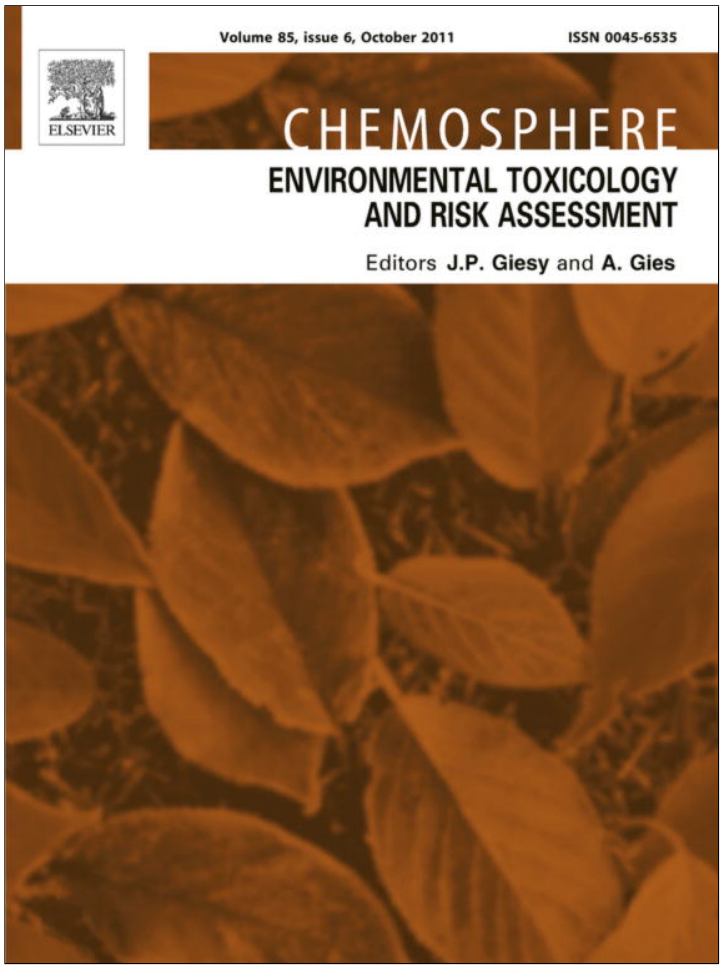

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Comparing the sensitivity of soil invertebrates to pesticides with that of *Eisenia fetida*

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ABSTRACT

The sole routine testing of the standard earthworm *Eisenia fetida* for the terrestrial risk assessment of pesticides has been under much debate since other soil invertebrates may be more sensitive than this standard test species. However, the very low availability of laboratory toxicity data for taxa other than *E. fetida* has greatly hampered sensitivity comparisons. In the present study, the relative tolerance (T_{rel}) approach was used to enable comparing toxicity thresholds obtained from the US-EPA ECOTOX database, for main terrestrial taxonomic groups and pesticidal types of action (insecticides, fungicides, herbicides, and other) separately. Analyses confirmed previously reported lower and higher sensitivity of collembolans to fungicides and insecticides, respectively. However, various other discrepancies in susceptibility relative to *E. fetida* were encountered as indicated by species sensitivity distributions and/or calculated 95% confidence intervals of T_{rel} values. Arachnids and isopods were found to be more sensitive to insecticides, and nematodes to fungicides, as compared to *E. fetida*. Implications of study findings for the terrestrial risk assessment of pesticides are discussed.

1. Introduction

The first-tier ecotoxicological effect assessment of pesticides is usually based on toxicity values derived from laboratory toxicity tests using a limited number of standard test organisms (e.g., Solomon et al., 2008). These organisms are intended to serve as sensitive surrogates for all species in a given environmental compartment, and were chosen based on their sensitivity to a wide range of compounds, well-known biology, and ease to keep/culture in the laboratory, among other reasons (e.g., Van Leeuwen, 1995). For example, current pesticide risk assessments for soil invertebrates in the EU are largely based on routine testing of earthworms (EC, 2002a; EPPO, 2003). Earthworms have indeed been considered as the most important invertebrates in most soils worldwide, standardized sampling methods are available, and their taxonomy is well known (Römbke et al., 2005). However, after reviewing laboratory studies into the effects of pesticides on soil invertebrates, Frampton et al. (2006) concluded that the standard test earthworm *Eisenia fetida* sensu lato (*E. fetida* and *Eisenia andrei*) was the least sensitive species to insecticides based on acute mortality (i.e., LC50 values). Soil arthropods (e.g., the standard collembolan test species *Folsomia candida*) appeared to be more sensitive to compounds with a broad range of (especially insecticidal) toxic modes of action, indicating that soil arthropods should also be tested routinely in regulatory risk assessments (Frampton et al., 2006).

Frampton et al. (2006) conducted their study by constructing species sensitivity distributions (SSD) based on a minimum of five species. Availability of toxicity data for soil invertebrates is very limited with a low number of species tested. Furthermore, the type of toxicity value and/or the unit in which they are expressed vary substantially among studies (see e.g. Fig. 1). Subsequently, SSDs could only be constructed for 11 (two herbicides, two fungicides and seven insecticides) out of the total of 250 pesticides for which toxicity data was available (Frampton et al., 2006). Furthermore, only acute mortality data (i.e., LC50) sufficed to construct SSDs and these could also not be constructed for individual taxonomic groups (e.g., Collembola, Lumbricidae and Nematoda) separately.

