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EFFECTS OF PESTICIDES ON SOIL INVERTEBRATES IN LABORATORY STUDIES: A REVIEW AND ANALYSIS USING SPECIES SENSITIVITY DISTRIBUTIONS

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Abstract—Species sensitivity distributions (SSD) and 5% hazardous concentrations (HC5) are distribution-based approaches for assessing environmental risks of pollutants. These methods have potential for application in pesticide risk assessments, but their applicability for assessing pesticide risks to soil invertebrate communities has not been evaluated. Using data obtained in a systematic review, the present study investigates the relevance of SSD and HC5 for predicting pesticide risks to soil invertebrates. Altogether, 1,950 laboratory toxicity data were obtained, representing 250 pesticides and 67 invertebrate taxa. The majority (96%) of pesticides have toxicity data for fewer than five species. Based on a minimum of five species, the best available endpoint data (acute mortality median lethal concentration) enabled SSD and HC5 to be calculated for 11 pesticides (atrazine, carbendazim, chlorpyrifos, copper compounds, diazinon, dimethoate, γ-hexachlorocyclohexane, lambda-cyhalothrin, parathion, pentachlorophenol, and propoxur). Arthropods and oligochaetes exhibit pronounced differences in their sensitivity to most of these pesticides. The standard test earthworm species, *Eisenia fetida* sensu lato, is the species that is least sensitive to insecticides based on acute mortality, whereas the standard Collembola test species, *Folsomia candida*, is among the most sensitive species for a broad range of toxic modes of action (biocide, fungicide, herbicide, and insecticide). These findings suggest that soil arthropods should be tested routinely in regulatory risk assessments. In addition, the data indicate that the uncertainty factor for earthworm acute mortality tests (i.e., 10) does not fully cover the range of earthworm species sensitivities and that acute mortality tests would not provide the most sensitive risk estimate for earthworms in the majority (95%) of cases.

Keywords—Acute to chronic ratio Environmental risk assessment Hazardous concentration Probabilistic risk assessment Soil fauna

INTRODUCTION

To predict effects of pollutants on soil invertebrate communities, information must be extrapolated from a small subset of the species, because it is impractical to conduct a large number of tests, the diversity of soil fauna is not precisely known [1], and not all the known species are amenable to testing. For species exposed to the same pollutant, a species sensitivity distribution (SSD) may be used to estimate the chemical concentration at which a certain proportion x (%) of the species would be affected (the x% hazardous concentration [HCx]) [2–6]. The hazardous concentration predicts the risk to ecological structure using information from a subset of the species. The certainty of the risk estimate may be indicated by the slope and confidence interval of the SSD. An HCx value typically used for the maximum permissible environmental concentration is the HC5 (i.e., the concentration that would affect no more than 5% of the species, giving a 95% protection level [3,6]. The choice of protection level is arbitrary [7], but it also reflects a compromise between statistical considerations (risks may not be predicted reliably if HCx is very small), environmental protection (HCx should be as small as possible [8]), and crop protection (exposure of some nontarget species to certain pesticides may be unavoidable). The use of SSD to estimate HC5 is based on several assumptions, including that an appropriate mathematical distribution is used to fit the sensitivity data and that the species are a random subset of those in the ecological community to be protected. Critics of the SSD approach question the validity of these assumptions [9] and the 95% protection goal, which has been interpreted as meaning that a fraction of the species may be considered not worthy of protection [8].

In Europe, only tests with earthworms, using the test species *Eisenia fetida* sensu lato (*Eisenia fetida* and *E. andrei*), are strictly required for assessing pesticide risks to soil invertebrates, although supplementary tests with Collembola or Enchytraeidae may be carried out on a case-by-case basis depending on the nature of the pesticide and its pattern of use [10]. The standard laboratory test methods currently available are an acute test for assessing earthworm mortality (*E. fetida* [11]) and chronic tests for assessing reproduction of earthworms (*E. fetida* [12,13]), Collembola (*Folsomia candida* [14]), and Enchytraeidae (*Enchytraeus albidus* [15]). A fourth test, for assessing reproduction of predatory mites (Acari: *Hypoaspis aculeifer*), is under development [16]. Risk is evaluated in these laboratory (lower-tier) tests by comparing the toxicity endpoint (e.g., median lethal concentration [LC50], median effective concentration [EC50], or no-observed-effect concentration [NOEC]) with the predicted environmental con-

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centration of the pesticide. If risk is identified, further testing under more realistic, higher-tier exposure conditions is triggered [10]. At present, the only standard higher-tier test available for soil invertebrates is an earthworm field test [17].

The current risk assessment is deterministic, in which a point estimate of toxicity from the lower-tier test is compared against a point estimate of the predicted environmental concentration to assess the risk. A key problem is the determination of how far below the toxic concentration the predicted environmental concentration should be for the risk to be considered as acceptable. An arbitrary uncertainty factor represents uncertainty in the risk estimate (e.g., as a result of the differences in pesticide sensitivity between the standard test species and other species that are not tested).

Deterministic estimates of risk have the advantage that they are easy to calculate and standardize. However, they do not make use of all available data and have been criticized as lacking transparency in their derivation ([18]; http://www.eupra.com/report.pdf). It has been acknowledged during several international risk assessment workshops that improvements would be made if risk estimates could take better account of the uncertain relevance of the standard test species, such as by using distribution-based risk assessment approaches ([18,19]; http://www.epa.gov/oppefed1/ecorisk/terrreport.pdf). Sensitivity distributions already are used to support other areas of pesticide regulatory risk assessment ([20]; http://europa.eu.int/comm/food/fs/ph_ps/pro/wrkdoc/wrkdoc10_en.pdf) and have been proposed for risk assessment of contaminated land [21–23]. Evaluations of the SSD approach have been attempted with soil invertebrates (e.g., with industrial chemicals [24] and pesticides [25]), but these have been ad hoc studies involving relatively few chemicals and species.

