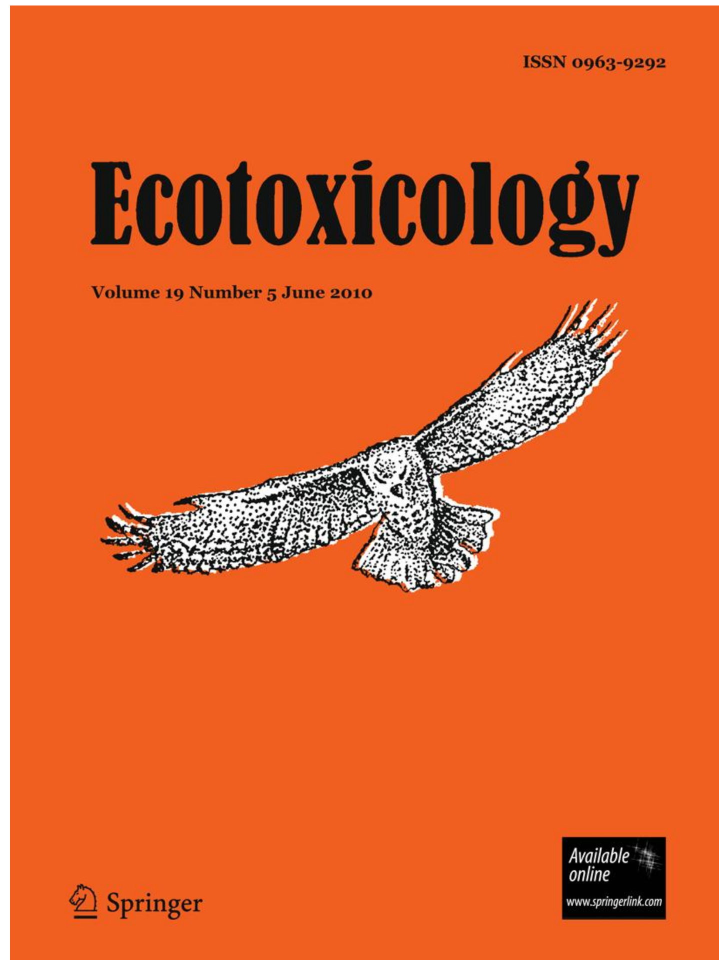
ISSN 0963-9292, Volume 19, Number 5



This article was published in the above mentioned Springer issue.
The material, including all portions thereof, is protected by copyright;
all rights are held exclusively by Springer Science + Business Media.
The material is for personal use only;
commercial use is not permitted.
Unauthorized reproduction, transfer and/or use
may be a violation of criminal as well as civil law.

Ecotoxicology (2010) 19:917–927
DOI 10.1007/s10646-010-0473-4

Author's personal copy

Toxicity of abamectin to the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea)

Lucija Kolar · Anita Jemec · Cornelis A. M. van Gestel · Janez Valant · Rok Hrženjak · Nevenka Kožuh Eržen · Primož Zidar

Accepted: 16 February 2010 / Published online: 9 March 2010
© Springer Science+Business Media, LLC 2010

Abstract To determine effects of the antiparasitic veterinary drug abamectin on the isopod *Porcellio scaber*, animals were exposed for 21 days to Lufa 2.2 soil spiked at concentrations of 3–300 mg/kg dry soil. After exposure, abamectin residues in the isopods were analysed using a novel analytical method. Toxicity was evaluated on different levels of biological organisation: biochemical, cellular and the individual organism. Measurements included glutathione S-transferase (GST) activity and stability of cell membranes in the digestive gland, animal mass gain or loss, food consumption, behaviour and mortality. LC50 for the effect of abamectin on survival of *P. scaber* was 71 mg/kg dry soil. The most obvious sublethal effects were reduced food consumption and decreased body mass (NOEC 3 mg/kg dry soil). Additionally, loss of digging activity and reduced GST activity (NOEC 30 mg/kg dry soil) and cell membrane destabilization (NOEC 10 mg/kg dry soil) were recorded. Abamectin only slightly accumulated in the isopods, with bioaccumulation factors always being <0.1. Based on these results and current information on environmental levels of abamectin, it is not likely that isopods will be affected by abamectin, but further studies with exposure through faeces are recommended.

Keywords Avermectins · Biomarker · Multi-level approach · Soil invertebrates

L. Kolar (✉) · R. Hrženjak · N. K. Eržen
Veterinary Faculty, Forensic and Administrative Veterinary Medicine, Institute of Pathology, University of Ljubljana, Gerbičeva 60, SI-1000 Ljubljana, Slovenia
e-mail: lucija.kolar@gmail.com

A. Jemec
National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

C. A. M. van Gestel
Institute of Ecological Science, VU University, De Boelelaan 1085, Amsterdam, 1081 HV, The Netherlands

J. Valant · P. Zidar
Biotechnical Faculty, Department of Biology, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia

Introduction

Abamectin is a natural fermentation product of the soil bacterium *Streptomyces avermitilis*. It is a mixture of two avermectins B1a and B1b with very similar biological and toxicological properties. Abamectin is widely used in veterinary medicine to control nematodes, sucking lice and ticks. It is also used to control the motile stages of insect and mite pests on a range of agronomic, fruit, vegetable and ornamental crops and for the control of fire ants (Adams 2001; EFSA 2008). Concerns about the use of abamectin have been raised, because its physico-chemical properties (non-volatile, low water solubility and strong affinity for lipids and organic matter). The high excretion rate of the parent compound from treated animals enables it to enter and persist in various environmental compartments. As the major part of the dosage is excreted unmetabolized with the faeces, abamectin along with other avermectins may pose a threat to the terrestrial environment (Sun et al. 2005).

Only recently a few toxicity studies on abamectin toxicity to soil invertebrates were published, including earthworms, springtails and enchytraeids (Wislocki et al. 1989; Sun et al. 2005; Diao et al. 2007; Jensen et al. 2007; Kolar et al. 2008). However, its effects on terrestrial isopods are not well known (Kolar et al. 2008). Terrestrial isopods are

abundant in different ecosystems and habitats, and have an important ecological role as macro-decomposers. *Porcellio scaber* Latreille, 1804 is a synanthropic species, in central Europe frequently found around stables, what makes it likely to come in direct contact with veterinary pharmaceuticals. These animals have been recognized as useful for the characterisation of chemical toxicity (Drobne 1997; Hornung et al. 1998; Walker et al. 2001), because they are easy to sample, handle and culture, and large enough to perform a variety of sub-organism studies. A multi-level approach in toxicity testing with terrestrial isopods has previously been successfully used to identify the hazard of different pesticides and nanomaterials (Stanek et al. 2006; Drobne et al. 2009). This approach enables to follow a variety of responses to pollutants in individual animals, ranging from biochemical to behavioural level responses. Data derived in this way are relevant to assess animal health status and link it with a tested chemical.