The first aim of the present study was to evaluate the sensitivity of *E. fetida* relative to other soil invertebrates for a greater number of compounds and endpoints using (an adapted version of) the relative tolerance (T_{rel}) approach as used by Wogram and Liess (2001) to compare sensitivity of aquatic macroinvertebrates with that of *Daphnia magna*. T_{rel} was calculated by dividing the toxicity threshold value of a particular species with that of *E. fetida*. A T_{rel} of one thus indicates a relative tolerance equal to that of *E. fetida*. For species more sensitive than *E. fetida*, T_{rel} is less than one and for less sensitive species it is greater than one.

The development and application of several basic environmental risk evaluation concepts has often been discussed to be focussed on the aquatic compartment (e.g., Tarazona et al., 2000; Baird and Van den Brink, 2007; Jänsch et al., 2007). Therefore, a second aim of the present paper was to evaluate the applicability of various concepts developed in aquatic risk evaluation studies for the terrestrial

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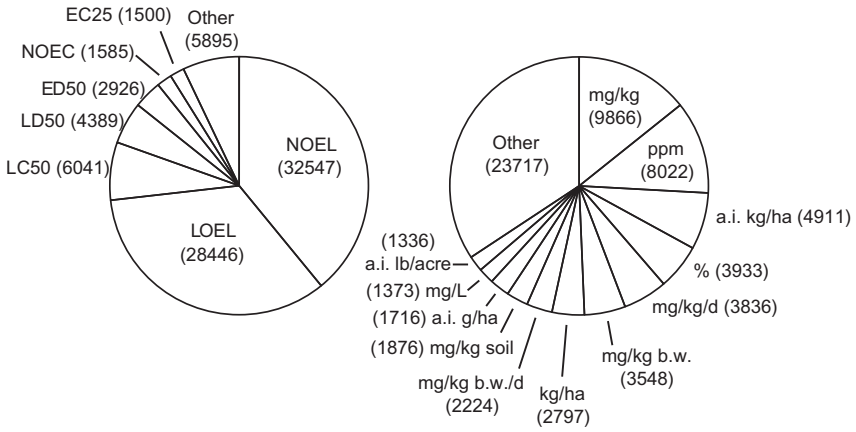

Fig. 1. Variety in threshold value type (left) and unit (right) of the data entries in the reconstructed US-EPA terrestrial ECOTOX database (after exclusion of those entries for which species Latin name, threshold type or unit was not recorded). Threshold types and units for which less than 1000 entries were encountered, were included in "other". Number of entries are provided in brackets. NOEL = no-observed-effect-level; LOEL = lowest-observed-effect-level; LC50 = lethal concentration to 50% of the test organisms; LD50 = lethal dose to 50% of the test organisms; ED50 = effective dose for 50% of the test organisms; NOEC = no-observed-effect-concentration; EC25 = effective concentration to 25% of the test organisms.

compartment. Thirdly, implications of study findings for the environmental risk assessment of soil invertebrates are discussed. This includes an evaluation of the protectiveness of predicted no effect concentrations (PNECs) based on one or more standard test organisms for other (non-standard) species using the T_{rel} approach.

2. Materials and methods

2.1. Database construction

Toxicity data were obtained from the US Environmental Protection Agency (US-EPA) ECOTOX database (http://cfpub.epa.gov/ecotox/), the largest database of its kind available. On 29 November 2009, the entire database (date of last update by EPA on 16 September 2009) was downloaded as several delimited ASCII data files and subsequently reconstructed into one Microsoft Excel spreadsheet. Database reconstruction was successfully verified for 10 random compounds by comparing results from the reconstructed database with online database queries. Subsequently, data for which no dose unit and/or Latin species name was recorded, and/or resulting from tests not carried out in the laboratory, were omitted.

2.2. Representativeness of the database

The extent by which the taxonomic diversity in the database corresponded with that in natural terrestrial ecosystems was evaluated as done by Baird and Van den Brink (2007) for the aquatic ECOTOX database of US-EPA. To this end, the relative number of species tested from a given taxonomic group was compared with the relative abundance of species in nature based on estimates given in Wilson (1992). Since many species within a taxonomic group may have been tested few times and/or few species tested often, the same was done for the relative number of toxicity values generated per taxonomic group as to obtain an estimate for how often taxonomic groups were evaluated.

2.3. Relative tolerance calculations

To enable a comparison of threshold values from different compounds, the threshold concentrations had to be "normalised". This was done by transforming these concentrations to relative tolerance (T_{rel}) values by dividing them by the (geometric mean of) threshold value(s) of *E. fetida* sensu lato. To this end, the following steps were undertaken:

1. In accordance with Jänsch et al. (2006), only data for euedaphic (soil-dwelling) invertebrates were accepted.
2. The resulting database was divided in four separate spreadsheets, separating no-observed-effect thresholds (i.e., NOEL and NOEC) from thresholds indicating 50% population effect (e.g., ED50), and sublethal (e.g., avoidance behaviour, growth) from lethal (i.e., mortality) endpoints. Data for other thresholds (e.g., LOEL and EC25) were omitted and the four spreadsheets were analysed separately (see legend of Fig. 1 for spelled-out acronyms).
3. T_{rel} values were calculated by dividing the lowest geometric mean (gm) toxicity value of a non-standard test species by the lowest gm toxicity value of *E. fetida* sensu lato. Subsequently, toxicity data for compounds for which no toxicity data were available for *E. fetida* sensu lato and at least one non-standard test species were omitted.
4. T_{rel} values were only calculated by dividing toxicity data of standard and non-standard taxa if expressed in the same dose units. In this regard, values expressed in kg ha^{-1} were converted to mg kg^{-1} using the equation reported in Jänsch et al. (2006): MC5 = 1.33D, where MC5 is the maximum concentration of a compound in the top 5 cm soil (in mg kg^{-1}) and D is the application concentration (in kg ha^{-1}). Subsequently, if no toxicity data for the standard taxon (or taxa) and a given non-standard taxon with comparable dose units were available for a given compound, no T_{rel} was calculated.
5. When multiple datapoints were available for the same taxon, compound and with the same dose unit, the gm of those values was taken.
6. If more than one T_{rel} could be calculated for the same taxon and compound, e.g. since both standard and non-standard taxa had toxicity values with more than one comparable dose unit (i.e., toxicity values were available for both *E. fetida* sensu lato and another soil invertebrate expressed in for example mg kg^{-1} dry soil and ppm), only the lowest T_{rel} was included.
7. After finishing the analysis of the four spreadsheets (see step 2), calculated T_{rel} values were pooled and presented collectively.