The present paper reports an evaluation of the applicability of the SSD and HC5 approaches to soil invertebrate pesticide toxicity data that was carried out in three steps. First, relevant pesticide effects data were collated by conducting a systematic review of soil invertebrate laboratory toxicity studies. Second, SSD curves were fitted to the data and factors that affect the soil invertebrate HC5 estimates were investigated. Third, the implications of these findings for the regulatory assessment of pesticide risks to soil invertebrates were examined, including a comparison of acute and chronic toxicity endpoints. Validation of the risks predicted by these HC5 estimates is explored in a related study [26] based on a detailed review of higher-tier data.

MATERIALS AND METHODS

Data collection

A systematic search strategy was used to locate relevant pesticide effects literature and data by searching the Biological Abstracts® (Thomson Scientific, Philadelphia, PA, USA), Institute for Scientific Information Web of Knowledge (Thomson Scientific), Pesticide Action Network (Europe, London, UK, and North America; San Francisco, CA, USA), Dutch National Institute for Public Health and the Environment (RIVM) E-toxBase (Bilthoven, The Netherlands), U.S. Academia EXTOXNET (Corvallis, OR, USA), U.S. Environmental Protection Agency ECOTOX (Duluth, MN, USA) database, International Centre for Pesticides and Health Risk Prevention SEEM (Milan, Italy) database, and the German Federal Soil Society Bundesverband Boden (BVB) SoilValue (St. Augustin, Germany) database. Information also was obtained by searching regulatory documents in the public domain, principally the United Kingdom Advisory Committee on Pesticides Disclosure Documents (York, UK), ecotoxicology journal tables of contents, and the World Wide Web, and by contact with ecotoxicologists in academia, regulatory organizations, contract testing laboratories, and the agrochemicals industry. During discussions with members of the agrochemicals industry, concerns about the confidentiality of commercially sensitive data were raised. Accordingly, only the data available in the public domain are reported here.

Pesticide active substances (excluding microorganisms and fungi used as biological pesticides) were considered to be relevant if they are currently approved, or have been approved previously, for use in European agriculture. The pesticides comprised acaricides, fungicides, herbicides, insecticides, molluskicides, nematicides, and broad-spectrum biocidal compounds. Copper compounds (acetates, carbonates, chlorides, nitrates, oxides, oxychlorides, and sulfates) are included (grouped together) as fungicides, because they are the group of pesticides that have been tested most widely on soil invertebrates. However, most of the copper data are from ad hoc studies, with only one commercial formulation (containing copper oxychloride) represented. Data also were extracted for some other pesticide-related substances that have been tested extensively on soil invertebrates. These include 4-nitrophenol (a metabolite of the insecticide parathion), 3,4-dichloroaniline (a metabolite of the herbicide diuron), and monochlorobenzene (a pesticide formulation additive).

Data were extracted into a Microsoft Excel® (Microsoft, Redmond, WA, USA) database if a relevant measurement endpoint (e.g., acute or chronic LC50, EC50, or NOEC) was given, together with sufficient supporting information to allow the endpoint to be interpreted. Data were accepted for any euedaphic (soil-dwelling) invertebrate groups other than microorganisms. All data were checked for potential duplication, because several of the databases reported primary studies that already had been located in the literature, while some overlap of data among databases also was observed.

Heterogeneity of sensitivity data

When comparing the sensitivity (indicated by the toxicity endpoint) of different species to pesticides, sensitivity may be confounded with exposure if the test conditions are dissimilar. To reduce the possibility of confounding, comparisons may be restricted to data obtained under the same set of test conditions (such that exposure is assumed to be similar for all the tests performed). Three approaches for selecting data were evaluated: First, a comparable-data approach included only those data that conform to an accepted test procedure (e.g., in the case of earthworms, only data acquired according to the standard guidelines 207 and 222 of the Organization for Economic Co-operation and Development [OECD] [11,13] were used). These tests use OECD artificial soil [11] with pH 5.4 to 7.5, an organic matter content of approximately 10%, and a moisture content of 40 to 60% of maximum water-holding capacity. Second, an all-data approach utilized all available data for a given test species and pesticide, without restriction to standard test conditions. Third, a noncomparable-data approach used the remaining data not assigned to the comparable-data approach. For each approach, if several comparable toxicity (e.g., LC50) values were available for the same species, the geometric mean was used.

SSD and HC5 estimation

So far, SSD have been based most frequently on the lognormal distribution [27], although some studies have not assumed a distribution but, instead, have used a bootstrap approach to estimate a hazardous concentration or community NOEC [28,29]. In a comparison of several distributions to fit SSD to pesticide effects data for aquatic organisms, Maltby et al. [30] found the lognormal distribution to be appropriate for most of the pesticides tested. For the present purposes, SSD for the terrestrial invertebrate data also were based on the lognormal distribution, subject to a test (Anderson-Darling) for goodness-of-fit.

An SSD can, in theory, be derived from as little as one toxicity value provided that an estimate of variation is available (e.g., from other data sets) [31], but to improve precision, at least 10 to 15 toxicity data (depending on the pesticide) are recommended [27]. The number of data required depends on the taxonomic resolution, the homogeneity of data, and the pesticide mode of action. For example, an arbitrary minimum of data for six species has been used to derive SSD and HC5 estimates for arthropods in aquatic risk assessment studies [30,32]. Because of limitations of the soil invertebrate data (see *Results* section), an arbitrary minimum of data for five species was used for the present study in calculating SSD and estimates of HC5.

