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Time-related survival effects of two gluconasturtiin hydrolysis products on the terrestrial isopod *Porcellio scaber*

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HIGHLIGHTS

- 2-Phenylethyl ITC and 3-proprionitrile have toxic effects on *Porcellio scaber*.
- Effects on survival could be observed after only a few days of exposure.
- Two mechanisms affect survival simultaneously; internal concentration and damage.
- The two compounds have the same effect patterns shown using a TKTD approach.
- The TKTD approach accounts for fast dissipation of the natural toxins from soil.

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ABSTRACT

Glucosinolates are compounds produced by commercial crops which can hydrolyse in a range of natural toxins that may exert detrimental effects on beneficial soil organisms. This study examined the effects of 2-phenylethyl isothiocyanate and 3-phenylpropionitrile on the survival and growth of the woodlouse *Porcellio scaber* exposed for 28 d. 2-Phenylethyl isothiocyanate dissipated from the soil with half-lives ranging from 19 to 96 h. Exposure through soil showed toxic effects only on survival. The LC50s after 28 d were significantly different at 65.3 mg kg^{-1} for 2-phenylethyl isothiocyanate and 155 mg kg^{-1} for 3-phenylpropionitrile. A toxicokinetic–toxicodynamic (TKTD) approach, however, revealed that both compounds in fact have very similar effect patterns. The TKTD model was better suited to interpret the survival data than descriptive dose–response analysis (LC_x), accounting for the fast dissipation of the compounds in the soil. Found effects were within environmentally relevant concentrations. Care should therefore be taken before allowing these natural toxins to enter soil ecosystems in large quantities.

1. Introduction

Litter decomposition is one of the most important processes for proper soil ecosystem functioning and depends on the physiochemical soil environment, litter quality and detritivore community composition. Litter-feeding soil fauna, e.g. millipedes and isopods, have extensive impact on decomposition by performing essential 'ecological services' such as litter fragmentation (Wardle, 2002; Hattenschwiler et al., 2005). Certain toxic compounds, such as abamectin and doramectin, are known for their adverse effects on litter decomposers (Hornung et al., 1998; Kolar et al., 2008). Most ecotoxicological studies focus on anthropogenic contaminants. Toxins are, however, also synthesized by organisms, so-called natural toxins. Examples can be found in various organisms such as mycotoxins originating from fungi (Bennett and Klich, 2003) or plant derived phytoanticipins or phytoalexins (Morant et al., 2008).

Glucosinolates (GSLs) are sulphated aldoximes (aldoxime-N-sulphates), derived from amino acids and are compounds which can hydrolyse in a range of natural toxins. They are found in many *Brassica* species, including agriculturally important plants such as broccoli, oilseed rape and the scientifically important model *Arabidopsis thaliana* (Wittstock and Halkier, 2002; Traka and Mithen, 2009). Evolved as anti-herbivore defence mechanism, glucosinolates are hydrolysed by the enzyme myrosinase (β-thioglucoside glucohydrolases) upon tissue disruption, such as chewing by herbivores (Wittstock and Halkier, 2002; Morant et al., 2008; Bednarek and Osbourn, 2009). After forming unstable aglucones,

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a spontaneous rearrangement occurs into various metabolites, such as isothiocyanates (ITCs) (Wittstock et al., 2003; Bones and Rossiter, 2006; Morant et al., 2008). Although ITCs are the predominantly formed metabolites, various *Brassica* species are also able to produce simple nitriles, epithionitriles or organic thiocyanates instead of ITC, especially when protein factors such as nitrile-specifier proteins (NSPs) and epithiospecifier proteins (ESPs) are present (Wittstock et al., 2003). Simple nitriles can also be formed in the absence of ESP under conditions of low pH values (<pH 5) or high concentrations of iron ions (Wittstock and Burow, 2007).