Studies using toxicity data sets often apply additional selection criteria besides those mentioned under (2) and incorporated under (4) (e.g., Daam et al., 2010) to their data as to account for differences

in experimental conditions (e.g., exposure duration, determined endpoints) under which the data were generated. No such additional selection criteria were used in the present study, since (i) data availability for soil invertebrates was already rather low; (ii) Frampton et al. (2006) reported little influence of data selection approaches on LC50 estimates of *E. fetida*; and (iii) including all data has the advantage (over e.g. only including data applying standard test procedures) that it includes (the range of) more ecologically representative soils and exposure conditions (Frampton et al., 2006).

2.4. T_{rel} PNEC

In the environmental risk assessment (ERA) procedure in the EU, uncertainty factors of 10 and 5 are applied to the acute and chronic toxicity values of *E. fetida*, respectively (EC, 2002a), to establish the predicted-no-effect-concentration (PNEC). To evaluate whether these uncertainty factors suffice to protect all other taxa included in the analyses, "T_{rel} PNECs" were calculated accordingly, i.e. by dividing toxicity values of non-standard test species for the different compounds by their corresponding PNEC values. In accordance with the ERA procedure in the EU, these PNECs were calculated by dividing the acute and chronic toxicity data for *E. fetida* with 10 and 5, respectively. A T_{rel} PNEC based on for example chronic NOEC data would thus be calculated using the following formula:

$$T_{rel}\ \text{PNEC} = \text{gmNOEC non-standard test species} / (\text{gmNOEC } E.\ fetida / 5)$$

Hence, a T_{rel} PNEC greater than 1 for a given non-standard test species indicates that the uncertainty factors applied to the toxicity data of *E. fetida* sufficiently protects this species, whereas a T_{rel} PNEC lower than 1 indicates that this may not be the case.

In addition, T_{rel} PNECs were calculated by considering the sensitivity of both *E. fetida* and *F. candida*, i.e. by using the lowest toxicity value of these organisms. In other words, the gmNOEC value of *E. fetida* in the previous formula would be replaced by that of *F. candida* if the gmNOEC value of *F. candida* was lower than that of *E. fetida*. Although the PNEC in the EU risk assessment is strictly based on lethal (mortality) acute data and sublethal (reproduction) chronic data, both lethal and sublethal data were included in the analysis since number of data points would otherwise be rather low. However, analysis of the data was done separately for no-observed-effect thresholds and thresholds indicating 50%, as well as sublethal and lethal endpoints, in the same way as described in Section 2.3. Since no uncertainty factors are defined in EU legislation for laboratory threshold values of *F. candida*, the same uncertainty factors as established for *E. fetida* were applied.

2.5. Species sensitivity distributions

The T_{rel} values calculated as described above were grouped for compound type (insecticides, fungicides, herbicides, and other) and taxonomic groups as used by Frampton et al. (2006; Acari, Chilopoda, Coleoptera, Collembola, Diplopoda, Enchytraeidae, Isopoda, Lumbricidae and other earthworm families, and Nematodes). Subsequently, if more than five T_{rel} values were available for a given taxonomic group and compound type (e.g., T_{rel} based on insecticides for Collembola), distribution curves of these T_{rel} values were constructed as described in Daam et al. (2010). In brief, log-normal distributions of the T_{rel} values were derived using the ETX computer program version 2.0 (Van Vlaardingen et al., 2004). If lognormality was not accepted by the Anderson–Darling Test included in the ETX software package, the BurrliOz program (Campbell et al., 2000) was used to fit a Burr type III distribution that best fitted the available data (log-logistic, log-normal, log-triangular, Weibull). The BurrliOZ software calculates confidence intervals for hazard concentrations (HC) values using a bootstrap technique, implying that confidence intervals may vary with subsequent re-runs (Hose, 2005). Therefore, each HC limit (i.e., lower and upper limits of HC5 and HC50) was estimated 10 times using 1000 permutations (separately for lower and upper limits) and the geometric mean of those 10 calculations was used as a best estimate (after Hose and Van den Brink, 2004). BurrliOZ does not include software to indicate how well the datapoints fit the curves. Hence, in accordance with Daam et al. (2010), r^2 values were calculated by applying linear regression in Microsoft Excel on PAF (potentially affected fraction) values indicated by the curve and actual PAF values of the individual T_{rel} values as a measure of how well the curve fitted the datapoints.

