SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 24, No. 2, pp. 379–388, 2005

Printed in the USA
0730-7268/05 $12.00 + .00

INSECTICIDE SPECIES SENSITIVITY DISTRIBUTIONS: IMPORTANCE OF TEST SPECIES SELECTION AND RELEVANCE TO AQUATIC ECOSYSTEMS

LORRAINE MALTBY,*† NAOMI BLAKE,†‡ THEO C.M. BROCK,§ and PAUL J. VAN DEN BRINK§
†Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, United Kingdom
‡Cambridge Environmental Assessments, Battlegate Road, Boxworth, Cambridge, CB3 8NN, United Kingdom
§Alterra Centre for Water and Climate, Wageningen University and Research Centre, P.O. Box 47, 6700 AA Wageningen, The Netherlands

(*Received* 20 *January* 2004; *Accepted* 21 *July* 2004)

Abstract—Single-species acute toxicity data and (micro)mesocosm data were collated for 16 insecticides. These data were used to investigate the importance of test-species selection in constructing species sensitivity distributions (SSDs) and the ability of estimated hazardous concentrations (HCs) to protect freshwater aquatic ecosystems. A log-normal model was fitted to a minimum of six data points, and the resulting distribution was used to estimate lower (95% confidence), median (50% confidence), and upper (5% confidence) 5% HC (HC5) values. Species sensitivity distributions for specific taxonomic groups (vertebrates, arthropods, nonarthropod invertebrates), habitats (saltwater, freshwater, lentic, lotic), and geographical regions (Palaearctic, Nearctic, temperate, tropical) were compared. The taxonomic composition of the species assemblage used to construct the SSD does have a significant influence on the assessment of hazard, but the habitat and geographical distribution of the species do not. Moreover, SSDs constructed using species recommended in test guidelines did not differ significantly from those constructed using nonrecommended species. Hazardous concentrations estimated using laboratory-derived acute toxicity data for freshwater arthropods (i.e., the most sensitive taxonomic group) were compared to the response of freshwater ecosystems exposed to insecticides. The sensitivity distributions of freshwater arthropods were similar for both field and laboratory exposure, and the lower HC5 (95% protection with 95% confidence) estimate was protective of adverse ecological effects in freshwater ecosystems. The corresponding median HC5 (95% protection level with 50% confidence) was generally protective of single applications of insecticide but not of continuous or multiple applications. In the latter cases, a safety factor of at least five should be applied to the median HC5.

Keywords—Ecological risk assessment Pesticides Aquatic

INTRODUCTION

The challenge faced by ecological risk assessors is to derive threshold concentrations for environmental contaminants that protect species diversity and the functional attributes of natural ecosystems. Species vary markedly in their sensitivity to environmental contaminants, and this variation can be described by constructing a species sensitivity distribution (SSD). The SSD is a statistical distribution estimated from a sample of toxicity data and visualized as a cumulative distribution function [1]. Species sensitivity distributions are used to calculate the concentration at which a specified proportion of species will be affected, referred to as the hazardous concentration (HC) for *p* (%) of species (HC*p*) [2]. The most frequently estimated HCs are the HC5 and HC10, with the HC5 derived from a SSD of no-observed-effect concentration (NOEC) values being used to define the maximum permissible concentration for environmental contaminants in The Netherlands [3].

The use of SSDs in the derivation of threshold concentrations originally was proposed during the late 1970s in the United States [4] and the mid-1980s in Europe [5]. Species sensitivity distributions have been used to assess the ecological risk posed by metals [6–8], surfactants [9,10], biocides [11], herbicides [12–14], insecticides [15,16], and general organic and inorganic substances [17,18]. Despite its widespread use, the SSD approach has been—and continues to be—the focus of debate and discussion [1].

The use of a HC in risk assessment assumes that test species can be conceived of as random selections from a specified distribution [19]. This assumption of randomness has been criticized, and some have been argued that because most toxicity test data are generated using sensitive species, estimated HCs will be overprotective [20]. Moreover, the use of species that are not representative of the ecosystem to be protected (e.g., combining data for freshwater and saltwater species when assessing the risk to freshwater ecosystems) has been criticized as well [21]. Proponents of the SSD approach have recognized that test species do not represent a random sample from a local or target community [5]. Although data often are pooled across taxonomic groups (e.g., invertebrates, fish, algae, vascular plants), habitats (e.g., freshwater, saltwater, ponds, rivers), and geographical regions (e.g., temperate, tropical), Posthuma et al. [22] suggest that SSDs should be tailored by selecting data for those species that occur in the ecosystem under consideration (e.g., using only Nearctic freshwater lotic species for an assessment of risk to North American stream ecosystems). Such a degree of data selection often is not possible, however, because of the limited amount of available toxicity data—and even if sufficient data are available, what difference would this tailoring make to the estimation of risk?

The first aim of the present study was to address the following questions: To what extent do the identity and source of the species used to construct SSDs influence the assessment of risk, and are some groups of species more appropriate than others for assessing risk? The impact of the taxonomy, habitat (i.e., freshwater or saltwater, lentic or lotic), and geographical distribution (i.e., zoogeographical range, temperate or tropical) of species used to construct SSDs on the derived HCs was

* To whom correspondence may be addressed (l.maltby@sheffield.ac.uk).

investigated. In addition, the sensitivity distributions of species recommended in toxicity test guidelines were compared to those of species not recommended.

Other assumptions of the SSD approach that have received criticism are that laboratory conditions do not greatly influence the sensitivity of species and that the HC derived from the SSD will protect ecosystems [20]. Sensitivity distributions are constructed using data from single-species laboratory toxicity tests, but the derived HC is applied to multispecies assemblages in natural ecosystems. By definition, single-species tests exclude interactions between species; therefore, their use in risk assessment assumes that interactions among species in communities also can be ignored or accounted for. This assumption has been criticized by Forbes and Forbes [23]. Thus, the second aim of the present study was to assess how SSDs derived from single-species laboratory toxicity data could be used to protect species assemblages in aquatic ecosystems. This was achieved by comparing SSDs constructed from natural and artificial assemblages exposed in (micro)mesocosms and single-species laboratory tests, respectively. The HCs derived from single-species SSDs were then compared to threshold levels for the direct toxic effects of insecticides in freshwater (micro)mesocosms.

Both aims of the present study were addressed using aquatic toxicity data for 16 insecticides, including organophosphate, pyrethroid, organochlorine, carbamate, and benzylphenyl urea compounds. Sensitivity distributions were constructed using acute toxicity data and described by a log-normal model [24]. Previous studies have used chronic toxicity data to construct SSDs [17]; however, acute toxicity data have a number of advantages over chronic toxicity data. First, for most chemicals, insufficient chronic toxicity data are available to generate appropriate SSDs, but there may be sufficient acute toxicity data. Second, whereas acute toxicity data relate to a limited number of responses and time scales (e.g., 96-h median lethal concentrations), chronic toxicity data include a wide range of responses and test durations, thereby introducing additional variability into the SSD. Third, pesticide risks often are short-term and, therefore, are more appropriately assessed using acute toxicity data.

MATERIALS AND METHODS

Data selection and SSD generation

The present study focused on 16 insecticides: Seven organophosphates (azinphos-methyl, carbofuran, chlorpyrifos, diazinon, fenitrothion, parathion-ethyl, parathion-methyl), five pyrethroids (cypermethrin, deltamethrin, fenvalerate, lambda-cyhalothrin, permethrin), two organochlorines (lindane, methoxychlor), a carbamate (carbaryl), and a benzylphenyl urea (diflubenzuron). Single-species acute toxicity data and (micro)mesocosm data were collated from existing toxicity databases (e.g., Rijksinstituut voor Volksgezondheid en Milieu [RIVM] database [25]), published literature, and unpublished industry data. Criteria used to select single-species toxicity data were related to test endpoint and duration, and outliers were checked using original publications. Selected endpoints were median lethal concentration or median effect concentration (EC50) regarding immobility for animals and EC50 regarding biomass or growth for plants. Test duration was 2 to 21 d for fish, 1 to 7 d for invertebrates, 2 to 28 d for macrophytes, and 1 to 7 d for algae. The geometric mean was calculated when more than one toxicity value was reported for a species, and a genus-specific geometric mean was used when no specific names were provided. Selection criteria for (micro)mesocosm data followed those described by Brock et al. [26].