ITCs are the most common products following hydrolysis of GSL and their toxic effects have been extensively studied. These compounds have detrimental effects on a wide range of organisms, including bacteria, fungi and invertebrates (reviewed in: Wittstock et al. (2003)). For example, 11 μM 2-phenylethyl ITC caused 50% mortality of the population of the root-knot nematode *Meloidogyne incognita* (Lazzeri et al., 2004). In like manner, species that do not directly interact with the plants (non-target species) can be affected. For example, 65 nmol benzyl ITC per gram soil caused a 50% reduction in reproduction of the non-target soil arthropod, *Folsomia fimetaria* (Jensen et al., 2010). However, less is known about effects of simple nitriles on invertebrates and even fewer studies have directly compared the effects of different hydrolysis products derived from the same glucosinolate (Wittstock et al., 2003; Wittstock and Burow, 2010). In general, nitriles are considered to be less toxic than ITC (Wittstock et al., 2003, Table 5.4) as was for instance shown for *Caenorhabditis elegans*, where the ITC was almost 100 times more toxic than the nitrile (Donkin et al., 1995).

The aim of this study was to investigate the effects of two hydrolysis products of the GSL gluconasturtiin, 2-phenylethyl GSL, on the survival and growth of the terrestrial isopod *Porcellio scaber*. This root GSL is studied especially for its ITC hydrolysis product which is a potential anti-cancer agent (Jeffery and Araya, 2009; Traka and Mithen, 2009). Isopods are macro-detritrivores and occur abundantly in various ecosystems and habitats (Drobne, 1997). Their litter fragmentation activity causes an increased moisture retention ability of litter, which favours microbial growth and results in enhanced nutrient mineralisation (Bradford et al., 2002; Wardle, 2002). Moreover, isopods such as *P. scaber* are recognised as useful test organisms for the characterisation of chemical toxicity (Drobne, 1997; Hornung et al., 1998; Kolar et al., 2010). 2-Phenylethyl isothiocyanate and 3-phenylpropionitrile were investigated individually. Lethal (mortality) and sub-lethal (growth) effects were measured through time and analysed using dose–response curves and a toxicokinetic–toxicodynamic (TKTD) model derived from the general unified threshold model for survival (GUTS, Jager et al., 2011). To investigate which route of exposure was most important, exposure was investigated using both contaminated soil and contaminated food. As ITCs are reported to be readily biodegradable (Brown and Morra, 1997; Jensen et al., 2010) concentrations of 2-phenylethyl ITC in the soil were measured over time. This is the first study comparing effects of different hydrolysis products derived from the same glucosinolate on a beneficial non-target soil invertebrate.

2. Material and methods

2.1. Animals

The common litter fragmenter, the woodlouse *P. scaber* (Hopkin, 1991), was used in the present study. Lab-cultured first generation of *P. scaber* specimens were used, originating from two field locations (courtesy of C.A.M. van Gestel): parental individuals for the soil exposure experiments were sampled at an allotment garden nearby Utrecht in the Netherlands and parental individuals for the food exposure experiments were retrieved from a compost heap in a garden near Utrecht. Lab-cultured animals were kept in climate chambers at 20 °C, 75% relative humidity and a 12:12 h light: dark regime. Housing was provided in glass aquaria with a layer of plaster of Paris, moistened potting soil, with frequently replenished leaf litter (mainly *Populus sp.* and *Acer sp.* leaves) and dry cat food as food source. Test animals had a body length ⩾15 mm and a body weight of 20–30 mg. The sex ratio of female:male was 6:4 for all experiments. Pregnant females were excluded from tests.

2.2. Experimental soil, compounds and spiking

LUFA 2.2 soil (Speyer, Germany) was used for all experiments, which has a pH-value (±SD) of 5.5 ± 0.1 and an organic C content (in %) of 2.09 ± 0.40. The soil was dried at 60 °C for 24 h before usage. 2-Phenylethyl ITC [CAS: 2257-09-2] and 3-phenylpropionitrile [CAS: 645-59-0] were obtained from Sigma Aldrich (www.sigmaaldrich.com) as a liquid solution (⩾99% pure). The compounds were spiked into the soil or food using acetone as a solvent using 1 mL acetone for each g dry weight (DW) soil (Brinch et al., 2002). Ten percent of the total amount of soil or food needed for each treatment was spiked with the desired concentration, shaken thoroughly and incubated for 24 h in preservation jars. Afterwards, jars were left open overnight under a fume hood to facilitate evaporation of the acetone, thereafter the remaining 90% of the total soil or food was added, mixed together thoroughly. Finally, the soil was moistened to 50% of the water holding capacity (WHC) of 45.2%, corresponding to 22% water of the soil DW. Food was moistened to 50% moisture relative to DW. For the soil exposure tests the soil was spiked only once, at the start of the experiment. For the food experiment, at the start of every week, freshly spiked (or non-spiked) food was made and used during one week, as the food was prone to fungal growth very quickly. Spiked food was kept in dry condition and was only moistened when needed for feeding.

