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Fate, Uptake and Distribution of Nanoencapsulated Pesticides in Soil-Earthworm Systems and Implications for Environmental Risk Assessment

Mohd Firdaus M. A.[1,2], Agatz. A.[1], Hodson M. E.[1], Omar S. A. Al-Khazrajy[1,3], Boxall A. B. A.[1*]

[1]Environment Department, Wentworth Way, University of York, Heslington, York, YO10 5NG, United Kingdom

[2]Department of Land Management, Faculty of Agriculture, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

[3]Department of Chemistry, College of Ibn al-Haitham, University of Baghdad, Baghdad, Iraq

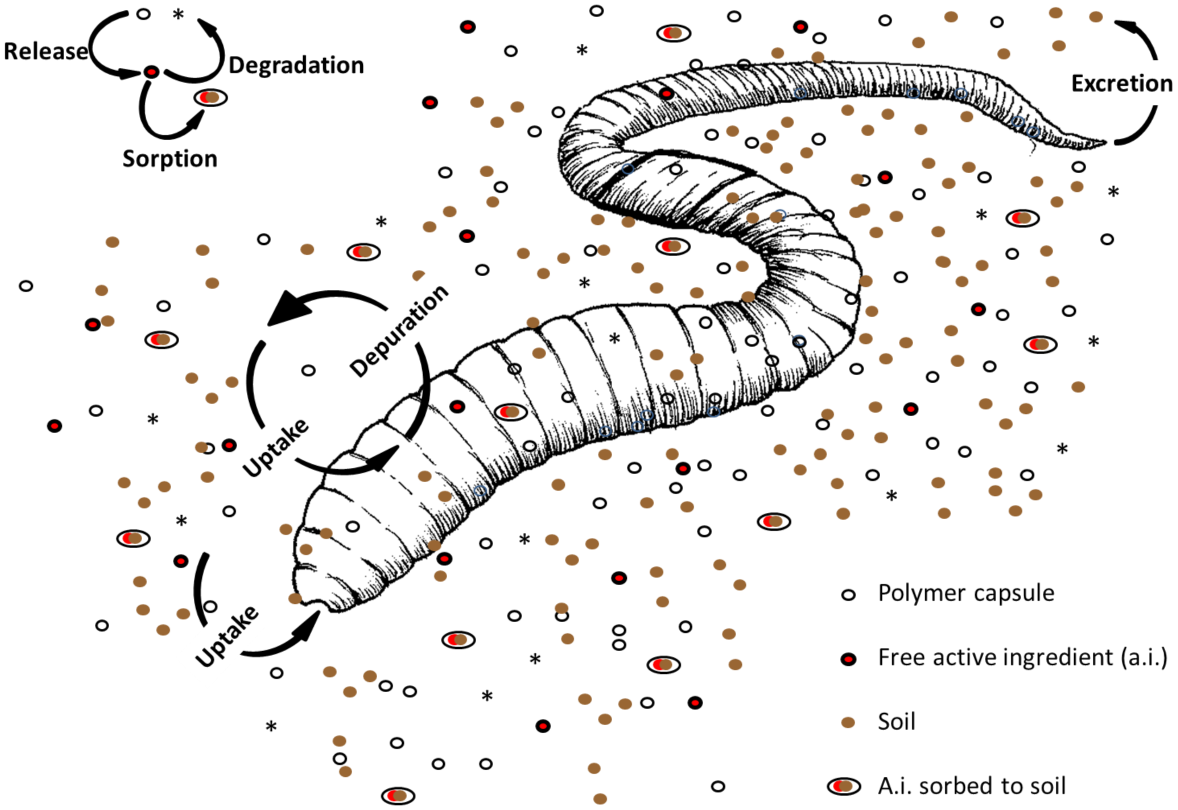
* - corresponding author, email: Alistair.boxall@york.ac.uk

Abstract

Nanopesticides are novel plant protection products offering numerous benefits. As nanoparticles behave differently from dissolved chemicals, environmental risks of these materials could differ from conventional pesticides. Here we used soil-earthworm systems to compare the fate and uptake of analytical grade bifenthrin to that of bifenthrin in traditional and nano-encapsulated formulations. Apparent sorption coefficients for bifenthrin in the nano-treatments were up to 3.8 times lower than in the non-nano treatments whereas dissipation half-lives of the nano-treatments were up to two time longer. Earthworms in the nano-treatments accumulated around 50% more bifenthrin than those in the non-nano treatments. In the non-nano treatments, most of the accumulated material was found in the earthworm tissue while in the nano-treatments, the majority resided in the gut. Evaluation of toxicokinetic modelling approaches showed that models incorporating the release rate of bifenthrin from the nanocapsule and distribution within the earthworm provided the best estimations of uptake from the nanoformulations. Overall, our findings indicate that the risks of nanopesticides may be different from conventional formulations. The modelling presented here provides a starting point for assessing risks of these materials but needs to be further developed to better consider the behaviour of the nanoencapsulated pesticide within the gut system.

Keywords: Nanopesticides; Synthetic pyrethroids; Nanoencapsulation; Earthworms; Toxicokinetic modelling, Eisenia fetida, Lumbricus terrestris

Graphical abstract



Introduction

Recently, novel pesticide products have been developed that employ nanotechnology (Kah et al., 2013; Kah, 2015). These so called ‘nanopesticides’ comprise either nanoparticulate forms of a pesticide active ingredient or nanocapsules containing an active ingredient (a.i.). Nanopesticides offer a range of advantages over conventional pesticides in that they may increase efficacy of the a.i. and/or enhance the environmental and human health safety profiles of the products (Kah et al., 2013; Kookana et al., 2014). However, there is recognition that the application of nanotechnology could also have negative and unanticipated impacts on the environment so it is also possible that nanopesticides could pose a greater risk than equivalent conventional pesticide products. The applicability of existing environmental risk assessment approaches for pesticides to nanoformulations has also been questioned (Kah, 2015).

One group of organisms that will be exposed to nanopesticides are terrestrial invertebrates such as earthworms. Earthworms are known to bio-magnify inorganic and organic soil contaminants, including pesticides, polycyclic aromatic hydrocarbons, brominated flame retardants, and metals (Heikens et al., 2001; Matscheko et al., 2002; Langdon et al., 2005). Earthworms being at the base of a food chain hold an integral position. Uptake and accumulation of contaminants into earthworms not only poses a risk to the earthworm directly, but bioaccumulation and contaminant transfer through the food chain to top predators such as birds has the potential to result in secondary poisoning (Spurgeon and Hopkin, 1996).

Data for other non-pesticide nanoparticles shows that these materials can be taken up by earthworms (Kwak and Youn-Joo, 2005). Investigations determining distribution of nanoparticles show that highest concentrations of accumulated materials are associated with the earthworm gut (Unrine et al., 2010; Waissi-Leinonen et al. 2012). Adverse effects have also been reported in earthworms following exposure to carbon-based and metal and metal oxide nanoparticles (Kwak and Youn-Joo, 2005; Scott-Fordsmand et al. 2008).