For each pesticide that met the minimum requirement of providing five relevant effects concentration estimates, an SSD and estimate of the HC5 were calculated using the method described by Aldenberg and Jaworska [33].

Relevance of the data to risk assessment

For earthworm risk assessment in the European Union, uncertainty factors of 10 and 5 are used for acute mortality and chronic reproduction tests [10], respectively, meaning that the predicted environmental concentration must be at least 10-fold smaller than the acute toxic concentration, or at least fivefold smaller than the chronic toxic concentration, for an active substance to be classified as low or negligible risk [10]. In the case of the earthworm acute mortality test, any differences in sensitivity between the standard test species (*E. fetida*) and other worms are assumed to be less than one order of magnitude (because the uncertainty factor is 10). Comparisons were made between acute and chronic toxicities for *E. fetida* sensu lato to investigate whether these uncertainty factors are supported by the available data. Comparisons of the sensitivities of two standard test species (*E. fetida* sensu lato and *F. candida*) also were made to clarify whether earthworm and/or Collembola tests are appropriate for assessing risks of particular chemicals or toxic modes of action.

RESULTS

Availability of data

The systematic search for information about pesticide effects on soil invertebrates yielded 1,950 toxicity endpoint data sets, representing 250 pesticide substances and 67 taxonomic groups of invertebrates (see Tables S1 and S2 in SETAC Supplemental Data Archive, Item ETC-25-09-004; http://etc.allenpress.com). Approximately 48 and 52% of the lower-tier data originate from acute and chronic studies, respectively. Lumbricidae, Collembola, and Enchytraeidae have been the soil invertebrates tested most frequently in laboratory studies. These invertebrates contribute 90% of the data. The standard test species (*E. fetida* sensu lato, *F. candida*, *E. albidus*, and *H. aculeifer*) together comprise 60% of the data overall. The acute LC50 has been the most frequently reported measurement endpoint, representing 32% of the data, followed by the chronic NOEC (17%) and the acute NOEC, chronic lowest-observed-effect concentration, and chronic EC50 (each ~11%). Despite the large number of toxicity data available, very few pesticides, when the data are broken down into their component variables (pesticide, measurement endpoint, and species studied), have data that are comparable across different tests or for different species. Only 45 (18%) of the 250 pesticides with laboratory toxicity data have comparable higher-tier (model ecosystem or field) effects data [26]. For copper compounds, which have the largest number of laboratory toxicity data, no comparable higher-tier effects data for soil invertebrates are available [26].

Effect of data selection on LC50 estimates

Only *E. fetida* LC50 data are available for comparison of the data selection approaches, because too few comparable NOEC values or data for other species were found. No consistent effect resulted from selecting only comparable data as opposed to using all available data to estimate an acute LC50. For all pesticides except benomyl, the difference is less than a factor of two (benomyl, 2.7) (Table 1). The noncomparable LC50 data also differ from the comparable data by a relatively small factor (≤2.5) except for benomyl (9.0). Confidence intervals indicate that none of the three data selection approaches consistently yielded the most precise estimates of LC50, although the noncomparable data usually gave the least precise estimates (Table 1).

Effect of data selection on SSD and HC5

Because no single data selection method appears to be preferable for estimation of the LC50, sensitivity distributions were calculated using both the comparable-data and all-data approaches. Based on the minimum of five species, the SSD can be derived for only four pesticides using the comparable-data approach: Chlorpyrifos, copper compounds (grouped), dimethoate, and pentachlorophenol (Table 2). When all the available data are used, the SSD for a further seven pesticides can be calculated: Atrazine, carbendazim, diazinon, γ-hexachlorocyclohexane (HCH), lambda-cyhalothrin, parathion, and propoxur (Table 2). With the exceptions of chlorpyrifos and parathion (see below), the pesticides pass goodness-of-fit tests (Anderson-Darling) for the lognormal distribution.

Estimates of HC5 for copper, dimethoate, and pentachlorophenol are lower if based on all available data than if based only on comparable data. For chlorpyrifos and parathion, the HC5 is highly sensitive to the inclusion of Collembola data (Table 2). Confidence intervals of HC5 do not indicate a consistent effect of data selection on the precision of the HC5 estimates. Although using all available data gives a more conservative estimate of risk (i.e., lower HC5) in the majority of comparisons, these data are too limited to use in drawing general recommendations about the choice of data selection when calculating SSD and HC5 for soil invertebrates.

Relative sensitivities of invertebrates in relation to pesticide mode of action

Oligochaetes and nematodes are more sensitive than arthropods to the fungicide carbendazim, whereas arthropods are more sensitive than oligochaetes to the insecticides diazinon,

Table 1. The effect of three data selection approaches on pesticide acute median lethal concentration estimates for *Eisenia fetida* (mg/kg dry wt soil)[a]