The aim of the present paper was to identify the hazard and the bioaccumulation potential of the antiparasitic abamectin to the non-target terrestrial invertebrate *P. scaber*. A battery of lethal (mortality) and sublethal responses (activity of glutathione S-transferase in digestive gland, stability of digestive cell membranes, animal mass gain or loss, food consumption and behaviour) due to exposure to soil contaminated with abamectin were assessed. Results will contribute to the assessment of the impact of abamectin on soil biota.

Materials and methods

Test species

Specimens of *Porcellio scaber*, fourth generation laboratory raised, with body weights of 18–30 mg, were used in the experiments. The original population was obtained from an unpolluted environment in the vicinity of Ljubljana, Slovenia. Prior to the experiment, the test animals were kept in a climate chamber at 20 ± 1°C with a 16/8 h day/night photo period, caged in glass containers with moist sand and peat on the bottom. They were fed with leaves from various trees (hazel, birch, maple, poplar), with periodic addition of apples, potatoes, carrots and commercial food designed for experimental animals (Altromin 1324, Germany).

Chemicals

Analytical grade abamectin was obtained from Dr. Ehrenstorfer Laboratory, Germany. The following chemicals were purchased from Sigma–Aldrich (Munich, Germany): dibasic and monobasic potassium phosphate, 1-chloro-2,4-dinitrobenzene and L-glutathione (reduced form). All chemicals were of the highest commercially available grade, typically 95% or higher. Acridine orange (AO) and ethidium bromide (EB) were purchased from Merck (Darmstadt, Germany). They were dissolved in a physiological solution (248 mM NaCl, 8 mM KCl, 5 mM $MgCl_2$, 5 mM glucose and 10 mM Tris in bidistilled water) (Hagedorn and Ziegler 2002).

Methods of exposure

The tests were performed using Lufa 2.2, a standardized natural soil having 3.7% organic matter and 6.8% clay. The test substance was introduced into the soils using acetone as a solvent. A small portion of the soil (approx. 25%) was spiked with the acetone solution containing abamectin (25 ml acetone per 30 g soil), thoroughly mixed and incubated over night in a fume cupboard. After evaporation of the acetone, the remainder of the soil was added, carefully mixed, and moisture content was adjusted to 40–50% of Water Holding Capacity (WHC). The test concentrations in soil were 0, 3, 10, 30, 100 and 300 mg/kg dry soil of abamectin. Besides a water control, we had two additional controls: a solvent control with acetone and a control with animals deprived of food. The latter was included to elucidate whether the observed effect was due to chemical action or starvation of isopods. The average pH (0.01 M $CaCl_2$) of Lufa 2.2 soil was 4.5 for the control and 4.4 for the soil samples treated with abamectin.

Experimental design

Glass jars (100 ml) were carefully filled with approx. 30 g moist soil, and five isopods were introduced. There were six replicates for each treatment and the controls. Isopods received food pellets, consisting of hazel litter (50%), commercial food mixture Altromin 1324, Germany (40%) and potato powder (10%). Food was offered on a plastic dish with a diameter of 12 mm and 3 mm height, to separate it from the contaminated soil. Test jars were covered with perforated aluminium foil, and placed on trays in a climate chamber at 21°C, with over 75% RH and a 16/8 h light/dark cycle. Twice a week the number of animals on the soil surface was counted and dead animals removed. At the same time uneaten food was replaced with a new pellet. Soil moisture content was checked weekly by weighing the containers and replenishing the water loss with deionised water.

The animals were extracted from the tested substrates after 21 days of exposure by emptying the test jars, hand sorting the test substrates and counting the surviving animals. Only surviving animals were used for further analyses.

Activity of glutathione-S-transferase

After the experiment, 6–8 surviving animals per treatment were dissected and the digestive gland (hepatopancreas) of each animal was isolated. The digestive gland consists of a pair of bi-lobed structures, which look like four blind-ending tubes. One tube was used to assess cell membrane stability of the digestive gland cells, the other three were prepared for enzyme analysis. At the highest concentration tested (300 mg/kg dry soil) only two animals survived, but due to handling errors one sample was lost. The remainder of test animals (not used in the described test) were frozen for abamectin residue analysis.

The three digestive gland tubes of each individual animal were homogenized in 800 μl of 50 mM phosphate buffer pH 7.0 for 3 min. GST activity was measured on microtiter plates (Bio-Tek® Instruments, USA; PowerWave™ XS) as previously described by Jemec et al. (2007). GST activity was expressed in nmoles of conjugated GSH/min/mg animal fresh weight at the end of experiment (extinction coefficient $\varepsilon_{340} = 9600\ M^{-1}\ cm^{-1}$). Usually enzyme activities are expressed per mg of protein in the reaction mixture, but they cannot be used as a reference when the treatment itself affects their content. It this experiment, the amount of proteins in digestive gland changed significantly in animals exposed to the highest two concentrations, animals exposed in the solvent control and in starved animals, thus rendering the use of protein content as a reference (discussed in detail in Jemec et al. 2008).

Stability of digestive gland cell membranes

Stability of the cell membranes in the digestive gland was assessed with the AO/EB (acridine orange/ethidium bromide) assay. Changes in cell membrane integrity result in differences in permeability for AO and EB dyes and therefore nuclei are differentially stained. Acridine orange is taken up by cells with intact and destabilized cell membranes. The dye emits green fluorescence as a result of intercalation into double-stranded nucleic acids. Ethidium bromide is taken up only by cells with destabilized cell membranes, and it emits red fluorescence after intercalation into DNA (McGahon et al. 1995).

A digestive gland tube was placed on a microscope slide immediately after isolation, and examined using an Axioimager.Z1 fluorescent microscope (Zeiss) equipped with two different sets of filters (Zeiss, Axioimager.Z1). The complete digestive gland was photographed and examined by the same observer twice at intervals of at least 24 h. Cell membrane integrity was determined visually and classified from 1 to 10 according to a predefined scale, corresponding to the percentage of nuclei stained with EB (Valant et al. 2009). The hepato-pancreatic tubes where no nuclei were stained with EB were classified as 1, those with the highest portion of EB-stained nuclei as 10.

