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Fungicide Risk Assessment for Aquatic Ecosystems: Importance of Interspecific Variation, Toxic Mode of Action, and Exposure Regime

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The risk assessment of fungicides in Europe uses information from ecotoxicity studies performed on vertebrates, invertebrates, and primary producers, but not nontarget fungi. But which toxicity data should be used to assess risk and how important are modes of action and exposure regimes? A data set was compiled comprising acute single-species toxicity data for 42 fungicides, semifield data for 12 fungicides, and covering seven toxic modes of action and different exposure regimes. Most fungicides were general biocides and data from all taxonomic groups were used to construct species sensitivity distributions (SSDs) and assess risk. The derived lower-limit HC5 values and HC1 values were always protective of adverse ecological effects in semifield studies and HC5 values were protective for at least 3 of the fungicides. Expanding the analysis to include insecticides and herbicides, the following threshold values, derived from SSDs based on the most sensitive taxonomic group, are proposed to protect against adverse ecological effects from pesticide exposure: (i) the HC5 can be used for short-term exposures; (ii) the HC5 divided by 1.5 can be used for medium-term exposures; (iii) either the HC1 or the HC5 divided by 3 can be used for long-term exposures.

Introduction

Fungicides play a crucial role in modern agriculture and act by either inhibiting sterol biosynthesis (e.g., demethylation inhibitors (DMI)), energy production (e.g., quinone outside inhibitors (QoI)), amino acid synthesis (e.g., aniline-pyrimidines), or cell division (e.g., benzimidazoles), or have multiple sites of action (e.g., dithiocarbamates, chloronitriles, and dinitroanilines) (*1*). Whereas fungicides are designed to control fungal pathogens, their modes of action are not specific to fungi (*2*). The processes of energy production and cell division are highly conserved and sulphydryl (SH) are components of numerous enzymes. Consequently, fungicides targeting these processes or functional groups will be toxic to a wide range of organisms. Even fungicides that inhibit the production of the fungal sterol ergosterol (i.e., DMI fungicides), interact with an enzyme (14-α-demethylase or CYP51) that is highly conserved across fungi, plants, and animals (*3*).

The regulatory risk assessment process for fungicides in Europe uses information from ecotoxicity studies ranging in complexity from standardized single-species toxicity tests to semifield studies (*4, 5*). Extrapolating data obtained on a few species to all species that may be exposed to a chemical is highly uncertain and is particularly problematic for fungicides that may have a broad spectrum of activity. One approach for reducing this uncertainty is to characterize interspecific variation in toxicant sensitivity by constructing species sensitivity distributions (SSDs) (*6*). Previous studies with insecticides and herbicides have demonstrated that SSDs based on the most sensitive taxonomic groups can be used to determine threshold levels that are protective of communities in semifield studies (*7, 8*). Whereas it is clear that the sensitive taxonomic groups for insecticides and herbicides usually comprise arthropods and primary producers respectively, it is not clear which of the three taxonomic groups used in pesticides risk assessment—vertebrates (fish), invertebrates, primary producers—should be the focus of attention for studies with fungicides. The few published semifield studies with fungicides do not suggest one common sensitive group (*9*−*14*).

Species sensitivity distributions are used to estimate the hazardous concentration affecting a specific proportion (p%) of species (HCp) (*15*). The median (50% confidence) and lower limit (95% confidence) HC5 estimates, generated using acute toxicity data, have been used to derive threshold levels for insecticides and herbicides that are protective of adverse effects in semifield studies: median HC5 values being protective under short-term exposure regimes and lower limit HC5 values being protective under long-term exposure regimes (*7, 8*). The value of the lower limit HC5 depends on how well the data fit the model and will tend toward the median HC5 as the number of taxa used to derive the SSD increases (*16*). With very large data sets, the lower limit and median HC5 values will not differ much and hence the distinction between the threshold values for different exposure regimes will be reduced, although differences in effect would remain. Whereas it is highly unlikely that this will be an issue for regulatory risk assessments, due to the lack of data on a sufficient number of species, a more robust approach would be to use different median HCp estimates to derive threshold values for different exposure regimes.