To date, the focus of research into bioconcentration and impacts of nanoparticles on earthworms has been on metals and metal oxides (Kwak and Youn-Joo, 2005), carbon nanotubes (Petersen et al., 2008, Petersen et al., 2011, Scott-Fordsmand et al. 2008) and fullerenes (Li et al. 2010, Kelsey and White 2013). To the best of our knowledge, no-one has explored the uptake of nanopesticides, even though it is inevitable that earthworms will be exposed to these products during use. Therefore, here we investigate the effects of nanoencapsulation on the fate, uptake, depuration and distribution of a pesticide a.i. in soil-earthworm systems. The nanoencapsulated materials used in the study were developed by Vive Crop Protection Inc and comprise bifenthrin encapsulated in a polymer nanoparticle with the aim to better target the active ingredient to the pest species. We compare the fate, uptake and distribution of the analytical grade a.i. with that of conventional and nanoformulated products for the two earthworm species Eisenia fetida and Lumbricus terrestris. The findings are used to explore the suitability of existing and novel toxicokinetic models to better characterise the environmental risks of nanoencapsulated substances in the future.

Materials and methods

Chemicals, soils and organisms

Analytical PESTANAL® grade bifenthrin was purchased from Sigma-Aldrich (Dorset, UK), formulated bifenthrin (Capture LFR) was obtained from FMC Corporation (Philadelphia, USA). Two nanoencapsulated formulations of bifenthrin (Nano A and B) were obtained from Vive Crop Protection Inc. (Toronto, Canada). The precise make-up of these materials is proprietary but both formulations employ an acrylate copolymer to encapsulate the bifenthrin but contain different co-formulants. Acetonitrile (99.9%) was purchased from Fisher Scientific (Loughborough, UK). Details of the bifenthrin treatments are provided in the Supporting Information.

A sandy loam soil was obtained from Landlook (Midlands, UK). Prior to use, the soil was air dried, sieved to ≤ 2 mm to ensure homogeneity within the soil matrix and stored at room temperature. Characteristics of the study soil are provided in the Supporting Information.

Eisenia fetida and Lumbricus terrestris were obtained from Blades Biological Ltd. (Kent, UK). The earthworms were cultured in a medium of horse manure and peat (50:50) for E. fetida, and in moist soil for L. terrestris. They were kept moist with deionized water under laboratory conditions (20 ± 3 ℃). The horse manure used in this culture was collected from horses that were not under medication to avoid any toxic effects on the earthworms. E. fetida were fed twice weekly with homogenized mashed potato powder which was added to the surface of the culture and L. terrestris were fed with dead birch leaves distributed on the surface of the moist soil.

Uptake and depuration studies

Uptake and depuration experiments followed OECD Guideline 317 'Bioaccumulation in Terrestrial Oligochaetes' and used only E. fetida (OECD, 2010). Experiments were performed in glass jars at a concentration of 10 µg/g of active ingredient where each jar contained 50 ± 1 g of test soil and kept in an incubator at 20 ± 2 °C, using a 16:8 light/dark cycle. Assuming a mixing depth of 20 cm, concentrations expected in the environment from the use of Capture would be expected to range from 35 - 100 µg g^{-1}. The test concentration was one order of magnitude lower than the concentration we used previously to assess the toxicity of the different bifenthrin treatments to E. fetida. At 100 µg g^{-1}, no mortality, a slight increase in growth and a small decrease in cocoon production were observed (Anuar, unpublished data). Before the earthworms were exposed to the different treatments, they were acclimated to the experimental conditions in the incubator for 48 h using non-treated soil. The different bifenthrin treatments were then mixed with the soil using deionized water as solvent carrier to achieve a moisture content between 60-70% of the maximum water holding capacity (MWHC). Treated soil was left for 24 h before adding the earthworms.

For each bifenthrin treatment (analytical grade, conventional and two nanoformulations), 45 glass jars of treated soil were prepared. At the start of the uptake phase, one mature adult E. fetida with a visible clitellum was added to each glass jar. Glass jars were then covered with garden fleece (to prevent earthworms from escaping while allowing sufficient air supply to be maintained) attached with an elastic band. The uptake phase of the experiment lasted for up to 21 d with triplicate samples being taken at 0 and 6 h and 1, 3, 7, 10, 14, 21 d. E. fetida in the remaining glass jars were then transferred to clean soil for up to another 21 d of depuration with samples being taken at 6 h and 1, 3, 7, 10, 14, 21 d after transfer. At each time point in both phases, the earthworm weight and mortality were recorded. Soil moisture content in each glass jars was monitored throughout both phases, and adjusted, where necessary, by adding deionized water so that it remained between 60-70% of the MWHC. The pH of the soils was measured at the beginning and end of the uptake phase and at the end of the depuration phase. Earthworms were fed weekly with mashed potato powder.

Once samples were collected, earthworms were removed, rinsed with deionized water, blotted dry, weighed and then placed for 48 h on moist filter papers to allow the earthworms to purge their gut contents (Dalby et al., 1996). The moist filter papers were changed twice a day (in the morning and evening). The earthworms were then frozen prior to analysis. Soil samples were taken for chemical analysis and to extract soil pore water.

Distribution of bifenthrin in earthworms

The distribution of bifenthrin following exposure to the different treatments was assessed using both E. fetida and L. terrestris. Experiments were performed at the same concentration and conditions as used in the uptake and depuration studies. E. fetida, were exposed to 50 ± 1 g of soil treated with each treatment or soil only while L. terrestis were exposed to 350 ± 5 g of treated soil or soil only. The duration of the uptake phase was 10 d while the depuration phase lasted for 7 d. There were six replicates per treatment and sampling point. Soil, faeces and earthworm samples were taken at the end of each phase for analysis.

The removed earthworms were placed on a dissecting tray with their dorsal side facing upwards. Using a pair of dissecting scissors, an opening cut was made below the clitellum. A straight line cut was made from the opening cut down to the posterior. The cut was made carefully and not too deeply to avoid damage to the internal organs. The skin was pulled apart using forceps and pinned back using dissecting pins. The earthworms were then separated into skin, gut and other tissue (hereafter referred to as ‘tissue’) for L. terrestris. Separation of E. fetida tissues proved challenging so it was only possible to separate these samples into gut + tissue and skin. Prior to analysis, samples were washed with distilled water and centrifuged at 3000 rpm for 15 min. Samples and washing water were analysed separately for bifenthrin residues.

Sample extraction and HPLC analysis

Soil (5 ± 0.5 g) was extracted by adding 15 mL acetonitrile and then shaking the mixture on an orbital shaker (250 oscillations min^{-1}) at room temperature (20 ± 2 °C) for 2 h. Samples were then allowed to settle and 2 mL aliquots of supernatant were taken for analysis. Soil pore water was obtained by placing 10 ± 1 g of soil into a glass syringe with a layer of 3 cm of glass wool inserted into the bottom. The syringe was inserted into a glass centrifuge tube and centrifuged for 20 min at 2016 g to separate soil and soil pore water. The difference in density of the polymer capsules and water is small so centrifugation would not be expected to affect the recovery of the bifenthrin from the nano-treatments compared to the non nano-treatments (Kah et al., 2016).

