Biol Fertil Soils (2005) 41: 135–143
DOI 10.1007/s00374-004-0829-0

ORIGINAL PAPER

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Lethal and sublethal effects of imidacloprid on two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*)

Received: 1 June 2004 / Revised: 6 December 2004 / Accepted: 9 December 2004 / Published online: 22 January 2005

Abstract The chemical imidacloprid is the major component of many widely used insecticides and is relatively persistent in soils. A set of experiments was carried out to estimate the lethal (mortality) and sublethal (weight loss) effects of one of these insecticides, Confidor, on two earthworm species commonly found in agricultural soils. A preliminary experiment in the absence of earthworms showed that imidacloprid was not rapidly degraded, with a decrease of less than 10% after 2 weeks, and that it was distributed in a reasonably homogeneous manner throughout the soil (less than 10% of variation between samples). The LC_{50} of imidacloprid for the anecic species *Aporrectodea nocturna* and the endogeic species *Allolobophora icterica* was between 2 and 4 mg kg^{-1} dry soil. This result is consistent with previous findings obtained with other earthworm species and natural soils. When sublethal effects were examined, significant decreases in weight were observed at concentrations of 0.5 and 1 mg kg^{-1} dry soil for the two earthworm species whereas no effect was observed at a concentration of 0.1 mg kg^{-1} dry soil (NOEC value). These concentrations are close to 0.33 mg kg^{-1} which is the Predictive Environmental Concentration. Weight loss appears to be a valuable endpoint that can be used with worms freshly collected in the field as long as variability in the response of a control is taken into account.

Keywords Spiking protocol · Weight loss · Sublethal endpoint · Imidacloprid

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Introduction

All pesticides found on the market have been evaluated by sets of standardized protocols (a so-called a priori evaluation). One of the aims of these standardized tests is to evaluate the negative effects of pesticides on terrestrial and aquatic ecosystems. Since the direct impact on ecosystems is difficult to study, these tests are based on detrimental effects seen for a set of model organisms, which play key roles in ecosystem structure and function. However, although the EEC encouraged development of tests determining sublethal effects on model organisms, most of these protocols focussed on mortality. The EEC recognized the importance of sublethal tests for earthworms in particular when the active substance is potentially persistent in soils or multi-applicated (EEC 2003).

Imidacloprid is a relatively new systemic insecticide and was the first member of a new family called neonicotinoids. It acts as an agonist of acetylcholine (Bai et al. 1991) and is therefore effective on many insects currently resistant to carbamates, organophosphates and pyrethroids. It is widely used in agriculture for controlling sucking insects, as a seed dressing, for soil treatment and as a foliar treatment in a variety of crops and orchards. It is also used for controlling cockroaches and termites and is found in many domestic products used for pets and in the garden (Cox 2001).

To date, studies on imidacloprid have focussed on its metabolism and behaviour in soils (Rouchaud et al. 1996; Cox et al. 1997; Oi 1999; Oliveira et al. 2000; Capri et al. 2001; Sarkar et al. 2001; Ambrust and Peeler 2002; Gupta et al. 2002; Nemeth-Konda et al. 2002). It can be concluded from these studies that the behaviour of this insecticide in soil varies depending on the formulation used, the soil type, the time of ageing of soil as well as its organic matter content. For example, the half-life of imidacloprid was found to range from 28.7 to 47.8 days in three different soil types (Sarkar et al. 2001).

In contrast, few studies have examined the effects of imidacloprid on soil organisms. Tu (1995) found no effect on microbial enzyme activity in soil treated with this

insecticide although short-lived inhibitory effects were observed. Idinger (2002) found a limited effect of imidacloprid on the reproduction rate of *Folsomia candida*. Luo et al. (1999) and Zang et al. (2000) working on *Eisenia fetida* showed that after 14 days the LC_{50} for imidacloprid was 2.3 mg kg^{-1} dry soil (or ppm). These authors also found sperm deformities in *E. fetida* at concentrations as low as 0.5 mg kg^{-1} dry soil whereas the predictive environmental concentration (PEC) for imidacloprid is between 0.33 and 0.66 mg kg^{-1} dry soil (Oi 1999; Mostert et al. 2000). More recently, Mostert et al. (2000, 2002) showed that the LC_{50} for worms of the *Pheretima* group was 3 mg kg^{-1} dry soil after 7 days and no effect was observed on earthworm weight at 0.66 mg kg^{-1} dry soil. All these studies were carried out under laboratory conditions but Lal et al. (2001) observed a decrease in the production of earthworm casts over 120 days in field conditions (treated seeds).