The method of Aldenberg and Jaworska [24], as incorporated in the ETX software [27], was used to generate SSDs and associated lower (95% confidence), median (50% confidence), and upper (5% confidence) HC5 estimates. A log-normal distribution model was fitted to a minimum of six data points, with model fit being evaluated using the Anderson–Darling goodness-of-fit test. Toxicity data reported as greater-than values were not used to generate a SSD.

Species selection

Separate SSDs were produced for arthropod and vertebrate species for each of the 16 insecticides and HC5 values estimated. In addition, SSDs for nonarthropod invertebrates and algae were generated for those compounds having sufficient data. The influence of habitat on HC5 estimates was investigated by comparing freshwater and saltwater arthropods and, within freshwater arthropods, by comparing lentic and lotic species. Only species that were endemic to one of the habitats being compared were used in the analysis. Sufficient data were available to perform the freshwater–saltwater comparison for 10 compounds (azinphos-methyl, carbaryl, chlorpyrifos, cypermethrin, fenitrothion, fenvalerate, lindane, methoxychlor, parathion-methyl, permethrin) and the lentic–lotic comparison for eight compounds (carbaryl, chlorpyrifos, diazinon, fenitrothion, lambda-cyhalothrin, lindane, parathion-ethyl, permethrin). Most saltwater arthropods are crustaceans, whereas freshwater arthropods include both crustaceans and insects. Because this taxonomic difference may confound the comparison of freshwater and saltwater arthropod SSDs, the comparison was restricted to crustaceans and run again for all 10 compounds.

Freshwater arthropods also were classified according to their geographical distribution (i.e., their zoogeographical region and whether they are from temperate or tropical zones). Many species, however, are cosmopolitan and occur in several regions. To minimize any confounding effects caused by widely dispersed species, only data for species that occurred uniquely in one of the categories being compared were used. A comparison of temperate and tropical species was possible for three compounds (carbofuran, chlorpyrifos, fenitrothion), whereas a comparison of Palaearctic and Nearctic species was possible for four compounds (chlorpyrifos, diazinon, fenitrothion, lindane).

Several of the arthropod species in the database have been recommended for use in toxicity test guidelines produced by the Organisation for Economic Cooperation and Development (OECD), U.S. Environmental Protection Agency (U.S. EPA), American Society for Testing and Materials (ASTM), and Environment Canada. Sufficient data were available to compare HC5 estimates for recommended and nonrecommended arthropod species for all compounds except diflubenzuron.

The HC5 estimates were compared both within and across compounds. Within compounds, the criterion to determine whether median HC5 values were significantly different was nonoverlapping HC5 estimates (i.e., lower to upper), and sensitivity distributions were compared using the two-sample Kolmogorov–Smirnov test (S-Plus 6.1; Insightful, Seattle, WA, USA). Comparing across compounds, the statistical significance of differences in HC5 estimates derived using species from different taxonomic groups, habitats, geographical re-

Table 1. Median (50% confidence) hazardous concentration for 5% of species (HC5; μg/L) calculated from species sensitivity distributions constructed for aquatic arthropods, nonarthropod invertebrates, vertebrates, or algae exposed to pesticides in single-species acute toxicity tests[a]

	Taxonomic group			
Insecticide	Algae	Arthropods	Nonarthropod invertebrate	Vertebrates
Azinphos-methyl		0.04 (0.01, 0.14)		0.36 (0.07, 1.18)
Carbaryl		2.67 (1.26, 4.85)	314 (64, 902)	450 (246, 718)
Carbofuran		0.23 (0.03, 0.90)		68 (28, 128)
Chlorpyrifos		0.07 (0.04, 0.11)		0.58 (0.15, 1.59)
Cypermethrin		0.003 (0.001, 0.007)		0.17 (0.05, 0.42)
Deltamethrin		0.009 (0.003, 0.18)		0.21 (0.1, 0.38)
Diazinon		0.36 (0.13, 0.77)	229 (34, 723)	52 (17, 117)
Diflubenzuron		0.05 (0.001, 0.42)		23,176 (2,202, 66,435)
Fenitrothion	790 (305, 1,379)	0.32 (0.15, 0.59)	8.16 (0.85, 37.8)	53 (19, 117)
Fenvalerate		0.013 (0.003, 0.036)		0.19 (0.06, 0.41)
Lambda-cyhalothrin		0.003 (0.001, 0.006)		0.08 (0.03, 0.16)
Lindane		0.79 (0.34, 1.53)	278 (68, 707)	4.84 (2.39, 8.51)
Methoxychlor		0.47 (0.21, 0.84)		4.56 (2.48, 7.22)
Parathion-ethyl		0.23 (0.11, 0.41)	176 (15, 631)	93 (30, 196)
Parathion-methyl		0.31 (0.08, 0.81)	754 (231, 1,589)	1,471 (926, 2,033)
Permethrin		0.096 (0.04, 0.19)		0.39 (0.05, 1.6)

[a] Lower (95% confidence) and upper (5% confidence) HC5 estimates are given in parentheses.

gions, and whether species were recommended or not was assessed using the Wilcoxon paired-sample test, with estimates being paired within compounds.

Comparison with ecosystem studies

Species sensitivity distributions were constructed for freshwater arthropods exposed to chlorpyrifos or lambda-cyhalothrin in multispecies (micro)mesocosms. Chlorpyrifos data were taken from Van Wijngaarden et al. [28] and lambda-cyhalothrin data from Schroer et al. [29]. (Micro)mesocosm SSDs were then compared to those constructed using single-species laboratory toxicity data using the two-sample Kolmogorov–Smirnov test. Laboratory data were restricted to freshwater arthropod toxicity values of less than or equal to 50% of the highest test concentration used in the (micro)mesocosm studies (i.e., 44 μg/L for chlorpyrifos and 250 ng/L for lambda-cyhalothrin).

The relationship between HC values derived from short-term, single-species toxicity data and effects observed in (micro)mesocosm studies was investigated for all 16 insecticides. Data from a recent review of (micro)mesocosm studies published between 1980 and 2001 [30] were used as the basis of this analysis. This review focused on neurotoxic insecticides and was supplemented by data from Brock et al. [26] for nonneurotoxic insecticides (i.e., diflubenzuron, lindane, methoxychlor). For each insecticide, studies were classified as single application or as multiple or continuous application, and (micro)mesocosm responses observed for the most sensitive endpoint at each exposure concentration were assigned to one of five effect classes following the method of Brock et al. [26]. The $NOEC_{eco}$ (i.e., highest test concentration at which class 1 effects were observed) and $LOEC_{eco}$ (i.e., lowest test concentration at which slight and transient effects [class 2] were observed) were determined for each compound and exposure scenario. These ecosystem threshold levels were compared to estimates of HC5 and HC10 derived from single-species acute toxicity data for freshwater arthropods.

RESULTS

Species selection

Toxicity data were collated for a total of 467 animal taxa exposed to 16 insecticides: 258 Arthropods, 153 vertebrates (fish and amphibians), and 56 nonarthropod invertebrate taxa. The dataset also contained toxicity information on 20 species of algae, a macrophyte, and a bacterium. Median HC5 values derived from arthropod SSDs were significantly lower than those derived from vertebrate (Wilcoxon paired test: $W = 136$, $n = 16$, $p < 0.001$) or nonarthropod invertebrate ($W = 28$, $n = 7$, $p < 0.05$) SSDs. The difference in median HC5 values ranged from less than one order of magnitude (e.g., chlorpyrifos) to more than five orders of magnitude (i.e., diflubenzuron) (Table 1). Forty-three percent of arthropod species in the toxicity dataset were crustaceans. Of these, more than 60% were malacostracans (Amphipoda, Isopoda, Decapoda), 20% branchiopods (Anostraca, Cladocera, Notostraca), and the remainder copepods (Cyclopoida, Calanoida), ostracods, and mysids. The most abundant insect orders in the dataset were Diptera (40%), Plecoptera (12%), Ephemeroptera (12%), Trichoptera (9%), Odonata (9%), Hemiptera (8%), and Coleoptera (8%).