Earthworm samples were homogenized for 5 minutes using a LabGen Series 7 homogenizer with 5 mL of acetonitrile. The suspension was transferred, with rinsing using an additional 5 ml of acetonitrile, to a glass vial. The extracts were centrifuged for 20 min at 2016 g. The samples were then filtered using 0.45 µm nylon filters and a 2 mL aliquot of the supernatant was taken for further analysis.

Soil and earthworm extracts and pore water were analysed using High-performance Liquid Chromatography (HPLC; Perkin Elmer, Flexar) coupled with photodiode array detection. More detail on the methods used are provided in the Supporting Information. The limits of detection and quantification were 1.2 and 3.7 ng mL^{-1} for the analytical grade, 1.5 and 4.7 ng mL^{-1} for the conventional formulation, 1.9 and 5.9 ng mL^{-1} for nano A and 2.1 and 6.5 ng mL^{-1} for nano B. Recoveries for analytical method ranged from 90-107% for water, 88-103% for soil, 84-

102% for E. fetida and 90-107% for L. terrestris and recoveries of the filtration method ranged from 87-100% (see supporting information).

Data analysis

Determination of sorption coefficient, k_d

Sorption coefficient, k_d values were calculated at each time point (Equation 1) where: C_{water} and C_{soil} are the concentrations of bifenthrin in soil pore water (µg mL^{-1}) and soil (µg g^{-1}), MWHC is the maximum water holding capacity of the soil (%), and %water is the moisture content of the soil (%). Averages of k_d-values were then determined.

$$k_d = \frac{C_{soil}}{C_{water} * (\frac{\%water}{MWHC})} - 1 \qquad (1)$$

Kinetic modelling

We wanted to evaluate whether data on the uptake and depuration characteristics could be used to inform the uptake and depuration of bifenthrin resulting from exposure to Capture LFR and the two nano formulations. Three models were explored with increasing complexity. Data from the analytical grade bifenthrin treatment was always used to parameterise the models.

Model 1 was the first order one compartment toxicokinetic model outlined by Ashauer et al. (2010) (Equation 2).

$$\frac{\mathrm{d}C\mathrm{organism}}{\mathrm{d}t} = k\mathrm{in} * C\mathrm{water}\,(t) - k\mathrm{out} * C\mathrm{organism}\,(t) \qquad (2)$$

Where: $C_{organism}$ is the internal concentration (µg g^{-1}); C_{water} is the concentration in the pore water (µg mL^{-1}); and k_{in} and k_{out} are the uptake rate constant (mL g^{-1} h^{-1}) and the depuration rate constant (h^{-1}), respectively.

Model 2 was designed for estimating uptake of an active ingredient from a nonencapsulated treatment. This model is an adaptation of Model 1 modified to account for the release of bifenthrin from the polymer capsules into the soil pore water (Equation 3).

$$\frac{\mathrm{d}C\mathrm{organism}}{\mathrm{d}t} = k\mathrm{in} * C\mathrm{water}_2\,(t) - k\mathrm{out} * C\mathrm{organism}\,(t) \qquad (3.1)$$

with

$$\frac{dC\text{water}_2}{\text{d}t} = (C\text{water (t)} - C\text{water}_2\ (t)) * k\text{r} \qquad (3.2)$$

Where: $C\text{water}_2$ is the concentration of the compound in the pore water released from the nanoformulation (µg g^{-1}) and k_r is the release rate of the nanoformulation (h^{-1}). The release rate can be calculated by comparing the degradation rate of the bifenthrin a.i. with the degradation rate of bifenthrin in the nanoencapsulated formulation (Kah et al., 2016). A full description of the approach for estimating release rate is provided in Kah et al. (2016).

Model 3 was used for the distribution studies. This model extends either Model 1 or 2 to account for the distribution of the compound in gut, skin and tissue (Equations 4).

$$C\text{skin} = C\text{organism}\ (t) * a \qquad (4.1)$$

$$C\text{tissue} = C\text{organism}\ (t) * b \qquad (4.2)$$

$$C\text{gut} = C\text{organism}\ (t) - C\text{skin} - C\text{tissue} \qquad (4.3)$$

Where: Cskin, Cgut and Ctissue are the concentration of the compound in skin, gut and tissue (µg g^{-1}), and a and b are distribution coefficients between the total internal concentration and the skin, the total internal concentration and the tissue. The distribution coefficients are obtained using studies on analytical grade a.i..

Statistical analysis

Statistical analysis was performed using SigmaPlot (Version 13.0; Systat Software, San Jose, CA). Data were tested performing one-way - or two-way- Analysis of Variance (ANOVA) via the Holm-Sidak pairwise comparison method with the Shapiro-Wilk test for normality of data and the Brown-Forsythe test for equal variance of data. Modelling was conducted in OpenModel V 2.4.2. (http://openmodel.info/) using the Runge-Kulta (4th Order) ordinary differential equation method with Monte Carlo simulations to obtain the 95% confidence interval and the Nash–Sutcliffe Efficiency calculation for goodness of fit indication. Nash–

Sutcliffe values (hereafter called Nash index) between 0 and 1 represent an acceptable fit of the model to the data.

Results and discussion

Fate of bifenthrin in soil

Generally, throughout the uptake phase, there was a decrease in concentration of bifenthrin in the soil and soil pore water which was associated with an increase in the concentration of bifenthrin in the earthworms (Data are summarised in the Supporting information). At the end of the uptake phase, 65-82% of bifenthrin was extractable and associated with the soil particles, 16-33% had dissipated/degraded, around 1% was present in the pore water and < 1% was taken up by the earthworms (Figure 1 in the Supporting Information). Apparent half-lives for bifenthrin in the different treatments increased in the order analytical grade bifenthrin – Capture LFR – Nano B – Nano A. The observed DT_{50} for the analytical grade bifenthrin is at the lower end of the values reported in the field for bifenthrin and is lower than reported in laboratory studies (Pesticide Properties Database, 2017). Half-lives are also lower than those obtained by Kah et al. (2016) in similar investigations into the differences in persistence of analytical grade bifenthrin and the a.i. in Capture LFR and nanoencapsulated treatments, although the order of half-lives is the same (Kah et al., 2016). Release rates and associated release times (RT_{50}) for the nanoformulations (Table 1) are lower than those found by Kah et al. (2016). Overall, these results indicate that even traditional formulations can affect the persistence of an active ingredient but this impact is more enhanced in the nano-encapsulated treatments, possibly due to the nanocapsules ‘shielding’ the unreleased a.i. from the degrading microbes.