3. Results and discussion

3.1. Data availability

After omitting those data for which no species name, dose unit and/or threshold type were recorded, the reconstructed US-EPA terrestrial ECOTOX database yielded 83 229 entries. The variety in reported threshold values and units of these entries is visualized in Fig. 1. Interestingly, although in aquatic studies availability of NOEC values is often reported to be very limited (e.g., Daam et al., 2010), NOEL was the most reported toxicity threshold for the terrestrial database (Fig. 1). Furthermore, a great variety in units used to express toxicity thresholds was noted (Fig. 1), which was not the case for the part of the aquatic US-EPA ECOTOX database used to conduct the study described in Daam et al. (2010), where "$\mu g\ L^{-1}$" was the unit used to express the vast majority of toxicity values. Evidently, this great variety in both threshold types and their units greatly hampers construction of "traditional" SSDs, i.e. based on different taxa with the same threshold type and unit for the same compound, as a result of incompatibility of the toxicity data, even though some toxicity values expressed in different units could be converted (e.g., a.i. $g\ ha^{-1}$ and a.i. $kg\ ha^{-1}$). In the present study, this limitation was intended to be significantly reduced by applying the T_{rel} approach as to allow incorporating as much data as possible. Indeed, since Frampton et al. (2006) only considered LC50 data and constructed SSDs for individual compounds, no separate SSDs for the different taxonomic groups could be included. Hence, reported greater or lower sensitivity of a given taxonomic group was based on the fact that a limited number of datapoints were positioned in the lower or upper tail, respectively. In the present study, however, separate SSDs could be constructed for various taxonomic groups to compare sensitivity to compounds grouped for toxic type of action (insecticidal, herbicidal, fungicidal, and other; see below). In addition, SSDs could be constructed based on three to five times as many different compounds compared to the relatively low number of compounds included in the analysis by Frampton et al. (2006): 21 *versus* 7 insecticides, 7 *versus* 2 fungicides, and 11 *versus* 2 herbicides, respectively (Table 1).

3.2. Limitations of the analysis

The representativeness of the database in terms of taxonomic composition was evaluated by comparing the relative number of invertebrate species tested and toxicity data generated within the database for main taxonomic groups with those known to occur in nature (after Wilson, 1992). As can be seen in Fig. 2, insects are clearly under-represented in the database. As also discussed by Baird and Van den Brink (2007) for the aquatic US-EPA ECOTOX database, this is evidently not intended as a criticism towards US-EPA, but simply reveals the lesser attention that has (erroneously, as will be discussed below) been attributed to establishing toxicity values for insects. This is also reflected in the data that could be used to calculate T_{rel}. Almost half of all T_{rel} values (110

Table 1
Total number of relative tolerance (T_{rel}) values (calculated by dividing the threshold value of a given species by the threshold value of *Eisenia fetida* sensu lato for the same compound) that could be calculated in the present study, sorted by compound type and taxonomic groups (after Frampton et al., 2006).

Sorted by	Type/taxonomic group	No. different pesticides/taxa	Total no. T_{rel} values
Compounds	Insecticides	21	58
	Fungicide	7	59
	Herbicide	11	20
	Other	35	112
	Total	74	249
Taxa	Acari	4	7
	Chilopoda	–	–
	Coleoptera	3	3
	Collembola	9	62
	Diplopoda	–	–
	Enchytraeidae	4	30
	Isopoda	3	10
	Lumbricidae	21	110
	Nematoda	18	27
	Total	62	249

out of 249) were calculated for earthworms (Lumbricidae), for which also the greatest number of different taxa (21) were included (Table 1; Fig. 2). Interestingly, although T_{rel} values could be obtained for a relatively great number of nematode taxa, total number of T_{rel} values were relatively low for this taxonomic group, indicating that many nematode species are tested very few times. Contrarily, only four enchytraeid taxa (*Cognettia sphagnetorum*, *Enchytraeus albidus*, *Enchytraeus crypticus*, and *Enchytraeus* sp.) were in total tested 30 times (Table 1). For arthropods, only collembolans were tested relatively frequently, whereas for other groups (including the insect order Coleoptera) very few or no toxicity data were available that were suitable for T_{rel} calculations (Table 1). As discussed by Wogram and Liess (2001), this indicates that species for which an above-average number of T_{rel} values could be calculated are overemphasized. Similarly, compounds that have been tested more frequently have a greater weight in the overall analysis of the pesticide type to which they belong. However, Wogram and Liess (2001) also concluded that the error introduced by alternatively taking a secondary mean at the order level to outweigh frequently tested taxa would probably be greater than the error resulting from overweighing individual species.

Due to the relatively low data availability and the great variety in test conditions (e.g., test duration, organism strain, and sublethal endpoints), no additional selection criteria were applied after separating the dataset in sublethal/lethal and 50% effect/no-observed-effect thresholds. Evidently, differences in experimental design will ultimately influence threshold levels. For example, Frampton et al. (2006) discussed that standard OECD soil has a higher organic content than most natural soils, implying a lower bioavailability and hence higher threshold values. Contrarily, longer exposure durations will logically lower threshold concentrations. To obtain an idea of the variation in toxicity values in the database as a result of differences in experimental design, the spread in toxicity values was evaluated by applying the method used by Brock et al. (2008) and Daam et al. (2009) to calculate the spread in NOECecosystem values derived in model ecosystem studies. To this end, 95% confidence intervals were calculated for those data for which at least three toxicity values, derived for the same species and compound but under different experimental conditions, were available. Subsequently, the ratio of the upper and lower limits of these intervals was used as an indication of the spread in toxicity values for that taxon-compound combination. Resulting average spreads (with 95% confidence intervals) were 5.3 (3.6–7), 8.5 (−1.6–19) and 7.1 (2.7–12) for 50% effect thresholds indicating mortality, 50% effect thresholds indicating sublethal effects and no-observed sublethal effect thresholds, respectively. For no-observed lethal effect thresholds not enough data were available to calculate a spread. These high values are not surprising considering that a ringtest with earthworm toxicity tests based on 18 participating laboratories, all using the same experimental conditions, resulted in a spread in LC50 values of up to a factor 5 (Moser et al., 2009). To date, only few studies have been performed to clarify the influence of soil properties on the fate (e.g., bioavailability) and toxicity of organic chemicals to soil invertebrates (Sousa et al., 2000; Frampton et al., 2006; Römbke et al., 2007; Chelinho et al., 2011). The need for such studies appears evident given the spreads in toxicity values discussed above, and may be further stressed by the indication given in the Sixth Community Environment Action Programme that regional and local environmental differences should be considered in the Community's environmental policy-making (EU, 2002).

