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EFFECTS OF PESTICIDES ON SOIL INVERTEBRATES IN MODEL ECOSYSTEM AND FIELD STUDIES: A REVIEW AND COMPARISON WITH LABORATORY TOXICITY DATA

STEPHAN JÄNSCH,*† GEOFF K. FRAMPTON,‡ JÖRG RÖMBKE,† PAUL J. VAN DEN BRINK§|| and JANECK J. SCOTT-FORDSMAND#
†ECT Oekotoxikologie, Böttgerstraße 2-14, D-65439 Flörsheim, Germany
‡Ecology and Evolutionary Biology Group, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, United Kingdom
§Alterra, Wageningen University and Research Centre, P.O. Box 47, 6700 AA Wageningen, The Netherlands
||Wageningen University, Department of Aquatic Ecology and Water Quality Management, Wageningen University and Research Centre, P.O. Box 8080, 6700 DD Wageningen, The Netherlands
#Department of Terrestrial Ecology, National Environment Research Institute, Vejlsøvej 25, P.O. Box 314, 8600 Silkeborg, Denmark

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Abstract—A systematic review was carried out to investigate the extent to which higher-tier (terrestrial model ecosystem [TME] and field) data regarding pesticide effects can be compared with laboratory toxicity data for soil invertebrates. Data in the public domain yielded 970 toxicity endpoint data sets, representing 71 pesticides and 42 soil invertebrate species or groups. For most pesticides, the most frequent effect class was for no observed effects, although relatively high numbers of pronounced and persistent effects occurred when Lumbricidae and Enchytraeidae were exposed to fungicides and when Lumbricidae, Collembola, and Arachnida were exposed to insecticides. No effects of fungicides on Arachnida, Formicidae, or Nematoda or of herbicides on Lumbricidae, Formicidae, or Nematoda were observed in any studies. For most pesticides, higher-tier no-observed-effect concentration or lowest-observed-effect concentration values cannot be determined because of a lack of information at low pesticide concentrations. Ten pesticides had sufficient laboratory data to enable the observed higher-tier effects to be compared with 5% hazardous concentrations (HC5) estimated from acute toxicity laboratory data (atrazine, carbendazim, chlorpyrifos, diazinon, dimethoate, γ-hexachlorocyclohexane, lambda-cyhalothrin, parathion, pentachlorophenol, and propoxur). In eight cases, higher-tier effects concentrations were within or below the 90% confidence interval of the HC5. Good agreement exists between the results of TME and field tests for carbendazim, but insufficient information is available for a comparison between TME and field studies for other pesticides. Availability and characteristics (e.g., taxonomic composition and heterogeneity) of the higher-tier effects data are discussed in terms of possible developments in risk assessment procedures.

Keywords—Hazardous concentration Lumbricidae Risk assessment Species sensitivity distributions Terrestrial model ecosystems

INTRODUCTION

Pesticide risk assessment procedures focus on the species that have been studied most intensively and that are most amenable to toxicological assessment (i.e., species that are easy to culture and maintain under standardized test conditions and, ideally, that exhibit sensitivity to a wide range of pesticides). An important conceptual element of the risk assessment is that the wider environmental risks of pesticide use can be predicted effectively for soil organisms using a small set of standard soil invertebrate test species. In Europe, where regulatory risk assessment conforms to the guidance of the Authorizations Directive (Directive 91/414/EEC, as amended [1]), adequately developed test methods are available for assessing effects of pesticides on three soil invertebrate species: *Eisenia fetida* (earthworm: Lumbricidae), *Folsomia candida* (springtail: Collembola), and *Enchytraeus albidus* (potworm: Enchytraeidae). A fourth method, using the predatory mite *Hypoaspis aculeifer* (Acari), is under development [2]. With the exception of earthworms (for which a field test guideline has been available since 1994), soil invertebrate tests are confined to the laboratory. Terrestrial model ecosystems (TME) have been proposed but currently are not required as part of the standard registration process. In regulatory risk assessment, opportunities to validate the predictions of lower-tier tests are limited by the lack of field data. Accordingly, investigations into relationships between the results of laboratory toxicity studies and effects of pesticides on soil invertebrate populations or communities [3] so far have been restricted almost entirely to earthworms.

Risk assessment of pesticides employs a tiered, stepwise approach, starting with relatively simple single-species tests carried out under (assumed) worst-case exposure conditions in laboratory studies. If laboratory studies indicate unacceptable risk, further testing under more ecologically realistic conditions is carried out, such as in extended laboratory, semifield, TME, or field tests. Ideally, the predictive ability of the laboratory (lower-tier) studies should be validated against pesticide effects data obtained under more ecologically realistic (higher-tier) conditions.

The current, deterministic pesticide risk assessment scheme for soil invertebrates assumes that effects on soil invertebrate communities may be predicted using single species tested under a narrow range of exposure conditions. To account for uncertainty in the ecological relevance of the test species and test conditions compared to real ecosystems, arbitrary uncertainty factors are used for the tiered testing scheme. The earth-

* To whom correspondence may be addressed (s-jaensch@ect.de).

worm (*E. fetida*) acute mortality test [4], for example, employs a factor of 10 such that further, higher-tier testing is triggered unless the median lethal concentration (LC50) is at least 10-fold higher than the predicted environmental concentration [5]. In this case, by definition, the range of sensitivities of the nontarget soil invertebrate community to pesticides is assumed not to exceed one order of magnitude.

The deterministic risk assessment approach has the advantages of being straightforward, easily harmonized, and applicable to a small amount of input data. However, the use of arbitrary application factors with uncertain margins of protection has been criticized as imparting a lack of transparency to the risk estimation. Recent workshops have recommended that deterministic and alternative probabilistic approaches to risk assessment should be compared to evaluate whether probabilistic approaches could improve the current risk assessment procedure ([6]; http://www.eupra.com/report.pdf). However, only in a few cases [7] have probabilistic risk assessment approaches been applied to investigate effects of chemicals on soil fauna.

If the sensitivity to a pesticide is known for a range of species, then parameters of the species sensitivity distribution (SSD) may be used to predict the concentration of the substance at which a certain proportion x (%) of the species would be affected (i.e., the hazardous concentration [HCx]) [8]. This concept is recognized as a potentially useful tool in environmental protection and risk assessment. The determination of the hazardous concentration enables (at least in theory) the risk to a community of species to be predicted from information based on a small subset of the species while also providing an indication regarding the certainty of the risk estimate (indicated by confidence bounds). The current convention is to use the median HC5 as a benchmark for the maximum permissible environmental concentration (i.e., the concentration that would affect no more than 5% of the species, giving a 95% protection level) [8].

The HC5 is a controversial approach [9] and is not formally specified as a risk estimate for pesticides at present under the European pesticide risk assessment scheme for soil invertebrates. However, the HC5 has been used in higher-tier assessment of pesticide risks to aquatic invertebrates [10]. Distribution-based trigger values also have been proposed for contaminated land assessment [11].

In the work reported here, a systematic review was carried out to investigate the extent to which higher-tier (TME and field) data regarding pesticide effects can be compared with laboratory toxicity data for soil invertebrates. To our knowledge, this compilation provides the most detailed review to date of higher-tier pesticide effects data for soil invertebrates. Where possible, both laboratory chronic effects data and HC5 values estimated from LC50 data [12] are compared with the higher-tier effects data to investigate the predictive accuracy of the laboratory toxicity data. Comparisons also are made between effects on soil invertebrates observed in TME and in field studies.