Sorption coefficients (k_d), based on the soil and soil-pore water concentrations ranged from 154 to 585 L kg^{-1} and increased significantly in the order Nano A = Nano B < Capture LFR < analytical grade bifenthrin (Table 1; Two-Way ANOVA Holm-Sidak method $P < 0.001$). Sorption coefficients are lower than previously reported for the a.i. which range from 882 to 6000 mL g^{-1} in different soil types (Pesticides Property Database, 2017). Sorption coefficients are also lower than those observed by Kah et al. for bifenthrin a.i. and bifenthrin in traditional and nanoencapsulated formulations (Kah et al., 2016). The mismatch is possibly explained by the fact that we derived k_d values based on pore water measurements, which is arguably more realistic than the batch equilibrium approach employed in previous studies. The differences might be explained by dissolved organic carbon in the pore water which may act as an additional sink for the bifenthrin or due to differences in the nature of the organic carbon in the soils used in the different studies.

The observations for Capture LFR demonstrate that even traditional co-formulants can affect the distribution of the bifenthrin in soils although the effect is more enhanced in the nano-encapsulated treatments. Other studies have explored the effects of formulation on pesticide behaviour. In studies with chlorsulfuron, co-formulants reduced sorption (Foldenyi et al., 2013) while studies with propyzamide (Khan and Brown, 2017) showed sorption to increase and studies with triticonazole, cyprodinil, propetamphhos and fludioxinil showed sorption to increase (Beigel et al., 1998; Beigel and Barriuso, 2000; Garcia-Ortega et al., 2006; Pose-Juan et al., 2011). The impacts of co-formulants therefore likely depend on the active ingredient and the nature of the co-formulants used in a product. The observed reduction in the k_d values for the nano-encapsulated materials is likely due to a combination of the co-formulant effects and the fact that the polymer capsule shielded the bifenthrin from sorption sites on the soil surface.

Uptake and depuration behaviour

Uptake and depuration in E. fetida

No mortality was recorded and the studies passed the validity criteria (based on earthworm growth and mortality) according to the principles outlined in the OECD 317 (OECD, 2010). Lower uptake and depuration was seen for bifenthrin in the analytical and Capture LFR treatments compared to the two nanoformulation treatments (Figure 1). At the end of the depuration phase, in the analytical grade and Capture LFR treatments, bifenthrin was still detectable in the earthworms whereas for the two nanoformulation treatments, it was not detectable. The pattern of uptake and depuration between the non-nano and nano treatments was also different. The non-nano exposures were characterised by a steady uptake and elimination of bifenthrin over time whereas in the nano treatments, an initial rapid period of uptake or elimination was observed and this then tailed off (Figure 1).

The first order one-compartment model (Model 1) was successfully fitted to the data from the analytical grade bifenthrin treatment (Nash index = 0.94) obtaining an uptake- and depuration - rate constant of 0.222 ± 0.009 mL g^{-1} h^{-1} and 0.0036 ± 0.0002 h^{-1}. Use of the uptake and depuration rates in the model to simulate the uptake and depuration bifenthrin from the Capture LFR formulation worked well (Nash index = 0.68) but failed to acceptably simulate the uptake of Nano A and Nano B (Nash index < -0.01) (Figure 1). The results for the Capture modelling do, however, suggest that it may be possible to extrapolate from studies into the uptake of analytical grade materials to estimate uptake of a.i.'s from traditional formulations.

Model 2, which incorporates the release rate of bifenthrin from the nanocapsule, underestimated uptake and depuration of the bifenthrin from the two nano treatments (Nash index < 0; Figure 1). Closer inspection of the simulation however revealed that this model more accurately simulated the internal concentration at the end of the depuration phase. The differences in kinetic patterns and model fits suggests that the nanoencapsulated bifenthrin was accumulated via a different mechanism than in the analytical grade material and Capture LFR treatments. As previous studies with earthworms have shown that other nanoparticles accumulate in the earthworm gut rather than the actual tissue (Unrine et al., 2010; Waissi-Leinonen 2012), we performed studies to explore whether there were any differences in the distribution of the bifenthrin in earthworms exposed to the different treatments. Here, we not only used E. fetida but also L. terrestris due to its larger size and consequent ease of handling.

Distribution studies

Concentrations of bifenthrin in L. terrestis were significantly greater than in E. fetida for all treatments (P < 0.001). Within each species, uptake was significantly different between the nano formulated and non-nano treatments, while within the non-nano and nano treatments no significant difference in uptake was observed (P<0.001). These differences in uptake might be explained by differences in the way the two earthworm species process soil organic matter, their ecological strategy and/or lipid content (Kelsey et al., 2005). Eisenia fetida is smaller than L. terrestris and is an epigeic species living primarily at or near the soil surface and consumes coarse particulate organic matter and surface litter. L. terrestris is an anecic species that lives in deep burrows and comes to surface to feed on surface litter [Bouche, 1983]. Interestingly, the interspecies difference in uptake that we see is the opposite to that observed in a similar study using pharmaceuticals covering a range of physico-chemical properties (Carter et al., 2016).

Significant differences were also seen in the depuration of bifenthrin by the two species (P < 0.001). For the analytical grade and Capture LFR treatments, L. terrestris still contained 57 - 59% of the accumulated bifenthrin after the 7 d depuration phase while concentrations in E.fetida were significantly lower (43-47%; P < 0.001). Depuration of bifenthrin from the two nano treatments was faster with L. terrestris containing 20-22% of accumulated bifenthrin after 7 d depuration while E. fetida contained only 10-13% of the accumulated mass. Bifenthrin from the nanoformulations was eliminated 2.8 and 4 times more quickly than from the non-nano treatments (P < 0.001).

In L. terrestris, following the uptake phase, concentrations of bifenthrin from the analytical grade and Capture LFR treatments was significantly higher (P < 0.01) in the tissue compared to the gut and skin which had similar (P = 0.6) bifenthrin concentrations (Figure 2). In contrast, for the nanoformulation treatments significantly (P < 0.001) higher concentrations were observed in the gut of the earthworms compared to the skin and tissue. Concentrations in skin and tissue were also significantly different (P < 0.001). The concentration in the gut of the nano- exposed animals was significantly higher (P < 0.001) than the non-nano exposed earthworms even though the concentration of bifenthrin in the soil was the same. A significant difference was also observed between the two nanoformulations (P = 0.019). This might indicate that the earthworms are selectively consuming the polymer capsules and/or that the capsules are becoming ‘trapped’ in the gut of the animal and are eliminated more slowly than the bulk soil. This seems to be nanoformulation specific. While it is not known whether earthworms are able to select finer material from coarser particles, there is a body of evidence indicating that they do exhibit preferences for different food types (Curry and Schmidt, 2007).

At the end of the 7 d depuration phase, for the analytical grade and Capture LFR treatments, highest concentrations of bifenthrin in L. terrestris were seen in the tissue while for the nanoformulation treatments highest concentrations were seen in the gut (Figure 2). The observation that nanopesticide treatments result in highest bifenthrin concentrations in the gut are similar to findings from a previous study into the uptake and distribution of C60 and Au nanoparticles into earthworms (Unrine et al., 2010; Waissi-Leinonen 2012; Petersen et al., 2008, 2011). We found that gut associated bifenthrin was generally less eliminated via the gut for the non-nanoformulation treatments compared to the nanoformulated treatments (Figure 3). Furthermore, a temporal shift in elimination of the nanoformulated bifenthrin occurred. Gut associated elimination of bifenthrin was greatest for the non-nanoformulation treatments whilst the earthworms were still in soil during the elimination phase of the experiment whilst elimination for the nanoformulated treatments was greatest when the organisms were on the filter paper after the elimination phase of the experiment.