Eighty-four percent of arthropod species were from freshwater habitats, and sufficient data were available to compare freshwater and saltwater taxa for 10 compounds. No significant difference was found in median HC5 values estimated from SSDs constructed using either freshwater or saltwater taxa ($W = 43$, $n = 10$, $p = 0.14$), although median HC5 estimates for saltwater arthropods tended to be less than those derived using freshwater arthropods (Fig. 1). Comparing within compounds, the sensitivity distributions for freshwater and saltwater arthropods were significantly different for permethrin (Kolmogorov–Smirnov test: $ks = 0.7$, $n_1 = 27$, $n_2 = 6$, $p = 0.006$) and chlorpyrifos ($ks = 0.45$, $n_1 = 81$, $n_2 = 11$, $p = 0.03$), although this difference was removed when the analysis was restricted to crustaceans (permethrin: $ks = 0.59$, $n_1 = 12$, $n_2 = 6$, $p = 0.12$; chlorpyrifos: $ks = 0.29$, $n_1 = 19$, $n_2 = 10$, $p = 0.53$) (Fig. 2). Approximately 50% of the freshwater test species were from lentic habitats, 31% from lotic habitats, and the remaining 17% from both lotic and lentic habitats. Comparing among compounds, no significant difference was found in median HC5 estimates derived using lentic or lotic species ($W = 25$, $n = 8$, $p = 0.36$). No consistent pattern in relative values was observed, and the differences noted between lentic and lotic median HC5 estimates were less than one order of magnitude (Fig. 1).

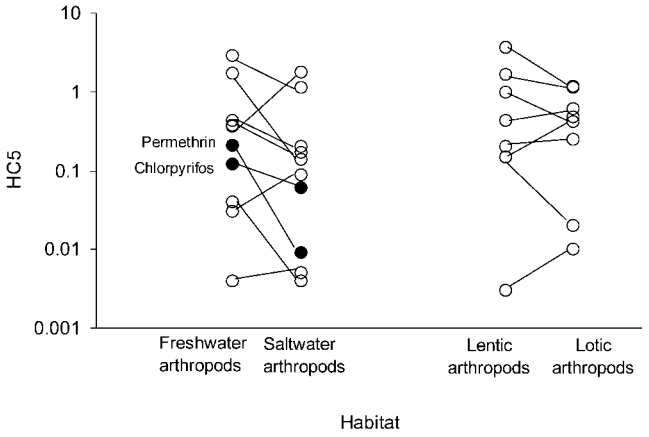

Fig. 1. Distribution of median hazardous concentration for 5% of species (HC5; μg/L) estimated from species sensitivity distributions constructed using arthropods from different habitats: Freshwater and saltwater or lentic and lotic. Each point represents a single compound, and lines link HC5 estimates within compounds. Ten insecticides were used for the freshwater–saltwater comparison and eight for the lentic–lotic comparison. The HC5 estimates for freshwater and saltwater arthropods exposed to permethrin or chlorpyrifos are highlighted.

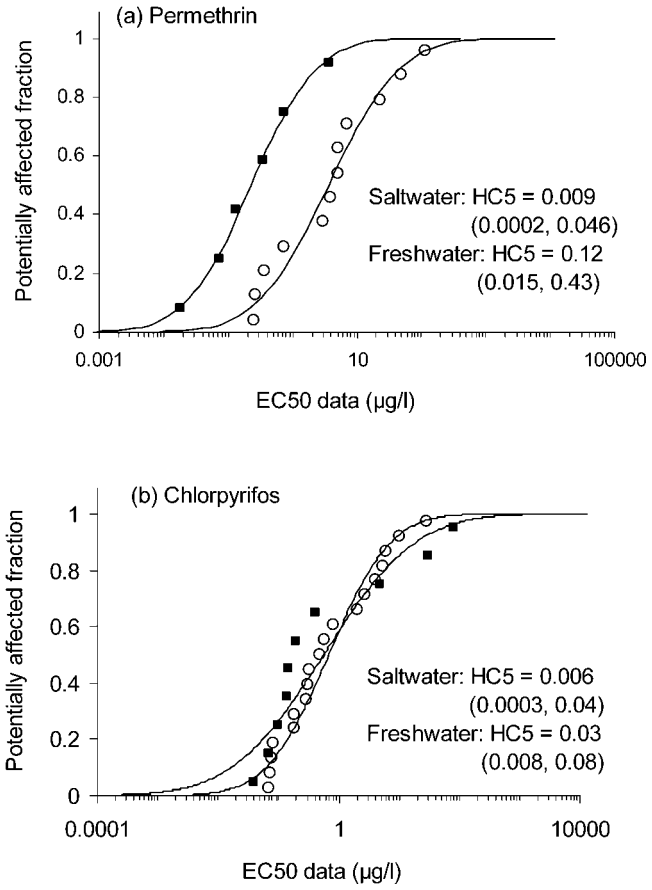

Fig. 2. Species sensitivity distributions for saltwater (solid symbols) or freshwater (open symbols) Crustacea exposed to (**a**) permethrin or (**b**) chlorpyrifos. Median hazardous concentrations for 5% of species (HC5; μg/L) are presented for each distribution with lower (95% confidence) and upper (5% confidence) estimates given in parentheses. Median effect concentrations (EC50s) are derived from single-species laboratory studies.

The majority (69%) of freshwater arthropod species tested were restricted to temperate zones. Of these, 23% were restricted to the Palaearctic region and 64% to the Nearctic region. Only 11% of the species tested were restricted to tropical zones, and more than 50% of these were from the Oriental region, primarily India. Although median HC5 values derived from Palaearctic species generally were lower than those derived using Nearctic species (Fig. 3), sensitivity distributions for species from Palaearctic or Nearctic regions were not significantly different ($ks \leq 0.62$, $n_1 \leq 13$, $n_2 \leq 36$, $p \geq 0.051$). Similarly, no significant difference was found in the sensitivity distributions of tropical and temperate species within a compound ($ks \leq 0.38$, $n_1 \leq 46$, $n_2 \leq 12$, $p \geq 0.15$) (Fig. 4), nor was a significant difference observed in median HC5 estimates across compounds ($W = 6$, $n = 3$, $p = 0.18$).

Thirty-eight aquatic arthropod species have been recommended in toxicity test guidelines published by the OECD, U.S. EPA, ASTM, and Environment Canada, and a comparison of SSDs generated using either recommended or nonrecommended arthropod species was possible for all insecticides except diflubenzuron. For 13 of the 15 insecticides investigated, HC5 estimates for recommended or nonrecommended arthropods overlapped, and no significant difference was found in median HC5 estimates across compounds ($W = 40$, $n = 15$, $p = 0.27$). However, carbaryl and chlorpyrifos had a significant difference in sensitivity distributions of recommended and nonrecommended species (carbaryl: $ks = 0.67$, $n_1 = 15$, $n_2 = 48$, $p < 0.001$; chlorpyrifos: $ks = 0.51$, $n_1 = 24$, $n_2 = 64$, $p < 0.001$), with recommended species having the smaller HC5 estimates (Fig. 5). Thirty-two of the 38 recommended arthropods are crustaceans, but nonrecommended arthropods are a mixture of crustaceans and insects. When SSDs for carbaryl and chlorpyrifos were constructed using recommended and nonrecommended crustaceans only, the differences in sensitivity distributions persisted ($ks \geq 0.71$, $n_1 \geq 11$, $n_2 \geq 14$, $p \leq 0.06$), but the HC5 estimates were no longer distinct (Fig. 5).

Comparison with ecosystem studies

Comparisons between the responses of freshwater arthropods in single-species laboratory tests and in multispecies assemblages in (micro)mesocosms were possible for two compounds (chlorpyrifos, lambda-cyhalothrin). In both cases, the SSDs were similar for laboratory and field data ($ks \leq 0.38$, $n_1 \leq 82$, $n_2 \leq 14$, $p > 0.05$), and the HC5 estimates overlapped (Fig. 6).