Pesticide	Comparable-data analysis (standard test conditions only)	Noncomparable-data analysis	All-data analysis
Atrazine[b]	101 (46–222) (n = 2)	41 (2–848) (n = 2)	64 (32–128) (n = 4)
Benomyl[b]	21 (14–29) (n = 6)	185 (56–611) (n = 5)	56 (38–83) (n = 11)
Carbendazim	5.8 (5.6–5.9) (n = 2)	4.1 (n = 1)	5.1 (4.2–6.3) (n = 3)
Chloroacetamide	31 (29–32) (n = 31)	22 (12–44) (n = 5)	29 (28–30) (n = 36)
Chlorpyrifos[b]	1,077 (n = 1)	— (n = 0)	1,077 (n = 1)
Copper compounds	899 (820–986) (n = 8)	1,182 (723–1,932) (n = 5)	999 (902–1,107) (n = 13)
Diazinon[b]	251 (4–16,721) (n = 2)	99 (80–123) (n = 5)	130 (93–180) (n = 7)
Dimethoate[b]	171 (97–304) (n = 2)	94 (83–107) (n = 2)	127 (97–165) (n = 4)
Lambda-cyhalothrin[b]	100 (n = 1)	140 (n = 1)	118 (88–158) (n = 2)
γ-HCH[b]	110 (62–196) (n = 3)	177 (15–2,096) (n = 2)	133 (91–194) (n = 5)
Parathion[b]	195 (139–274) (n = 3)	— (n = 0)	195 (139–274) (n = 3)
Propoxur	26 (16–44) (n = 4)	— (n = 0)	26 (16–44) (n = 4)
Pentachlorophenol[b]	44 (37–52) (n = 7)	17 (6–52) (n = 7)	27 (19–39) (n = 14)

[a] When the number of toxicity data (n) exceeded one, the geometric mean is given with 95% confidence limits in parentheses. The all-data analysis used all available data. The comparable-data analysis used only data obtained from comparable standard test conditions. The noncomparable data are the data not included in the comparable-data approach. HCH = hexachlorocyclohexane.
[b] Pesticides with log K_{ow} >2.

dimethoate, γ-HCH, lambda-cyhalothrin, and the biocide pentachlorophenol (Figs. 1 and 2). With the exception of one mite species (Acari), arthropods tend to be less sensitive than oligochaete worms and nematodes to copper. Although arthropod sensitivity to insecticides is expected, sensitivity distributions for the broad-spectrum insecticides chlorpyrifos, parathion, and propoxur can be fitted only for oligochaetes (Lumbricidae). The chlorpyrifos and parathion SSD fail goodness-of-fit tests if Collembola (*F. candida*) data are included (Table 2). This reflects the considerably lower sensitivity of earthworms

Table 2. Estimates of the 5% hazardous concentration (HC5; with 90% confidence limits [CL]) obtained from species sensitivity distributions based on comparable data only or all available data[a]

Data source	Pesticide	No. of data	Median HC5 (mg/kg dry wt soil)	Lognormal goodness of fit[b]	$LC50_{Eisenia}$ (mg/kg dry wt soil)	$LC50_{Eisenia}$ *HC5*
Comparable data	Chlorpyrifos	6	94.9 (15.6–213.1)	Accepted	1,077	11.3
	Copper compounds	12	352.5 (141.3–610.5)	Accepted	555	1.6
	Dimethoate	13	0.39 (0.05–1.34)	Accepted	142	364.1
	Pentachlorophenol	7	4.18 (0.44–12.47)	Accepted	27	6.5
All data	Atrazine	7	5.39 (0.76–13.97)	Accepted	15	2.8
	Carbendazim	10	0.75 (0.04–3.90)	Accepted	4.1	5.5
	Chlorpyrifos[c]	7	0.37 (0.001–6.040)	Rejected	1,077	2,910.8
	Chlorpyrifos[d]	6	124.9 (25.8–252.8)	Accepted	1,077	8.6
	Copper compounds	17	183.3 (80.3–316.3)	Accepted	453	2.5
	Diazinon	5	0.06 (0–1.07)	Accepted	63.3	1,055
	Dimethoate	14	0.30 (0.05–0.95)	Accepted	90	300
	γ-HCH	8	0.21 (0.01–1.36)	Accepted	59	281
	Lambda-cyhalothrin	5	0.09 (0–0.84)	Accepted	23.9	265.6
	Parathion[c]	7	0.27 (0–2.77)	Rejected	148	548.1
	Parathion[d]	6	57.3 (25.9–81.7)	Accepted	148	2.6
	Propoxur	5	0.36 (0.01–1.51)	Accepted	10	27.8
	Pentachlorophenol	9	3.74 (0.43–12.10)	Accepted	0.011	0.003

[a] Also given for comparison is the geometric mean lethal concentration for *Eisenia fetida* ($LC50_{Eisenia}$). HCH = hexachlorocyclohexane.
[b] Anderson-Darling test (α = 0.05).
[c] Including Collembola data.
[d] Excluding Collembola data.