Unfortunately, it was not practically possible to separate out the internal organs of E. fetida from the tissue so we could only distinguish between bifenthrin in the skin and in tissue combined with the gut (Figure 2). For the analytical grade and Capture LFR treatments, concentrations in the gut + tissue and in the skin were significantly different after the uptake phase (Figure 2; $P < 0.001$) with higher concentrations being seen in the skin. For the nanoformulation treatments, concentrations of bifenthrin accumulated in the gut combined with the internal organs were significantly higher than in the skin ($P < 0.001$). Following the depuration phase, concentrations of bifenthrin in the gut + tissue and skin in all treatments were similar (Figure 2) and not significantly different within all treatment ($P > 0.1$) except for the analytical grade treatment (P = 0.003). As we were unable to fully characterise the distribution of the a.i. in E. fetida our modelling efforts focused on the L. terrestris studies.

Model 1, and combinations of models 1 and 3 and models 2 and 3 were used to simulate uptake and depuration in the different L. terrestris treatments (Figure 4). Model 1 performed very well for estimating the concentrations of the a.i. in the whole organism for the analytical grade and Capture LFR treatments (Nash Index > 0.90). Predictions for the nano treatments were also good (Nash Index > 0.48) although the model overestimated whole organism concentrations at the end of the depuration phase (Figure 4). When model 1 was combined with model 3 to simulate distribution of the a.i. between gut- skin- and remaining-tissues, good predictions were obtained for the analytical grade and Capture LFR treatments for all tissues (Nash Index > 0.76) and for the skin in the two nano treatments (Nash Index > 0.39). Underestimates of concentrations in the gut and overestimates of concentrations in the tissue by a factor of 8-11 were obtained using a combination of models 1 and 3 for both nano treatments (Nash Index < 0.05). The fact that the two models worked well for estimating behaviour in the Capture treatment is encouraging and suggests that estimates of uptake and distribution based on analytical grade material can be used to extrapolate to behaviours in traditional formulations. The approach worked less well for the nanoformulations so we then extended the modelling to factor in the effect of the release rate from the capsule.

Incorporation of the a.i. release from the nanocapsule into the modelling of the nanoformulations (i.e. model 2 and model 3 were used) resulted in predictions that fitted the whole organism data and the skin data well (Nash Index > 0.58). This approach underestimated concentrations in the gut at the end of the uptake phase while predictions of concentrations at the end of the depuration phase were close to the measured data. This is a direct result of the model assumption that compound distribution between different tissues (skin, gut and remaining tissue) is instantaneous and fixed by distribution factors and a temporal change in gut clearance between nano and non-nano formulations (Figure 3). Nonetheless, inclusion of the release rate resulted in better predictions, compared to the approach not considering release, of internal tissue concentrations of the a.i. with concentrations being a factor of 3.8 (nano A) and 5.1 (nano B) of measured data at the end of the uptake phase and within a factor of 5.7 (nano A) to 7.5. (nano B) at the end of the depuration phase. While the predictions were not perfect, these results indicate that to model internal tissue exposure, which will likely represent the toxicologically important fraction of the accumulate a.i., it is necessary to factor in the release rate of the a.i. from the nanocapsule into the toxicokinetic modelling.

Implications for risk and a potential modelling approach

We have demonstrated that nanoencapsulation will affect the behaviour and uptake of pesticides in soil. For bifenthrin, it will decrease the sorption of the active ingredient to soils, increase the apparent persistence of the compound and alter the uptake behaviour of the active ingredient into earthworms and the subsequent distribution. Consequently, the risk of nanoencapsulated bifenthrin to earthworms will be different from a conventional product. Whether nanoencapsulation increases, decreases or has no effect on risk is difficult to establish at this stage. While nanoencapsulated bifenthrin is taken up more quickly by the earthworms, from the L. terristris studies, it appears that the majority of the bifenthrin taken up is contained in the gut so the internalised concentration is lower than in earthworms exposed to the analytical grade substance and a conventional formulation. If less active ingredient is internalised, one would assume that less of the active ingredient will reach the site of toxic action so the effects of the nanoformulation will be lower. However, nanoencapsulation also increases the apparent persistence of the active ingredient which will lengthen the exposure duration of the earthworms to the active ingredient in the nanoformulation compared to a conventional ingredient. The increased efficacy of the nanoformulation compared to conventional formulations could mean that application rates to field are decreased which will also affect risk. The risks to birds and mammals feeding of earthworms could also be altered. If a nanoformulation is applied at the same rate as a conventional product then the oral exposure of these organisms will increase but differences in the bioaccessibility of nanoencapsulated bifenthrin compared to free bifenthrin could be lower meaning less is internalised. Again the duration of exposure will increase.

To answer some of these questions around the implications of changes in fate and uptake or effects, a toxicokinetic toxicodynamic modelling approach is probably required (Ashauer and Escher, 2010). In Figure 5, we present a conceptual model, based on our experimental findings and investigations into the performance of the different toxicokinetic modelling approaches, that could be used to model the toxicokinetics of a nanoencapsulated active ingredient. The model assumes sorption to soil is instantaneous following release of pesticide from the capsule and that it is the free (i.e. dissolved pore water) pesticide that is taken up into the earthworm tissue – this assumption is supported by our distribution studies in L. terrestris and the testing of Models 2 and 3. The internal concentration in the earthworm tissue over time, needed for toxicokinetic toxicodynamic modelling of the effects, are then calculated based on the release rate from the capsule, the soil-water distribution coefficient and the uptake and depuration rates of the free active ingredient into/out of the earthworm. To estimate oral exposure of birds and mammals, the mass concentration of the active ingredient in the gut also needs to be considered and this is estimated based on the feeding rate of the earthworm on whole soil and on the nanoparticles.

We believe that this conceptual model is a useful first step towards developing improved environmental risk assessment approaches for estimating the uptake and effects of nanoencapsulated pesticides in earthworms. The approach might also be applicable to other materials (e.g. nanoencapsulated pharmaceuticals) and other organisms. In the future, we recommend that the model be further parameterised for bifenthrin. We also recommend that studies of the type reported here are done on a wider range of organisms using other pesticide active ingredients with different persistence and physico-chemical properties contained in a wider range of nanocarrier materials in order to evaluate the broader applicability of the model.

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Data Availability

The experimental data on which this manuscript is based can be obtained, on request, from the corresponding author.

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Table 1: Sorption coefficients (k_d), dissipation half-lives (DT50), release half times (RT50), bioconcentration factors (BCFs) and rates for release, uptake and depuration (k) for the different bifenthrin treatments studied in the Eisenia fetida and L. terrestris studies.