Freshwater arthropod data were used to estimate HC5 and HC10 values for each of the 16 insecticides studied, and these values were then compared to ecological threshold levels for freshwater assemblages exposed to either single or multiple applications (Table 2). Precise $NOEC_{eco}$ values were available for 10 of the 16 insecticides, and these are presented with the median (50% confidence) and lower (95% confidence) HC5 values in Figure 7. The HC5 is considered to be protective when it is smaller than the $NOEC_{eco}$ or $LOEC_{eco}$, because in the present study, the latter represents the concentration at which only transient, slight effects are observed.

For single, multiple, and continuous applications, the lower HC5 estimate was less than the $LOEC_{eco}$ of all 11 insecticides for which this comparison was possible (Table 2) and lower than the $NOEC_{eco}$ for 9 of the 10 insecticides for which a precise $NOEC_{eco}$ was available, with the exception being lindane (Fig. 7). A comparison of the median HC5 estimate and

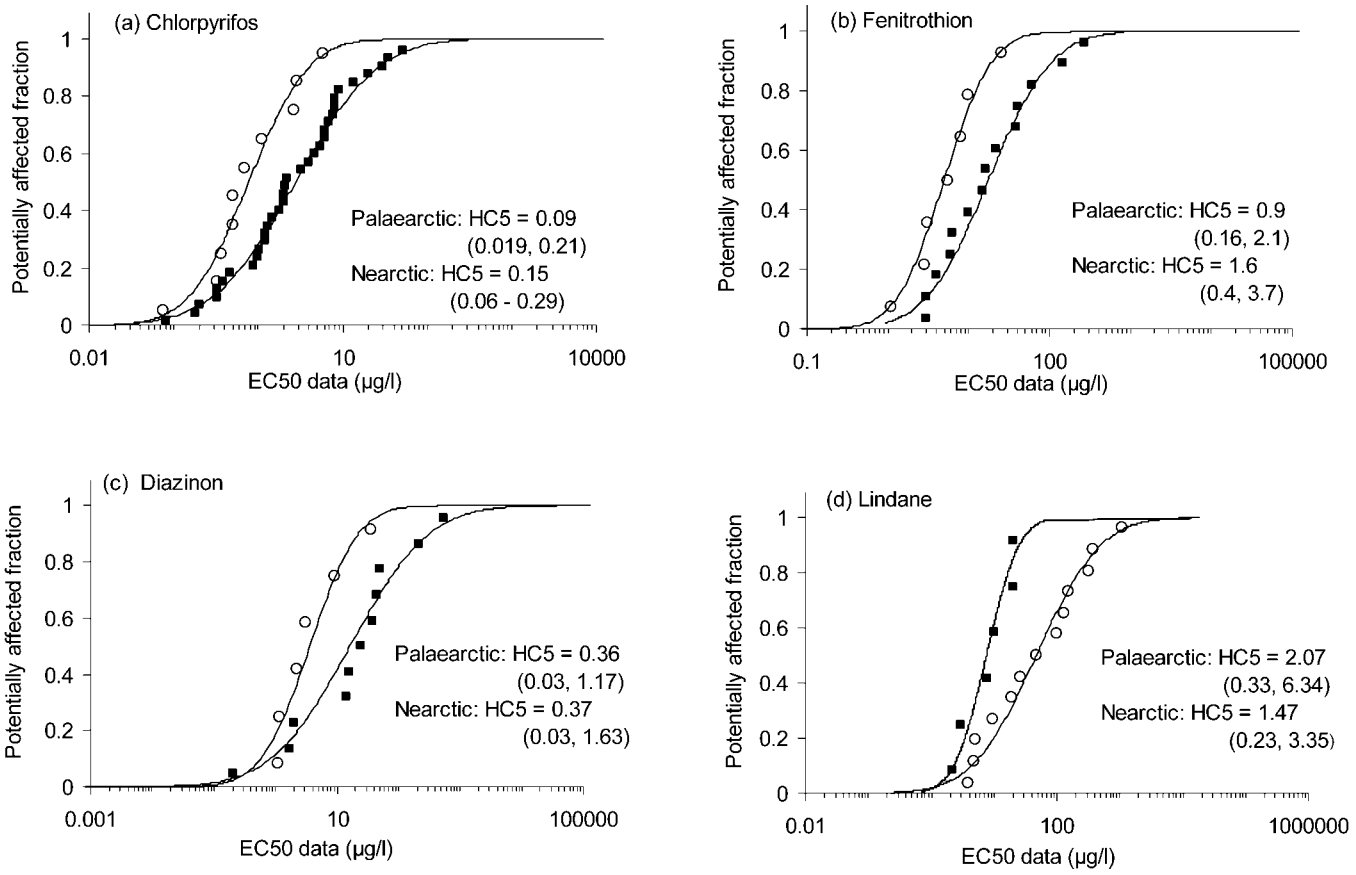

Fig. 3. Species sensitivity distributions for Palaearctic (open symbols) or Nearctic (solid symbols) arthropods exposed to (**a**) chlorpyrifos, (**b**) fenitrothion, (**c**) diazinon, or (**d**) lindane. Median hazardous concentrations for 5% of species (HC5; µg/L) are presented for each distribution, with lower (95% confidence) and upper (5% confidence) estimates given in parentheses. Median effect concentrations (EC50s) are derived from single-species laboratory studies.

either the $LOEC_{eco}$ or $NOEC_{eco}$ from single-application (micro)mesocosm studies was possible for eight insecticides (azinphos-methyl, carbaryl, carbofuran, chlorpyrifos, diflubenzuron, fenitrothion, fenvalerate, methoxychlor). The median HC5 estimate exceeded the $LOEC_{eco}$ for carbaryl and the $NOEC_{eco}$ for carbaryl and fenvalerate. A comparison of the median HC5 estimate and either the $LOEC_{eco}$ or $NOEC_{eco}$ from multiple- or continuous-application (micro)mesocosm studies was possible for six insecticides (azinphos-methyl, diflubenzuron, fenvalerate, lindane, parathion-ethyl, lambda cyhalothrin). The median HC5 estimate exceeded the $LOEC_{eco}$ for lindane and fenvalerate (Table 2) and was lower than the $NOEC_{eco}$ for azinphos-methyl and diflubenzuron (Fig. 7). Regarding the remaining three insecticides for which a precise $NOEC_{eco}$ was available (lindane, parathion-ethyl, lambda cyhalothrin), the median HC5 estimate was within one order of magnitude of the $NOEC_{eco}$.

Comparisons between median HC10 estimates and single-application $LOEC_{eco}$ values were possible for eight insecticides (azinphos-methyl, carbaryl, carbofuran, chlorpyrifos, diflubenzuron, fenitrothion, fenvalerate, methoxychlor), and comparisons between HC10 estimates and multiple- or continuous-application $LOEC_{eco}$ values were possible for five insecticides (azinphos-methyl, chlorpyrifos, fenvalerate, lambda-cyhalothrin, lindane). Median HC10 estimates exceeded $LOEC_{eco}$ values for single applications of carbaryl and fenvalerate and for multiple or continuous applications of chlorpyrifos, fenvalerate, and lindane (Table 2).

Regarding the compounds for which neither precise $NOEC_{eco}$ nor $LOEC_{eco}$ values have been determined (cypermethrin, deltamethrin, diazinon, parathion-methyl, permethrin), more severe effects (classes 3–5) have been reported at concentrations greater than the median HC10 and at least one order of magnitude greater than the median HC5. The exception is permethrin, for which severe effects have been reported at concentrations twice the median HC5.