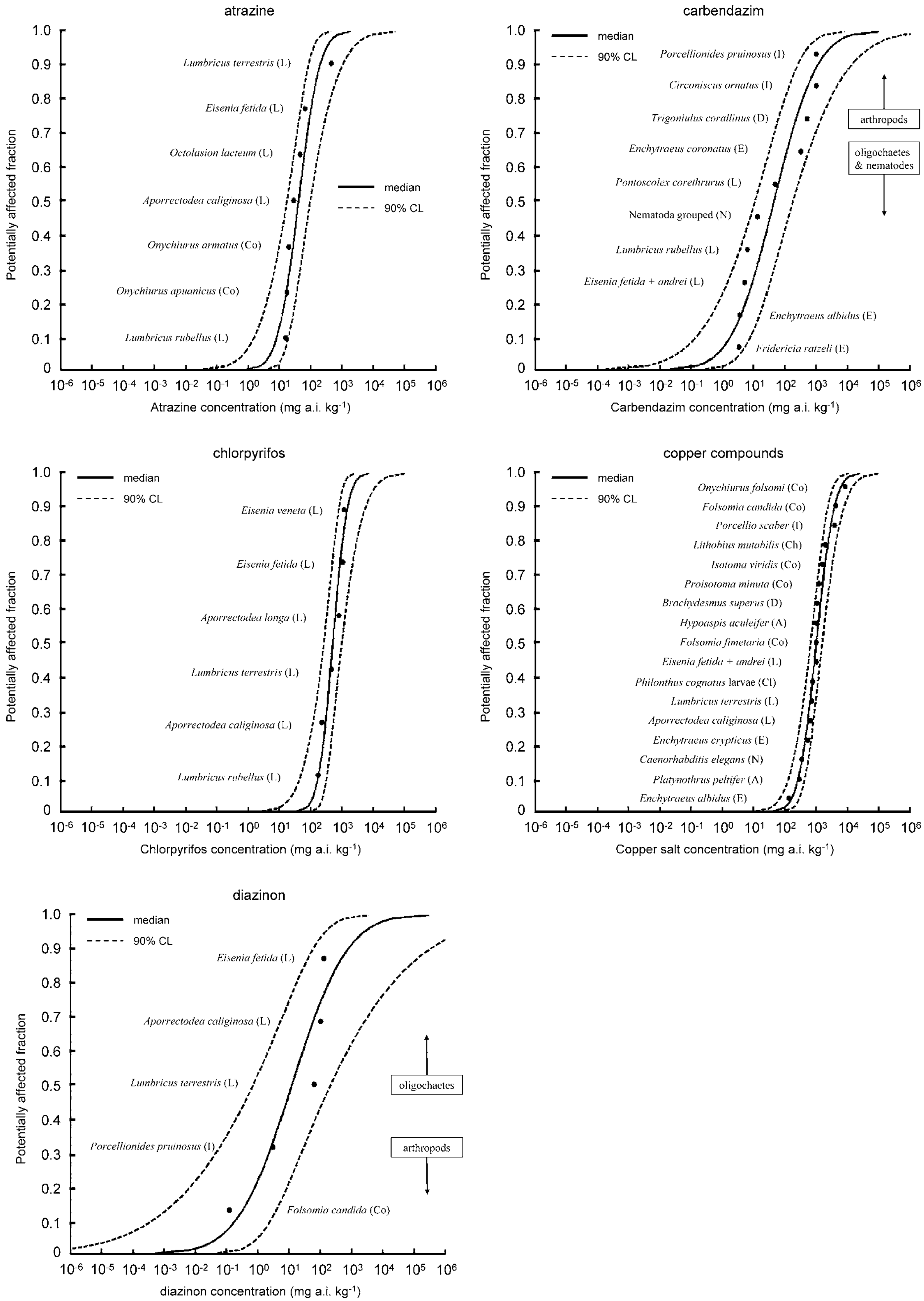

Fig. 1. Species sensitivity distributions (SSD) with 90% confidence limits (CL) for atrazine, carbendazim, chlorpyrifos, copper compounds, and diazinon based on 50% lethal concentration data. The SSD for chlorpyrifos excludes collembolan data (see Table 2). A = Acari; Ch = Chilopoda; Cl = Coleoptera larvae; Co = Collembola; D = Diplopoda; E = Enchytraeidae; I = Isopoda; L = Lumbricidae and other earthworm families; N = Nematoda.