MATERIALS AND METHODS

Data compilation

The methodology of data compilation has been reported in detail by Frampton et al. [12]. Pesticide active substances (excluding microbial and fungal) were considered to be relevant if they are currently approved, or had been approved previously, for use in European agriculture. The pesticides comprised acaricides, fungicides, herbicides, insecticides, molluskicides, nematicides, and broad-spectrum biocidal compounds [12]. Two types of data were extracted from the literature and databases: Lower-tier data (from laboratory tests), and higher-tier data (from TME or field tests).

Lower-tier (laboratory test) data

The laboratory pesticide effects data for soil invertebrates reported here are those compiled by Frampton et al. [12]. Data were extracted into a Microsoft Excel® database (Microsoft, Redmond, WA, USA) if at least one relevant measurement endpoint (e.g., acute or chronic LC50, median effective concentration [EC50], or no-observed-effect concentration [NOEC]) was given, together with sufficient supporting information to allow the endpoint to be interpreted (i.e., information regarding the species and test conditions, pesticide formulation type, application rate, and type and duration of exposure). Data were accepted for any euedaphic (soil-dwelling) invertebrate group other than microorganisms.

Higher-tier (TME and field) data

Data from TME, semifield, and field studies were extracted into a second Excel database if all the following criteria were met: The data concerned euedaphic invertebrates (excluding microorganisms), were reported for an euedaphic life stage (e.g., the larvae of certain flies and beetles), or were obtained by soil sampling; the measurement endpoints given were relevant to field populations or communities; effects of specified individual pesticides were not confounded with other variables (e.g., other chemical applications); the spatial and temporal characteristics of the reference (control) treatment were appropriate for interpreting pesticide effects; and the study design and analysis were reported in sufficient detail to enable quantitative interpretation of pesticide effects (i.e., based on adequate replication and statistical evaluation).

Classification of higher-tier effects

Because of the heterogeneity of higher-tier studies, which vary considerably in their aims, methods, experimental designs, and how they report their results (e.g., in the amount of detail given), no single effect measure could be clearly compared across all studies. To overcome this problem, the results for each pesticide concentration tested were assigned to one of five pesticide effect classes, which cover the range from no observed effect (class 1) to a pronounced long-term effect (class 5). These classes are based on initial recommendations by Brock et al. [13] for assessing higher-tier effects of pesticides in aquatic systems and have since been adopted by the European Union (EU) for regulatory risk assessment in aquatic systems ([14]; http://europa.eu.int/comm/food/fs/ph_ps/pro/wrkdoc/wrkdoc10_en.pdf). The only quantitative information given in the definition of the effect classes is that a period of 100 d is recommended as a threshold for determining recovery. This originates from the EU definition of persistence [15], which is ecologically arbitrary (i.e., not related to ecological considerations such as species life cycle) and serves primarily to standardize the reporting of recovery. The magnitude of effects was assessed according to guidance for nontarget arthropods, in which clear pesticide effects are (arbitrarily) defined as those differing by more than 30% compared to a control treatment [16]. Here, the 30% threshold is used to distinguish slight and pronounced effects, whereas statistical significance is used primarily to distinguish slight effects from noneffects.

The following higher-tier effects classes for soil invertebrates were thus distinguished. In class 1, no effect could be demonstrated: No clear causal relationship was observed between pesticide and control treatments (primarily based on statistical significance). In class 2, a slight effect was observed: Effects of small magnitude (≤30%) and short duration (<100 d). In class 3, a pronounced short-term effect was observed: Effects of large magnitude (>30%) but short duration (<100 d). In class 4, a pronounced effect in a short-term study was observed: Effects of large magnitude (>30%) but too short a study (or too long a sampling interval) to demonstrate complete recovery within 100 d. In class 5, a pronounced long-term effect was observed: Effects of large magnitude (>30%) and long duration (≥100 d).

For cases in which no distinct classification of effects was possible (e.g., when no statistical significance testing was reported or in studies of behavior), expert judgment was applied. In borderline cases, effects usually were assigned to the higher effect class to remain on the protective side when considering risk to soil invertebrates.

The biological data were grouped as Arachnida, Chilopoda, Collembola, Enchytraeidae, Formicidae, Lumbricidae, and Nematoda. The assessed endpoints were abundance, biomass, mortality, behavior (e.g., surface migration), and development. Higher-tier risk was explored by plotting the higher-tier effects class against the pesticide concentration.

Comparison of effects in TME and field studies with laboratory effects data

To enable comparison of field observations with the results of laboratory tests, the exposure units required standardization. This was achieved by converting application rates in kg/ha to mg/kg using the following equation:

$$\mathrm{MC5} = F \cdot D / \Delta z / \rho \quad (1)$$

where MC5 is the maximum concentration of pesticide in the top 5 cm of soil (mg/kg), F is the factor for conversion from kg/ha to mg/m^2 (100 mg/m^2/kg ha), D is the nominal treatment (the application concentration; kg/ha), Δz is the layer thickness (0.05 m), and ρ is the dry bulk density (kg/m^3). This conversion follows the current pesticide regulatory risk assessment for soil invertebrates in assuming that the top 5 cm of soil is the relevant exposure depth and that a standard soil has a bulk density of 1,500 kg/m^3 [5]. Using the described parameter values, Equation 1 becomes

$$\mathrm{MC5} = 1.33D \quad (2)$$

When availability of data for a pesticide permitted, laboratory-derived HC5s, which were each based on a minimum of five species [12], were compared directly with the higher-tier pesticide effect concentrations and (if available) with higher-tier NOEC estimates.

RESULTS

Availability of lower-tier effects data

The systematic search for laboratory effects data yielded 1,950 toxicity endpoint data sets, representing 250 pesticide active substances and 67 soil invertebrate species or groups [12]. Despite the large number of pesticide substances and taxonomic groups, relatively few effects data were found for each pesticide, meaning that SSD and HC5 could be calculated only for 11 pesticides using acute mortality data. Too few data (i.e., for less than five species per pesticide) were available to permit calculation of the HC5 based on sublethal endpoints [12].

Availability of higher-tier effects data

The systematic search for higher-tier effects data yielded 970 toxicity endpoint data sets, of which 762 and 208 originate from field and TME studies, respectively, representing altogether 71 pesticide active substances and 42 soil invertebrate species or groups. Most higher-tier data are of low taxonomic resolution; for example, 58% of the Collembola and 69% of the Lumbricidae data sets are stated only as belonging to the broad groups Collembola and Lumbricidae, respectively. The standard collembolan test species, *F. candida* [17], accounted for only 3% of the higher-tier Collembola data, whereas the standard earthworm test species, *E. fetida* [18], was not represented at all in the higher-tier data (because of its specific ecological requirements [19]).

The fungicide carbendazim has been the best investigated compound by far in higher-tier studies of soil invertebrates, followed by the insecticide carbofuran, the fungicide benomyl, and the herbicide atrazine; all other compounds have less than 30 data sets. Only 14 of the 71 pesticides in the higher-tier database have more than 10 toxicity data sets. It is notable that despite the intensive study of carbendazim, almost all the studies with this compound have involved only three groups of soil invertebrates: Lumbricidae, Nematoda, and Enchytraeidae. This reflects the use of carbendazim in an extensive TME ring-testing and field-validation study that focused mainly on these groups [20]. For Nematoda and Enchytraeidae in particular, a strong bias exists toward higher-tier testing with carbendazim compared to other pesticides. For Collembola, which are highly sensitive to insecticides in laboratory studies, the best availability of higher-tier data is for an herbicide (atrazine). Overall, the higher-tier data are dominated by older pesticides, such as carbamate, organophosphorous, and organochlorine insecticides. Synthetic pyrethroid insecticides, which currently are the most widely used insecticides in Europe, each have fewer than five data sets. Strobilurin fungicides, which represent a relatively new mode of action and include replacements for carbendazim, are not represented at all among the higher-tier data (see Table S1 in SETAC Supplemental Data Archive, Item ETC-25-09-005; http://etc.allenpress.com).