Due to the discussed low data availability, differences in experimental conditions under which the toxicity data were derived could not be accounted for in the presented analyses. Hence, sensitivity comparisons as visualized in Fig. 3 would have been biased by such differences in case experimental conditions of a certain taxonomic group would as a rule differ from that of *E. fetida*. For example, consider the case where exposure durations of the tests evaluating insecticides conducted with collembolans are significantly longer than those carried out with *E. fetida*. This would indicate that the differences between collembolans and *E. fetida* (Tables 2 and 3; Fig. 3) did not result from a greater sensitivity of the former, but would merely be the result of these differences in experimental design. Although there is no direct reason that

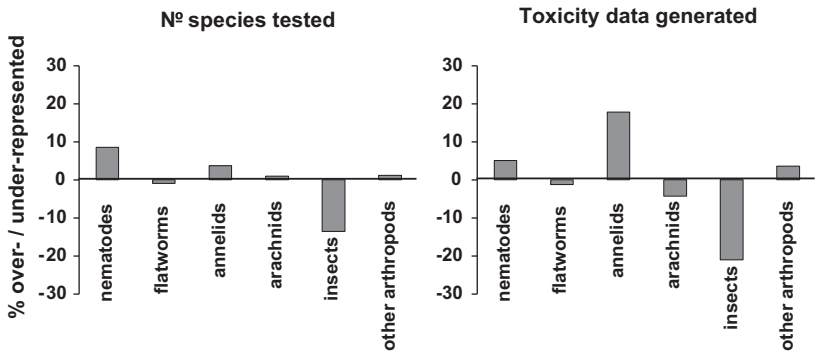

Fig. 2. Visualization of the relative number of invertebrate species tested (left) and toxicity data generated (right) in the US-EPA terrestrial ECOTOX database as compared to the relative abundance of species in nature as estimated by Wilson (1992). A negative percentage indicates that a group is under-represented in the database, whereas a positive percentage indicates that a group is over-represented (after Baird and Van den Brink, 2007).

indicates that this would be the case, it was verified for the T_{rel} calculations of Lumbricidae (both insecticides and fungicides) since the greatest differences with *E. fetida* were obtained for this taxonomic group. The exposure duration and organic matter content in studies used to calculate the T_{rel} values were verified as potential confounding parameters by dividing the values for collembolans by those of *E. fetida*. Average (with 95% confidence interval) ratios for exposure duration were 2.3 (0.4–4.2) for fungicides and 1.3 (0.8–1.8) for insecticides. Data to calculate this ratio for organic matter were only available for fungicides: 1.3 (0.5–2.1). As anticipated, no consistent trend could be demonstrated, although exposure duration appears slightly higher for collembolan tests evaluating fungicides. However, this would imply lower toxicity values, whereas a lower sensitivity of collembolans for fungicides was noted. Hence, difference in sensitivity between collembolans and *E. fetida* to fungicides might have been even slightly greater than indicated by the presented analysis (Fig. 3; Tables 2 and 3) if similar test conditions would have been considered.

3.3. Sensitivity of E. fetida sensu lato compared to other soil invertebrates

In Fig. 3, the sensitivity of soil invertebrates by taxonomic group are compared with that of *E. fetida* sensu lato. The greater and lower sensitivities of collembolans to insecticides and fungicides, respectively, as noted by Frampton et al. (2006; laboratory single species tests) and Jänsch et al. (2006; (semi) field tests), are confirmed (see also Tables 2 and 3). However, overall greater sensitivity of the standard collembolan *F. candida* to a broad range of toxic modes of action (e.g., herbicidal), as discussed by Frampton et al. (2006), could not be demonstrated. This may be partly due to the fact that only 4 T_{rel} values could be calculated for collembolans based on herbicides. Although paraquat dichloride (T_{rel} = 0.0014) and pendimethalin (T_{rel} = 0.42) indicated a greater sensitivity of collembolans, they appear less sensitive to pentachlorophenol (T_{rel} values of 1.5 and 8). Contrarily, the SSD constructed by Frampton et al. (2006) for the latter compound indicated a (slightly) greater sensitivity for collembolans as compared to *E. fetida*. This may be related with the fact that Frampton et al. (2006) constructed their SSD based on LC50 data, whereas the two T_{rel} values for pentachlorophenol in the present study were based on sublethal NOEC and EC50 values.