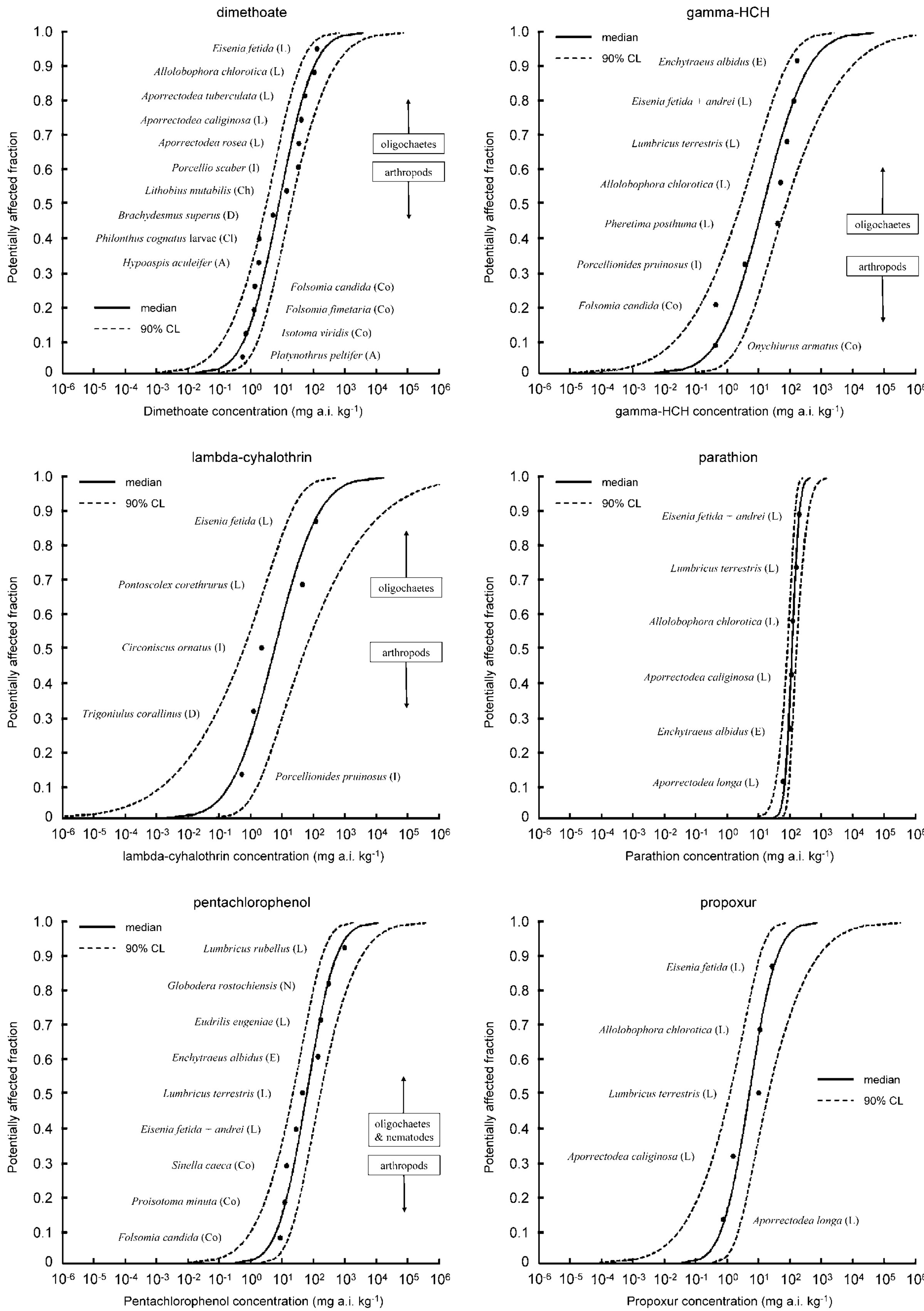

Fig. 2. Species sensitivity distributions (SSD) with 90% confidence limits (CL) for dimethoate, γ-hexachlorocyclohexane (HCH), lambda-cyhalothrin, parathion, pentachlorophenol, and propoxur based on 50% lethal concentration data. The SSD for parathion excludes collembolan data (see Table 2). A = Acari; Ch = Chilopoda; Cl = Coleoptera larvae; Co = Collembola; D = Diplopoda; E = Enchytraeidae; I = Isopoda; L = Lumbricidae and other earthworm families; N = Nematoda.

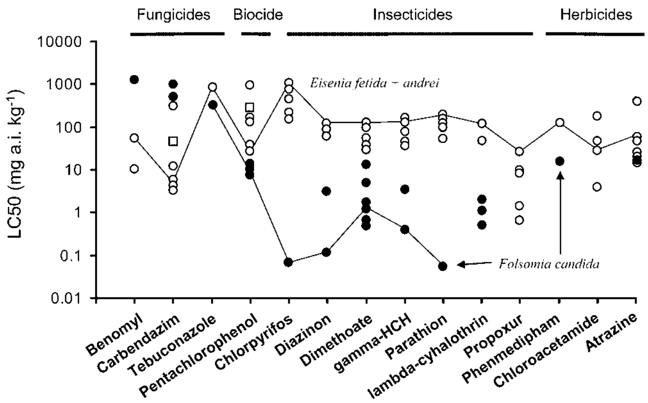

Fig. 3. Sensitivities of different soil invertebrates (○ = oligochaetes; □ = nematodes; ● = arthropods) to pesticides illustrated with acute mortality data. Each data point represents the geometric mean of the available median lethal concentration (LC50) data for one species. Data points for the standard test species *Eisenia fetida* plus *Eisenia andrei* (Lumbricidae) and *Folsomia candida* (Collembola) are connected by lines.

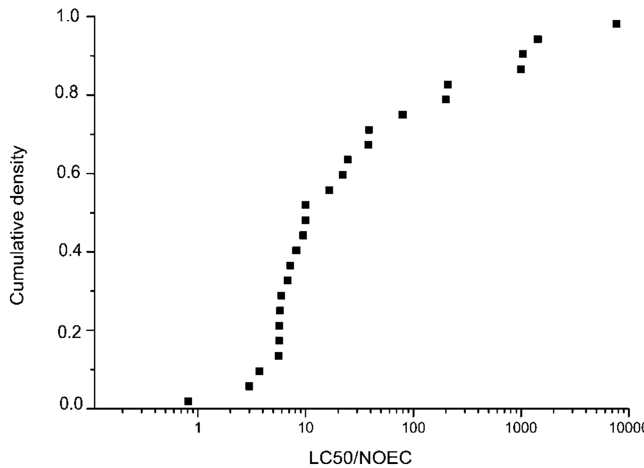

Fig. 4. Pesticide acute to chronic ratios for earthworms (*Eisenia fetida*) plotted as a cumulative density function. LC50 = lethal concentration; NOEC = no-observed-effect concentration.

compared with Collembola to these organophosphorous insecticides and the limited Collembola data ($n = 1$) compared to Lumbricidae ($n = 6$). Exclusion of *F. candida* data raises the HC5 for chlorpyrifos and parathion and increases the precision of their HC5 estimates (indicated by narrower confidence intervals) by up to four orders of magnitude (Table 2). However, these Lumbricidae-based HC5 values would not be protective for arthropods.

Acute mortality LC50 data for the standard earthworm test species, *E. fetida* sensu lato, are available for 131 pesticides, but only 12 of these pesticides have LC50 data for more than two other species for comparison. Sensitivities of the standard test species *E. fetida* sensu lato and *F. candida* can be compared for eight pesticides. In all cases, *F. candida* is the more sensitive species (Fig. 3). For most of these compounds, *E. fetida* sensu lato is one of the least sensitive species, with the exception of two fungicides and a biocide. The data show that *F. candida* can be more sensitive than *E. fetida* to chemicals in each of four main pesticide groups (biocide, herbicide, fungicide, and insecticide) (Fig. 3).