Endpoint	Study	Unit	Bifenthrin	Capture LFR	Nano A	Nano B
k_d	Uptake and depuration	L Kg^{-1}	550 ± 21	394 ± 15	186 ± 4	233 ± 17
k_d	Distribution study E.fetida	L Kg^{-1}	494 ± 71	371 ± 47	154 ± 7	163 ± 9
k_d	Distribution study L. terrestris	L Kg^{-1}	585 ± 92	488 ± 32	274 ± 42	251 ± 31
DT_{50}	Uptake and depuration	d	25 - 27	33-35	49 - 50	38 - 40
Release rate (k_r)	Uptake and depuration	h^{-1}	NA	NA	0.104 ± 0.008	0.182 ± 0.016
RT_{50}	Uptake and depuration	d	NA	NA	6 - 7	3 - 4
k_{in} E.fetida	Uptake and depuration	$LKg^{-1}h^{-1}$	0.222 ± 0.009	NA	NA	NA
k_{out} E.fetida	Uptake and depuration	h^{-1}	0.0036 ± 0.0002	NA	NA	NA
BCF E.fetida	Uptake and depuration	-	61.7	NA	NA	NA
k_{in} L. terrestris	Uptake and depuration	$LKg^{-1}h^{-1}$	0.7021 ± 0.0336	NA	NA	NA
k_{out} L. terrestris	Uptake and depuration	h^{-1}	0.0033 ± 0.0003	NA	NA	NA
BCF L. terrestris	Uptake and depuration	-	212	NA	NA	NA

[1] Determined from data presented here with additional data on soil concentrations over time in four other soils (unpublished).

Figure Legends

Figure 1: Measured (dots) and predicted (lines) total internal concentration of different formulations of bifenthrin in E. fetida over time using Model 1 (black) and Model 2 (grey). Dotted lines indicate the 95% confidence interval for the predictions. Model parameterisation was conducted with data from bifenthrin a.i..

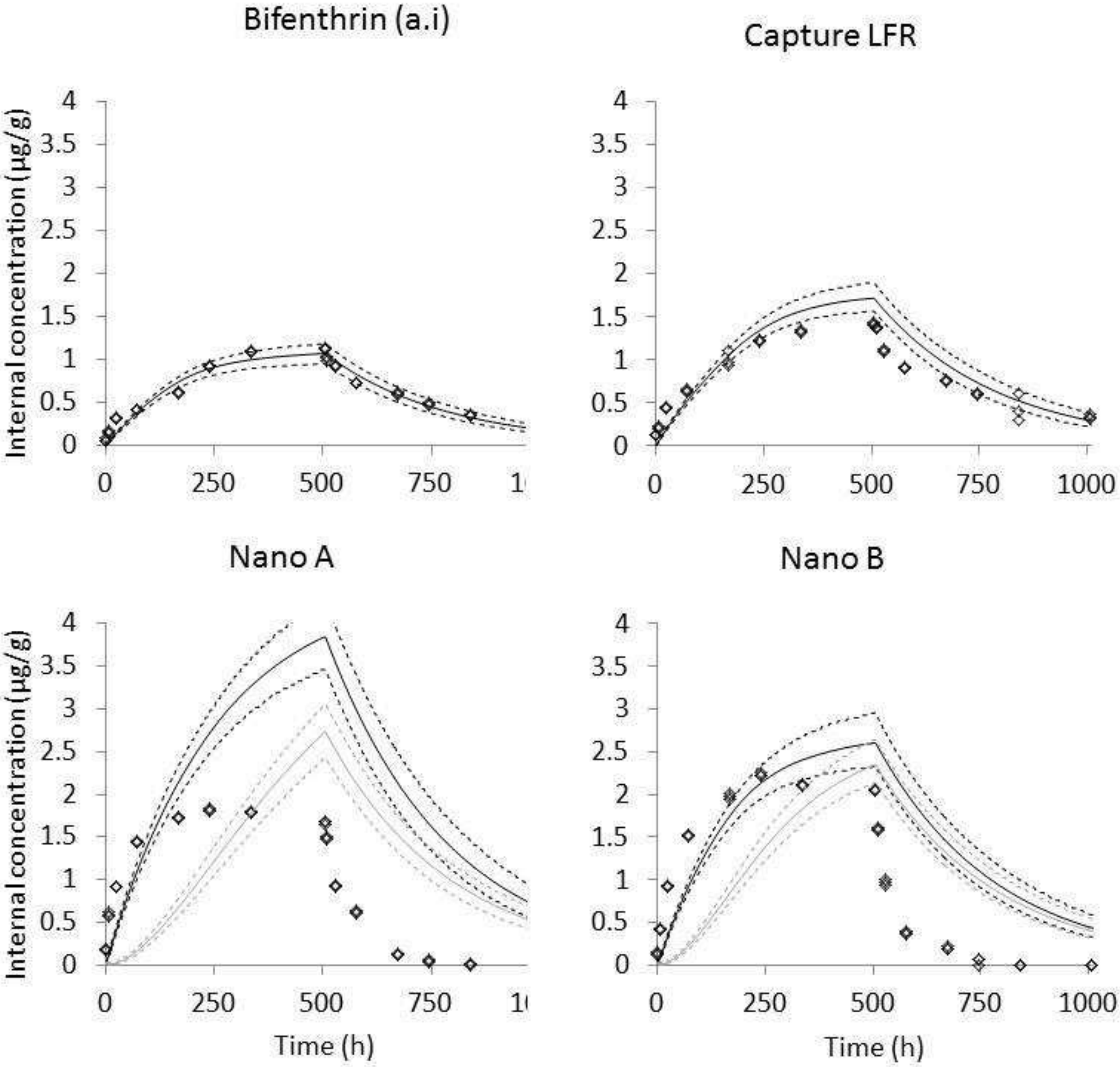
Figure 2: Proportion of total internal concentration of different formulations of bifenthrin in earthworms after 10 d uptake and 7 d depuration as average ± SD in relation to the internal concentration at the end of the uptake phase.

Figure 3: Proportion of the gut concentration at the end of the uptake phase of different formulations of bifenthrin recovered from L. terrestris faeces samples as average ± SD.

Figure 4: Measurements (dots) and predictions from different model combinations (lines) of internal concentration of different formulations of bifenthrin in whole organism and different compartments of L. terrestris over time. Grey backgrounds indicate that predictions account for the released fraction of bifenthrin from the nanoformulation. Dotted lines indicate the 95% confidence intervals for the predictions.