Whole-organism endpoints

Mortality of the test animals was followed throughout the experiment. The animals were weighted before and after 21 days of exposure, and mass change was calculated. The dry weight of food pellets was recorded before and after each replacement of food (twice a week). The difference between these two weights was regarded as consumed food, expressed per live weight of surviving animals. At the end of the experiment the total amount of food consumed in 21 days was estimated. Impact on animal behaviour was followed by observing digging activity. Digging activity was considered to be absent when at least two-thirds of an animals' body surface including the head was still on the soil surface and was expressed as percentage of live animals in the jar.

Bioaccumulation

Analyses of samples for bioaccumulation were performed using a modification of the procedure developed by Kolar et al. (2004) for the analysis of faeces. All samples were analyzed in four parallel determinations. Isopod samples (blank) were fortified with abamectin standard at concentration of 10, 25, and 50 ng/g. Standard solutions of abamectin at concentration of 100, 200 and 500 mg/ml were also injected into the system. Recovery of the method was above 85%. Measured peak areas at retention time of abamectin as indicated by standards were used to calculate the actual abamectin concentration in samples.

Frozen organisms were first weighed and then ground in liquid nitrogen for 2 min. The homogenized samples were extracted with 30% acetonitrile (ACN) under ultrasonic treatment followed by centrifugation at 3000 rpm for 20 min. The supernatant was applied to Octyl (C8) solid phase extraction (SPE) columns that had previously been activated with 10 ml ACN and conditioned with 10 ml 30% ACN. After applying the sample, the column was washed with 3 ml of 50% ACN and eluted with 5 ml ACN. The eluate was collected and evaporated to complete dryness under a constant flow of nitrogen at 60°C. After derivatization, samples were analysed by HPLC according to De Montigny et al. (1990).

Bioaccumulation factors for abamectin were calculated in two ways: (1) as a ratio between the internal concentration of abamectin per dry body weight and exposure concentration in mg/kg dry soil, and (2) ratio between internal concentration of abamectin per animal lipid content and exposure concentration in mg/kg organic carbon (OC).

Data analysis

LC50, the concentration causing a 50% reduction in survival, was estimated applying the trimmed Spearman-Karber method (Hamilton et al. 1977/1978). NOEC and LOEC values for all sublethal parameters were calculated as a statistically significant response in relation to the control group, using a nonparametric statistic (Mann–Whitney test). To define the general trends of the animals' sublethal responses to abamectin in soil, linear regression was used. For that purpose, abamectin concentrations were logarithmically transformed, and so were data on the behavioural response and GST activity. No data transformation was applied when calculating the trend of increase of internal abamectin concentrations. Results on the strength of correlations are presented as r^2 and P values. Calculations were done using the software package SPSS for Windows (Microsoft, USA, 2002). All LC50, LOEC, and NOEC values are based on nominal concentrations in the Lufa 2.2 test soil. Due to the routine procedure performed in our group (Kolar et al. 2004, 2008), we might expect that actual concentrations do not differ by more than 10% from the nominal concentrations.

Results

Isopod survival in water and solvent controls was 100 and 93%, respectively. Survival of food-deprived control animals was 90%. LC50 for the effect of abamectin on isopod survival in Lufa 2.2 soil was 71 (95% confidence interval: 48–93) mg/kg dry soil.

Mass gain of *P. scaber* decreased with increasing abamectin concentration in soil ($r^2 = 0.458$; $P < 0.001$) (Fig. 1). The lowest concentration of abamectin that significantly (Mann–Whitney test, $P < 0.05$) decreased body mass gain was 10 mg/kg dry soil, so NOEC was 3 mg/kg dry soil. Mass gain did not differ between the two controls (water and solvent treated), while food-deprived animals gained significantly (Mann–Whitney test, $P < 0.05$) less mass compared to the controls. Mass change did not significantly differ between food-deprived animals and those exposed to 10, 30, 100 and 300 mg abamectin/kg dry soil.

Food consumption rate decreased with increasing abamectin concentration in soil ($r^2 = 0.801$; $P < 0.001$) (Fig. 2), and was significantly reduced from 10 mg/kg dry soil onwards (Mann–Whitney test, $P < 0.05$). The NOEC value was 3 mg abamectin/kg dry soil.

With increasing abamectin concentration in soil the percentage of animals found on the soil surface increased ($r^2 = 0.638$; $P < 0.001$) (Fig. 3). This behavioural response was observed already at 30 mg abamectin/kg dry soil, but was only statistically significant at 100 and 300 mg abamectin/kg dry soil (Mann–Whitney test, $P < 0.05$). No differences were found between the controls.

The membrane stability of digestive gland cells gradually decreased with increasing concentrations of abamectin ($r^2 = 0.484$; $P < 0.001$), significant decrease was detected

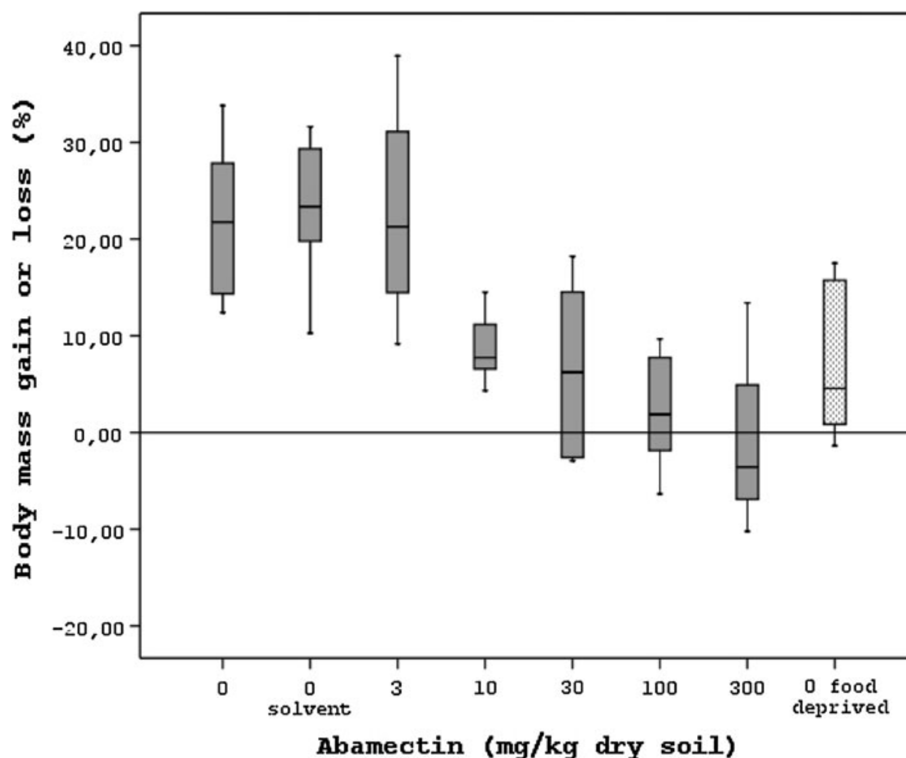


Fig. 1 Mass gain or loss (in % of initial mass) of the terrestrial isopod *Porcellio scaber* after 21 days exposure to Lufa 2.2 soil treated with abamectin. $n = 6$ per concentration. *Box* represents first quartile, median value, and third quartile