2.3. Measuring dissipation in soil

In a separate experiment, without the presence of *P. scaber*, the rate of dissipation of 2-phenylethyl ITC from the soil was measured for three concentrations falling within the ecotoxicological test range: 25, 100 and 400 mg kg^{-1} soil. From the spiked soils 5 g samples were taken at time 0 (hydrated soil) and 1, 3, 5, 24, 48, 72, 168 h after initial spiking. The samples were extracted by adding 5 mL ethyl acetate and 100 μL benzyl ITC solution (500 μmol L^{-1} in ethyl acetate) as analytical internal standard (IS) to the samples which were then stored at −18 °C in darkness. Prior to analysis, samples were vortexed and the ethyl acetate phase was filtered and dried using Pasteur pipettes packed with quartz wool (inactivated, silica treated) and 2.0 g anhydrous Na_2SO_4. This procedure was repeated with an additional 5 mL of ethyl acetate added to the initial soil. The eluate was then evaporated with nitrogen to nearly dryness (less than 200 μL), transferred to GC-vials, and later analysed by gas chromatography tandem mass spectrometry as described in Van Ommen Kloeke et al. (2012). Calibration curves of both benzyl and 2-phenylethyl ITC were used to calculate the factual concentrations of 2-phenylethyl ITC present in the soil samples. First-order degradation kinetics was assumed to estimate the dissipation half-lives, using SPSS 15.0. Dissipation data for the concentration 3.28 mg kg^{-1} (20.1 nmol kg^{-1}), measured in a former GC–MS experiment (Van Ommen Kloeke et al., 2012), was added to complement the dataset needed for an integrated model analysis with the general unified threshold model for survival (GUTS, see below).

2.4. Ecotoxicological experiments

Two tests were performed for each compound: exposure through soil and exposure through food. The method for ecotoxicological testing on *P. scaber* of Hornung and co-authors (1998) was used to determine effects on the survival and growth. Treatments for both exposure tests were 3.9, 15.6, 62.5, 250 and 1000 mg kg^{-1} DW complemented by a normal control (C) using only LUFA 2.2 soil or food and an acetone control (AC) with LUFA 2.2 soil or food spiked with only acetone. All treatments comprised five biological replicates. Each replicate, consisting of 150 g moist soil and ten isopods, was kept in 600 mL glass jars and incubated at 20 °C, 75% relative humidity in a 12:12 h light: dark regime. Test food consisted of *Populus sp.* leaves, commercial rabbit food and potato powder ground together in a 50-40-10% ratio (Hornung et al., 1998). Before usage, leaf material was dried overnight at 60 °C, after which it was frozen for a day at −20 °C. Food was presented *ad libitum* in small plastic dishes (18 mm diameter) and refreshed three times a week. To maintain 50% WHC of the soil, moisture content of the soil was checked once a week and replenished when needed. For shelter three moistened pieces of roof tile were put on top of the soil surface. Survival was checked three times a week and dead individuals were removed from the jar. The mass of all living individuals was measured per replicate once a week on a microbalance.

2.5. Dose response curves

Median and 10% lethal effect concentration (LC50 and LC10, respectively) values were calculated using the log–logistic response model after Haanstra et al. (1985):

$$Y(c) = \frac{Y_{\max}}{1 + \frac{x}{(100-x)}\left(\frac{c}{LC_x}\right)^b}$$

In which Y is the percentage of survival as a function of the concentration c. $Y_{\max}$ is the estimated survival in the untreated control, LC_x is the estimated concentration for the selected percentage of effect x (here either 10 or 50) and b the slope parameter of the dose–response curve. SPSS 15.0 was used to fit the model by least–squares analysis. To investigate if the LC values differed significantly between the two compounds, a generalised likelihood ratio test was performed (Sokal and Rohlf, 1995).