Earthworms, as "ecosystem engineers" (Jones et al. 1994), play a major role in many soil ecosystems (Lavelle 1997). In brief, their activities (creation of burrows and burial of organic matter) influence biological (other parts of the soil ecosystem from micro-organisms to roots), chemical (biogeochemical cycles) and physical (transfer properties) functions in soils. Emphasis has to be placed on the fact that the beneficial role of earthworms is highly dependent on their activities. Therefore it is crucial that the sublethal effects of insecticides that could reduce earthworm activity are also studied. Due to their key role in soil ecosystems, earthworms have been classed as a model organism in biomonitoring processes by the OECD (1984) and EEC (1984). For reasons of simplicity of use and supply, *Eisenia fetida* and *Eisenia andrei* are the classical earthworm species used in standardized tests (Ribera and Saint Denis 1999). The biological relevance of these species is still, however, open to debate (Bouché 1992) since they are often less susceptible to pollutants than other species (Edwards and Coulson 1992; Spurgeon and Weeks 1998) and rarely found in agricultural soils.

In a previous experiment (Capowiez et al. 2003), we investigated the effects of imidacloprid on several biochemical markers (specific activity of acetylcholinesterase and glutathione-*S*-transferase) and on the burrowing behaviour of two earthworm species that belong to two different "ecological types" (sensu Bouché1977): *Apporectodea nocturna*, an anecic species and *Allolobophora icterica*, an endogeic species. These two earthworm species are currently found in many European soils, especially in orchards (Paoletti 1999). The behaviour of earthworms was significantly altered (decrease in burrow length) for concentrations of imidacloprid between 0.5 and 1 mg kg^{-1} dry soil. During this experiment, we also observed that earthworms lost more weight in the presence of imidacloprid but this trend was not found to be statistically significant. In the present work, we designed specific experiments to analyse whether imidacloprid could indeed cause earthworm weight loss. As natural soil was used and spiked manually for these experiments, particular attention is given to the dynamics of the pollutant in the soil (without the presence of worms) and to the quality of our protocol used to spike the soil.

Material and methods

Soil and earthworms

Soil (23.4% clay, 57% silt, 19.6% sand, 28.3 g kg^{-1} organic matter, pH 8.3) and earthworms (*Aporrectodea nocturna* and *Allolobophora icterica*) were collected on different dates (for each replicate) from an apple orchard abandoned for at least 5 years in Montfavet near Avignon, France. These two earthworm species were chosen because they were present in great numbers in the abandoned orchard, they are common in agricultural soils (Paoletti 1999) and represent different ecological types (anecic for *A. nocturna* and endogeic for *A. icterica*). Worms were collected manually on the day of use and kept in a cold dark chamber for no longer than 12 h. Since a large number of worms was necessary for the study, adults (presence of clitellum) as well as subadults (absence of clitellum but adult weight) were collected. As a consequence, the weight of individuals was quite variable: 2.462±0.657 g (mean ± standard deviation) for *A. nocturna* and 0.645±0.165 g for *A. icterica*. Thus, when earthworms were subjected to the treatment, care was taken to ensure that the total weight of earthworms in each treatment was similar using a block procedure (McIndoe et al. 1998).

Imidacloprid and soil spiking

Imidacloprid was supplied by Bayer as Confidor solution (200 g l^{-1} in 100% DMSO) without additive. Soil was polluted by manually spraying a solution of 40 ml on 1 kg soil at 20% gravimetric water content (i.e. 800 g dry soil). To increase the homogeneity of the pollution, (1) each kg of soil was placed as a fine layer (of about 40×50 cm in area) and (2) the pollution (spraying) was done three times with the soil being thoroughly mixed in between and then repacked as a fine layer. To obtain the desired concentrations dilutions of imidacloprid were made first in DMSO (1/10) then in distilled water. To obtain a final concentration of 1 mg imidacloprid per kg dry soil, we used a 40 ml solution that contained 0.05% DMSO. Therefore a second control was prepared without pesticide but with this concentration of DMSO. The 1.04 kg wet soil was then split between ten petri dishes (diameter 10 cm, height 3 cm) that each contained 100 g soil. A single earthworm was placed in each petri dish to limit interspecific interactions and to prevent cascade deaths (Sheppard and Evende 1992). Dishes were then placed in a dark cold chamber (12±1°C) for 14 days, the duration of the experiment.