DISCUSSION

The present study addressed two questions: Do the identity and source of the species used to construct SSDs influence the assessment of hazard, and can SSDs derived from single-species laboratory acute toxicity data be used to protect species assemblages in aquatic ecosystems. These questions are fundamental to the use of the species sensitivity approach in aquatic risk assessment, and they were investigated with reference to insecticides. The major conclusions of the present study are as follows: First, whereas the species used to construct SSDs do affect hazard assessment, the habitat and geographical distribution of species generally do not have a significant influence. Second, the lower HC5 estimate (i.e., 95% protection level with 95% confidence) derived from acute toxicity data for the most sensitive taxonomic group is protective of adverse ecological effects in aquatic model ecosystems even with repeated insecticide applications. Third, the median HC5 estimate (i.e., 95% protection level with 50% confidence) derived from acute toxicity data for the most sensitive taxonomic group

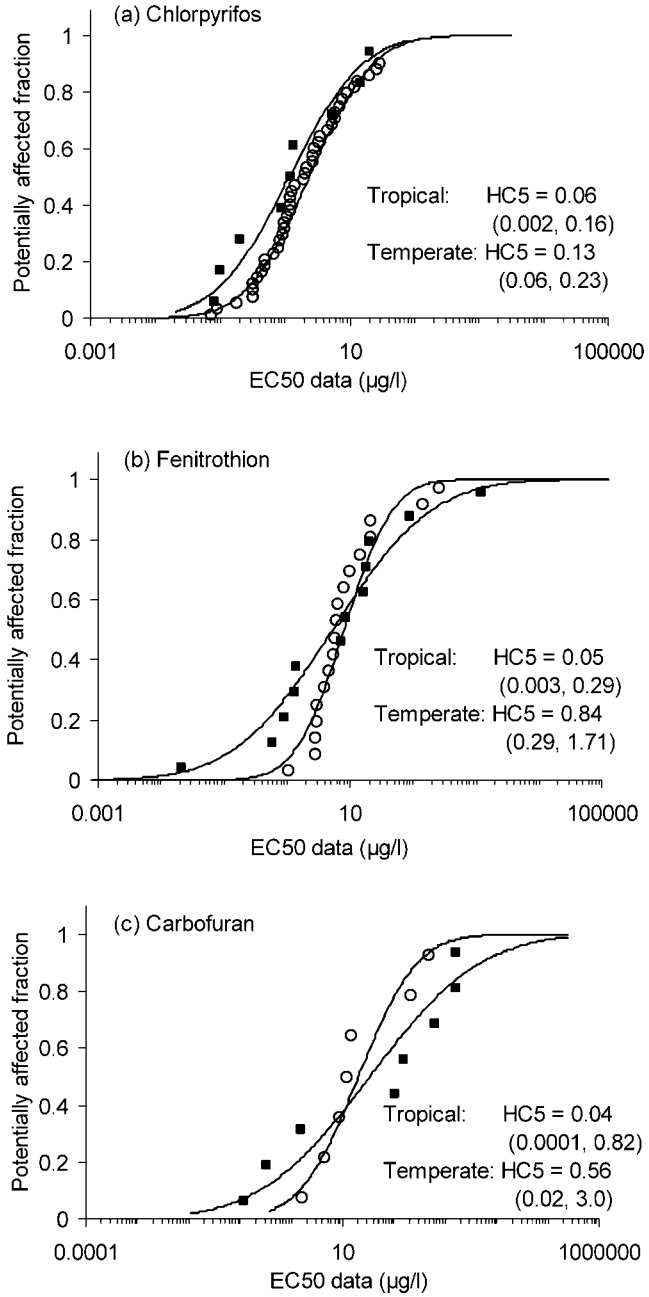

Fig. 4. Species sensitivity distributions for tropical (solid symbols) or temperate (open symbols) arthropods exposed to (**a**) chlorpyrifos, (**b**) fenitrothion, or (**c**) carbofuran. Median hazardous concentrations for 5% of species (HC5; µg/L) are presented for each distribution, with lower (95% confidence) and upper (5% confidence) estimates given in parentheses. Median effect concentrations (EC50s) are derived from single-species laboratory studies.

is generally protective of single applications of insecticide in aquatic model ecosystems but not of continuous or multiple applications. In these latter cases, a safety factor of at least five should be applied to the median HC5 to ensure that the threshold level derived using the SSD approach is lower than the $NOEC_{eco}$ derived from (micro)mesocosm studies.

All 16 insecticides investigated were more toxic to arthropods than vertebrates (fish and amphibians) or nonarthropod invertebrates (i.e., Mollusca, Annelida, Platyhelminthes, Rotifera, Protozoa). The magnitude of difference between median HC5 values derived from vertebrate or arthropod SSDs ranged

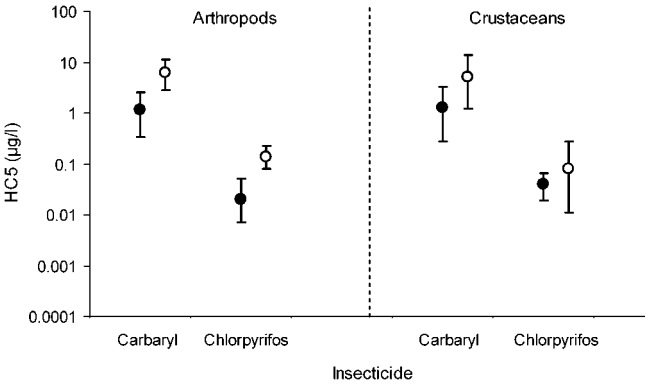

Fig. 5. Median hazardous concentration for 5% of species (HC5) estimated from species sensitivity distributions constructed using recommended (solid symbols) or nonrecommended (open symbols) arthropods or crustaceans. Bars denote the range between lower (95% confidence) and upper (5% confidence) HC5 estimates.

from a factor of four (permethrin) to a factor of 4×10^5 (diflubenzuron). The increased sensitivity of arthropods to insecticides is not surprising given the toxic mode of action of these compounds. For instance, diflubenzuron inhibits chitin production and, therefore, is highly toxic to arthropods, but it has low toxicity to nonarthropod invertebrates and vertebrates.

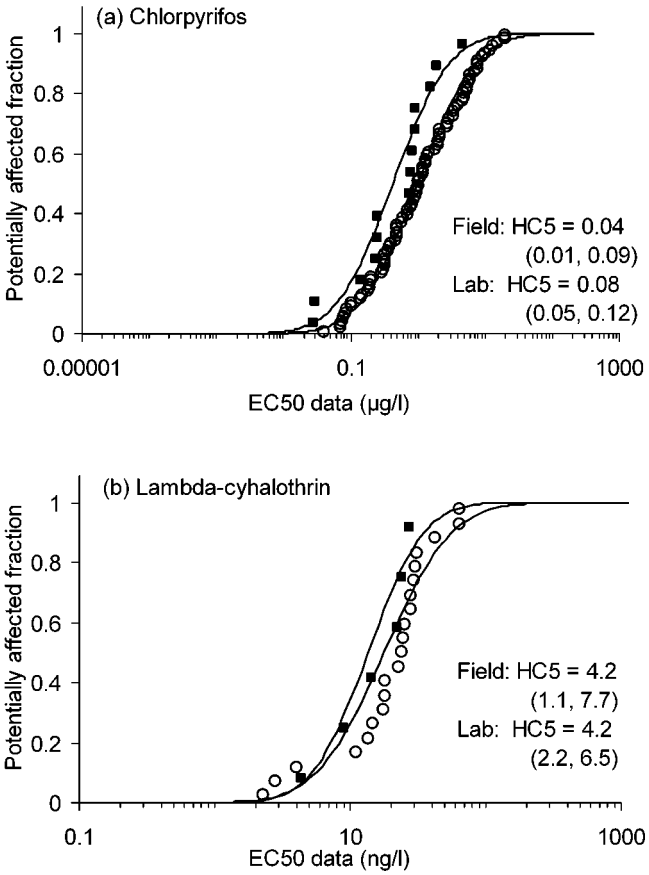

Fig. 6. Species sensitivity distributions for freshwater arthropods exposed to (**a**) chlorpyrifos or (**b**) lambda-cyhalothrin in single-species laboratory tests (open symbols) or in (micro)mesocosms (solid symbols). Median hazardous concentrations for 5% of species (HC5) are presented for each distribution with lower (95% confidence) and upper (5% confidence) estimates given in parentheses. Median effect concentrations (EC50s) are derived from single-species laboratory studies.