2.6. Time course modelling - GUTS

To analyse the time course of the toxic effects on the organisms, the survival data were further analysed using the GUTS model (Jager et al., 2011) with the assumption of stochastic death. This toxicokinetic–toxicodynamic (TKTD) framework makes use of all data for survival over time and also allows accounting for the dissipation of the test compounds (see Fig. 1). The simplest model in which hazard is linked to a single mechanism based on scaled internal concentration, was not able to fit the data satisfactorily, i.e. the single mechanism model was not able to capture the individual treatments. The slowly building up of the effects at low concentrations could not be reconciled with the rapid and strong effects at the highest doses (see *Supporting* information, Fig. S1). Therefore, a second mechanism was added using a stage of damage. The scaled damage is calculated from the scaled internal concentration, making the damage double scaled, i.e. it obtained the dimensions of an external concentration (see Jager et al., 2011).

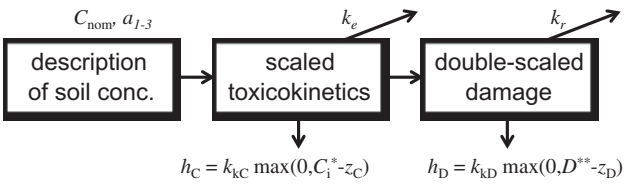

Fig. 1. Schematic representation of the model used to analyse the survival data for *Porcellio scaber* exposed to two gluconasturtiin hydrolysis products in LUFA 2.2 soil. The soil concentration over time is described empirically with three coefficients (a_1–a_3) and the nominal concentration (C_{nom}). This concentration is used to calculate the scaled internal concentration C_i^*, which links to a hazard rate (h_C). The scaled internal concentration is subsequently used to calculate a double-scaled damage (D^{**}), also linking to a hazard rate (h_D). The parameters k_e and k_r describe the two rate constants, z_C and z_D two thresholds and k_{kC} and k_{kD} two killing rates. Model parameters are explained in Table 2.

Consequently, GUTS was adapted to account for two hazard rates for toxicant effects; one calculated from the scaled internal concentration and one from the damage stage. Two mechanisms of toxicity were thus assumed: one related to the body residues and, linked to that, another related to the damage caused by the body residue. Apart from the fact that the body residues determine the damage levels, the two causes of death are assumed to be independent, so their associated hazard rates can be added.

This resulted in two rate constants (k_e and k_r), two thresholds (z_C and z_D) and two killing rates (k_{kC} and k_{kD}). Adding a Weibull function for background mortality (S_b), which increased slightly in time, improved the fit to the data. An accurate description of the background mortality can help to identify deviations from the control:

$$S_b = \exp(-(h_b t)^F)$$

In which h_b is the hazard rate in the control, t is time and F is the shape coefficient.

Overall survival was calculated by multiplying S_b with the survival fractions calculated from the sum of the hazards due to the chemical. Finally, the model was fit to the survival data for both compounds simultaneously using maximum likelihood estimation (Jager et al., 2011), and confidence intervals were calculated using the profile likelihood (Meeker and Escobar, 1995).

As the model accounts for dissipation of the test compounds, the analysis of the survival data required an adequate description of the actual exposure concentration over time. The measured concentrations of 2-phenylethyl ITC in LUFA 2.2 soil over time did, however, not match the nominal test concentrations. Therefore, the actual soil concentration at each time point was estimated using a model fit on the measured dissipation data. As a consequence, the dissipation rate constant (k_d) decreased with increasing initial concentration in soil (C_0), which could be described with a log-linear function:

$$k_d = a_1 - a_2 \log C_0$$

The initial concentration is assumed to be a fixed fraction of the nominal concentration (C_{nom}):

$$C_0 = a_3 C_{nom}$$

The three coefficients a_1–a_3 were estimated from the complete set of measured dissipation data, four concentrations combined, using maximum likelihood estimation and assuming independent and normally distributed errors after log transformation. Confidence intervals were calculated by profiling the likelihood function (Meeker and Escobar, 1995). The best estimates were used in the survival analysis for both compounds, as there is no dissipation data available for 3-phenylproprionitrile. The complete set of equations of the model can be found in the supporting information.

Alternatively to stochastic death, the GUTS framework can use individual tolerance as a limit case (Supporting information, Fig. S2). The current data set is, however, not strong enough to distinguish between stochastic death and individual tolerance.

