than the animals at the lower concentrations and the controls.

Effect on reproduction
Interesting is that at the four highest treatments the animals had laid eggs already after one day, while the lower treatments did not show any eggs until day 2. Since affected mobility was also shown only at the four highest treatments after one day, it indicates some kind of reproduction stress in the animals induced by the test compound.

At day 14 juveniles had hatched, so no significant delay in hatching was observed. The increase in number of juveniles at the concentration of 3.3 µg/g from days 14 to 15 is fully normal since not all eggs are laid on the same day. But it may also indicate a slight delay in hatching at the higher concentrations. It was not examined if all the eggs laid also hatched, and the number of eggs laid in each treatment was not counted, so it remains unclear if the animals at the higher concentrations experienced a decrease in reproduction due to the production of fewer eggs or because fewer eggs hatched.

Juvenile mortality was, as assumed for the adult animals, probably also affected by the change in moistening regime. A significant difference was shown between the higher treatments and the lower; therefore, the juveniles seemed to be more resistant to changes in the moistening regime.

Evaluating the condition of the antennas

The number of animals with affected antennas should be viewed with some caution. Just before moulting, the antennas seem to show more weakness and to be more easily stuck together for a couple of days. But that does not mean all the animals in this test only show an effect because they are about to moult. When the animals are exposed to stress, like the insecticide for example, the antennas tend to be adversely affected. And the distinct increase of impact on the antennas over time in this experiment shows a clear effect of the insecticide.

Toxicity to non-target organisms and environmental safety

Toxicity and selectivity of chlorantraniliprole

The toxicity and toxicodynamic tests performed in this study show a clear toxicity of chlorantraniliprole to *F. candida*, which has in other studies shown to be very safe for other organisms, like mammals, birds and fish. Experiments on rats and rabbits demonstrate that chlorantraniliprole has a low acute dermal, oral and inhalation toxicity to mammals (LD_{50}: >5000 mg/kg bw/d) (*4, 8, 10, 11*), and the insecticide shows minimal toxicity to mammals even after long-term exposure (*1, 4, 8*). The only notable effect seen in mammalian studies (rats) is an increased grade of microvesiculation (formation of small vesicles) of the adrenal cortex after long-term dermal exposure or dietary intake (*1, 4*). So how come it is so safe for mammals?

Mammals and other vertebrates possess three isoforms of the ryanodine receptor (RyR) that regulates the release of intracellular calcium stores critical for muscle contraction. Insects have only one RyR. And it is the low binding of all the anthranilic diamides to vetebrate RyRs that determines the extremely low toxicity to mammals and other vertebrates (*5-7, 9, 10*). At concentrations below 1 µM, chlorantraniliprole is inactive against mammalian cell lines of various RyR isoforms (*11*). The most potent anthranilic diamide compound has more than 500 times higher selectivity to insect receptors than to mammalian (*7, 9*), chlorantraniliprole is ~350-fold more selective (*8, 11*).

Environmental impact and insect resistance

The selectivity between non-target organisms and pests is important when evaluating the environmental safety of a pesticide (*16*). The low impact of chlorantraniliprole on non-target organisms makes it an ideal tool in integrated pest management (IPM) programs (*5, 6, 9*). It also has a low impact on the environment, and a long lasting crop protection (*3, 5*). The low toxicity towards non-target organisms together with low use-rates (*5, 9, 11*) makes it a safer pesticide for agricultural workers and for consumers than other pesticides (*5, 9*). This means

that CAP has attractive toxicological and ecotoxicological attributes, which makes it a very interesting and suitable pesticide on the market.

The importance of Collembola in the environment makes the finding of a target-specific pesticide highly desirable. Collembola can also affect the growth of agricultural crops and should therefore be of economical importance to farmers.

The ability of insects to develop resistance against pesticides poses a problem for pest management, thus, the importance to create new pesticides with alternative modes of action is an inevitable necessity. Chlorantraniliprole, with its biochemical target for the ryanodine receptor and regulation of calcium channels represents a new mode of action, and is therefore an important and highly needed discovery (*10*). Because of its recent introduction to the market and lack of cross-resistance with other insecticides, no resistance in the field is developed against it yet (*4, 5*).

Cautions with laboratory testing

Finally, some care must be taken when extrapolating the results from laboratory testing to field conditions because of the artificial conditions of the test procedures. The animals are restrained within the test vessels, temperature and humidity are constant and there are no predators. In natural conditions, the animals can move away from a less pleasant site to a more favourable one, if necessary, and normally an organism is not only exposed to one chemical, but to mixtures of several chemicals (*27*). Further problems with laboratory testing is that species of Collembola differ in sensitivity from each other, and the testing results from one species do not automatically give a general view of all species (*12*). Nonetheless, laboratory testing is still better than no testing at all, but as far as it is possible, applied field testing should be preferred.

Another question is if low genetic variances in parthenogenetic species make them more sensitive to pesticides than sexually reproducing species (*12*). If so, toxicity tests with *F. candida* for example should be even more carefully extrapolated upon other species of Collembola. However, low genetic variability provides individuals of the same species with less difference in sensitivity between them. Therefore, they will make for better test species since they will react more similar when exposed to the same chemical (*13*).

CONCLUSIONS

The test species, *Folsomia candida* Willem, was significantly adversely affected by the insecticide chlorantraniliprole (CAP). *F. candida* is a non-target organism with some ecologically important attributes. Hence, they may be beneficial in agricultural soils, which make this kind of research important from both economical and ecological aspects. It is also important to determine toxicity of new compounds and to evaluate their fate in the environment.

The reproduction tests in the four soils of different organic matter content showed a difference in toxicity of CAP between the high-organic and the low-organic soils, and EC_{50} values were significantly higher in the high-organic soils. EC_{10} values also showed a clear decrease in toxicity for the high-organic soils. When OM content was increased more than two times, the difference between the lowest and the highest EC_{50} and EC_{10} values was a factor of 5.3 and 8.4, respectively.

pH of the different soils did not seem to significantly affect the toxicity of CAP, but might have influenced the total number of juveniles produced. Organic matter content did not seem to affect the total number of juveniles.

The toxicodynamic test demonstrated a fast mode of action of chlorantraniliprole to *F. candida*, with decreased mobility of the animals in the four highest treatments already after one day, and paralyzed animals in the two highest. However, it did not show any fast mortality, and after almost three weeks still no significant deaths were recorded.

The animals in the four highest treatments showed a possible compound induced stress to lay eggs earlier than the animals in the lower treatments. The number of juveniles produced was also affected, with a decline in total amount of juveniles in the five highest treatments.

An effect on the antennas was also recorded during the test, and the number of animals with affected antennas increased steadily over time.

In general, the two tests showed expected results. An interesting addition to the toxicodynamic test would have been to do more surveillance on the time of egg laying and hatching, and to see if any of the animals stopped to lay eggs at some point. Another interesting parameter would have been to see if, and in that case how fast, the exposed animals had recovered if they had been transferred to fresh uncontaminated soil.

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