Table 2. Median hazardous concentrations for 5% (HC5) and 10% (HC10) of potentially affected species estimated from species sensitivity distributions constructed using freshwater arthropod single-species acute toxicity data[a]

Pesticide	HC5	HC10	$NOEC_{eco}/LOEC_{eco}$	
			Single application	Multiple or continuous application
Azinphos-methyl	0.1 (0.02)	0.21	0.2/0.72	0.22/<0.95
Carbaryl	2.94 (1.3)	6.22	<2/2	
Carbofuran	0.22 (0.03)	0.63	5/<25	
Chlorpyrifos	0.08 (0.05)	0.18	0.1/0.3	<0.1/<0.1
Cypermethrin	0.004 (0.001)	0.009		<0.07/<0.07
Deltamethrin	0.015 (0.006)	0.027	<0.2/<0.2	
Diazinon	0.26 (0.09)	0.56		<2.4/<2.4
Diflubenzuron	0.06 (0.005)	0.19	0.3/0.7	0.1/<1
Fenitrothion	0.44 (0.19)	0.88	1.1/<18.7	<14.3/<14.3
Fenvalerate	0.042 (0.007)	0.088	0.01/<0.05	<0.01/0.01
Lambda-cyhalothrin	0.003 (0.001)	0.005		0.0016/0.01
Lindane	1.7 (0.74)	8.2		0.25/1
Methoxychlor	0.37 (0.14)	0.64	3/5	
Parathion-ethyl	0.24 (0.03)	0.47		0.2/<0.5
Parathion-methyl	0.38 (0.09)	0.76	<10/<10	
Permethrin	0.21 (0.09)	0.41	<0.5/<0.5	

[a] Ecosystem no-observed-effect concentrations ($NOEC_{eco}$) and lowest-observed-effect concentrations ($LOEC_{eco}$) are nominal concentrations at which class 1 and class 2 effects [26] were observed in (micro)mesocosm studies with either a single or multiple/continuous insecticide application. The lower (95% confidence) estimate of the HC5 is given in parentheses. All concentrations are given in μg/L.

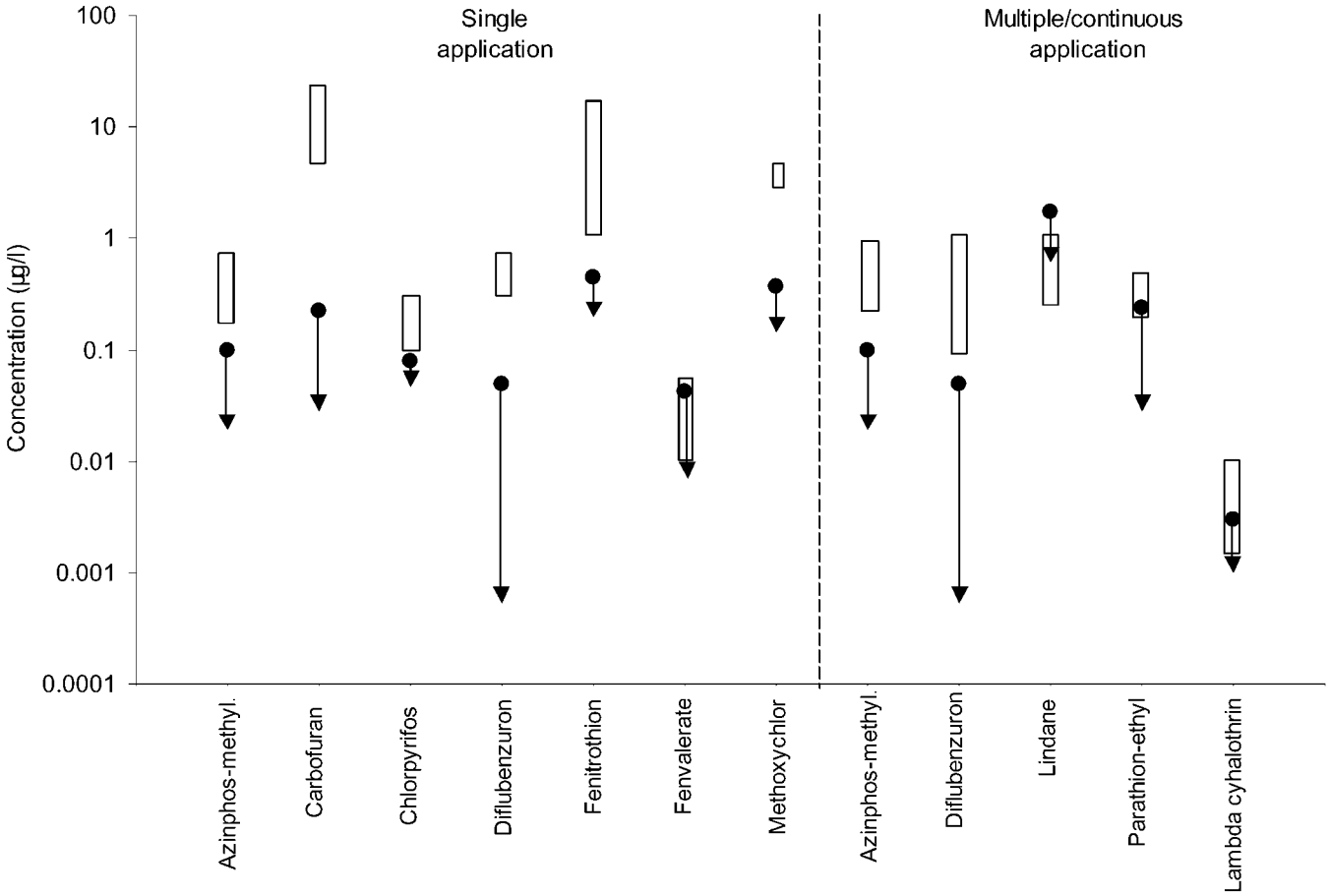

Fig. 7. Comparison of single-species hazardous concentration for 5% of species (HC5) estimates and response of multispecies assemblages in (micro)mesocosms exposed to insecticides. Median HC5 estimates (solid circles) and lower (95% confidence) estimates (arrow) were calculated using freshwater arthropod acute toxicity data. Rectangles represent the ecosystem lowest-observed-effect concentration ($LOEC_{eco}$; class 2) to ecosystem no-observed-effect concentration ($NOEC_{eco}$; class 1) concentration range derived from (micro)mesocosm studies.

These results are consistent with those of Solomon et al. [16], who reported a one order of magnitude difference in median HC5 values for fish and those for arthropods using short-term toxicity data for cypermethrin, permethrin, and fenvalerate. The results of the present study also support previous recommendations [31] that the toxic mode of action of pesticides should be taken into account when deciding which taxonomic groups are most appropriate for assessing risk, which in the case of insecticides are the arthropods.

Even within the arthropods, however, taxonomic composition may influence hazard assessment, as is the case when comparing the sensitivity distributions of freshwater and saltwater species. Several authors have reported that saltwater species are more sensitive than freshwater species to insecticides [16,18,32], although a study of 160 compounds, including 92 pesticides, found no significant difference in the average sensitivity of freshwater and saltwater species [25]. We compared the sensitivity distributions of freshwater and saltwater arthropods for 10 insecticides and found no significant overall difference in median HC5 estimates. However, for permethrin and chlorpyrifos, saltwater arthropods were significantly more sensitive than freshwater arthropods. With the exception of the dipteran *Chironomus salinarius*, all saltwater arthropods tested were crustaceans, whereas the majority of freshwater arthropods tested were insects. No significant difference was found in the sensitivity distributions of freshwater or saltwater crustaceans to either chlorpyrifos or permethrin, suggesting that the apparent increased sensitivity of saltwater arthropods resulted from differences in the taxonomic composition of the two datasets being compared rather than from a fundamental difference in the responses of freshwater and saltwater taxa to insecticides.