Author's personal copy

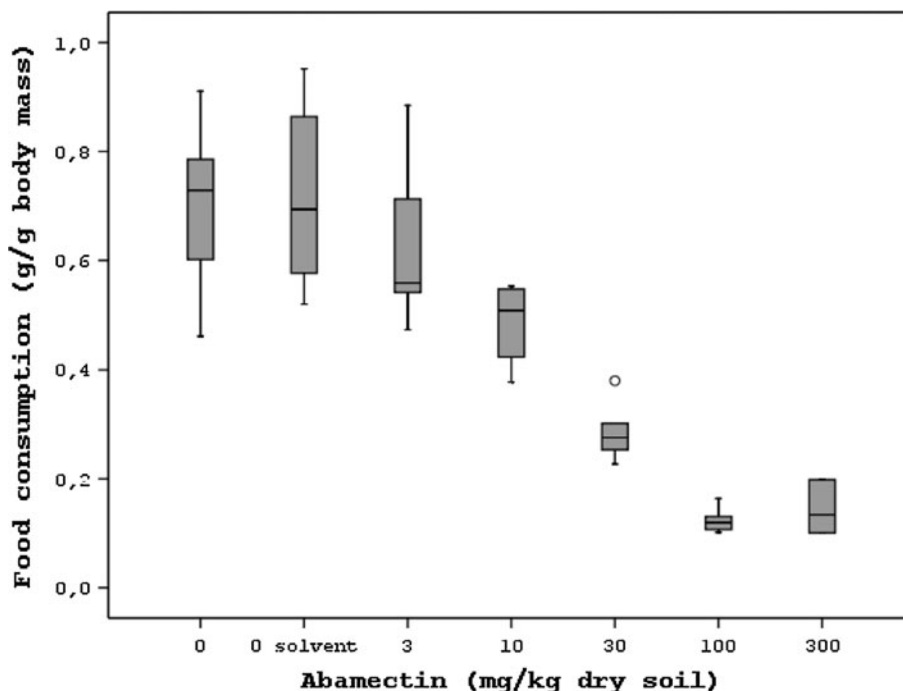


Fig. 2 Food consumption of *Porcellio scaber* (g dry weight of uncontaminated food consumed per g fresh animal body mass) during 21 days exposure to Lufa 2.2 soil treated with abamectin. *o* outlier (value less than or equal to 3 and more than 1.5 times the interquartile range outside the quartile). $n = 6$ per concentration. *Box* represents first quartile, median value, and third quartile

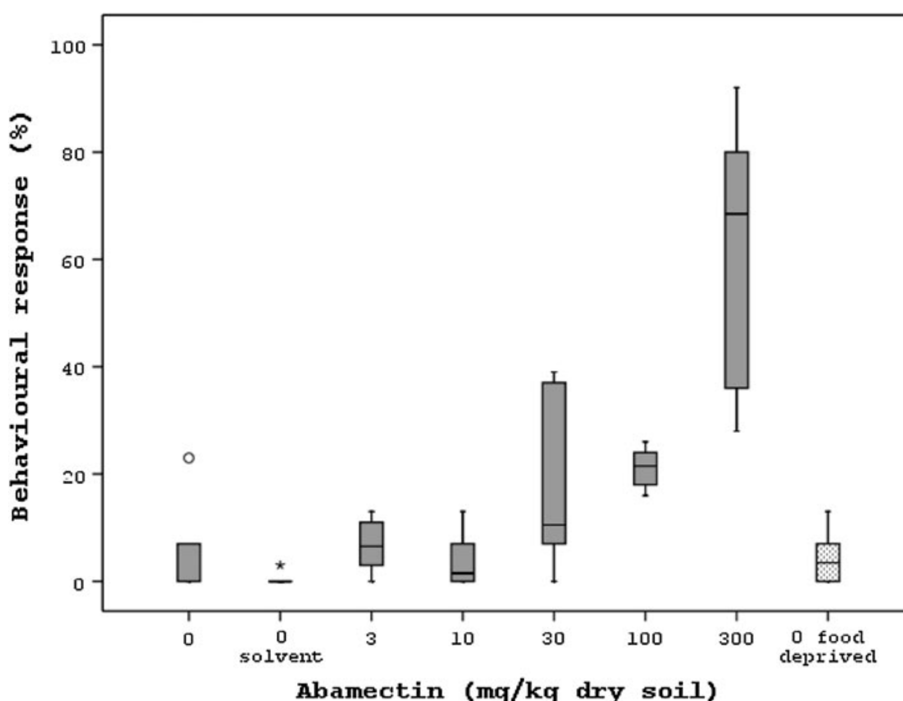


Fig. 3 Behavioural response of *Porcellio scaber*, expressed as the percentage of animals that was found on the soil surface (suggesting impaired digging ability), during 21 days exposure to Lufa 2.2 soil treated with abamectin. *o* outlier (value less than or equal to 3 and more than 1.5 times the interquartile range outside the quartile); * extreme (value more than 3 times the interquartile range outside the quartile). $n = 6$ per concentration. *Box* represents first quartile, median value, and third quartile

in animals exposed to 30, 100 and 300 mg abamectin/kg dry soil compared to control animals (Mann–Whitney test, $P < 0.05$; Fig. 4). No such changes in comparison to control were observed in animals exposed to 3 and 10 mg abamectin/kg dry soil and in food-deprived animals. The NOEC for cell membrane destabilisation therefore was 10 mg abamectin/kg dry soil.