To ensure the homogeneity of the pollution and to study the dynamics of imidacloprid in our soil, an experiment was carried out without any earthworms using 1 kg soil (noted A) that was manually polluted so that the nominal

concentration of imidacloprid was 1 mg kg^{-1} dry soil. One hundred grams of soil was placed in each of ten petri dishes (A1 to A10) and placed in the same dark chamber. In order to measure the actual concentration and to evaluate the homogeneity of the pollution, subsamples of 10 g soil were collected the day of the pollution and 2, 4, 7 and 14 days after the imidacloprid treatment from dishes A1, A5 and A10 and immediately put in a cold chamber (2°C). We observed in a preliminary study that samples could be conserved at 2°C without decreasing recovery values. To estimate the actual concentration of imidacloprid, the sample of 10 g wet soil was put into 50 ml acetonitrile. The mixture was homogenized for 1 min and filtered under vacuum through a Büchner funnel using a Whatman GF/A glass fibre filter. The residue of filtration was mixed with a further 50 ml acetonitrile, homogenized again and filtered in the same way. The acetonitrile was evaporated from the filtrate using a rotary vacuum evaporator. Dichloromethane (50 ml) was added to the dry residue and put into a separating funnel. One hundred millilitres of deionized water was added after washing the evaporation flask with it, then 25 ml saturated aqueous sodium sulfate solution was added before liquid-liquid partitioning. The partitioning was repeated twice with two further parts of dichloromethane. All the organic phases were combined, dried over sodium sulphate and evaporated to dryness with a rotary vacuum evaporator. The residue was dissolved into 10 ml HPLC mobile phase. This solution was filtered through a 0.45-µm PTFE filter and diluted ten times with HPLC mobile phase prior to injection. Imidacloprid was analysed by HPLC using a Hewlett Packard system (Model 1050) with a UV/vis detector operating at 270 nm. Acquisition of chromatographic data was performed with HP Chemstation software. The mobile phase was acetonitrile/water (30:70 by volume) at a flow rate of 0.6 ml/min. The column used was a C18 Interspher ODS2 (Interchim; length 25 cm, diameter 4.0 cm, particle size 5.0 µm). Imidacloprid was eluted after about 9 min. Before the experiment, four control samples were spiked at 1 mg kg^{-1} and two at the limit of determination 0.05 mg kg^{-1} (wet soil) with analytical grade imidacloprid. Recovery values ranged between 78% and 89% showing that this method can be used for determining imidacloprid concentrations from soil.

Lethal and sublethal effects

A first experiment (with two replicates) was carried out to determine the LD_{50} of imidacloprid for the two earthworm species. For each replicate, ten worms of each species were placed in increasing concentrations of imidacloprid (50, 10, 5, 2.5, 1, and 0.1 mg kg^{-1} dry soil). Above 1 mg kg^{-1} some worms of the two species died but none died at this concentration or below. As a consequence, only three concentrations of imidacloprid (1, 0.5 and 0.1 mg kg^{-1} dry soil) were studied for the weight loss experiment. Since endogeic and anecic worms are not easy to rear and their growth is slow, it is difficult and tedious to detect effects of pesticides on growth for these species. Instead, we studied weight loss as an indicator of the general health of the earthworms (Bauer and Römbke 1997; Kula 1998; Leland et al. 2001; Vermeulen et al. 2001; Ma et al. 2002; Zwahlen et al. 2003). Whatever the causes, it is likely that significant weight loss can impair their behaviour and therefore affect the soil ecosystem. Earthworms were weighed to the nearest milligram at the beginning of the experiment and then 7 and 14 days later. Each dish was also weighed to ensure that no water loss occurred. After 14 days, we observed that worms in the control sample lost weight due to starvation. We therefore only ran the experiment for this period. Before weighing, worms were rinsed rapidly in distilled water and gently dried with filter paper. We did not attempt to remove soil from the earthworm intestines by, for example, putting worms in water for 24 h, as an increase in the water content of worms could decrease or erase negative effects on weight (recovery; Dalby et al. 1996). As a consequence, worm weight was seen to vary depending on the amount of soil in the intestines. Weight loss was expressed as a percentage of the initial weight. For weight measurements, three replicates were carried out during the period of activity of the two worm species from November to March.

Statistical analyses

In our case, a replicate consisted of measurements for ten worms at each concentration of imidacloprid and each control. The mortality experiment was done in two replicates (n =2*10 for each species and each concentration). Estimates of the LC_{50} and their 95% confidence intervals were obtained using a probit computer program (Raymond et al. 1993). For weight loss experiments, weights were measured in triplicate (n =3*10 for each species and each concentration) and because these replicates were carried out on different dates (from November to March), it must be noted that the worms had been exposed to different climatic situations (temperature and humidity). Because worms were not reared but collected in the abandoned orchard the day of the experiment, their age and initial weight also varied. This variability may have influenced the weight loss measured and so a "block" design was used (each repetition is a block). Moreover, data (weight, activity) were not normal and variances were heterogeneous. Therefore we chose to use non-parametric statistical methods (rank tests) to analyse the data. Overall, a Friedman test was used for each measurement with a non-parametric multiple comparison test (Zar 1984; Skillings and Mack 1981) with a significance level of 5%.