One of the criticisms made about the use of SSDs in risk assessment is that the species assemblage used to construct the sensitivity distribution is not representative of the ecosystem to be protected [21]. As discussed above, we have no evidence that taxonomically similar freshwater and saltwater species differ in their sensitivity to insecticides, but does freshwater habitat matter? Freshwater ecosystems are divided into lentic (ditches, ponds, lakes) and lotic (rivers, streams) habitats, each with their own distinct community. Half the freshwater arthropods in the toxicity dataset were restricted to lentic habitats, and a third were restricted to lotic habitats. The sensitivity distributions of lentic and lotic arthropods were compared for eight insecticides, and no evidence of a significant difference among or within compounds was found.

Environmental hazard assessment for freshwater environments has primarily used species native to Europe or North America, and this is reflected in the dataset compiled here. More than two-thirds of the species in the dataset were restricted to temperate zones, and of these, a quarter were restricted to the Palaearctic zoogeographical region, which includes Europe, and two-thirds to the Nearctic zoogeographical region, which includes North America. The relevance of using species from one geographical region to assess the hazard posed to species in a different region has been questioned [33], and differences in the sensitivity of cold-water, temperate, and tropical fish species have been reported [34]. The importance of geographical distribution was evaluated by comparing the sensitivity of Palaearctic and Nearctic arthropods as well as the sensitivity of tropical and temperate arthropods. Sufficient data were available to conduct the temperate–tropical comparison with three insecticides and the Palaearctic–Nearctic comparison with four compounds. Palaearctic species tended to have lower HC5 values than Nearctic species and tropical species to have lower HC5 values than temperate species, but the differences were not statistically significant.

On the basis of this analysis, insecticide SSDs therefore can be constructed using arthropods from any freshwater habitat and, as long as taxonomic composition is taken into account, may be constructed using both freshwater and saltwater arthropods. Furthermore, there is no evidence to suggest that the use of northern hemisphere temperate species in hazard assessment places tropical or southern hemisphere freshwater ecosystems at undue risk from insecticides, a conclusion also reached by Hose and Van den Brink [35] for endosulfan. However, what about the range of species tested? Toxicity datasets are dominated by a limited number of species, usually those recommended in national and international test guidelines. Thirty-eight aquatic arthropod species have been recommended in test guidelines produced by the OECD, U.S. EPA, ASTM, and Environment Canada, and of these, 32 are crustaceans. Species are recommended on the basis of their sensitivity, suitability for laboratory culture/maintenance, and ecological or economic importance. It has been argued that by focusing on sensitive species, SSDs that are generated using recommended species may overestimate hazard to natural communities and, hence, be overprotective [20]. The SSDs and HC5 estimates for recommended and nonrecommended arthropods were compared for 15 insecticides. For carbaryl and chlorpyrifos, recommended arthropods were significantly more sensitive than nonrecommended arthropods, but the difference in median HC5 estimates was not significant when the analysis was restricted to crustaceans, again highlighting the importance of taxonomy in assessing hazard.

Species sensitivity distributions are used to estimate HCs that can be used to protect natural ecosystems. This approach has been criticized for depending on data from single-species laboratory toxicity tests conducted with a haphazard collection of species [20,21,23]. Despite these apparent deficiencies, SSDs constructed for chlorpyrifos and lambda cyhalothrin using laboratory acute toxicity data for freshwater arthropods did not differ significantly from those produced using acute toxicity data from (micro)mesocosm studies. Therefore, SSDs generated from a ''haphazard'' collection of species were representative of the sensitivity distribution of a functioning assemblage of species exposed in the field to the same compound under a similar exposure regime, thus extending the contention that similar or related species do not have different sensitivities under field or laboratory conditions [29,36,37].

The lower HC5 estimate from laboratory SSDs was less than the $LOEC_{eco}$ from (micro)mesocosm studies examining all 11 insecticides for which the comparison was possible, irrespective of the exposure regime. Furthermore, the corresponding median HC5 estimate was less than the $LOEC_{eco}$ from acute (single-application) (micro)mesocosm studies for all but one (carbaryl) of the eight insecticides for which comparison was possible. Considering chronic (multiple- or continuous-application) (micro)mesocosm studies, the median HC5 exceeded the $LOEC_{eco}$ for two (fenvalerate and lindane) of the six insecticides for which comparison was possible. The $LOEC_{eco}$ is the lowest concentration at which a slight effect (class 2 [26]) was observed on the most sensitive endpoint, structural or functional, whereas the $NOEC_{eco}$ is the highest concentration at which no treatment effect was demonstrated. Again, except for carbaryl, lindane, and fenvalerate, the lower HC5 estimate was less than the $NOEC_{eco}$

for both acute and chronic insecticide exposures. The median (lower) HC5 estimate for lindane of 1.71 (0.74) μg/L compares to reported values of 3.2 (1.9) μg/L calculated using all freshwater lethality data [18] and of 0.45 (0.34) μg/L calculated using freshwater and saltwater arthropod lethality data [38]. The lower HC5 estimate for carbaryl (1.3 μg/L) is comparable to published values of 0.43 to 0.66 μg/L calculated using the concentration lethal to 1% of test organisms for stream invertebrates [39], and the HC10 for fenvalerate (i.e., 0.088 μg/L) is comparable to a reported value of 0.04 μg/L calculated using acute toxicity data from freshwater arthropods [16].

Previous studies have demonstrated that the median HC5 based on single-species NOEC data is protective of ecosystem-level effects [17,36]. However, most chemicals have insufficient chronic toxicity data to generate appropriate sensitivity distributions. Acute toxicity data are more abundant, and in the present study, we have demonstrated that SSDs constructed using acute toxicity data can estimate threshold concentrations that are protective of the adverse ecological effects of insecticides in aquatic systems. Although the potential of this approach has been demonstrated previously for individual chemicals [38,40], to our knowledge this is the first demonstration of the general application of this approach to insecticide risk assessment.

In summary, the results of the present investigation of 16 insecticides indicate that acute toxicity data for freshwater arthropods from different geographical regions and different freshwater habitats may be combined within a single SSD. If necessary, data from freshwater and saltwater habitats also can be combined, but it is important to be aware of differences in taxonomic composition and possible consequences for any threshold concentrations that are calculated. The lower HC5 (i.e., 95% protection level with 95% confidence [24]) estimated using acute toxicity data for freshwater arthropods provides a conservative estimate of the ecosystem threshold concentration for both acute and multiple or continuous applications of insecticide. The median HC5 estimate (i.e., 95% protection level with 50% confidence) based on acute toxicity data for freshwater arthropods is generally protective of single insecticide applications and of continuous or multiple applications when a safety factor of at least five is applied.

Acknowledgement—This research was funded jointly by the UK Department of Environment, Food, and Rural Affairs (Defra) and the Dutch Ministry of Agriculture, Nature, and Food Safety (DLO/PO Research Program 416). The authors are indebted to representatives of RIVM (Dick de Zwart) and the chemical industry (Syngenta and Bayer CropScience) for kindly providing additional toxicity data.