3. Results and discussion

3.1. Dissipation of 2-phenylethyl ITC in soil

At the start of the experiment ($t = 0$), recovery rates of 2-phenylethyl ITC varied between 69.7% and 100%. 2-Phenylethyl ITC dissipated rapidly in natural LUFA 2.2 soil, showing an exponential decrease over time (Fig. 2). Half-lives were 19.4 (±std error (SE): 1.86) h for 3.28 mg kg^{-1}: 32.5 (±2.33) h for 25 mg kg^{-1}: 60.9 (±4.73) h for 100 mg kg^{-1} and 95.8 (±12.5) h for 400 mg kg^{-1} soil. Rate constants decreased with increasing initial concentration, giving rise to four different dissipation models. To be able to use the dissipation data for the GUTS model (at different initial concentrations) the dissipation data was fitted into a single descriptive model (see Section 2.6). The resulting fit is shown in Fig. 2, and the parameter estimates (with corresponding 95% confidence intervals) were $a_1 = 0.932$ (0.868–0.997), $a_2 = 0.144$ (0.129–0.159), and $a_3 = 0.729$ (0.663–0.803).

These dissipation patterns of 2-phenylethyl ITC in natural soil can also be found for lower concentration ranges. For instance, concentrations ranging between 0.5 and 3.28 mg kg^{-1} soil showed half-lives of around 16 h, depending on the initial concentration (Van Ommen Kloeke et al., 2012). Additionally, benzyl ITC, which has a chemical structure similar to 2-phenylethyl ITC, showed similar dissipation patterns in non-sterile or natural soil (Gimsing et al., 2009; Jensen et al., 2010), while in sterile soils the dissipation of benzyl ITC was much slower. Microbial degradation is therefore likely the main driver responsible for the natural dissipation process of ITCs (Gimsing et al., 2009).

3.2. Toxic effects through food exposure

Exposure via food did not affect survival or growth of *P. scaber*. The highest death rate was found for 3-phenylproprionitrile at the lowest concentration of 3.9 mg kg^{-1} soil with 58% (±SE 15.9) survival. Glucosinolate hydrolysis products are known for their particular odour and taste (Fahey et al., 2001; Halkier and Gershenzon, 2006). It is therefore very likely that *P. scaber* was able to detect the compounds and avoided the spiked food at higher concentrations.

3.3. Toxic effects through soil exposure

Growth was measured throughout all the experiments, but did not show any coherent pattern and was therefore excluded from further analyses.

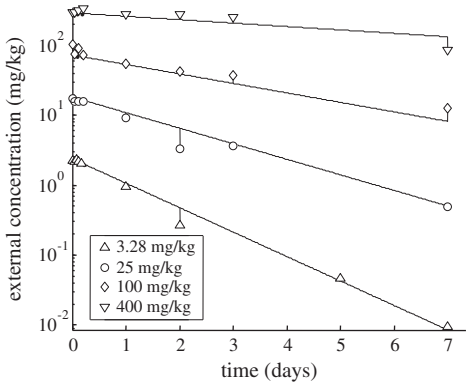

Fig. 2. Dissipation of 2-phenylethyl ITC (mg kg^{-1} soil) as a function of time (days) in moist LUFA 2.2 soil at 20 °C for four different starting concentrations. Model curves represent the simultaneous fit of an empirical model (see main text).

3.3.1. Log–logistic response modelling

Dose–response curves of the effects of 2-phenylethyl ITC and 3-phenylpropionitrile on survival of *P. scaber* after 7 d and 28 d exposure are presented in Fig. 3. On average, adult survival of *P. scaber* for the control (C) in the 2-phenylethyl ITC test was (±SE) 88 ± 3.7% and 80 ± 5.5% for the acetone control (AC) group, after 28 d of exposure. Adult survival of *P. scaber* for C in the 3-phenylpropionitrile test was on average 80 ± 4.5% and 88 ± 3.7% for AC, after 28 d of exposure. There were no significant differences between the C and AC group for each compound; $p = 0.262$ for to 2-phenylethyl ITC and $p = 0.207$ for 3-phenylpropionitrile. Moreover, C and AC did not differ between the two compounds.

Both 2-phenylethyl ITC and 3-phenylpropionitrile proved to be toxic for *P. scaber* when exposed through the soil (Fig. 3), with a mortality of 100% at 1000 mg kg^{-1} soil. Lethal concentrations (based on nominal concentrations) were calculated after 7 and 28 d and are shown in Table 1. For 2-phenylethyl ITC, the LC_x estimates differed substantially when calculated after 7 or 28 d, with higher LC_x estimates after 7 d. To be able to understand this difference in the LC_x estimates, the data was further analysed using time-course modelling (see below).