Results

Soil spiking

We adjusted the concentrations of imidacloprid by using the mean recovery values (80.47%) of our protocol:

$$X_{final} = X_{measured}/0.8047 \quad (1)$$

with X the concentration of imidacloprid.

At the beginning of the experiment, the adjusted concentration of imidacloprid in our samples ranged from 0.814 to 0.955 mg kg^{-1} dry soil (Fig. 1). Imidacloprid was not rapidly metabolised under our experimental conditions since the concentration of imidacloprid remained high after 14 days (between 0.724 and 0.881 mg kg^{-1} dry soil). The mean decrease in imidacloprid concentration was therefore 9.25%. The variability (coefficients of variation) between samples (A1, A5 and A10) at each date ranged from 5.77% to 9.76%.

Mortality and symptoms

The LC_{50} (and confidence intervals) for imidacloprid were found to be similar for the two species: 3.74 mg kg^{-1} dry soil (3.41–4.08) for *A. nocturna* and 2.81 (1.94–4.05) for *A. icterica* (Fig. 2). Clear symptoms of toxicity were observed for both species at concentrations above 1 mg kg^{-1} dry soil: some worms showed at least one but sometimes two zones of intense swelling in approximately ten segments located in the post-clitellar regions (Fig. 3). These zones led to a thinning out of the anterior part of the body, which sometimes burst leading to death. These morphological abnormalities generally appeared after 2 days of treatment and after 7 days most worms showed swelling. After 10 days of exposure, body constrictions were frequent and fragmentation often occurred. These symptoms, however, were transitory since they generally disappeared if the worms were placed in unpolluted soils for 2 days (results not shown).

Weight loss

Throughout the experiment, no significant water loss was observed from the dishes (mean =0.68 g, maximum =0.83 g water) after 14 days. Weight loss for the earthworms was expressed as a percentage of the initial weight. This is the usual approach when working with worms of similar weight but in our case (worms initially variable in weight), we needed to confirm that there is indeed a linear rela-

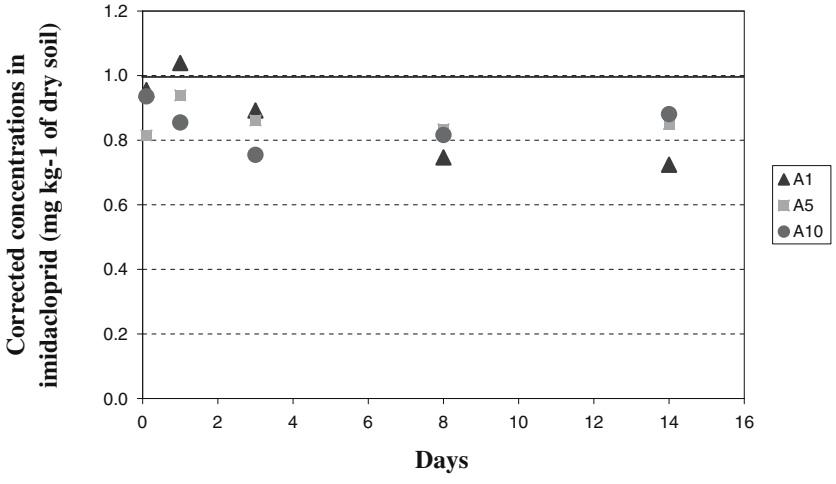

Fig. 1 Adjusted concentrations (according to mean recovery value) of imidacloprid as a function of time for three subsamples taken in three samples (A1, A5 and A10). The *horizontal plain line* is the nominal value (1 mg kg^{-1} dry soil)