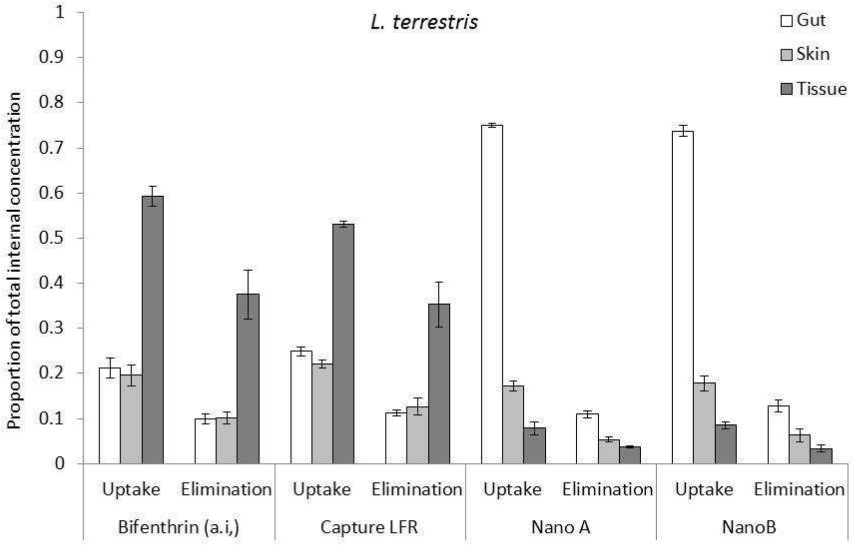
Figure 5: Conceptual model for estimating residues of active ingredients contained in nanoencapsulated formulations in terrestrial invertebrates over time.

Figure 1



Bifenthrin (a.i)
Capture LFR
Nano A
Nano B
Internal concentration (µg/g)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
250
500
750
1000
Time (h)

Figure 2


L. terrestris
Gut
Skin
Tissue
Proportion of total internal concentration
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Uptake
Elimination
Bifenthrin (a.i,)
Capture LFR
Nano A
NanoB

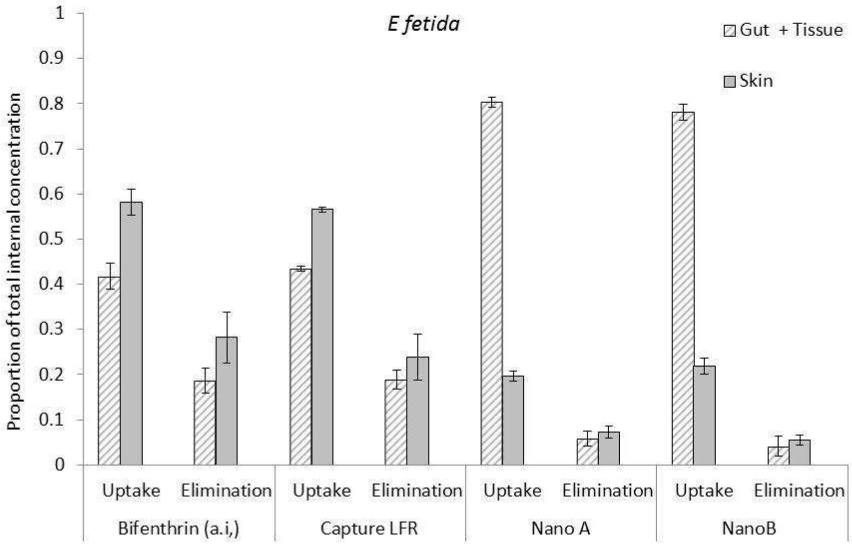

E fetida
Gut + Tissue
Skin
Proportion of total internal concentration
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Uptake
Elimination
Bifenthrin (a.i,)
Capture LFR
Nano A
NanoB

Figure 3

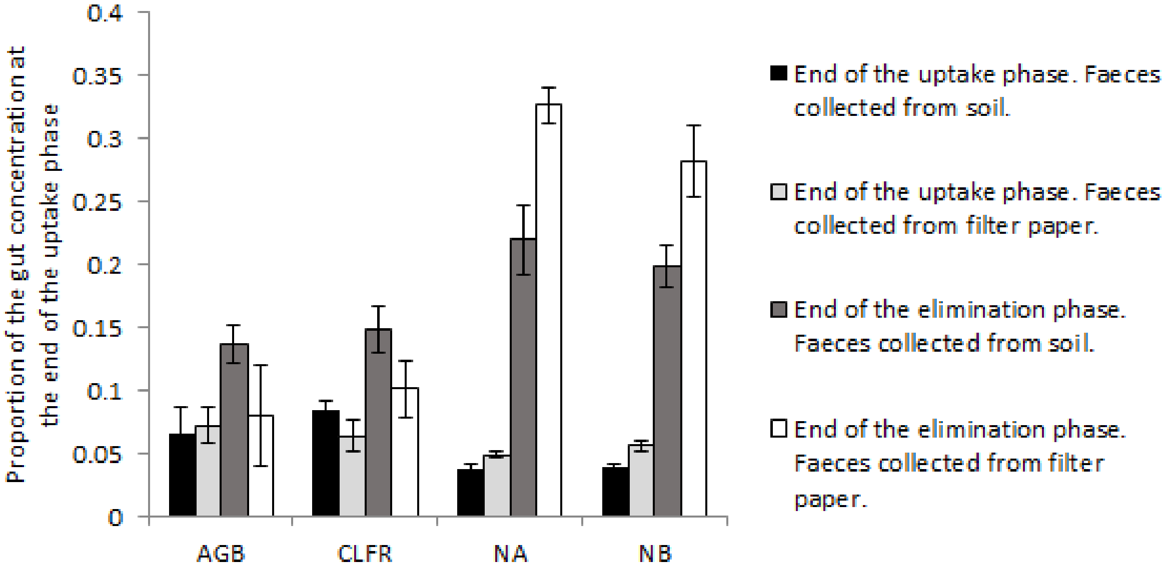

Proportion of the gut concentration at the end of the uptake phase
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
AGB
CLFR
NA
NB
■ End of the uptake phase. Faeces collected from soil.
□ End of the uptake phase. Faeces collected from filter paper.
▨ End of the elimination phase. Faeces collected from soil.
□ End of the elimination phase. Faeces collected from filter paper.