Overall, *P. scaber* seems relatively resilient to both hydrolysis products compared to other beneficial soil invertebrates. For instance the collembolan *Folsomia candida* and *Protaphorura fimata* showed an LC50 estimate of 2.51–2.48 mg kg^{-1} soil, more than 25 times lower, when exposed to 2-phenylethyl ITC after 28 d of exposure (Van Ommen Kloeke et al., 2012). The toxicity of 2-phenylethyl ITC to *P. scaber* is comparable to that of other natural toxins. For instance abamectin, a natural fermentation product of soil bacteria and known antiparasitic veterinary medicine, showed a LC50 of 71 mg kg^{-1} soil for *P. scaber* after 21 d of exposure (Kolar et al., 2010). Abamectin also rapidly degrades in soil partly due to photodegradation (Wislocki et al., 1989). Furthermore, both 2-phenylethyl ITC and 3-proprionitrile are known to cause inhibition of

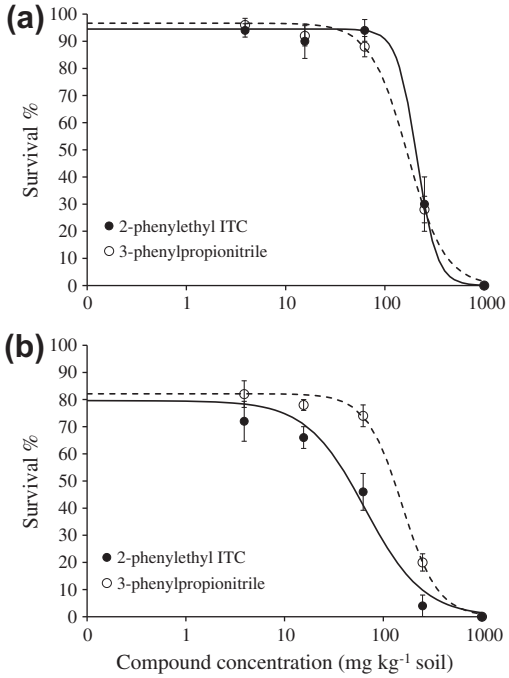

Fig. 3. Effects of 2-phenylethyl isothiocyanate and 3-phenylpropionitrile (nominal concentrations) on the survival of *Porcellio scaber* after: (a) 7 d and (b) 28 d exposure in LUFA 2.2 soil. Lines show the fit of the log–logistic dose response model to the data with continuous line: fit to 2-phenylethyl ITC and dashed line: fit to 3-proprionitrile. Error bars are standard errors ($n = 5$).

Table 1
LC-values for the effects of to 2-phenylethyl isothiocyanate and 3-phenylpropionitrile on survival of *Porcellio scaber* after 28 d exposure in LUFA 2.2 soil. Values are in µg compound per gram DW soil (nominal concentrations).

		2-Phenylethyl ITC	CI	3-Phenylpropionitrile	CI	χ^2
7 d	LC10	126	(0–431)	65.5	(42.7–88.3)	1.85
	LC50	210	(75.8–343)	169	(145–194)	1.79
28 d	LC10	14.6	(2.16–27.0)	62.0	(34.1–89.9)	5.00
	LC50	65.3	(42.0–88.7)	155	(123–188)	18.9

CI = 95% confidence interval. LC_x differences between 2-phenylethyl isothiocyanate and 3-phenylpropionitrile are deemed significant ($p < 0.05$) if $\chi^2 > 3.84$.

soil nitrifying bacteria communities with 2-phenylethyl ITC being most toxic (Bending and Lincoln, 2000). For 3-phenylpropionitrile no other toxicity data with regard to invertebrates or other soil organisms is known.

Comparing the toxicity of 2-phenylethyl ITC versus 3-phenylpropionitrile at the two different time points gave very different results. LC_x estimates calculated after 7 d of exposure did not significantly differ between 2-phenylethyl ITC and the 3-phenylpropionitrile while there was a significant difference in toxicity of the two compounds after 28 d, with the ITC being more toxic (Table 1). The majority of other studies did show a difference in toxicity between corresponding ITCs and nitriles (same precursor glucosinolate) with ITC being more toxic than the nitrile (reviewed in Wittstock et al., 2003). The results presented here are therefore only partly consistent with previous studies. The deviating effects of the different hydrolysis products depend, however, on species and exposure method (Wittstock et al., 2003).