Besides the anticipated differences in sensitivity between collembolans and *E. fetida* described above, the SSDs also revealed that isopods were more sensitive to insecticides, and nematodes to fungicides, as compared to *E. fetida* (Fig. 3; Table 2). Since SSDs could only be constructed for a limited number of taxonomic-compound group combinations, 95% confidence intervals (CI) of T_{rel} values from these combinations were calculated, which are presented in Table 3. These additional analysis also indicated significant (i.e., the value 1 is not covered by the 95% CI) greater sensitivity of Acari to insecticides, and nematodes to fungicides (Table 3). This greater vulnerability of arthropods to insecticides, as demonstrated for Acari, Collembola and Isopoda, and indicated by the single T_{rel} value of 0.29 for Coleoptera (Table 3), has also previously been demonstrated for aquatic organisms (e.g., Maltby et al., 2005). Logically, pesticides developed to kill insect pest organisms (e.g., by inhibiting acetylcholinesterase or chitin production) are also more likely to exert side-effects on non-target insects and taxonomically-related taxa. Similarly, the lower sensitivity of the arthropods, as indicated by the SSD of collembolans (Table 2; Fig. 3) and individual T_{rel} values for Acari and Isopoda (Table 3) compared to *E. fetida*, could also be anticipated based on aquatic studies into fungicide toxicity. For example, Van Wijngaarden et al. (1998) and Cuppen

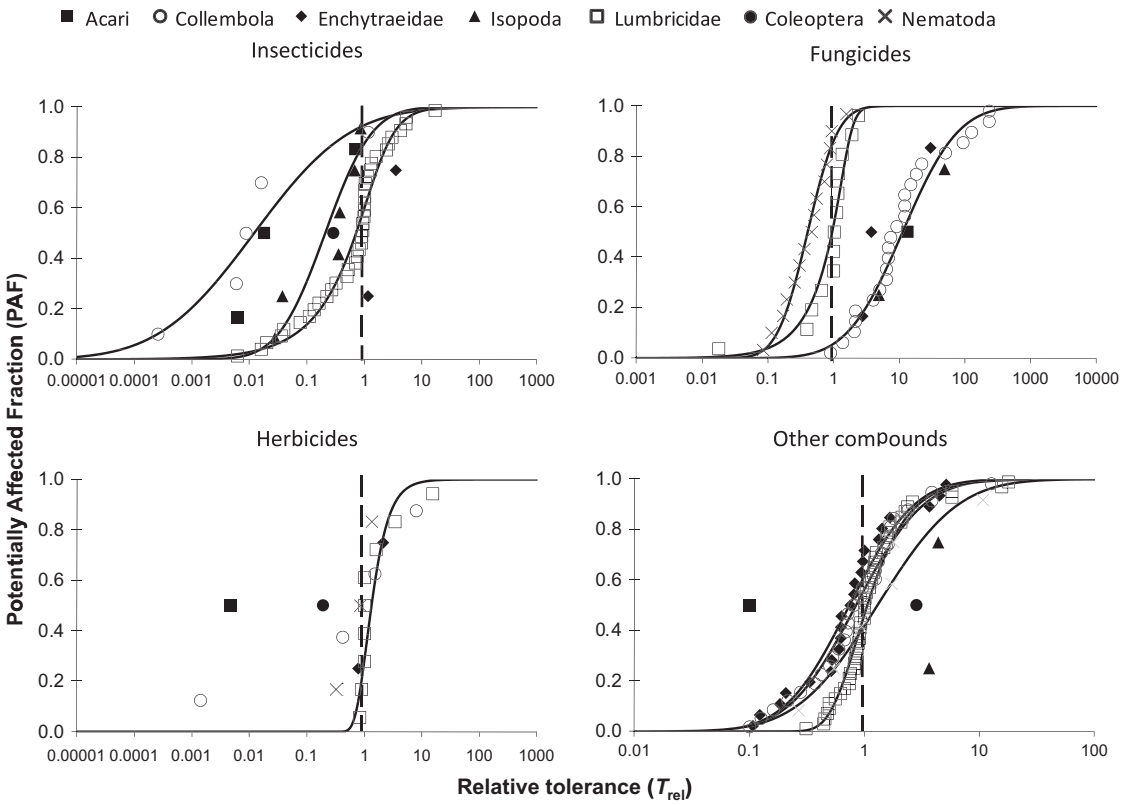

Fig. 3. Species sensitivity distributions (SSD) comparing the sensitivity of different taxonomic groups to insecticides, fungicides, herbicides and other compounds with that of *E. fetida* sensu lato using the toxic unit approach. The vertical dashed line at T_{rel} = 1 indicates the sensitivity of *E. fetida* sensu lato. A T_{rel} < 1 and a T_{rel} > 1 indicate a greater and lower sensitivity relative to *E. fetida* sensu lato, respectively. T_{rel} = relative tolerance.

Table 2
Estimates of the 5% (P5) and 50% (P50) percentiles (with 95% confidence intervals) derived from the species sensitivity distributions (SSDs) of the relative tolerance (T_{rel}) values. PAF = predicted affected fraction.