The majority of the higher-tier data sets are from grass (56%), followed by crop sites (32%) and forests (12%). Most studies had a duration of one to four months (43%), followed by studies lasting from four to 12 months (25%) and those lasting less than one month (20%). Only 12% of all data sets originated from studies that lasted more than one year. Nearly all studies were performed under temperate conditions, meaning that comparisons between climatic regions are not possible.

Lumbricidae, Collembola, Enchytraeidae, Arachnida, Nematoda, and Formicidae have been the most studied of the soil invertebrate groups, comprising 92% of the higher-tier data. The most frequent measurement endpoints (89% of the data) were abundance and/or biomass (often reported together, with biomass estimated from the abundance and mean weight per species), followed by mortality (10%). Very few studies assessed behavior or development (together accounting for less than 1% of the data).

In most cases, the modal effects class was class 1 (no effect observed), although relatively high numbers of pronounced

Table 1. Distribution of higher-tier pesticide effects among five effect classes for the six most intensively studied groups of soil invertebrates[a]

Mode of action	Effect class	Invertebrate group					
		Lumbricidae	Collembola	Enchytraeidae	Arachnida	Nematoda	Formicidae
Fungicides	1	52	6	40	5	50	5
	2	1	2	0	0	0	0
	3	4	0	11	0	0	0
	4	11	0	0	0	0	0
	5	38	1	20	0	0	0
Herbicides	1	12	11	0	6	2	4
	2	0	0	1	0	0	0
	3	0	0	1	3	0	0
	4	0	2	0	0	0	0
	5	0	7	2	4	0	0
Insecticides	1	32	26	7	24	1	11
	2	9	13	0	4	2	0
	3	20	0	0	2	0	0
	4	15	17	1	4	1	4
	5	16	11	1	10	1	0
Mixed action	1	7	7	4	2	4	0
	2	2	0	0	3	0	0
	3	4	4	1	3	0	0
	4	4	2	1	2	0	1
	5	6	5	3	3	1	0

[a] Effect classes are as follows: 1 = no observed effect; 2 = slight transient effect; 3 = pronounced transient effect; 4 = pronounced effect in a short-term study; 5 = pronounced long-term effect (full details are given in *Materials and Methods*). Pesticides are grouped according to either specific mode of action (fungicides, herbicides, or insecticides) or mixed action (combined acaricide, fungicide, herbicide, insecticide, and/or nematicide.

and persistent effects (class 5) occurred when Lumbricidae and Enchytraeidae were exposed to fungicides and when Lumbricidae, Collembola, and Arachnida were exposed to insecticides (Table 1). The large number of studies reviewed and the heterogeneity of study designs and the variety of pesticides within each mode of action (e.g., broad-spectrum and selective compounds) limit interpretation of the summarized data. However, the reported data indicate a general lack of higher-tier effects of fungicides on Arachnida, Nematoda, and Formicidae and of herbicide effects on Lumbricidae, Nematoda, and Formicidae (Table 1).

Forty-five of the 71 pesticides with higher-tier soil invertebrate effects data have corresponding lower-tier data. However, only 11 pesticides met the minimum lower-tier data requirement (five species) for estimating the HC5, in all cases using acute mortality (LC50) data [12] (atrazine, carbendazim, chlorpyrifos, copper compounds, diazinon, dimethoate, γ-hexachlorocyclohexane [HCH], lambda-cyhalothrin, parathion, pentachlorophenol, and propoxur) (Table 2). For copper compounds, no comparable higher-tier data were found, probably because monitoring studies of copper-contaminated sites involved multiple pollutants.

Availability of higher-tier effects data differs between the pesticides. Only a single effect record is available for dimethoate, whereas for carbendazim, γ-HCH, and pentachlorophenol, the effects concentrations range up to three orders of magnitude (Figs. 1 and 2). For most invertebrate groups, atrazine and carbendazim provide evidence for a concentration–response relationship, because no effects (class 1) were observed at the lowest concentrations tested, whereas pronounced long-term effects (class 5) occurred at the highest concentrations. However, pronounced long-term effects of atrazine on Collembola occurred at a relatively low concentration (Fig. 1A). This might reflect indirect effects of the herbicide (e.g., influencing soil vegetation or organic matter content). Insecticides (chlorpyrifos, diazinon, dimethoate, γ-HCH, lambda-cyhalothrin, parathion, and propoxur) have been tested on soil invertebrates at relatively few concentrations, and in nearly all cases, slight or pronounced effects (classes 2–5) occurred (Figs. 1 and 2). The biocide pentachlorophenol exhibits a mixture of no observed effects (class 1) and pronounced long-term effects (class 5) at most of the concentrations tested.

Comparison of higher-tier pesticide effects with lower-tier HC5 estimates

In aquatic ecotoxicology, it has been possible for some pesticides to validate the HC5 estimates obtained from mesocosm studies by comparing them with estimates of the higher-tier NOEC or lowest-observed-effect concentration (LOEC) [10]. However, for soil invertebrates, the higher-tier NOEC or LOEC can be estimated only for atrazine; for all other pesticides, effects occurred at the lowest concentrations tested (Figs. 1 and 2 and Table 2).

The median HC5 exceeds the higher-tier NOEC and LOEC estimates for atrazine and carbendazim. For all other substances, it lies below the lowest concentration at which higher-tier effects were observed (in the case of parathion, only very closely so). However, the estimates of HC5 are uncertain (as indicated by the 90% confidence interval). The taxonomic composition of the data differs both between study tiers and pesticides. For chlorpyrifos, parathion, and propoxur, HC5 estimates are based mainly on species of Lumbricidae (Table 2). In higher-tier studies, Collembola are more sensitive than Lumbricidae to chlorpyrifos (Fig. 1), whereas for parathion, Lumbricidae appear to be more sensitive (Fig. 2). The Collembola data (*F. candida*) has a large impact on the HC5 [12], because exclusion of the *F. candida* data would cause the HC5 (including its 90% confidence interval) to lie clearly above all tested higher-tier concentrations of chlorpyrifos and parathion (compare Figs. 1 and 2 and Table 2). With the Collembola

Table 2. Summary of lower-tier (laboratory) and higher-tier effects data for those pesticides with at least five data sets for calculation of 5% hazardous concentration (HC5) and at least one higher-tier data set for comparison[a]