The activity of GST gradually decreased with increasing concentrations of abamectin ($r^2 = 0.290$; $P = 0.007$), but was significantly (Mann–Whitney test, $P < 0.05$) affected only at the highest concentrations tested for enzyme activities (100 and 300 mg/kg dry soil) (Fig. 5). No difference in GST activity was observed between the water and solvent controls, but a significant

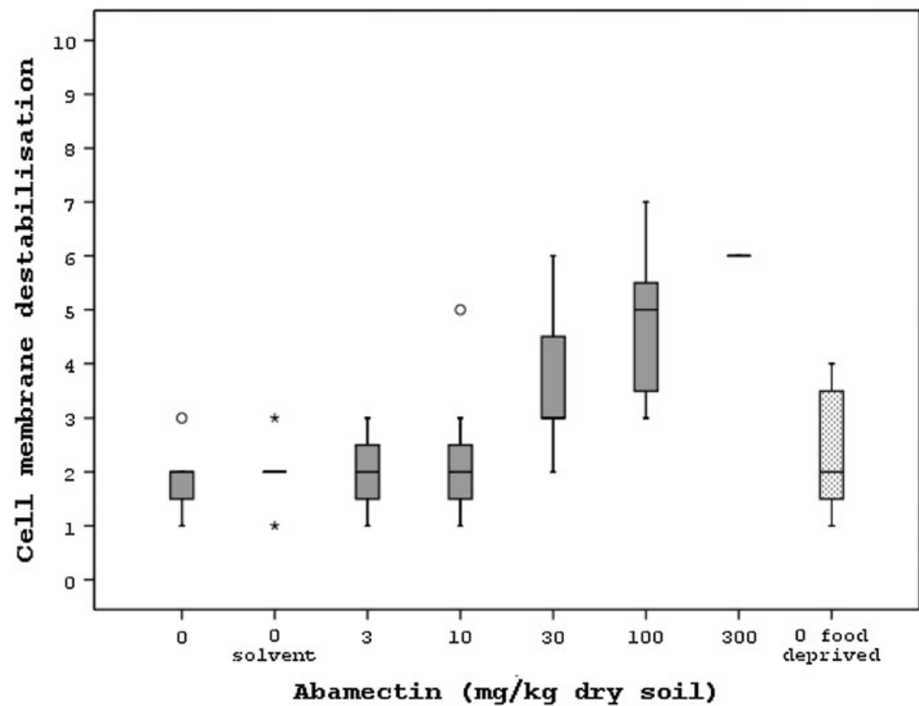


Fig. 4 Digestive gland cell membrane destabilization in *Porcellio scaber* exposed to abamectin in Lufa 2.2 soil for 21 days (10 grades scale). *o* outlier (value less than or equal to 3 and more than 1.5 times the interquartile range outside the quartile); * extreme (value more than 3 times the interquartile range outside the quartile). The number of animals analysed per concentration (in order from *left* to *right*): 6, 7, 8, 7, 7, 8, 1, and 7. *Box* represents first quartile, median value, and third quartile

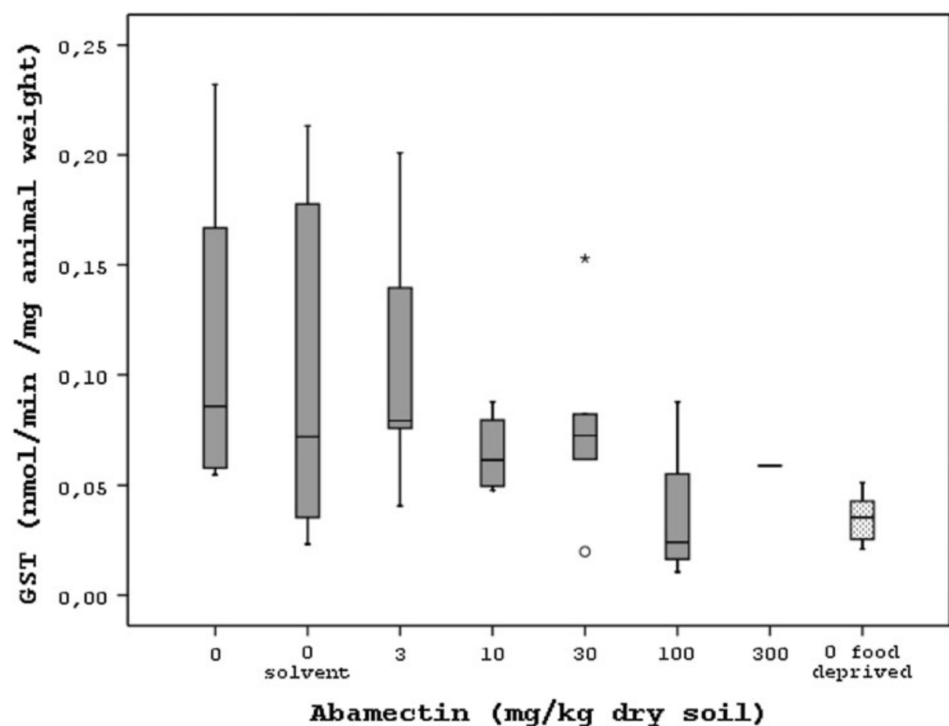


Fig. 5 Glutathione S-transferase (GST) activity in *Porcellio scaber* exposed to abamectin in Lufa 2.2 soil for 21 days. *o* outlier (value less than or equal to 3 and more than 1.5 times the interquartile range outside the quartile); * extreme (value more than 3 times the interquartile range outside the quartile). The number of animals analysed per concentration (in order from *left* to *right*): 6, 7, 8, 7, 7, 8, 1, and 7. *Box* represents first quartile, median value, and third quartile

decrease of GST activity was observed in starved animals.

Measured abamectin concentrations in *P. scaber* increased with increasing exposure concentrations ($r^2 = 0.942$; $P = 0.006$) (Table 1). At the highest exposure concentration (with only two survivors), abamectin internal concentration was 7.8 µg/kg fresh weight. Assuming a dry weight of 33.6% of fresh weight (van Brummelen et al. 1996), this corresponds with a concentrations 23 µg/g dry weight. An average lipid content of 2–3% of fresh weight has been reported for isopods used in laboratory studies or collected from the field, which may

Author's personal copy

Table 1 Measured internal concentrations of abamectin in the isopod *Porcellio scaber* after 21 days exposure to treated Lufa 2.2 soil

Exposure conc. mg/kg dry soil	Exposure conc. in mg/kg OC	No. of animals per sample	Internal conc. in mg/kg fresh body weight	Internal conc. in mg/kg dry body weight	Internal conc. in mg/kg lipid	BAF	BAF (kg lipid/kg OC)
3	140	6	0.021	0.06	2.1	0.021	0.015
10	465	6	0.033	0.10	3.3	0.010	0.0071
30	1395	4	0.12	0.35	12	0.012	0.0083
100	4651	4	0.61	1.8	61	0.018	0.013
300	13953	2	7.8	23	775	0.077	0.056

decrease to approx. 1% upon starvation (van Brummelen and Stuijfzand 1993; Lavy et al. 1997; Ribeiro et al. 2001). So, using the latter value, internal concentrations ranged from an estimated 2.1 to 775 μg/g lipid at exposure levels between 3 and 300 mg/kg dry soil. Corresponding values for the Bioaccumulation Factor (BAF) are 0.010–0.077 when expressed on the basis of dry weight or 0.071–0.056 kg OC/kg lipid, assuming soil organic carbon (OC) content is approximately 2.15%.