		P5	P50	PAF at T_{rel} = 1 (%)	SSD constructed with/fit to curve**
Insecticide	Lumbricidae	0.022 (0.0062–0.076)	0.71 (0.49–1.03)	60	BurrliOz Burr III/r = 0.98 (p < 0.01; n = 38)
	Collembola	0.000057 (0.000000038–0.0010)	0.012 (0.00068–0.21)*	93	ETX lognormal/accepted (n = 5)
	Isopoda	0.016 (0.00088–0.057)	0.020 (0.062–0.7)*	86	ETX lognormal/accepted (n = 6)
Fungicide	Lumbricidae	0.12 (0.018–0.59)	0.93 (0.62–1.25)	54	BurrliOz Burr III/r = 0.97 (p < 0.01; n = 13)
	Collembola	0.86 (0.32–1.73)	11 (6.4–19)*	6	ETX lognormal/accepted (n = 24)
	Nematoda	0.097 (0.046–0.16)	0.39 (0.27–0.58)*	87	ETX lognormal/accepted (n = 15)
Herbicide	Lumbricidae	0.66 (0.62–0.75)	1.3 (1.1–1.6)	29	BurrliOz Reciprocal Weibull/r = 0.89 (p < 0.01; n = 9)
Other compounds	Lumbricidae	0.45 (0.39–0.57)	1.05 (0.92–1.23)	47	BurrliOz Burr III/r = 0.99 (p < 0.01; n = 50)
	Collembola	0.14 (0.078–0.22)	0.84 (0.60–1.2)	57	ETX lognormal/accepted (n = 29)
	Enchytraeidae	0.13 (0.0069–0.22)	0.73 (0.51–1.1)	62	ETX lognormal/accepted (n = 23)
	Nematoda	0.14 (0.012–0.43)	1.29 (0.45–3.6)	42	ETX lognormal/accepted (n = 6)

* Considered significant since T_{rel} = 1 not in 95% CI.
** SSDs were constructed with the ETX program, which includes the Anderson–Darling Test to evaluate the fit to curve, or the BurrliOz software package, for which the fit to curve was determined by calculating the correlation coefficients (For details, please refer to the Section 2).

Table 3
Mean relative tolerance (T_{rel}) values (with 95% confidence intervals; CI) for the different taxonomic groups and compound types. – = No data; NP = not possible to calculate a 95% CI since not enough data available (<3 datapoints). In the latter case, the single or two T_{rel} values are presented.

	Insecticide	Fungicide	Herbicide	Other compounds
Acari	0.24 (−0.21–0.69)*	13 (NP)	0.0047 (NP)	0.1
Chilopoda	–	–	–	–
Coleoptera	0.29 (NP)	–	0.19 (NP)	2.8 (NP)
Collembola	0.24 (0.22–0.70)*	37 (9.9–64)*	2.5 (−1.2–6.2)	1.5 (0.65–2.4)
Diplopoda	–	–	–	–
Enchytraeidae	1.16; 3.51 (NP)	12 (−5.3–30)	0.78; 2.1 (NP)	1.2 (0.64–1.8)
Isopoda	0.39 (0.12–0.65)*	4.9; 49 (NP)	–	3.7; 4.4 (NP)
Lumbricidae	1.56 (0.65–2.48)	1.1 (0.71–1.39)	2.9 (−0.22–6.1)	1.9 (1.008–2.8)*
Nematoda	1.3 (0.72–1.9)	0.53 (0.33–0.73)*	0.84 (0.26–1.4)	2.7 (−0.54–5.9)

* Considered significant since T_{rel} = 1 not in 95% CI.

et al. (2000) reported greatest sensitivity of "worm-like" taxa to the fungicide carbendazim in single species tests and a microcosm study, respectively, although the underlying reason for this is unclear. Frampton et al. (2006) discussed that a surprising finding of their analysis was that SSDs for insecticides could only be calculated for oligochaets despite the expected greater sensitivity of arthropods. Similarly, much more T_{rel} values based on insecticidal toxicity data could be calculated in the present study for Lumbricidae than for arthropods (Fig. 3). Another surprising observation arising from Fig. 3 is that, despite the discussed greater sensitivity of collembolans to insecticides, T_{rel} availability for these organisms is approximately five times higher for fungicides than for insecticides. These findings thus imply an overall poor selection of test compound (or test species) in the soil toxicity assays included in the database.

The SSD of Nematodes indicated a greater sensitivity than *E. fetida* for fungicides, which was based on toxicity values of 14 nematode taxa to copper sulphate and cupric chloride. Interestingly, studies evaluating the sensitivity of a single nematode species to copper compounds reported that obtained toxicity values were comparable (Boyd et al., 2001), slightly lower (Kammenga et al., 1996) or even slightly greater (Peredney and Williams, 2000) than those of *E. fetida*. Korthals et al. (1996) derived toxicity thresholds for a total of 14 nematode taxa from different feeding and life-history strategy groups to copper. Based on these tests, they concluded that *K*-strategist nematodes were among the most sensitive taxa (Korthals et al., 1996). Interestingly, *E. fetida* has been considered a typical *r*-strategist in its life history traits (Lukkari et al., 2005), which may thus be related with its low sensitivity to copper as compared to nematodes. This appears not to hold true, however, for all compound types, since Kammenga et al. (1994) concluded that slow colonizing nematodes (*K*-strategists) were not more sensitive to cadmium and pentachlorophenol than opportunistic nematode species (*r*-strategists). Sensitivity of *E. fetida* sensu lato appeared to be similar or slightly greater (for herbicides) compared to other Lumbricidae (Fig. 3; Tables 2 and 3).

3.4. Implications for the terrestrial risk assessment of toxic compounds

After reviewing the sensitivity of soil arthropods in single species, model ecosystem and field studies, Frampton et al. (2006) and Jänsch et al. (2006) concluded that the standard collembolan test species *F. candida* should be included in regulatory risk assessments. Based on the analysis demonstrated in Fig. 4, the need for this seems justified: PNECs based on only *E. fetida* sensu lato do not fully protect a great number of other test organisms, whereas this is not the case when including *F. candida* in PNEC calculations (Fig. 4). Similarly, toxicity testing of a chironomid larvae (Insecta) is required in the aquatic environmental risk assessment of insecticides if side-effects on these organisms are to be expected (EC, 2002b).