Comparison of sensitivity data with uncertainty factors

In approximately half the analyses (in all cases, insecticides), the relative difference between the LC50 for *E. fetida* sensu lato and the median HC5 is more than a factor of 10 (Table 2), indicating that the range of sensitivities exceeds one order of magnitude (compare Fig. 3). The uncertainty factor of 10 used in the acute mortality test therefore does not cover the full range of soil invertebrate species sensitivities based on acute mortality. However, such a comparison is appropriate only if earthworm tests are used to predict risks to populations of nontarget organisms, as currently is done ([10,34]; http://europa.eu.int/comm/food/plant/protection/evaluation/guidance/wrkdoc09_en.pdf). An ecologically more relevant question is whether the earthworm uncertainty factor would be protective for earthworm species. Three pesticides (chlorpyrifos, parathion, and propoxur) have SSD based entirely on earthworm (Lumbricidae) data. For one of these (propoxur), the ratio of the *E. fetida* LC50 to the Lumbricidae HC5 also exceeds 10. The uncertainty factor of 10 thus does not cover the range of Lumbricidae species sensitivities for all pesticides.

Relationship between acute and chronic sensitivity of earthworms to pesticides

In European regulatory risk assessment of pesticides, earthworm (*E. fetida*) acute mortality (LC50) is assumed to be predictive of the sublethal sensitivity (NOEC) in chronic reproduction tests [34]. Previous work has investigated the relationships between acute and chronic endpoints for birds, mammals, fish, and aquatic invertebrates [35,36], but limited information is available for terrestrial invertebrates. For example, Lock et al. [37] reported γ-HCH acute to chronic ratios (ACR) for *F. candida* ($n = 3$ ACR values), *E. fetida* ($n = 3$), and *E. albidus* ($n = 3$), and a copper ACR for *E. albidus* ($n = 1$).

As the data reported by Lock et al. [37] suggest, too few paired acute mortality and chronic reproduction data are available to permit acute to chronic relationships to be investigated reliably for individual pesticide toxic modes of action. However, for *E. fetida* sensu lato, overall patterns can be investigated by plotting the cumulative distribution of the available ACR values, which range from one to several thousand, with a mean of approximately 10 (Fig. 4). Because the ratio of the uncertainty factors for acute and chronic studies is two (the factors are 10 and 5, respectively [10]), for pesticides with an acute LC50 just on the border of triggering a chronic test, the chronic test will be more than 10-fold as sensitive in 50% of cases and more than twice as sensitive in 95% of cases (Fig. 4). Overall, the acute mortality test would not provide the most protective risk assessment.

DISCUSSION

Data availability

Availability of data is a key issue when considering the use of sensitivity distributions for predicting pesticide hazardous concentrations. For the majority (>95%) of pesticides approved for commercial use in Europe, few input data (in many cases, only one species) would be available unless new data are generated. Standard test species, such as *E. fetida*, may not be ecologically relevant, because they are selected primarily for amenability to testing rather than for presence or ecological function in agricultural soils (where *E. fetida* is, in fact, absent) [38]. The arbitrary minimum of five species chosen here would ensure that at least some ecologically relevant species are included in a sensitivity distribution, because the required number of input data would exceed the number of available standard test species (*E. fetida*, *F. candida*, *E. albidus*, and *H. aculeifer*). The current review excludes data owned privately by agrochemical companies, but it is unclear whether the inclusion of such data would substantially affect the results. Much of the industry data are likely to be generated from standard regulatory testing protocols, which could increase the number of pesticides represented but probably would not involve new species.

A key assumption of SSD and HC5 approach is that the species form a representative sample of those in the environment to be protected [9]. The SSD presented here primarily reflect data availability. However, definition of protection goals is essential in regulatory risk assessment (see below). Soil invertebrate toxicity data obtained from the open literature clearly are dominated by the species used in regulatory testing. This implies that a large proportion of ecotoxicological data in the public domain originates from research connected with regulatory risk assessment. It also suggests that ecotoxicologists tend to work with species selected for their amenability to testing rather than for their ecological attributes. To improve the ecological realism of distribution-based risk assessments, it appears to be necessary to encourage research with more ecologically relevant soil invertebrate species.

Confounding of sensitivity with exposure and bioavailability

An SSD reflects not only the variation in sensitivity among species but also variation in exposure among tests. Variation in sensitivity is most likely to be confounded with variation in exposure when effects are compared for test organisms with different test conditions (e.g., *E. fetida* in a standard earthworm reproduction test compared with *F. candida* in a standard Collembola reproduction test). Such variability might be reduced by using only comparable data when deriving SSD, but the very principle of SSD—that it contains different species—means that some variability resulting from the unique test conditions required for each species cannot be excluded.