	Laboratory (SSD) data					Higher-tier data			
			HC5 (mg/kg)						
Pesticide	Data sets (*n*)	Taxonomic composition	Lower	Median	Upper	Data sets (*n*)	Taxonomic composition	NOEC (mg/kg)	Best LOEC estimate (mg/kg)
Atrazine	7	2 Collembola 5 Lumbricidae	0.76	5.39	13.97	29	6 Arachnida 13 Collembola 2 Coleoptera 2 Enchytraeidae 4 Lumbricidae 2 Nematoda	0.13	0.53
Carbendazim	10	1 Diplopoda 3 Enchytraeidae 3 Lumbricidae[b] 2 Isopoda 1 Nematoda	0.04	0.75	3.90	177	1 Collembola 67 Enchytraeidae 61 Lumbricidae 48 Nematoda	Not estimable	≤0.24
Chlorpyrifos	7	1 Collembola 6 Lumbricidae	0.001	0.370	6.040	11	1 Arachnida 6 Collembola 1 Formicidae 3 Lumbricidae	Not estimable	≤0.64
	6	6 Lumbricidae	25.8	124.9	252.8				
Diazinon	5	1 Collembola 3 Lumbricidae 1 Isopoda	0	0.06	1.07	16	4 Arachnida 4 Collembola 1 Formicidae 7 Lumbricidae	Not estimable	≤5.97
Dimethoate	14	2 Acari 3 Collembola 1 Chilopoda 1 Coleoptera 1 Diplopoda 5 Lumbricidae 1 Isopoda	0.05	0.30	0.95	1	1 Collembola	Not estimable	≤0.53
γ-HCH	8	2 Collembola 1 Enchytraeidae 4 Lumbricidae 1 Isopoda	0.01	0.27	1.66	5	1 Arachnida 1 Collembola 3 Nematoda	Not estimable	≤1.30
Lambda-cyhalothrin	5	1 Diplopoda 2 Lumbricidae[b] 2 Isopoda	0	0.09	0.84	4	4 Collembola	Not estimable	≤2
Parathion	7	1 Collembola 1 Enchytraeidae 5 Lumbricidae	0	0.27	2.77	13	4 Arachnida 4 Collembola 5 Lumbricidae	Not estimable	≤0.35
	6	1 Enchytraeidae 5 Lumbricidae	25.9	57.3	81.7				
Pentachlorophenol	9	3 Collembola 1 Enchytraeidae 4 Lumbricidae[c] 1 Nematoda	0.43	3.74	12.10	26	3 Arachnida 7 Collembola 5 Enchytraeidae 8 Lumbricidae 3 Nematoda	Not estimable	≤6.60
Propoxur	5	5 Lumbricidae	0.01	0.36	1.51	2	2 Lumbricidae	Not estimable	≤1.34

[a] The lower and upper estimates of the HC5 are the 90% confidence limits. The HC5 estimates for chlorpyrifos and parathion are given both with and without Collembola data being included. The HC5 data are from Frampton et al. [12]. SSD = species sensitivity distribution; NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; HCH = hexachlorocyclohexane.
[b] Including Glossoscolecidae.
[c] Including Eudrilidae.

data included, the median HC5 for chlorpyrifos and parathion lie below all recorded higher-tier effects concentrations and, thus, may appear to be protective for soil invertebrates. However, the HC5 is highly uncertain, as indicated by the 90% confidence interval, which spans nearly four orders of magnitude for chlorpyrifos (Fig. 1C) and is even wider for parathion (Fig. 2D). In both cases, the uncertainty range of the HC5 includes concentrations at which higher-tier effects occurred. For carbendazim and propoxur, neither the lower-tier HC5 (Table 2) nor the higher-tier effects data (Figs. 1B and 2F) include Collembola, whereas for γ-HCH, the taxonomic agreement between the lower-tier (Table 2) and higher-tier (Fig. 2B) data is limited.

Comparison of higher-tier pesticide effects with other lower-tier endpoints

Most chronic lower-tier data (NOEC, LOEC, and EC50) are available for the standard test organisms, *E. fetida* sensu

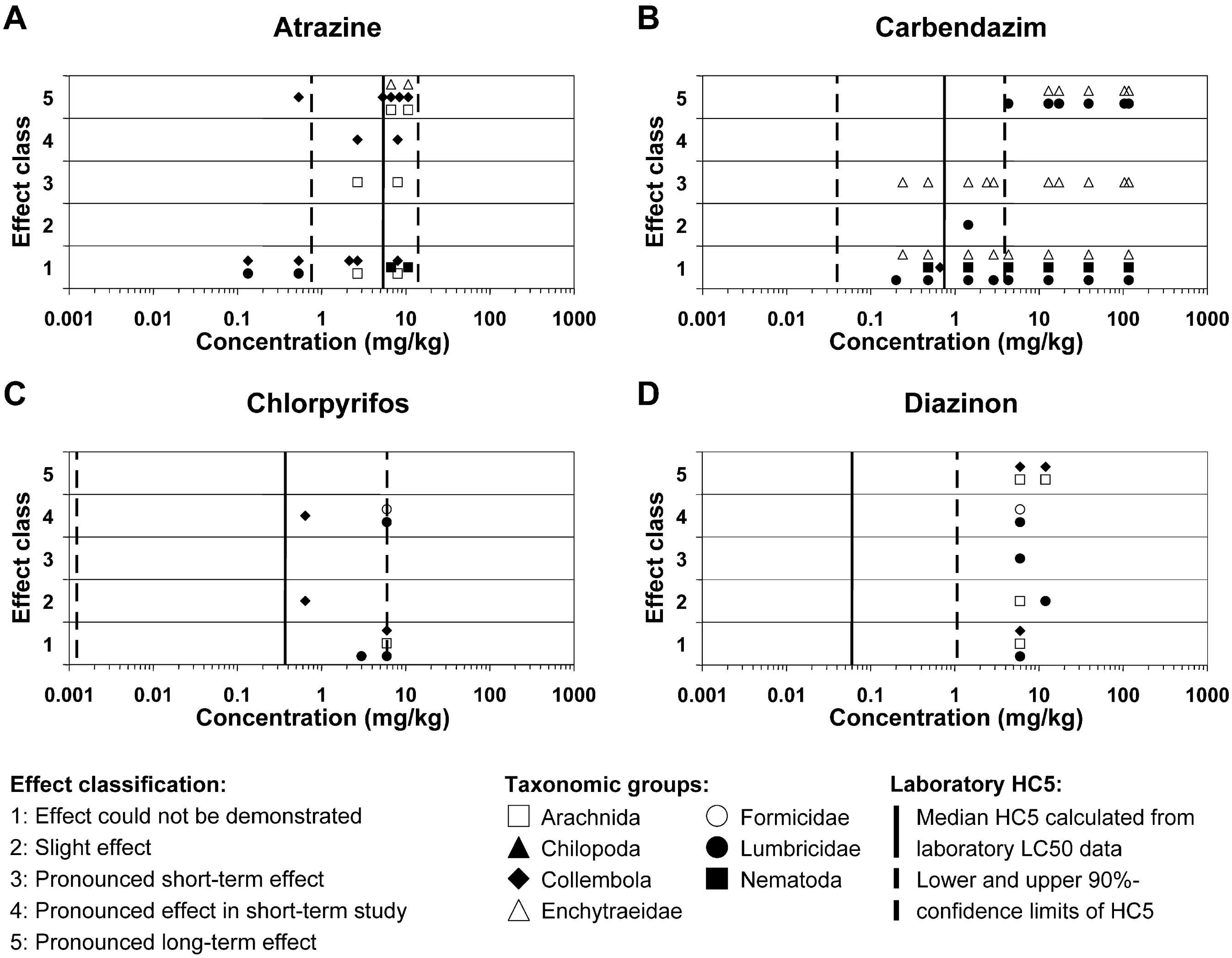

Fig. 1. Higher-tier effects on soil invertebrates of (**A**) atrazine (n = 29), (**B**) carbendazim (n = 177), (**C**) chlorpyrifos (n = 11), and (**D**) diazinon (n = 16). Data points represent concentrations for which at least one study has been conducted. The lower 90% confidence limit of the 5% hazardous concentration (HC5) is not shown for diazinon, because it was too low to fit the given scale (see Table 2). Legends for Figure 1 apply to all figures. LC50 = lethal concentration.

lato (*E. fetida* and *Eisenia andrei*) (Lumbricidae) and *F. candida* (Collembola). If no chronic data were found for these species, then data from other species were used in the comparison when possible. For diazinon and propoxur, no chronic data are available. For atrazine, only data for *F. candida* were found, whereas for carbendazim, chlorpyrifos, parathion, and pentachlorophenol, only chronic Lumbricidae data are available (Table 3).