3.3.2. Time course modelling – GUTS

LC_x estimates may be less relevant as measures of toxicity in studies experiencing rapid loss of the compound from the soil, as observed in this study (Fig. 2). Descriptive dose–response models are of limited use in handling time-varying exposure concentrations. The resulting LC_x is only a description for this particular situation (single dose, this dissipation pattern, and this exposure duration); extrapolation to other scenarios or comparison to other compounds (with different dissipation kinetics) is therefore impossible. The GUTS-based survival model is a TKTD approach and thus allows for the analysis of the survival data, accounting for the fact that the external concentrations were not constant (Fig. 4). The complete set of survival data for the two chemicals was estimated with a single parameter set (model parameters are given in Table 2 with their confidence intervals) as the survival patterns were actually very similar. Toxic effects of both compounds were especially rapid at the highest test concentration, resulting in death to all individuals in less than 2 d.

It is likely that two mechanisms are responsible for the toxicity of the compounds, i.e. the large effect of the two highest concentrations could not be reconciled with smaller effects at lower doses. To stay close to the GUTS model, a stage of damage was added with effects being linked both to the internal concentration and to the damage level (Fig. 1). However, the damage dynamics were very fast in this case, which implies that damage closely follows the internal concentration, or perhaps that the internal concentration itself affects two target sites. A possible justification for the existence of two mechanisms might thus be that there are two receptors for the compound, each producing a different pattern of effect. Alternatively, the parent compound might be metabolised into a transformation product that increases the probability of death through a different mechanism. The current data set is insufficient to explore these possibilities further. The most appropriate choice of mechanism requires a more dedicated study. The possible existence of a two-mechanism effect is not uncommon and was for instance also proposed for *F. candida* exposed to chlorpyrifos via food (Jager et al., 2007).

The same model parameters were used to fit the data for both compounds, assuming that the two compounds have similar effects on survival. This is in accordance with the logistic response modelling when using data for 7 d of exposure. As can be seen from Fig. 4, any possible difference in the effects on survival between the two compounds is largely driven by the difference in response at 62.5 mg kg^{-1} in the last week of exposure. The same diverting data points are also responsible for the different LC_x estimates found for 2-phenylethyl ITC calculated after 7 and 28 d.

At the moment it is unclear whether the two compounds really exert a comparable effect. The current data set does not allow a clear distinction, since soil concentrations or dissipation rates for 3-phenylproprionitrile were not available. However, the GUTS modelling does show the shortcomings of the descriptive dose–response modelling that is generally used in ecotoxicological studies. When the exposure concentration decreases during the test, LC_x estimates based on nominal concentrations will underestimate

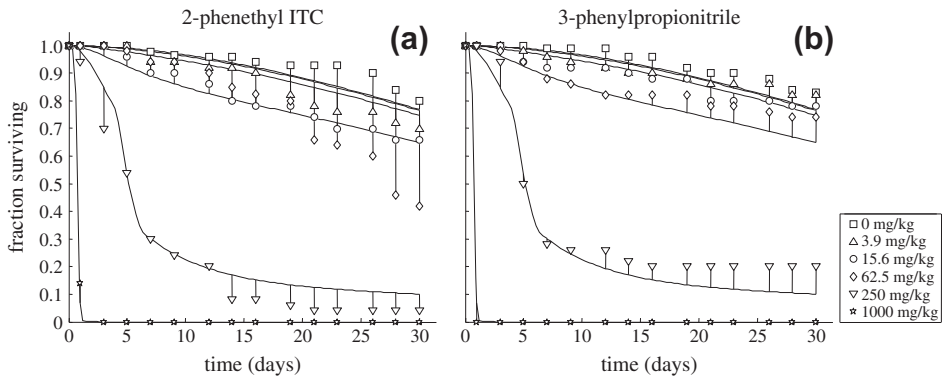

Fig. 4. Fit of the hazard model from GUTS (Fig. 1) to the observed data of the survival of *Porcellio scaber* over time upon exposure to: (a) 2-phenylethyl ITC and (b) 3-proprionitrile in LUFA 2.2 soil. The model is simultaneously fit to the data for both compounds. Parameter estimates are provided in Table 2. The legend provides nominal concentrations; calculations were performed using the estimated soil concentrations based on the fit in Fig. 2.