Artificial (OECD standard) soil has a higher organic matter content than most natural soils, which may result in lower bioavailability (resulting in higher LC50) being observed in standard soils for those pesticides that are readily adsorbed to organic matter. To adjust toxicity data for the soil organic matter content, the regulatory risk assessment guidance specifies a correction factor (0.5) for toxicity endpoints if an adsorptive pesticide (log $K_{ow} > 2$) is tested in an artificial soil [10]. The comparable LC50 data for *E. fetida* (Table 1) are, in all cases, obtained from standard tests with OECD soils. For most pesticides, the comparable data give the least sensitive toxicity estimates if no correction for soil organic matter is applied, but when corrected for soil organic matter, the comparable data give the most sensitive toxicity estimates (Table 1). Although the correction for the soil organic matter content yields a more conservative estimate of toxicity for most pesticides, the correction is deterministic and imprecise. The all-data approach has the advantage that it may include more ecologically representative soils and exposure conditions, enabling variation in toxicity because of soil characteristics to be represented in the SSD and HC5 instead of using a deterministic correction factor. At present, however, the available data for soil invertebrates are patchy, with some pesticides and species having been tested on a wider range of soils compared with others.

Relevance of pesticide mode of action for selection of test species

According to the European Union Terrestrial Guidance Document on Ecotoxicology [34], the protection goal of the ecological risk assessment for effects of pesticides on the soil environment is populations of nontarget organisms. Because of their ecological relevance and amenability to testing, earthworms were selected as representatives for the whole community (i.e., all populations) of soil invertebrates when setting up test requirements for pesticide registration in Europe [39]. Until now, only earthworm tests have been strictly required in the pesticide risk assessment process for soil invertebrates in the European Union [10,34]. However, the arthropod (*F. candida*) test is more sensitive than the earthworm test to a broad range of pesticide modes of action, suggesting that *E. fetida* is not the most appropriate test species in all cases and that arthropods should be tested routinely as well. The differing sensitivities of arthropods and oligochaetes to pesticides also have been observed in aquatic ecotoxicology [40]. The findings presented here are in agreement with recommendations arising from the aquatic research that separate sensitivity distributions should be used for different pesticide toxic modes of action [30,40]. A surprising finding is that for several broad-spectrum insecticides, SSD and HC5 can be calculated only for oligochaetes, despite the expected high sensitivity of arthropods.

Can SSD and HC5 assist regulatory risk assessment for soil invertebrates?

At present, the regulatory risk assessment for soil invertebrates does not support the use of a distribution-based approach to risk assessment, because only one species (*E. fetida* sensu lato) is used routinely in lower-tier tests, while most higher-tier data are not amenable to analysis using the SSD approach [26]. To use an SSD-based approach, either a very low number of data must be considered as acceptable, the additional species data must come from an external data set (e.g., the literature or archived data, such as the U.S. Environmental Protection Agency ECOTOX database), or they must be generated by testing additional species within the risk assessment procedure.

A possible application of HC5 in soil invertebrate regulatory risk assessment (subject to availability of data) could be to improve the ecological realism of toxicity to exposure ratios: The point (deterministic) toxicity estimate for a standard test species could be replaced by a taxonomically relevant HC5 (or distribution of the HC5, indicated by the confidence interval) based on a larger number of ecologically more relevant species. At present, however, SSD and HC5 that incorporate both oligochaetes and arthropods would not be directly applicable to the risk assessment scheme, in which risk is evaluated separately for earthworms and arthropods [10]. Possibly, separate Lumbricidae-based and arthropod-based HC5 values could be used to refine the estimates for the earthworm toxicity to exposure ratio and arthropod toxicity to exposure ratio, respectively, but clarification would be needed regarding how the small available data sets referring to earthworm and arthropod populations would address the overall protection goal (i.e., populations of nontarget soil invertebrates in general [34]). Validation of HC5 could be carried out by comparing HC5 estimates for individual pesticides with concentrations at which effects are observed in higher-tier studies. A detailed comparison of soil invertebrate HC5 with pesticide effects observed in higher-tier studies has been given by Jänsch et al. [26].

A limitation of the hazardous concentrations reported here is that they are based on acute mortality, which is not the most sensitive endpoint. The ACR could, in theory, be used to derive an extrapolation factor for the HC5 estimates, which would enable HC5 predictions to take more sensitive chronic endpoints (in particular, reproduction) into account. If the chronic reproduction test is assumed to be twice as sensitive as the acute test (as suggested by the ratio of acute to chronic safety factors), then such an extrapolation factor would only be appropriate for 5% of all pesticides (Fig. 4). On the other hand, an extrapolation factor that would be appropriate for the majority (95%) of all pesticides would need to be very large (>1,000) (Fig. 4) and might be considered as too conservative, suggesting that it is inappropriate to calculate a general deterministic extrapolation factor across all toxic modes of action. Arguably, a more appropriate course of action would be to encourage the generation of chronic sublethal toxicity data so that HC5 estimates may be based on the most sensitive toxicity data while, at the same time, excluding the uncertainty that would be introduced by use of a deterministic extrapolation factor.

CONCLUSION

Data availability is a limiting factor for the use of sensitivity distributions to predict ecological effects of pesticides on soil invertebrates. To overcome this problem, either the availability of toxicity data should be improved or the ecological relevance of sensitivity distributions based on few species should be validated. For most of the pesticides evaluated using sensitivity distributions, oligochaetes and arthropods clearly differ in their sensitivity. The available data highlight three limitations of the current regulatory risk assessment for soil invertebrates: First, the acute mortality uncertainty factor does not cover the range of sensitivities of all species for all chemicals. Second, earthworm acute mortality is not the most sensitive endpoint; chronic reproduction should be a preferred test. Third, the arthropod *F. candida* is a more sensitive test species than the earthworm *E. fetida* sensu lato for a broad range of pesticide modes of action (including biocide, fungicide, herbicide, and insecticide). These findings question the relevance of *E. fetida* for assessing the toxicity of some pesticide modes of action and suggest that arthropods (e.g., *F. candida*) should be tested routinely as well.

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