For atrazine, lambda-cyhalothrin, and parathion, the effects observed in higher-tier studies tend to occur at lower concentrations than the effects observed in lower-tier studies. However, for carbendazim, the EC50 for *E. fetida* reproduction clearly is below the concentration at which pronounced effects on lumbricids have been observed in higher-tier studies (Table 3). For carbendazim (only NOEC and LOEC), chlorpyrifos, dimethoate, and pentachlorophenol, effects occurred at similar concentrations at both lower and higher tiers. In the case of γ-HCH, no comparison is possible, because no concentrations in the range of the laboratory NOEC, LOEC, or EC50 have been tested in the field (compare Figs. 1 and 2 and Table 3).

Several pesticides have good availability of higher-tier effects data but insufficient lower-tier data for comparison based on HC5 estimates. Of these, bendiocarb, benomyl, carbaryl, carbofuran, diflubenzuron, halofenozide, imidacloprid, and phorate have the best higher-tier data, although for bendiocarb and halofenozide, this covers only one concentration (Fig. 3). For these pesticides, estimates of higher-tier NOEC and LOEC are not possible, either because slight effects (classes 2 and 3) or pronounced effects (classes 4 and 5) occurred at the lowest concentrations tested or, for halofenozide, because no higher-tier effects were observed (class 1 only).

Relevant lower-tier toxicity estimates for comparison with these higher-tier data are available primarily for the standard test Lumbricidae *E. fetida* sensu lato. No lower-tier arthropod toxicity data were found for the insecticides diflubenzuron and imidacloprid. No lower-tier data at all are available for the insecticides bendiocarb and halofenozide. For the fungicide benomyl, almost all the higher-tier data are for Lumbricidae (Fig. 3B).

For benomyl, the lowest concentration tested in higher-tier studies (0.2 mg/kg) is slightly lower than the lowest available laboratory NOEC (0.3 mg/kg) and LOEC (1.0 mg/kg), both of which are for *E. andrei* (production of fertile cocoons [21]). Pronounced and long-term (class 5), higher-tier effects of ben-

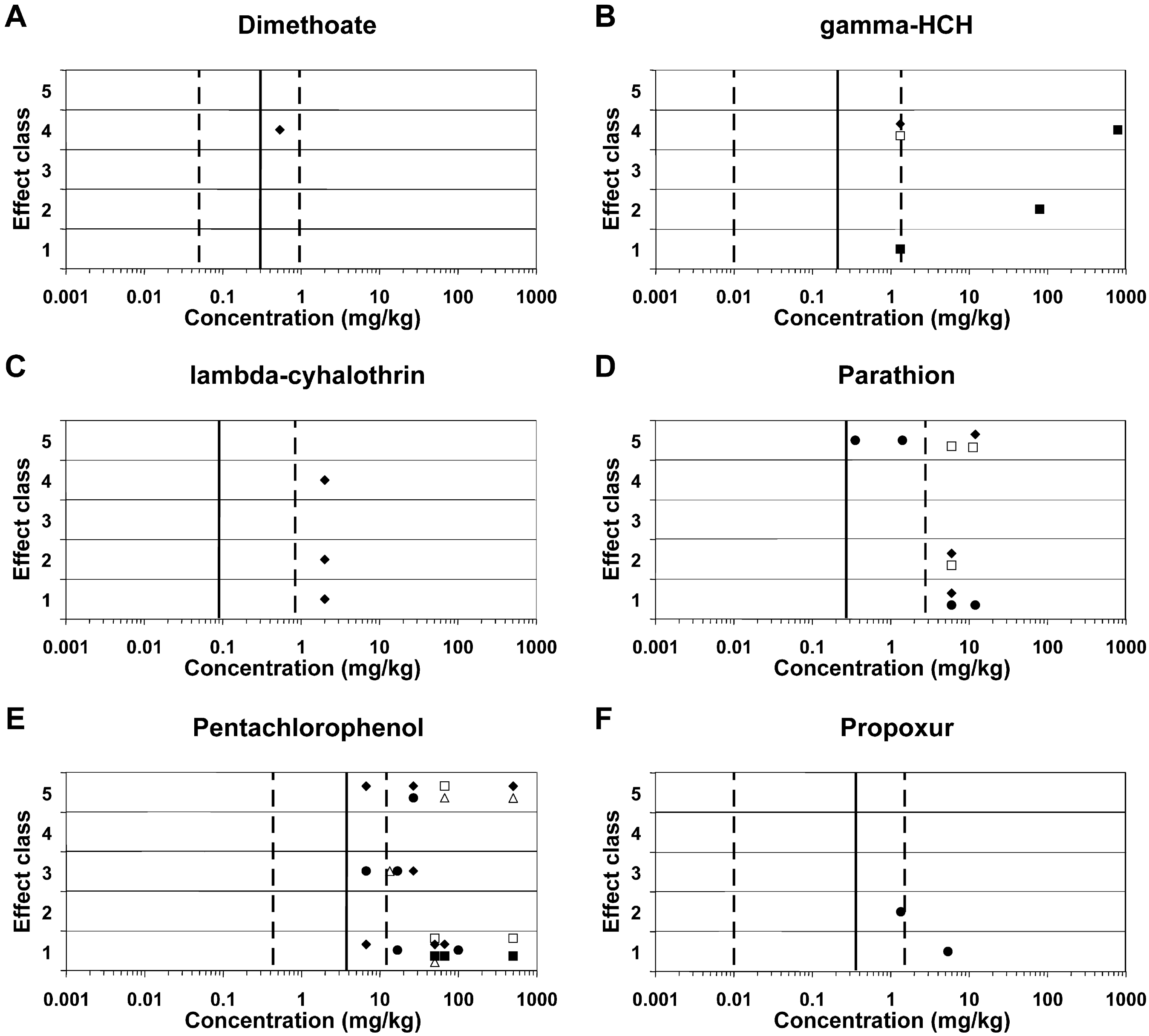

Fig. 2. Higher-tier effects on soil invertebrates of (**A**) dimethoate (n = 1), (**B**) γ-hexachlorocyclohexane (HCH; n = 5), (**C**) lambda-cyhalothrin (n = 4), (**D**) parathion (n = 13), (**E**) pentachlorophenol (n = 26), and (**F**) propoxur (n = 2). Data points represent concentrations for which at least one study has been conducted. Lower 90% confidence limits of the 5% hazardous concentration (HC5) are not shown for lambda-cyhalothrin and parathion, because they were too low to fit the given scale (see Table 2). For explanation of symbols and effect classification, see Figure 1.

omyl on Lumbricidae have been recorded at lower concentrations than those of its first metabolite carbendazim (in the range of 0.1–5.0 mg/kg, at which no pronounced effects of carbendazim are seen) (compare Figs. 1B and 3B). Collembola appear to be considerably less sensitive than Lumbricidae to benomyl, as indicated by both the (limited) higher-tier effects data (Fig. 3B) and the chronic laboratory NOEC and LOEC for reproduction of *Onychiurus folsomi* (200 and 300 mg/kg, respectively [Ecological Services Group (ESG) International. 2002. Assessment of the biological test methods for terrestrial plants and soil invertebrates: Pesticides. Report no. 99096 (March 3, 2002) for the Method Development and Applications Section, Environmental Technology Centre, Environment Canada, Ottawa, ON, Canada]).