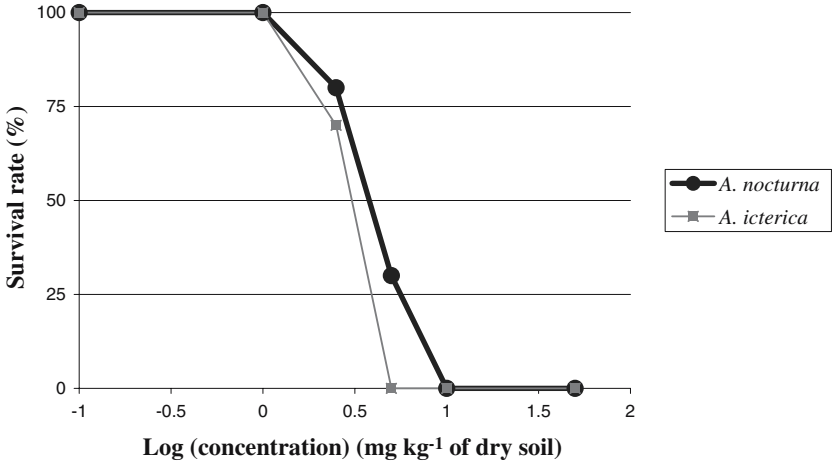

Fig. 2 Mortality of the two earthworm species as a function of imidacloprid concentration (logarithmic scale). Each value is the mean of two replicates

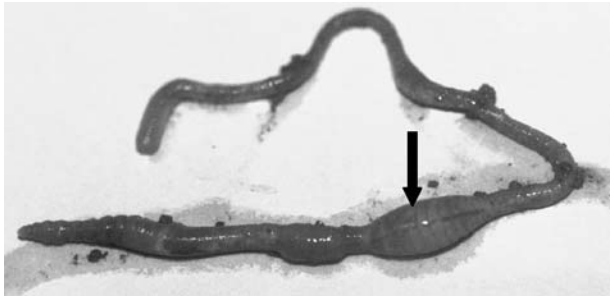

Fig. 3 Toxicity symptoms in *Allolobophora icterica* for imidacloprid concentrations greater than 1 mg kg^{-1} dry soil (*arrow* globular swelling)

tionship between weight loss and the initial weight of the worms. In polluted soil, this relationship does exist but the slopes are different for the two species (Fig. 4). Moreover this linear relationship appeared to be more marked when the concentration of imidacloprid was increased. The relationship was not linear for the control worms, instead showing the inherent variability of worm weight possibly due to the rate of intestine filling. Bearing this in mind, weight loss appeared to be variable (standard deviations of weight loss expressed as percentages are about 8%) especially after 7 days (Fig. 5). This variability was greater for *A. icterica* than for *A. nocturna*. After 14 days, all worms showed a decrease in weight (the means were approximately 10% for control to 30% for the highest concentration of imidacloprid). Nevertheless, this high degree of variability in weight loss between individuals did not mask differences between treatments. Indeed, for both earthworm species, imidacloprid had a significant effect on worm weight when used at the two highest concentrations (0.5 and 1 mg kg^{-1}). No significant effect was observed for 0.1 mg kg^{-1} which is therefore the NOEC value. DMSO did not affect worm weight (Fig. 5). Regarding variability between replicates, it was clear that control worms in the three replicates had different responses (weight losses; Fig. 6 for *A. nocturna*).

Discussion

In our spiking protocol, relatively large amounts of soil (1 kg) were spiked manually. Bearing this in mind, the efficiency of this spiking protocol is believed to be satisfactory. Indeed the initial mean concentration of imidaclo-