Table 2
Estimated model parameters of the fit (with 95% confidence intervals) to the data of the effect of 2-phenylethyl ITC and 3-proprionitrile on the survival of *Porcellio scaber* in LUFA 2.2 soil. See Fig. 4 for the corresponding model fits.

Parameter	Estimate (95% confidence)	Unit
Blank hazard rate (h_b)	0.0161 (0.0132–0.0189)	d^{-1}
Weibull factor (F)	1.84 (1.50–2.26)	(−)
Elimination rate body residue (k_e)	0.212 (0.184–0.250)	d^{-1}
Threshold for body residue (z_C)	70.0 (65.5–75.2)	$mg\ kg_{soil}^{-1}$
Killing rate for body residues (k_{kC})	0.136 (0.0892–0.200)	$kg_{soil}\ mg^{-1}\ d^{-1}$
Repair rate for damage (k_r)	9.76 (1.06–10)[a]	d^{-1}
Threshold for damage (z_D)	0 (0–4.50)	$mg\ kg_{soil}^{-1}$
Killing rate for damage (k_{kD})	0.00143 (0.00101–0.00195)	$kg_{soil}\ mg^{-1}\ d^{-1}$

[a] The repair rate of 10 per day is the maximum allowed in the analysis for numerical reasons. This value indicates very rapid equilibration of damage (damage kinetics thus follow the kinetics of the body residue).

the toxicity of the compound. Therefore, a TKTD approach should be preferred in these situations. However, even when exposure is constant, there are compelling reasons to prefer TKTD modelling (Ashauer and Escher, 2010; Jager et al., 2011). TKTK modelling is limited to situations where survival is followed over time (and preferably also the exposure concentrations), which limits its applicability. Even though the number of parameters is quite large (Table 2), the model fit describes all of the effects data over time, where the descriptive dose response would require three parameters per observation time. Furthermore, the GUTS parameters in principle allow extrapolation to other exposure scenarios such as pulsed exposures (Ashauer and Escher, 2010). However, in this particular case, the complexity of the mechanism (Fig. 1), and the number of parameters needed (Table 2, and a_1–a_3), does not seem to provide a solid basis for extrapolation.

In any case, ecotoxicological research should include environmental dissipation data in order to get a more realistic understanding of the acute or chronic effects of highly degradable compounds.

3.4. General discussion

The aim of this study was to demonstrate the toxic effects of two hydrolysis products of gluconasturtiin on the beneficial litter fragmenter *P. scaber*. These natural toxins clearly showed lethal effects on this isopod after only a few days of exposure to contaminated soil. ITC toxicity is expected to be due to irreversible and nonspecific reactions of the compounds with proteins and amino acids, which result in inactivation of enzymes (Brown and Morra, 1997). Toxic effects of nitriles are likely related to the cyano group, inactivating especially enzyme systems involved in cellular respiration such as cytochrome oxidase (Brown and Morra, 1997). Survival patterns for both compounds looked very similar for *P. scaber*. GUTS modelling gave more insight in the toxic effects through time and accounted for the fast dissipation rates of the compounds. These patterns suggest the presence of two effect mechanisms, operating in different concentration ranges. Thus, care has to be taken when extrapolating effects from short-term, high-exposure tests to long-term, low-exposure situations, and vice versa.

Recent interest in GSL containing crops surged due to the discovery of their potential as anti-cancer agent (Jeffery and Araya, 2009; Traka and Mithen, 2009), their natural ability for crop protection (Halkier and Gershenzon, 2006) and use as a natural pesticide in agriculture in the form of biofumigation (Morra and Kirkegaard, 2002). At present, concentrations up to 100 nmol g^{-1} for ITCs are found in laboratory experiments and in the field after using effective biofumigation strategies, which is equivalent to 16.3 mg kg^{-1} 2-phenylethyl ITC (Gimsing and Kirkegaard, 2009). The toxic ranges investigated in this study are therefore likely to be found, and even exceeded, in the field. The detrimental effects on beneficial soil invertebrates such as isopods can have serious repercussions on soil functioning. Care should therefore be taken before allowing natural toxins to enter the soil ecosystem at these levels.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.05.074.

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