Figure 4

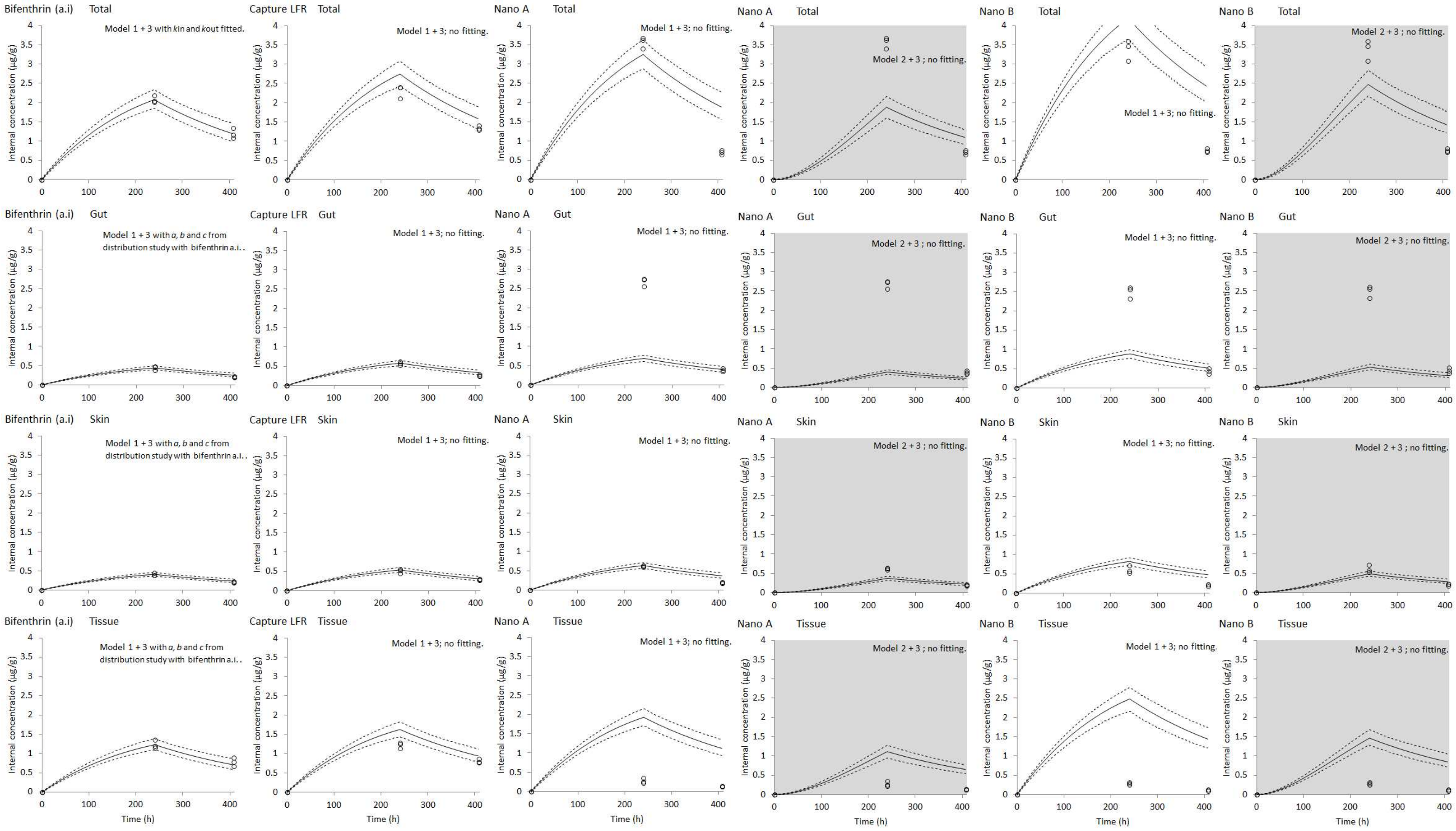
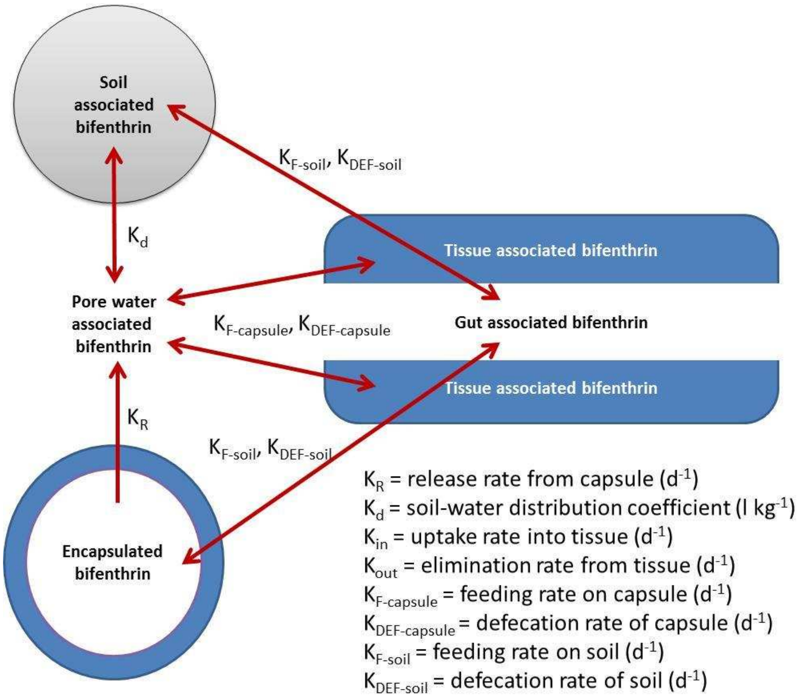

Bifenthrin (a.i) Total
Model 1 + 3 with kin and kout fitted.
Capture LFR Total
Model 1 + 3; no fitting.
Nano A Total
Model 1 + 3; no fitting.
Nano A Total
Model 2 + 3 ; no fitting.
Nano B Total
Model 1 + 3; no fitting.
Nano B Total
Model 2 + 3 ; no fitting.
Bifenthrin (a.i) Gut
Model 1 + 3 with a, b and c from distribution study with bifenthrin a.i..
Capture LFR Gut
Model 1 + 3; no fitting.
Nano A Gut
Model 1 + 3; no fitting.
Nano A Gut
Model 2 + 3 ; no fitting.
Nano B Gut
Model 1 + 3; no fitting.
Nano B Gut
Model 2 + 3 ; no fitting.
Bifenthrin (a.i) Skin
Model 1 + 3 with a, b and c from distribution study with bifenthrin a.i..
Capture LFR Skin
Model 1 + 3; no fitting.
Nano A Skin
Model 1 + 3; no fitting.
Nano A Skin
Model 2 + 3 ; no fitting.
Nano B Skin
Model 1 + 3; no fitting.
Nano B Skin
Model 2 + 3 ; no fitting.
Bifenthrin (a.i) Tissue
Model 1 + 3 with a, b and c from distribution study with bifenthrin a.i..
Capture LFR Tissue
Model 1 + 3; no fitting.
Nano A Tissue
Model 1 + 3; no fitting.
Nano A Tissue
Model 2 + 3 ; no fitting.
Nano B Tissue
Model 1 + 3; no fitting.
Nano B Tissue
Model 2 + 3 ; no fitting.
Internal concentration (µg/g)
0
0.5
1
1.5
2
2.5
3
3.5
4
0
100
200
300
400
Time (h)

Figure 5.



K_R = release rate from capsule (d^{-1})
K_d = soil-water distribution coefficient ($l\ kg^{-1}$)
K_{in} = uptake rate into tissue (d^{-1})
K_{out} = elimination rate from tissue (d^{-1})
$K_{F\text{-capsule}}$ = feeding rate on capsule (d^{-1})
$K_{DEF\text{-capsule}}$ = defecation rate of capsule (d^{-1})
$K_{F\text{-soil}}$ = feeding rate on soil (d^{-1})
$K_{DEF\text{-soil}}$ = defecation rate of soil (d^{-1})