REFERENCES

1. Posthuma L, Suter GW II, Traas TP, eds. 2002. *Species-Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, FL, USA.
2. Van Straalen NM, Denneman CAJ. 1989. Ecotoxicological evaluation of soil quality criteria. *Ecotoxicol Environ Saf* 18:241–251.
3. Sijm DTHM, van Wezel AP, Crommentuijn T. 2002. Environmental risk limits in the Netherlands. In Posthuma L, Suter GWI, Traas TP, eds, *Species-Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 221–253.
4. Suter GWI. 2002. North American history of species sensitivity distributions. In Posthuma L, Suter GWI, Traas TP, eds, *Species-Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 11–17.
5. Van Straalen NM, Van Leeuwen CJ. 2002. European history of species sensitivity distributions. In Posthuma L, Suter GW, Traas TP, eds, *Species-Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 19–34.
6. Hall LW Jr, Scott MC, Killen WD. 1998. Ecological risk assessment of copper and cadmium in surface waters of Chesapeake Bay watershed. *Environ Toxicol Chem* 17:1172–1189.
7. Crommentuijn T, Ploder M, Sijm D, de Bruijn J, van de Plassche E. 2000. Evaluation of the Dutch environmental risk limits for metals by application of the added risk approach. *Environ Toxicol Chem* 19:1692–1701.
8. Brix KV, DeForest DK, Adams WJ. 2001. Assessing acute and chronic copper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups. *Environ Toxicol Chem* 20:1846–1856.
9. Van de Plassche EJ, de Bruijn JHM, Stephenson RR, Marshall SJ, Feijtel TCJ, Belanger SE. 1999. Predicted no-effect concentrations and risk characterization of four surfactants: Linear alkyl benzene sulfonate, alcohol ethoxylates, alcohol ethoxylated sulfates, and soap. *Environ Toxicol Chem* 18:2653–2663.
10. Selck H, Riemann B, Christoffersen K, Forbes VE, Gustavson K, Hansen BW, Jacobsen JA, Kusk OK, Petersen S. 2002. Comparing sensitivity of ecotoxicological effect endpoints between laboratory and field. *Ecotoxicol Environ Saf* 52:97–112.
11. Klaine SJ, Cobb GP, Dickerson RL, Dixon KR, Kendall RJ, Smith EE, Solomon KR. 1996. An ecological risk assessment for the use of the biocide, dibromonitrilopropionamide (DBNPA), in industrial cooling systems. *Environ Toxicol Chem* 15:21–20.
12. Solomon KR, Baker DB, Richards RP, Dixon KR, Klaine SJ, La Point TW, Kendall RJ, Weisskopf CP, Giddings JM, Giesy JP, Hall LW Jr, Williams WM. 1996. Ecological risk assessment of atrazine in North American surface waters. *Environ Toxicol Chem* 15:31–76.
13. Campbell KR, Bartell SM, Shaw JL. 2000. Characterizing aquatic ecological risks from pesticides using a diquat dibromide case study. II. Approaches using quotients and distributions. *Environ Toxicol Chem* 19:760–774.
14. Brock TCM, Crum SJH, Deneer JW, Heimbach F, Roijackers RMM, Sinkeldam JA. 2004. Comparing aquatic risk assessment methods for the photosynthesis-inhibiting herbicides metribuzin and metamitron. *Environ Pollut* 130:403–426.
15. Giesy JP, Solomon KR, Coats JR, Dixon KR, Giddings JM, Kenaga EE. 1999. Chlorpyrifos: Ecological; risk assessment in North American aquatic environments. *Rev Environ Contam Toxicol* 160:1–129.
16. Solomon KR, Giddings JM, Maund SJ. 2001. Probabilistic risk assessment of cotton pyrethroids: I. Distributional analyses of laboratory aquatic toxicity data. *Environ Toxicol Chem* 20:652–659.
17. Versteeg DJ, Belanger SE, Carr GJ. 1999. Understanding single-species and model ecosystem sensitivity: Database comparison. *Environ Toxicol Chem* 18:1329–1346.
18. Wheeler JR, Leung KMY, Morritt D, Sorokin N, Rodgers H, Toy R, Holt M, Whitehouse P, Crane M. 2002. Freshwater to saltwater toxicity extrapolations using species sensitivity distributions. *Environ Toxicol Chem* 21:2459–2467.
19. Kooijman SALM. 1987. A safety factor for LC50 values allowing for differences in sensitivity among species. *Water Res* 21:269–276.
20. Smith EP, Cairns J Jr. 1993. Extrapolation methods for setting ecological standards for water quality: statistical and ecological concerns. *Ecotoxicology* 2:203–219.
21. Forbes VE, Calow P. 2002. Species sensitivity distributions revisited: A critical appraisal. *Human Ecol Risk Assess* 8:473–492.
22. Posthuma L, Traas TP, De Zwart D, Suter GWI. 2002. Conceptual and technical outlook on species sensitivity distributions. In Posthuma L, Suter GW Traas TP, eds, *Species-Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 475–508.
23. Forbes TL, Forbes VE. 1993. A critique of the use of distribution-based extrapolation models in ecotoxicology. *Funct Ecol* 7:249–254.
24. Aldenberg T, Jaworska JS. 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. *Ecotoxicol Environ Saf* 46:1–18.
25. De Zwart D. 2002. Observed regularities in species sensitivity distributions for aquatic species. In Posthuma L, Suter GWI, Traas TP, eds, *Species-Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, FL, USA, pp 133–154.
26. Brock TCM, Van Wijngaarden RPA, Van Geest G. 2000. Ecological risks of pesticides in freshwater ecosystems. Part 2—In-

secticides. Technical Report 089. Alterra Centre for Water and Climate, Wageningen, The Netherlands.
27. Van Vlaardingen P, Traas TP, Aldenberg T. 2003. Normal distribution based hazardous concentration and potentially affected fraction. ETX-2000. Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, The Netherlands.
28. Van Wijngaarden RPA, Van den Brink PJ, Crum SJH, Oude Voshaar JH, Brock TCM, Leeuwangh P. 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: I. Comparison of short-term toxicity between the laboratory and the field. *Environ Toxicol Chem* 15:1133–1142.
29. Schroer AFW, Belgers D, Brock TCM, Matser A, Maund SJ, Van den Brink PJ. 2004. Acute toxicity of the pyrethroid insecticide lambda-cyhalothrin to invertebrates of lentic freshwater ecosystems. *Arch Environ Contam Toxicol* 46:324–335.
30. Van Wijngaarden RPA, Brock TCM, Van den Brink PJ. 2005. Threshold levels for effects of insecticides in freshwater ecosystems. *Ecotoxicology* (in press).
31. Campbell PJ, Arnold DJS, Brock TCM, Grandy NJ, Heger W, Heimbach F, Maund SJ, Streloke M, eds. 1999. *Guidance Document on Higher-Tier Aquatic Risk Assessment for Pesticides (HARAP).* SETAC-Europe, Brussels, Belgium.
32. Hutchinson TH, Scholz N, Guhl W. 1998. Analysis of the ECETOC aquatic toxicity (EAT) database IV—Comparative toxicity of chemical substances to freshwater versus saltwater organisms. *Chemosphere* 36:143–153.
33. Davies PE, Cook LSJ, Goenarso D. 1994. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. *Environ Toxicol Chem* 13:1341–1354.
34. Dyer SD, Belanger SE, Carr GJ. 1997. An initial evaluation of the use of Euro/North American fish species for tropical effects assessment. *Chemosphere* 35:2767–2781.
35. Hose GC, Van den Brink PJ. 2004. Confirming the species sensitivity distribution concept for endosulfan using laboratory, mesocosm and field data. *Arch Environ Contam Toxicol* 47:511–515.
36. Emans HJB, Van de Plassche EJ, Canton JH, Okkerman PC, Sparenburg PM. 1993. Validation of some extrapolation methods used for effect assessment. *Environ Toxicol Chem* 12:2139–2154.
37. Van den Brink PJ, Brock TCM, Posthuma L. 2002. The value of the species sensitivity distribution concept for predicting field effects: (Non-)confirmation of the concept using semifield experiments. In Posthuma L, Suter GWI, Traas TP, eds, *Species-Sensitivity Distributions in Ecotoxicology.* Lewis, Boca Raton, FL, USA, pp 155–193.
38. Van den Brink PJ, Hartgers EM, Gylstra R, Bransen F, Brock TCM. 2002. Effects of mixtures of two insecticides in freshwater microcosms: II. Responses of plankton and ecological risk assessment. *Ecotoxicology* 11:181–197.
39. Peterson JL, Jepson PC, Jenkins JJ. 2001. Effect of varying pesticide exposure duration and concentration on the toxicity of carbaryl to two field-collected stream invertebrates, *Calineuria californica* (Plecoptera: Perlidae) and *Cinygma* sp. (Ephemeroptera: Heptageniidae). *Environ Toxicol Chem* 20:2215–2223.
40. Giddings JM, Solomon KR, Maund SJ. 2001. Probabilistic risk assessment of cotton pyrethroids: II. Aquatic mesocosm and field studies. *Environ Toxicol Chem* 20:660–668.