Discussion

In the present paper the toxicity of the antiparasitic abamectin to the non-target terrestrial invertebrate *Porcellio scaber* was assessed after 3 weeks of exposure in Lufa 2.2 soil. Exposure concentrations that significantly affected survival, feeding rate, body mass, animal behaviour, digestive gland cell membrane stability, and biotransformation enzyme activity ranged from 10 to 100 mg abamectin/kg dry soil.

The most obvious effects of abamectin on isopods were reduced food consumption and decreased body mass. Similar effects on the weight of the earthworms *Eisenia fetida and E. andrei* exposed to abamectin were previously observed (Table 2). According to abamectin's mode of action, this might be due to paralysis of muscles but it can also be explained by unspecific effects on metabolism, which is not an uncommon observation (Zidar et al. 2004). Literature data on target invertebrates (insects and nematodes) exposed to a compound with a similar mode of action—ivermectin, report ataxia, and paralysis, which occur through suppression of electrical activity in muscle and nerve cells by activation of irreversible chloride permeability (Ding et al. 2001). Impact on muscle activity might also explain why animals exposed to abamectin changed burying behaviour. Behavioural alterations were previously also reported for ivermectin, which reduced the sediment re-burying rate of the marine polychaete *Arenicola marina* (Thain et al. 1997) and significantly impaired the locomotor behaviour (e.g., swimming behaviour and crawling) of the freshwater oligochaete *Lumbriculus variegates* (Ding et al. 2001).

Abamectin-exposed animals also showed a significantly increased permeability of the digestive gland cellular membrane for ethidium bromide, which reflects membrane destabilization. Concentration-dependent cell membrane destabilization coincided with decreased body mass gain and food consumption, as well as a reduced digging ability. There are two possible mechanisms that could play an important role in cell membrane destabilization. Upon exposure to abamectin, the level of reactive oxygen species (ROS) could be increased through redox cycling and as a result lipid peroxidation could take place (Halliwell and Gutteridge 2007). Second, damaged lipid bilayers might also be a result of an inhibitory effect of abamectin on glutamate-gated chloride ion channels, as was suggested by Jensen et al. (2007) who found a similar decrease in lysosomal membrane stability of earthworm coelomocytes. Assessing the exact mechanism of membrane destabilization was not the prime aim of this study and still needs to be elucidated.

In the present work, the total GST activity in the digestive glands of *P. scaber* was not induced and one could speculate that GST had a minor role in the detoxification of abamectin. The activity was measured using the substrate 1-chloro-2, 4-dinitrobenzene (CDNB), which is conjugated by the majority of GST isoenzymes (Clark et al. 1973). It has previously been shown that the induction of GST activity upon chemical exposure is substrate-dependent, meaning that the response might be different if another substrate would be used (Hoarau et al. 2001). It may therefore be possible that other GST isoenzymes were changed in isopods, but these were not measured in this study. Contradictory results on total GST activity changes were reported in the literature. In abamectin-resistant strains of the spider mite *Tetranychus urticae* and the sweet-potato whitefly *Bemisia tabaci* GST activity against CDNB was increased (Stumpf and Nauen 2002; Wang and Wu 2007). On the contrary, total GST was not changed in Brazilian populations of the tomato leaf-miner *Tuta absoluta* (Siqueira et al. 2001). In this study, GST activity in the isopods decreased at the highest abamectin concentrations

Table 2 Toxicity data for abamectin and non-target terrestrial invertebrates

Test organism	Time (days)	Test substrate properties	End-point	Effective (lethal) conc. (mg/kg dry soil)[a]	Reference
Eisenia fetida	28	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Membrane stability[b]	NOEC < 0.25; EC50 = 0.99 (0.67–1.88)	Jensen et al. 2007
	28		Weight loss	NOEC = 0.25; EC50 = 0.46 (0.05–2.15)	Jensen et al. 2007
	28		Hatching	NOEC = 0.25; EC50 = 0.43 (0.13–1.38)	Jensen et al. 2007
	28		Reproduction	NOEC < 0.25; EC50 = 1.03 (0.75–1.19)	Jensen et al. 2007
	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Reproduction	NOEC < 0.25; EC50 = 0.39 (0.15–0.87)	Diao et al. 2007
	7	Data not available	Mortality	LC50 = 24	Sun et al. 2005
	14		Mortality	LC50 = 17	Sun et al. 2005
	28	Data not available	Mortality	LC50 = 38	Wislocki et al. 1989
	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Mortality	NOEC = 5; LC50 > 5	Diao et al. 2007
Eisenia andrei	28	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Weight loss	NOEC = 9,8	Kolar et al. 2008
	28	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Mortality	LC50 = 18	Kolar et al. 2008
Enchytraeus crypticus	28	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Reproduction	NOEC = 8; EC50 = 38	Kolar et al. 2008
	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Reproduction	NOEC = 10; EC50 = 23,7 (20.2–33.8)	Diao et al. 2007
	28	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Mortality	LC50 = 111	Kolar et al. 2008
	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Mortality	NOEC = 50;	Diao et al. 2007
Folsomia candida	28	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Reproduction	NOEC = 1.5; EC50 = 13	Kolar et al. 2008
	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Reproduction	NOEC = 0.25; EC50 = 0.68 (0.34–1.03)	Diao et al. 2007
	28	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Mortality	LC50 = 67	Kolar et al. 2008
	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Mortality	NOEC > 2.5	Diao et al. 2007
Folsomia fimetaria	70	Sandy–loamy soil (21% clay, 12% silt, 67% sand, 2.22% OC; pH 7.0)	Reproduction	NOEC < 0.25; LC50 = 0.33 (0.16–0.69)	Diao et al. 2007
	70		Mortality	NOEC = 0.5; LC50 = 0.81 (0.76–0.87)	Diao et al. 2007
Porcellio scaber	21	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	Mortality	LC50 = 69	Kolar et al. 2008
	21	Lufa 2.2, 3.7% organic matter, 6.8% clay; pH = 6.0	GST activity	NOEC = 30	This study
	21		Membrane stability[c]	NOEC = 10	
	21		Weight loss	NOEC = 3	
	21		Food consumption	NOEC = 3	
	21		Digging activity	NOEC = 30	
	21		Mortality	LC50 = 71	

[a] Data reported for soil exposure, [b] lysosomes of coelomocytes, [c] digestive gland

tested (100 and 300 mg/kg dry soil) and also in starved animals. In both cases feeding rate and animal weight were decreased. These results are in accordance with previous findings that antioxidant enzymes, such as GST, depend on the metabolic rate of animals, because the production of reactive oxygen species is dependent upon the respiration of mitochondria (Abele et al. 1998).