For carbaryl, no chronic laboratory data were found. The lowest LC50 values reported in the literature for Lumbricidae are 19.3 mg/kg for *Lumbricus terrestris* [22] and 174 mg/kg for *E. andrei* [23]. For *F. candida*, the lowest laboratory LC50 is 2 mg/kg [24], confirming a much higher sensitivity of Collembola to this insecticide. Almost all the concentrations of carbaryl tested in higher-tier studies have been below the laboratory LC50 values, with effects observed on Lumbricidae between 3 and 30 mg/kg, whereas no concentration in the range of the lowest laboratory LC50 for Collembola has been tested in the field. Effects on Collembola in the field occur at a concentration of 11.9 mg/kg (Fig. 3C).

For carbofuran, both the lowest-effect concentration in a chronic reproduction laboratory test using *F. candida* (LOEC, 0.1 mg/kg; NOEC, 0.08 mg/kg) [25] and the lowest LC50 (0.06 mg/kg) are considerably lower than those found for this insecticide in higher-tier studies (1–20 mg/kg). The same is true for chronic effects on the earthworm *Aporrectodea caliginosa* (LOEC, 0.1 mg/kg; NOEC, 0.05 mg/kg) [26]. The lowest LC50 found in the laboratory for Lumbricidae is 3.1

Table 3. Chronic laboratory toxicity of atrazine, carbendazim, chlorpyrifos, dimethoate, γ-hexachlorocyclohexane (HCH), lambda-cyhalothrin, parathion, and pentachlorophenol to *Eisenia fetida/andrei* (lumbricids) and *Folsomia candida* (collembolans)[a]

	Eisenia fetida/andrei						*Folsomia candida*					
	NOEC		LOEC		EC50		NOEC		LOEC		EC50	
	Value	Reference	Value	Reference	Value	Reference	Value	Reference	Value	Reference	Value	Reference
Atrazine	—	—	—	—	—	—	40[b]	[37]	80[b]	[37]	—	—
Carbendazim	0.5	[38]	1.3	[38]	0.6	[38]	—	—	—	—	—	—
Chlorpyrifos	<4[c]	[39]	4[c]	[39]	9.5[d]	[40]	—	—	—	—	—	—
Dimethoate	1	[41]	5	[42]	5.3	[41]	<0.1	[43]	0.1	[43]	0.3	[43]
γ-HCH	3.2	[44]	5.6	[44]	6.3	[44]	0.03	[44]	0.06	[44]	0.09	[44]
Lambda-cyhalothrin	3.6	[38]	10	[38]	7.7	[38]	7.6	[25]	13	[25]	—	—
Parathion	0.7	[21]	3.6	[21]	7.0	[21]	—	—	—	—	—	—
Pentachlorophenol	10	[45]	20	[45]	13	[46]	—	—	—	—	8	[46]

[a] NOEC = no-observed-effect concentration; LOEC = lowest-observed-effect concentration; EC50 = median effective concentration.
[b] *Orchesella cincta.*
[c] *Aporrectodea caliginosa.*
[d] *Lumbricus rubellus.*

mg/kg for *E. fetida* [27], which is above the lowest concentration at which pronounced long-term (class 5) effects on Lumbricidae are observed (Fig. 3D).

For diflubenzuron, no chronic toxicity data are available. The only LC50s found in the literature are greater than 47.7 and greater than 1,000 mg/kg for the Lumbricidae *L. terrestris* [28] and *E. fetida* (A.C. Grosscurt, Crompton Europe Limited, Berkshire, UK, unpublished data), respectively. This is not comparable with effects found in the field, all of which originate from investigations on arthropods (Fig. 3E).

For imidacloprid, effects are difficult to assess. The higher-tier data do not show lasting effects (none are above class 3), but the tests have been done only at a narrow range of low concentrations (0.4–0.6 mg/kg) (Fig. 3G), which are below the most sensitive laboratory toxic concentration found (LC50 for *E. fetida*, 10.7 mg/kg [29]; http://www.pesticides.gov.uk/psd_evaluation_all.asp).

For phorate, no chronic toxicity data are available. The lowest LC50 for *F. candida* found in the literature is 0.02 mg/kg [24], which is clearly below all the concentrations tested in the field. Lumbricid LC50 data are 19.8 to 59.4 mg/kg for *E. fetida* [30] (http://www.pesticides.gov.uk/psd_evaluation_all.asp) and 44.1 mg/kg for *L. terrestris* [28]. These values are clearly above the lowest concentration at which pronounced effects on lumbricids have occurred in the field (4.5 mg/kg) (Fig. 3H).

Although the availability of lower-tier toxicity data is rather variable, these results illustrate a tendency for higher-tier effects to occur at lower concentrations than those that would be predicted from the most sensitive available lower-tier toxicity data, assuming that no uncertainty factor is used. Especially in the cases of carbaryl, carbofuran, and phorate, the laboratory data show that the concentrations tested in the field have been too high to detect threshold effects concentrations.

In some cases, each effect class and pesticide concentration summarized in the charts (Figs. 1–4) is represented by multiple data. The largest overlap of data points is for carbendazim (177 data points summarized) (Fig. 1B). However, the hidden data are unlikely to bias the interpretations given above. For carbendazim, the data are evenly distributed across the range of tested concentrations, because the originating TME and field studies employed a concentration–response design [20].

Comparison of effects observed in mesocosm and field studies

For carbendazim only, enough higher-tier data are available to allow comparisons of effects in mesocosms (TME) with those observed in field studies. Both study types have yielded similar results, with a concentration–response pattern evident for Enchytraeidae and Lumbricidae, although both no effects (class 1) and slight effects (classes 2 and 3) have been observed at all the concentrations tested (Fig. 4). The only notable difference between the two study types is that pronounced long-term (class 5) effects on Enchytraeidae and Lumbricidae were observed at slightly lower concentrations in TME than in field studies (Fig. 4). Although the HC5 does not provide a sufficient level of protection against all effects, pronounced long-term (class 5) effects of carbendazim on Lumbricidae and Enchytraeidae occurred only above the upper confidence limit of the HC5. In this case, the HC5 appears to identify concentrations that are likely to trigger pronounced long-term effects.