Only few T_{rel} PNEC values could be calculated when considering both *E. fetida* sensu lato and *F. candida* due to constraints in data availability and the fact that at least three (*E. fetida* sensu lato, *F. candida* and a third species) toxicity values for the same

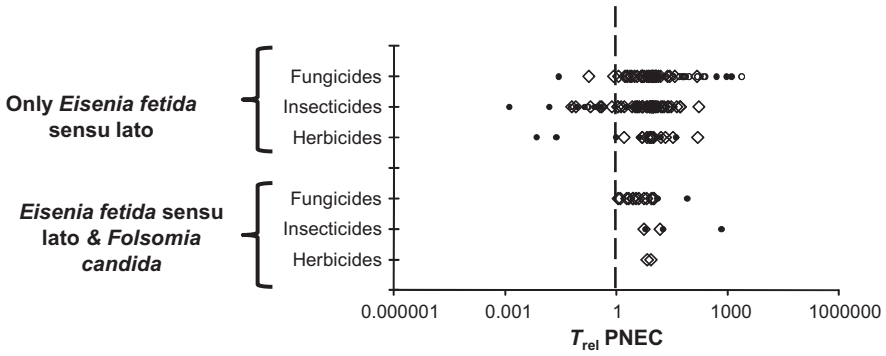

Fig. 4. Protectiveness of predicted no effect concentrations (PNEC) for *E. fetida* sensu lato alone, and in combination with *Folsomia candida*, for other test organisms included in the database (for details, see text). Taxonomic groups were grouped in arthropods (black dots) and annelids and nematodes (open diamonds). The vertical dashed line at T_{rel} PNEC = 1 indicates the PNEC of *E. fetida* sensu lato. A T_{rel} PNEC < 1 indicates that the corresponding PNEC value(s) for the standard test species considered is/are not sufficiently protective, whereas a T_{rel} PNEC > 1 indicates that the PNEC value(s) for the standard test species considered is/are sufficiently protective. T_{rel} = relative tolerance.

compound expressed in the same dose unit had to be available. Especially for arthropods few T_{rel} PNECs could be calculated, and was limited to a maximum of three values: Acari (1), Coleoptera (2), Collembola other than *F. candida* (3), and Isopoda (3). Furthermore, various T_{rel} PNEC values lay close to 1, especially for fungicides (Fig. 4), for which three T_{rel} PNEC values between 1 and 2 were obtained for three different nematode taxa. Furthermore, a T_{rel} PNEC of 0.96 was calculated for the enchytraeid *E. crypticus* exposed to manganese sulphate. Also considering that several T_{rel} < 1 were obtained for Acari, Isopoda and Nematoda (Fig. 3; Tables 2 and 3), it may thus be questionable whether sole testing of *E. fetida* sensu lato and *F. candida* for the first-tier risk assessment covers the range of other potentially sensitive taxa. For the same reason, a battery of tests using a range of test organisms has previously been recommended (e.g., Römbke et al., 2005; Jänsch et al., 2007). Representatives of the organism groups indicated in the present study to contain sensitive taxa, have also previously been recommended as test organisms in laboratory toxicity testing, e.g. predatory Acari (Frampton and Van den Brink, 2007; Jänsch et al., 2007), Isopoda (Caseiro et al., 2000; Ribeiro et al., 2001), Enchytraeidae (Jänsch et al., 2005), and Nematoda (Kammenga et al., 1996; Sochová et al., 2006). Regarding Nematodes, Boyd et al. (2001) reported that the nematode *Caenorhobditis elegans* is especially suitable to assess toxicity associated with porewater exposures because it resides in water within the soil matrix. As further discussed by Boyd et al. (2001), among other authors, soil sorption (i.e. the capacity of soil particles to bind chemical substances) may alter the bioavailability of contaminants in soils and soil porewaters and influence the results of soil toxicity tests. Furthermore, chemical bioavailability in Organisation for Economic Co-operation and Development (OECD) artificial soil may contrast with bioavailability in natural soils and produce ecotoxicological benchmarks that are not representative of species exposure conditions in the field, indicating that toxicity testing should include studies with natural soils in addition to OECD soil to better reflect exposure conditions in the field (Römbke et al., 2007; Chelinho et al., 2011). In these regards, it should be noted that in the present study the representativeness of standard test organisms was only studied on a first-tier level, i.e. by evaluating whether PNEC values for these species cover the sensitivity of other species tested in laboratory single species tests. Jänsch et al. (2006) made an effort to validate as to whether first-tier toxicity values suffice to protect terrestrial ecosystems under real-world (semi) field conditions. They concluded that for eight pesticides, higher-tier effect concentrations were within or below the 90% CI of the HC5 from SSDs constructed from first-tier toxicity values (Jänsch et al., 2006). However, in most cases there was insufficient data from field studies and/or insufficiently low test concentrations were included to allow NOEC estimations, hampering the validation of risk predictions based on first-tier testing. This emphasizes the urgent need for higher-tier studies into the risk evaluation of pesticides in terrestrial (model) ecosystems. Besides the reasons discussed above, the need for this may be further stressed by the importance to evaluate functional endpoints, which may be more sensitive than structural effects (Jänsch et al., 2007). Furthermore, only model ecosystem or field studies will allow (i) an environmental realistic evaluation of the influence of complex mixtures, usually present in natural contaminated soils (Sousa et al., 2008), and (ii) coping with interactions between species and the role of pesticide stress on this (indirect effects) as well as the recovery potential of affected terrestrial communities (Schäffer et al., 2010).

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