The two cellular biomarkers were affected at higher concentrations than the whole-organism effects, such as feeding rate and animal mass change, which is not a usual expectation for this kind of biomarkers (Adams 2002). This is due to the very specific mode of action of abamectin on the muscular-nervous system of isopods. This speaks in favour of our previous suggestion that an array of responses along levels of biological complexity is necessary to be able to detect the hazard of pollutants to organisms (Drobne et al. 2008).

Internal concentrations of abamectin in isopods increased with increasing exposure concentrations, which is in line with their ability to accumulate metals and organic compounds from food and soil (Loureiro et al. 2006). As abamectin is very lipophilic (log Kow = 4.0) we would expect high accumulation potential in tested organisms, but BAFs generally were well below 0.1, both when expressed on a dry weight basis and related to lipid content of the animals and organic carbon content of the soil. It therefore has to be concluded that bioaccumulation potential of abamectin in isopods is low. This is in agreement with the studies on earthworms by Sun et al. (2005) and on sturgeon by Shen et al. (2005). Sousa et al. (2000) demonstrated that exposure route is important in the kinetics of lipophilic chemicals in isopods. In the case of lindane, the assimilation rate was much greater when exposed to contaminated soil compared to contaminated food exposure (Sousa et al. 2000). As the food was not contaminated by abamectin, we might expect that most of the chemical was assimilated via the body surface. Nevertheless, this did not seem to be an effective uptake pathway. The low BAF might also be due to rapid metabolism of the parent substance, which could be confirmed by measuring metabolites, but this was not the scope of the present paper. Further studies on the exact pathway of abamectin uptake and possible metabolism are needed to clarify this issue.

Isopods seem less susceptible to abamectin than earthworms, and similarly or slightly more sensitive than springtails and enchytraeids, respectively (Table 2). However, a thorough comparison is not possible, because exposure periods in the tests with the other organisms were mostly longer than 21 days. Also the usage of toxicity data provided in Table 2 for species sensitivity distribution is limited, since the exposure conditions, such as test substrate properties and endpoints tested differ. From the data presented in Table 2, it is clear that the toxicity of abamectin for soil invertebrates depends on the species tested, which has previously also been concluded for ivermectin (Kövecses and Marcogliese 2005). Therefore, it is necessary that a variety of toxicity data for different terrestrial non-target organisms is available to accurately predict the risk of abamectin to soil biota.

Furthermore, most studies with terrestrial invertebrates have been performed using soil exposure, but exposure through faeces is also highly likely. Namely, avermectins undergo little metabolism in animals and are expelled relatively unaltered in the faeces (Sun et al. 2005). Isopods are frequently found around stables, where they feed on food with high organic matter contents and faeces as well. Further studies with isopods exposed through faeces are therefore needed.

The effect levels found in the presented study (the lowest NOEC = 3 mg/kg dry soil) are close to predicted levels in cow dung (9 mg/kg d.w) (Jensen et al. 2007), but are significantly higher than the exposure levels that can be predicted in soil based on current literature data. Namely, Kožuh Eržen et al. (2005) reported quite low environmental levels of abamectin in soil (1.4 μg/kg d.w.), soil-faeces with less than 1% faeces (22.8 ± 7.9 μg/kg d.w.), and sheep faeces (800 ± 46 μg/kg d.w.) after 6 days of pasture of Istrian Pramenka sheep, which received a single subcutaneous dose of 0.2 mg abamectin/kg b.w. The latter data suggest abamectin is not likely to affect isopods, whereas the study of Jensen et al. (2007) does indicate that there may be conditions at which environmental exposure concentrations are close to the NOEC and may pose a risk. More studies are therefore needed on the presence of abamectin in soil and faeces to enable a more accurate risk assessment for this species. Due to the increasing use of abamectin and its properties, which enable it to enter and persist in the environment, it is expected that the environmental levels of this antiparasitic drug will rise.

In conclusion, abamectin was shown to affect terrestrial isopods *Porcellio scaber* after 3 weeks soil exposure. The most sensitive endpoints were food consumption, body mass, and soil digging activity. Additionally, membrane stability of digestive gland cells was decreased. In comparison to other non-target terrestrial invertebrates, isopods seem less susceptible than earthworms, and similarly or slightly more sensitive than springtails and enchytraeids, respectively. Based on current information on environmental levels, abamectin is not likely to affect isopods, but we recommend further toxicity studies through faeces exposure. Also more data on environmental levels of avermectins in soil and faeces are needed to provide an accurate risk assessment for this species.

References

Abele D, Burlando B, Viarengo A, Pörtner HO (1998) Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antartic intertidal limpet *Nacella concinna*. Comp Biochem Physiol B 120:425–435

Adams HR (2001) Veterinary pharmacology and therapeutics. Iowa State University Press, Ames, Iowa, USA

Adams SM (2002) Biological indicators of aquatic ecosystem stress. American Fisheries Society. Bethesda, Maryland, USA

Clark AG, Smith JN, Speir TW (1973) Cross specificity in some vertebrate and insect glutathione S-transferases with methyl parathion (dimethyl p-nitrophenyl phosphorothionate), 1-chloro-2, 4-dinitrobenzene and S-crotonyl-N-acetylcysteamine as substrates. Biochem J 135:385–592

De Montigny P, Shim JSK, Pivnichny JV (1990) Liquid chromatographic determination of ivermectin in animal plasma with trifluoroacetic anhydride and N-methylimidazole as the derivatization reagent. J Pharm Biomed Anal 8:507–511

Diao X, Jensen J, Hansen AD (2007) Toxicity of the anthelmintic abamectin to four species of soil invertebrates. Environ Pollut 148:514–519

Ding J, Drewes CD, Hsu WH (2001) Behavioural effects of ivermectin in a freshwater oligocheate, *Lumbriculus variegates*. Environ Toxicol Chem 20:1584–1590

Drobne D (1997) Terrestrial isopods—a good choice for toxicity testing of pollutants in the terrestrial environment. Environ Toxicol Chem 16:1159–1164

Drobne D, Blažic M, Van Gestel CAM, Lešer V, Zidar P, Jemec A, Trebše P (2008) Toxicity of imidacloprid to the terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea). Chemosphere 71:1326–1334