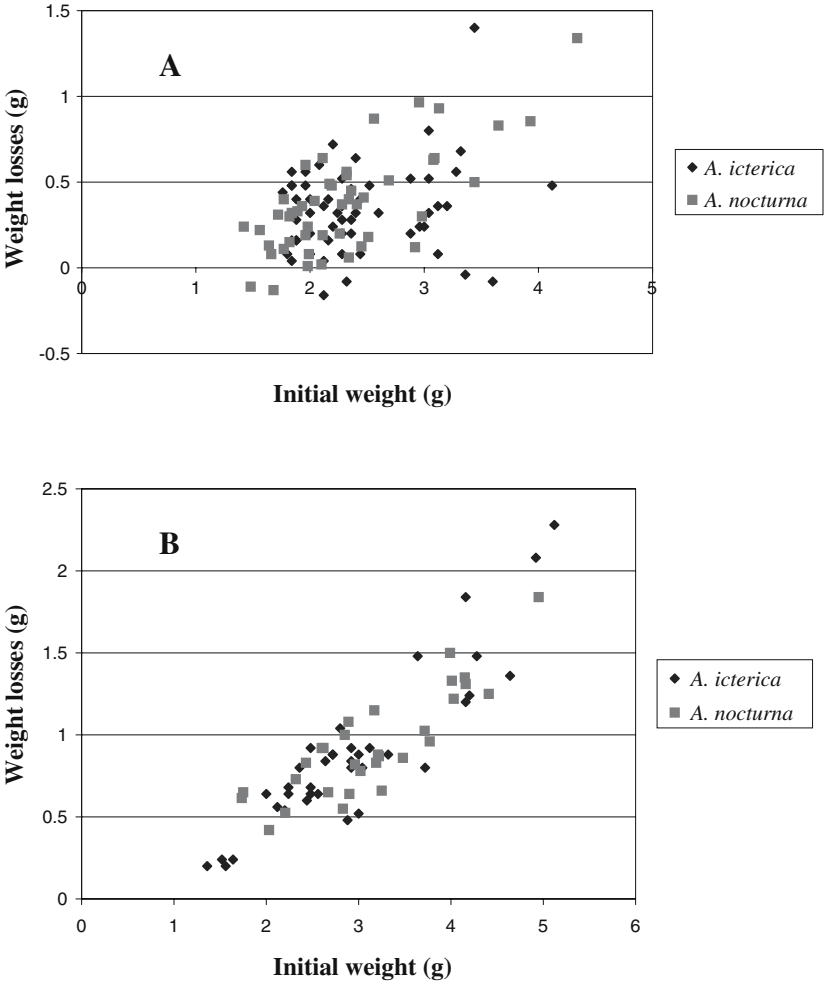

Fig. 4 Relation between initial weight and weight loss after 14 days for the two earthworm species at two imidacloprid concentrations: control (**a**) and 1 mg kg^{-1} dry soil (**b**). For each species, the values for the three replicates are shown (n =3*10). The determined weights of *Allolobophora icterica* were multiplied by 4

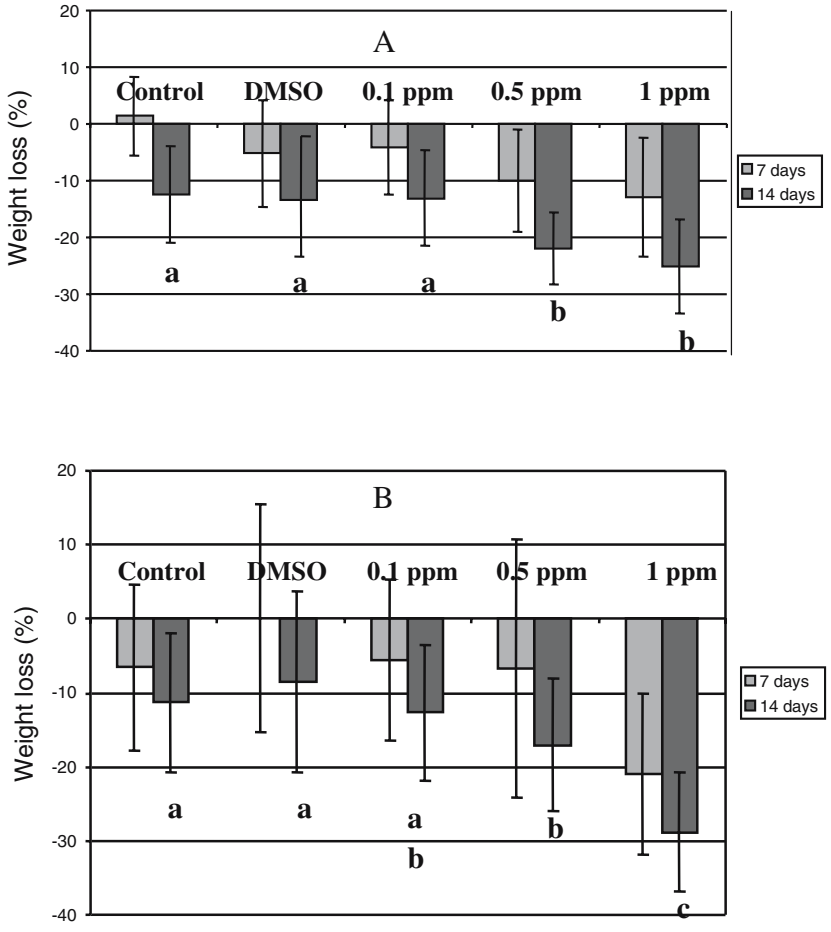

Fig. 5 Weight loss observed after 7 and 14 days for the two earthworm species: *Aporrectodea nocturna* (**a**) and *Allolobophora icterica* (**b**). Standard deviations are shown. *Bars with different letters* are significantly different at the 5% level