DISCUSSION

Availability of pesticide effects data for soil invertebrates

Lower-tier pesticide effects data can be generated relatively quickly under standardized test conditions. However, the availability of higher-tier data is more problematic, because field studies are long-lasting, expensive, and less easy to control. Their success is less predictable as well. Generally, higher-tier studies with soil invertebrates have been carried out with relatively few pesticide concentrations (often, only the recommended application rate has been tested), meaning that concentrations in the field relevant to risk assessment (e.g., a higher-tier NOEC) either cannot be determined or can be only roughly approximated. The present review shows that the availability of pesticide effects data for soil invertebrates often is biased strongly toward one tier of testing. For example, copper and dimethoate have detailed laboratory effects data but little or no higher-tier (TME or field) effects data, whereas the opposite is true for carbaryl, carbofuran, bendiocarb, and halofenozide. For cases in which both laboratory and higher-tier data are available, the taxonomic composition of the data often differs between the tiers. This raises the questions of whether currently available lower-tier data can be adequately

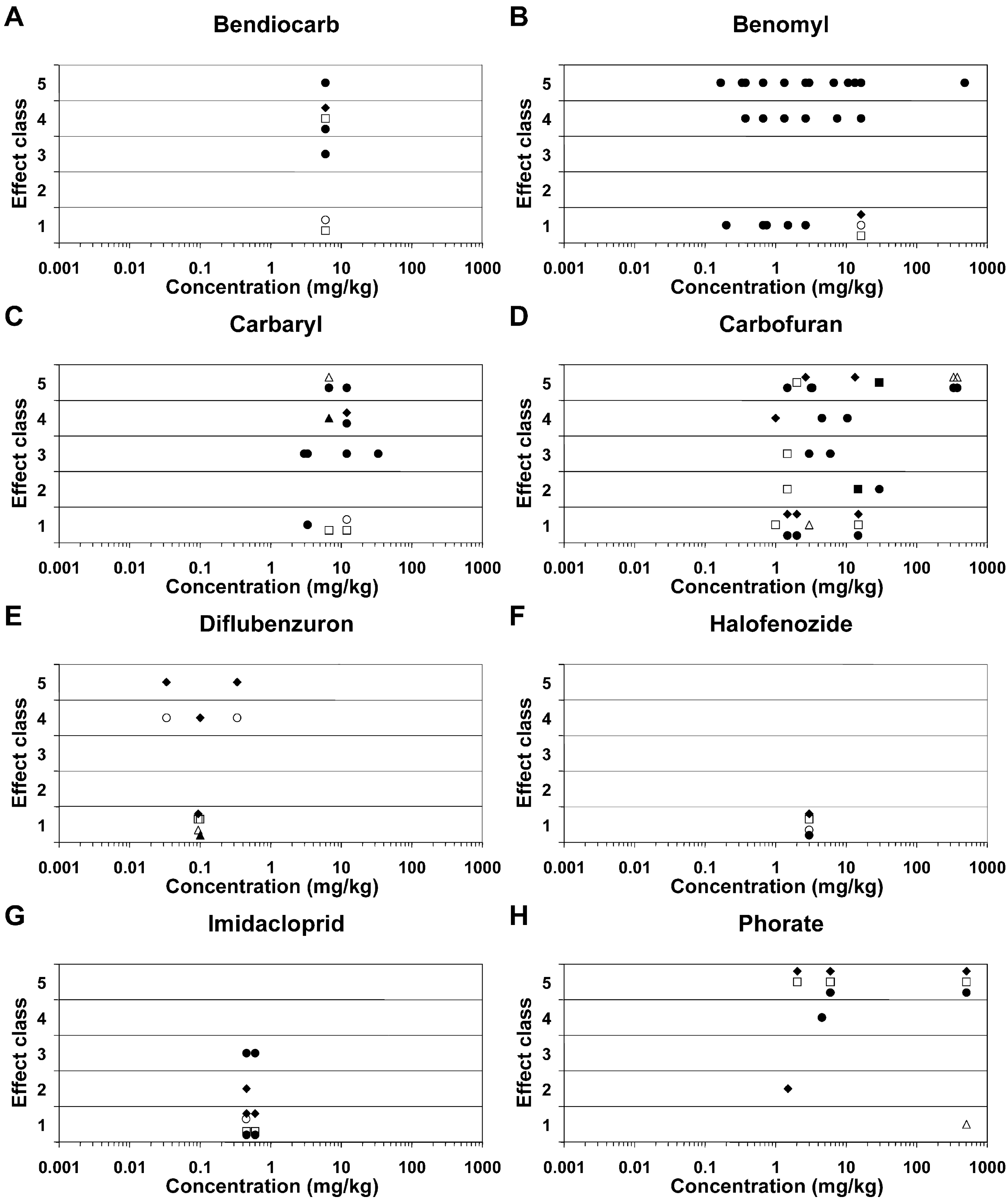

Fig. 3. Higher-tier effects on soil invertebrates of (**A**) bendiocarb (n = 15), (**B**) benomyl (n = 35), (**C**) carbaryl (n = 16), (**D**) carbofuran (n = 38), (**E**) diflubenzuron (n = 10), (**F**) halofenozide (n = 14), (**G**) imidacloprid (n = 20), and (**H**) phorate (n = 11). Data points represent concentrations for which at least one study has been conducted. For explanation of symbols and effect classification, see Figure 1.

predictive of higher-tier effects and whether, subsequently, such predictions can be validated. However, because of species interactions and the impact of environmental conditions that cannot be represented in lower-tier tests, the validity of such predictions will always have limitations, irrespective of the taxonomic composition of the available data [31].

This review is restricted to information in the public domain because of the commercial sensitivity of agrochemicals in-

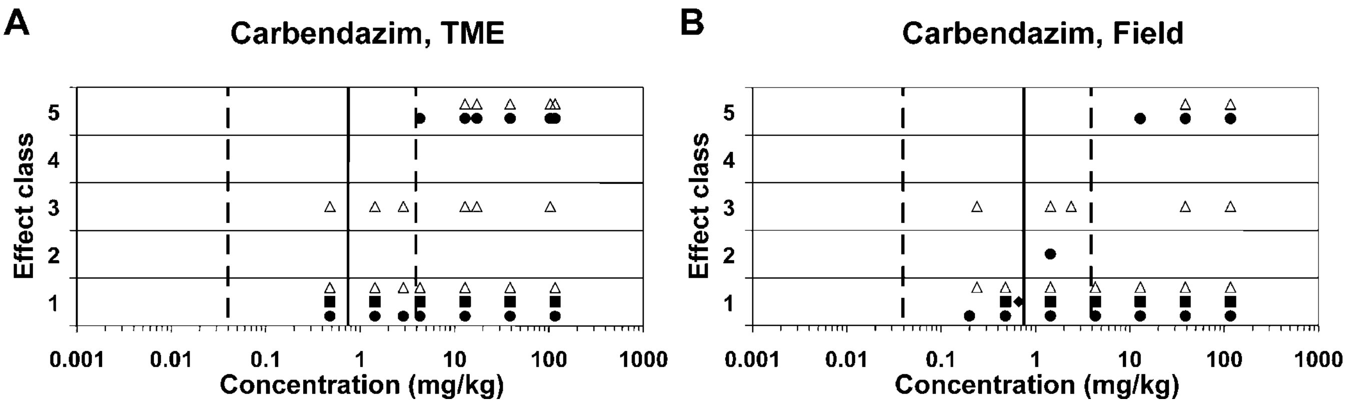

Fig. 4. Effects of carbendazim on soil invertebrates in (**A**) terrestrial model ecosystems (TME; n = 100) and (**B**) field studies (n = 77). Data points represent concentrations for which at least one study has been conducted. For explanation of symbols and effect classification, see Figure 1.

dustry–owned data [12]. As a result, most of the data obtained are for relatively old pesticides, including those no longer used routinely or no longer approved for use. It is not clear what difference the inclusion of industry data would make to the results, and it is not possible to comment on how soil invertebrates would respond to relatively new substances. Although the HC5 approach has the advantage that, being a statistical method, it can be applied to any active substance, it would seem to be unlikely, based on the findings for existing pesticides, that a new chemical substance would have both sufficient lower-tier data to generate a HC5 estimate and enough higher-tier data to enable validation of it.