Drobne D, Jemec A, Pipan-Tkalec Ž (2009) In vivo screening to determine hazards of nanoparticles: nanosized TiO_2. Environ Pollut 157:1157–1164

EFSA (European food safety authority) (2008) Peer review report on abamectin. Efsa Scientific report. Available online: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902028558.htm. Accessed 27 Nov 2009

Hagedorn M, Ziegler A (2002) Analysis of Ca^{2+} uptake into the smooth endoplasmic reticulum of permeabilized sternal epithelial cells during the molting cycle of the terrestrial isopod *Porcellio scaber*. J Exp Biol 205:1935–1942

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine. Oxford University Press, New York

Hamilton MA, Russo RC, Thurston RV (1977/1978) Trimmed Spearman-Karber method for estimating lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714–719 (Correction: Environ Sci Technol (1978) 12:417)

Hoarau P, Gnassia-Barelli M, Romeo M, Girard JP (2001) Differential induction of Glutathione S-transferases in the clam *Ruditapes Decussatus* exposed to organic compounds. Environ Toxicol Chem 20:523–529

Hornung E, Farkas S, Fischer E (1998) Tests on the isopod *Porcellio scaber*. In: Løkke H, Van Gestel CAM (eds) Handbook of soil invertebrate toxicity tests, 1st edn. Wiley, Chichester, pp 207–226

Jemec A, Tišler T, Drobne D, Sepčić K, Fournier D, Trebše P (2007) Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the microcrustacean *Daphnia magna*. Chemosphere 68:1408–1418

Jemec A, Drobne D, Tišler T, Sepčić K, Jamnik P, Roš M (2008) Biochemical biomarkers in chronically metal-stressed daphnids. Comp Biochem Phys C 147:61–68

Jensen J, Diao X, Scott-Fordsmand JJ (2007) Sub-lethal toxicity of the antiparasitic abamectin on earthworms and the application of neutral red retention time as a biomarker. Chemosphere 68:744–750

Kolar L, Kužner J, Kožuh Eržen N (2004) Determination of abamectin and doramectin in sheep faeces using HPLC with fluorescence detection. Biomed Chromatogr 18:117–124

Kolar L, Kožuh Eržen N, Hogerwerf L, van Gestel CAM (2008) Toxicity of abamectin and doramectin to soil invertebrates. Environ Pollut 151:182–189

Kövecses J, Marcogliese DJ (2005) Avermectins: potential environmental risks and impacts on freshwater ecosystems in Quebec. Scientific and technical report ST-233E. Environment Canada, Quebec region, environmental conservation, St. Lawrence Centre, 72 pp

Kožuh Eržen N, Kolar L, Kužner J, Cerkvenik Flajs V, Marc I, Pogačnik M (2005) Degradation of abamectin and doramectin on sheep grazed pastures. Ecotoxicology 14:627–635

Lavy D, Nedved O, Verhoef HA (1997) Effects of starvation on body composition and cold tolerance in the collembolan *Orchesella cincta* and the isopod *Porcellio scaber*. J Insect Physiol 43:973–978

Loureiro S, Sampaio A, Brandão A, Nogueira AJ, Soares AM (2006) Feeding behaviour of the terrestrial isopod *Porcellionides pruinosus* Brandt, 1833 (Crustacea, Isopoda) in response to changes in food quality and contamination. Sci Total Environ 369:119–128

McGahon AJ, Martin SJ, Bissonnette RP, Mahboubi A, Shi Y, Mogil RJ, Nishioka WK, Green DR (1995) The end of the (cell) line: methods for the study of apoptosis. Methods Cell Biol 46:153–185

Ribeiro S, Sousa JP, Nogueira AJA, Soares AMVM (2001) Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*. Ecotoxicol Environ Saf 49:131–138

Shen J, Zhang Q, Ding S, Zhang S, Coats JR (2005) Bioconcentration and elimination of avermectin B1 in sturgeon. Environ Toxicol Chem 24:396–399

Siqueira HAA, Guedes RNC, Fragoso DB, Magalhaes LC (2001) Abamectin resistance and synergism in Brazilian populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Int J Pest Manag 47:247–251

Sousa JP, Loureiro S, Pieper S, Frost M, Kratz W, Nogueira AJA, Soares AM (2000) Soil and plant diet exposure routes and toxicokinetics of lindane in terrestrial isopods. Environ Toxicol Chem 10:2557–2563

Stanek K, Drobne D, Trebše P (2006) Linkage of biomarkers along levels of biological complexity in juvenile and adult diazinon fed terrestrial isopod (*Porcellio scaber*, Isopoda, Crustacea). Chemosphere 64:1745–1752

Stumpf N, Nauen R (2002) Biochemical markers linked to abamectin resistance in *Tetranychus urticae* (Acari- Tetranychidae). Pestic Biochem Physiol 72:111–121

Sun Y, Diao X, Zhang Q, Shen J (2005) Bioaccumulation and elimination of avermectin B1a in the earthworms (*Eisenia foetida*). Chemosphere 60:699–704

Thain JE, Davies IM, Rae GH, Allen YT (1997) Acute toxicity of ivermectin to the lugworm, *Arenicola marina*. Aquaculture 159:47–52

Valant J, Drobne D, Sepčić K, Jemec A, Kogej K, Kostanjšek R (2009) Hazardous potential of manufactured nanoparticles identified by in vivo assay. J Hazard Mater 171:160–165

Van Brummelen TC, Stuijfzand SC (1993) Effects of benzo[a]pyrene on survival, growth and energy reserves in the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Sci Total Environ Suppl 1993:921–930

Van Brummelen TC, Verweij RA, Wedsinga SA, van Gestel CAM (1996) Polycyclic aromatic hydrocarbons in earthworms and

Author's personal copy

isopods from contaminated forest soils. Chemosphere 32: 315–341

Walker CH, Hopkin SP, Sibly RM, Peakall DB (2001) Principles of ecotoxicology. Taylor and Francis, London

Wang L, Wu Y (2007) Cross-resistance and biochemical mechanisms of abamectin resistance in the B-type *Bemisia tabaci*. J Appl Entomol 131:98–103

Wislocki PG, Grosso LS, Dybas RA (1989) Environmental aspects of abamectin: use in crop protection. In: Campbell WC (ed) Ivermectin and abamectin, 1st edn. Springer, New York, pp 182–200

Zidar P, Drobne D, Štrus J, Van Gestel CAM, Donker M (2004) Food selection as a means of Cu intake reduction in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda). Appl Soil Ecol 25:257–265