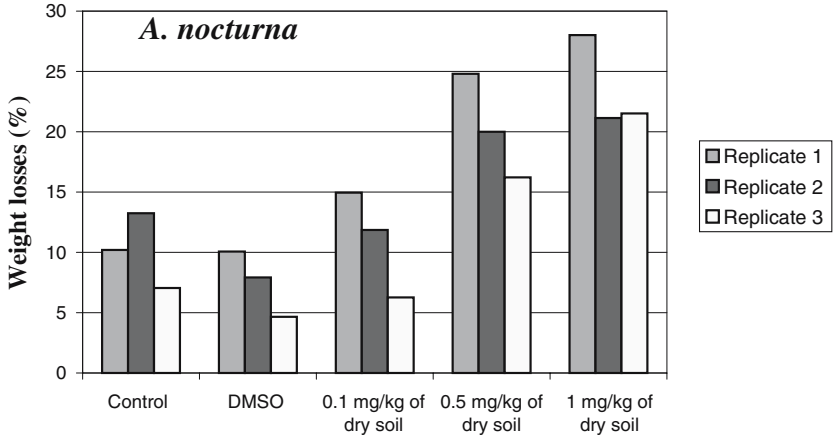

Fig. 6 Variability between replicates for mean weight losses after 14 days in *Aporrectodea nocturna*

prid measured in the soil (0.902 mg kg^{-1} dry soil) was not too far from the nominal concentrations (1 mg kg^{-1} dry soil). It is possible that the spiking of the soil was not completely homogeneous and that the 10 g of soil that we sampled contained slightly less pesticide than the rest of the soil. Otherwise this discrepancy could be due to either: (1) small inaccuracies in the dilution procedure and/or preparation of solutions; (2) small quantities of pesticide being lost on the walls of the big plate (40×50 cm) used to spike the soil; or (3) rapid degradation or immobilization (bound residues) of imidacloprid in the soil. This last assumption is not supported by previous studies since, for example, Sarkar et al. (2001) did not detect metabolites in three different soil samples from West Bengal before day 28 and in a separate study on three different soils from Minnesota, the percentages of bound residues were only 9%, 3% and 2% of the applied imidacloprid after 1 week (Cox et al. 1997). Obviously, further studies are needed to determine the exact cause of this discrepancy. Compared with other studies, the variability in imidacloprid concentration be-

tween soil samples using our protocol appears satisfactory, the coefficient of variations for samples (A1, A5 and A10) and subsamples (10 g at four dates) remaining acceptable at between 5% and 10%. In comparison, Northcott and Jones (2000a) calculated coefficients of variation that ranged from 3% to 10% when spiking wet soils with PAHs using a blender. However, as discussed by Northcott and Jones (2000b), it is difficult to discuss this issue in more detail since authors rarely describe their spiking procedures in enough detail and few have directly tested the quality of their procedure.

In this study, the sublethal effects of imidacloprid were assessed after 14 days, the same time span used in standardized protocols testing lethal effects on earthworms. In this period, imidacloprid was not significantly degraded or immobilized in our soil (in the absence of light) since the mean decrease in concentration was only 9.25%. This result is consistent with results of previous studies on imidacloprid breakdown within soils (Rouchaud et al. 1996; Sarkar et al. 2001). Therefore, we can conclude that under our experimental conditions the sublethal effects were caused by the presence of imidacloprid in the soil.

For the two earthworm species studied, the LD_{50} of imidacloprid is similar to that found for *Eisenia fetida* (Luo et al. 1999) and for worms of the *Pheretima* group (Mostert et al. 2002). These results seem to be rather independent of the soil characteristics showing the persistence of imidacloprid in soils for such short periods of time. *Aporrectodea nocturna*, which is two times bigger, is slightly less sensitive than *Allolobophora icterica*. The morphological abnormalities observed after imidacloprid treatment do not appear to be specific to this pesticide since they are similar to those described by Hans et al. (1990) after *Pheretima posthuma* was treated with lindane and by Venkateswara Rao et al. (2003a,b) after exposure of *E. fetida* to chlorpyrifos or lead oxide.