Taxonomic composition of the data

More than one-third (36%) of the higher-tier data are for earthworms. Although Lumbricidae are acknowledged to be ecologically important as ecosystem engineers and keystone species [32], the data show that they are not in all cases the most sensitive soil invertebrates to pesticides (for insecticides, the standard laboratory test earthworm *E. fetida* is consistently the least sensitive of the invertebrates tested). As expected, *E. fetida* (a habitat specialist) [19] is not represented in the higher-tier data. This illustrates the point that laboratory test species are chosen primarily for their amenability to laboratory testing rather than for their ecological relevance. The same is true for the Collembola test species *F. candida*, which accounts for only 3% of the higher-tier Collembola data. The taxonomic dissimilarity between lower and higher tiers of study has implications for validation of lower-tier predictions, because in some cases (e.g., for the insecticides chlorpyrifos and parathion), the most sensitive taxa (arthropods) also were underrepresented among the lower-tier data.

Classification of higher-tier effects

The only formal guideline used so far in higher-tier testing of pesticide effects with soil invertebrates is for the standard earthworm field test [33]. However, these tests usually are performed as part of the commercial pesticide registration process, meaning that their results usually are not available in the open literature. Generally, the reporting of pesticide effects in higher-tier studies is variable and not comparable across all the studies. The effects classification scheme used here, which is based on a system employed in aquatic risk assessment [13], appears to be promising as a means of overcoming the problem of incompatible reporting of effects. Such a classification scheme could be improved by replacing the current arbitrary thresholds that distinguish slight effects from pronounced effects and short-term from long-term effects with more ecologically meaningful (and, perhaps, taxonomically specific) thresholds (e.g., based on specific life history, population dynamics, and dispersal behavior attributes).

Are HC5 estimates protective for soil invertebrates?

The results clearly show that if distribution-based approaches are to be used in risk assessment for soil invertebrates, then the current availability of data would be a limiting factor. The estimation of hazardous concentrations was based on one of several possible ways to calculate HC5 (a minimum input data requirement of five species was assigned, and a lognormal sensitivity distribution was assumed [12]). If the HC5 estimates are not protective for soil invertebrates, it could be because they were not based on the most sensitive endpoint: Too few chronic and sublethal data were available, so only acute mortality could be used [12]. Effects in the field at concentrations less than the LC50-based HC5 would not be surprising if they originate from chronic physiological effects (e.g., the inhibition of reproduction). In some cases, the HC5 appears to be protective, but this is uncertain (with a wide confidence interval). However, in these cases (for chlorpyrifos, diazinon, dimethoate, γ-HCH, parathion, and pentachlorophenol), insufficient proof exists that no effects occurred below the HC5. It can be concluded that further information would be required to test properly the protective value of the HC5 for soil invertebrates.

Implications for pesticides regulatory risk assessment

According to the EU Terrestrial Guidance Document [34] (http://europa.eu.int/comm/food/plant/protection/evaluation/guidance/wrkdoc09_en.pdf), the aim of the ecological risk assessment for pesticides concerning the soil environment is the protection of populations of nontarget organisms. The data presented here and from the review of lower-tier studies [12] call into question whether the results from earthworm tests alone (the only ones strictly required as part of the current pesticide registration process in the EU) can fulfill this aim.

At present, the regulatory risk assessment for soil invertebrates does not support the use of a distribution-based approach. This is because only one species (*E. fetida*) is used routinely in lower-tier tests, whereas in higher-tier studies, too few pesticide concentrations usually are tested to determine concentration–response relationships. Furthermore, the current risk assessment involves separate analyses of risks to Lumbricidae and arthropods [34]. To use a SSD-based approach would require the additional testing of at least four species if a minimum number of five input data for SSD analysis is assumed (although this number is somewhat arbitrary and may depend on the mode of action [12]). Even if a consensus of agreement existed on which additional species should be tested (to generate either a Lumbricidae, arthropod, or combined SSD), the development of new standard methods and validated guidelines would take considerable time and effort.

Because the estimated hazardous concentrations are not based on the most sensitive lower-tier endpoint, their relevance in a tiered risk assessment pathway is unclear. A more promising direction for the development of SSD in soil invertebrate risk assessment could be to use TME to generate sensitivity distributions. This would have the advantages that individual test guidelines per species would not have to be developed and that test conditions for the different species would be more realistic and less variable. This approach, with SSD employed at a semifield tier, is broadly comparable with the use of SSD in aquatic risk assessment [35]. However, TME studies would need to incorporate a concentration–response design to allow the derivation of sensitivity distributions. As the data indicate, use of TME to predict field effects appears to be promising, but, so far, the TME approach has been developed and evaluated only for one pesticide (carbendazim) and using only a narrow range of invertebrates (principally Lumbricidae, Enchytraeidae, and Nematoda) [20].

Quality requirements of higher-tier data

In some of the studies reviewed, insufficient information was available to enable the reported effects to be interpreted with confidence. In particular, the estimation of soil concentrations of pesticides is difficult if only nominal application rates are reported without information regarding soil characteristics or pesticide deposition. Many reports missed information concerning important aspects of the study design, such as the soil characteristics, layout of experimental plots, or pesticide application technique. Recommendations for standardizing the reporting of information in higher-tier studies to improve comparison and interpretation of the results are proposed by Römbke et al. [36]. Standardizing higher-tier studies is more likely to be possible if the studies are already required to conform to a scheme, such as the pesticides regulatory risk assessment. However, many of the data obtained are from ad hoc studies with aims that were not related to regulatory risk assessment. It would be difficult to enforce standardization on ad hoc studies, but nevertheless the principles of good experimental practice, reporting, and analysis should apply [36]. When higher-tier studies are carried out as part of the risk assessment process, three recommendations based on the results of the present review can be made: First, higher-tier studies should employ lower concentrations of pesticides in addition to those already tested to enable acceptable environmental concentrations to be identified and, perhaps, validated. Second, as many relevant taxonomic groups as possible should be sampled, preferably identified to the species level. This will help to fill gaps in our knowledge concerning the sensitivity of taxa that currently are not monitored frequently. Third, concentration–response study designs would assist the generation and evaluation of SSD for a wider range of species and pesticides.

CONCLUSION

The HC5 approach appears to be a promising tool for validating predictions of pesticide risk for soil invertebrates, but its applicability is limited by data availability. In particular, lower-tier chronic test data for sensitive sublethal endpoints and higher-tier information at appropriate pesticide concentrations to enable calculation of effects thresholds are lacking. Validation of lower-tier risk predictions also is hindered by inconsistent taxonomic composition of laboratory and field data sets as well as by uncertainty regarding whether the taxonomic data appropriately reflect the protection goal. Improvements in higher-tier testing are recommended to address these limitations (e.g., standardization of guidance for selecting chemical concentrations and species for evaluation). Lumbricidae and Arthropoda differ in sensitivity to most pesticides; for many compounds, particularly insecticides, Lumbricidae alone would be insufficiently sensitive to indicate risk to a typical soil invertebrate community. Collembola are an appropriately sensitive arthropod group for inclusion in risk assessments to improve risk prediction for soil invertebrate communities. Although too few species currently are tested for distribution-based risk assessment to be applied routinely at a lower tier, TME could generate higher-tier multispecies data under controlled conditions and using a dose–response design. However, TME have been evaluated in detail for only one pesticide and require further validation before they can be considered in distribution-based risk assessment for soil invertebrates.

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