Regarding the observed sublethal effects (weight loss), we observed significant effects for imidacloprid concentrations between 0.5 and 1 mg kg^{-1} dry soil which is near the Predicted Environmental Concentration (PEC; 0.33 mg kg^{-1} dry soil; Oi 1999). The percentage of weight loss observed is statistically significant (mean of 29% for *A. icterica* and 25% for *A. nocturna*) compared to the control (mean of 11% and 12%, respectively) for 1 mg kg^{-1} dry soil after 14 days. These percentages are similar to those found by Mostert et al. (2000) studying the effect of imidacloprid on earthworms of the *Pheretima* group (a mean decrease of 21% after 14 days) but in this work the weight losses were not significant. However, it should be kept in mind that control worms also decreased in weight after 14 days, even if these weight losses were under the recommended threshold of 15–20% (Bembridge 1998). This indicated that either the quantity of soil per worm was insufficient or that the organic matter content of the soil was low. Weight loss is not only a general indicator of health, but it can influence the behaviour of earthworms since these animals are highly dependent on their hydraulic skeleton to burrow and move in the soil (Quillin 1999). Indeed, these results could explain the effects of imidacloprid on the burrowing behaviour of these species in laboratory conditions at the same concentrations (Capowiez et al. 2003) and the decreasing surface casting activities of earthworms in the field after normal imidacloprid application (Lal et al. 2001). Unfortunately, we can not confirm that worms that exhibited the greatest weight loss burrowed the least (and the contrary) since these results (weight and burrowing behaviour) were assessed in two different experiments. With regards to weight loss, *A. nocturna* again appeared to be less sensitive than *A. icterica* since *A. icterica* lost more weight during a shorter period (after 1 week) for 1 mg imidacloprid kg^{-1} dry soil (Fig. 5). However, because these earthworm species belong to different ecological types under natural conditions they are probably exposed to pesticides in different ways (Tomlin 1992). Anecic species may be exposed to pesticides through food or when the pollutant infiltrates into burrows if applied just before rain, whereas endogeic species tend to burrow continuously in the soil and are thus more likely to be exposed by directly ingesting polluted soil (Capowiez 2000; Capowiez and Belzunces 2001). It is difficult to predict which worm species will be more exposed since imidacloprid has a significant potential for leaching (Ndongo et al. 2000; Gupta et al. 2002). Furthermore, it should be noted that in this study imidacloprid was distributed homogeneously throughout the soil but this is not the case in natural conditions where actual exposure to imidacloprid may be decreased. Further work is required if we are to fully understand and predict the actual exposure of worms to pesticides in soils in the natural environment.

Weight loss appears to be a sensitive biomarker since we were able to detect significant weight losses for low concentration of imidacloprid (0.5 mg kg^{-1} dry soil). This biomarker is easy to use and does not require special equipment. As mentioned above, in a separate study (Capowiez et al. 2003), we explored the use of earthworm behaviour as a biomarker for sublethal effects of imidacloprid. Weight loss appears to be as sensitive as burrowing behaviour in detecting the sublethal effects of imidacloprid and since it is much easier to measure, it should be preferred. Nevertheless studies of burrowing behaviour are invaluable as they provide information that can be directly linked to the impact of a pesticide on the soil ecosystem and the soil properties (aeration, infiltration). Moreover, weight loss as a biomarker seemed to be robust regarding initial weight of earthworms, which was very variable in this study. As a consequence, it is possible to work with anecic species (such as *A. nocturna* or *Lumbricus terrestris*) whose field densities are generally low. One drawback with this biomarker is that initial conditions could vary if replicates are done at different dates. In our study, this resulted in variability between replicates regarding the weight loss observed for the control. This variability could be due to soil variability, different weather conditions or variations in animal history during the season. However by using a block design (each replicate being a block) and performing a Friedman test (Zar 1984) rather than a more classical Kruskal-Wallis test with two factors (treatment and dates) we were still able to produce statistically significant results.

Lastly, although we are convinced that weight loss is a relevant biomarker, the cause of this weight loss is not yet clear. Indeed, these decreases in weight could be caused by two different factors that are not exclusive: (1) inactivity, as a direct response to the insecticide or as a mechanism of avoidance, or (2) physiological causes such as less efficient assimilation or development of a costly mechanism of detoxification.

Conclusion

The protocol we designed to spike natural soils with imidacloprid was proven to be satisfactory. In our experimental conditions, the LD_{50} of imidacloprid for *Aporrectodea nocturna* and *Allolobophora icterica* were close to those found for other earthworm species and soil types. At concentrations above 1 mg kg^{-1} dry soil, non specific swelling was observed. Weight loss was shown to be a valuable and easy to measure endpoint. Using this measurement, imidacloprid was shown to produce sublethal effects at concentrations between 0.5 and 1 mg kg^{-1} dry soil; these values are near the predictive environmental concentrations (0.33 mg kg^{-1}). We showed that this criterion can be measured using natural populations of earthworms collected in the field. As it is likely that critical weight losses could have a drastic effect on earthworm activity, this measurement, which is now used in standardized tests, is important in order to evaluate the impacts of pollutants and pesticides on soil ecosystems.

Acknowledgements The authors are very grateful to Leigh Gebbie for editorial assistance.

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