The aims of the present paper are (a) to investigate the relative sensitivity of aquatic organisms to fungicides with different toxic modes of action, (b) to evaluate the predictive value of the SSD approach for assessing the ecological risk of fungicides to freshwater ecosystems, and (c) to determine the most appropriate HCp estimates for setting threshold values for different exposure regimes. Aims (b) and (c) are achieved by comparing SSD-derived threshold values with effects observed in microcosm and mesocosm experiments. The data set used for addressing aim (c) was extended to include data for herbicides and insecticides taken from (*7*) and (*8*). These evaluations were possible because pesticide companies kindly provided confidential data from both single-species and microcosm/mesocosm experiments.

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TABLE 1. Forty-two Fungicides Used in This Study Organized by Toxic Mode of Action (*38*) (Also Indicated Are the Numbers of Aquatic Taxa for Which Single-Species Acute Toxicity Data Met the Selection Criteria)

mode of action	chemical group	compound	no. taxa
amino acid synthesis	anilinopyrimidines	cyprodinil	18
cell division	benzimidazole	carbendazim	16
		benomyl	19
energy production	benzamides	flutolanil	10
	quinone outside inhibitor	azoxystrobin	17
		fluoxastrobin	14
		kresoxim-methyl	15
		picoxystrobin	19
		trifloxystrobin	23
	dinitrophenol	binapacryl	9
		dinitro-o-cresol (DNOC)	9
	dinitroaniline	fluazinam	27
	organotin	tributyltin oxide	54
		triphenyltin acetate	42
	aromatic	pentachlorophenol (PCP)	166
membrane integrity	phosphorothiolate	pyrazophos	14
	phenylpyrrole	fludioxonil	8
	quaternary ammonium	benzalkonium chloride	14
multisite	ethylene bisdithio-carbamate (EBDC)	mancozeb	18
		maneb	22
		metiram	23
		nabam	10
		zineb	7
	dimethyl dithiocarbamate	thiram	24
		ziram	12
	phthalimide	captan	24
		captafol	13
		folpet	21
	chloronitrile	chlorothalonil	46
	sulfamide	tolylfluanid	17
	guanidine	dodine	13
	triazine	anilazine	12
	quinone	dichlone	9
	anthraquinone	dithianon	11
	inorganic	mercuric chloride	151
nephrotoxic		hexachlorobutadiene (HCBD)	12
sterol biosynthesis	demethylation inhibitors	cyproconazole	9
		epoxiconazole	6
		fenbuconazole	8
		myclobutanil	8
		propiconazole	19
		tebuconazole	9

Materials and Methods

Aquatic single-species acute toxicity data and multispecies data from microcosm and mesocosm studies were collated from the open literature, existing toxicity databases (e.g., www.epa.gov/ecotox; (*17*)) and company reports. Data selection criteria followed those of Maltby et al. (*7*), where the end points used for animals were EC50 values for either mortality or immobilization, and for primary producers were EC50 values for biomass or growth. The test durations selected were 2−21 d for vertebrates, 1−7 d for invertebrates, 2−28 d for macrophytes, and 1−7 d for algae. Each species was represented only once per compound in the analysis. The following data manipulations were performed where there were multiple toxicity values for a taxon.

- The lowest value was selected where several duration values, temperatures, life stages, water types, etc., were studied in the same experiment.
- The geometric mean was taken for data for the same species (and end point), but from different experiments.
- Data reported as < or > were collated, but not used for the construction of SSD curves.

Genera data were only used if no species data were reported for a genus. The resulting data set is described in Table 1. Seventeen of the 42 fungicides in the data set exhibited multisite activity and these were further divided into ethylene bisdithiocarbamates (EBDC) and non-EBDC multisite fungicides for subsequent analyses.

SSD curves were generated where a minimum of six data points (i.e., taxa) were available. Initially, all available data for a compound were used to generate an SSD and the fit to a log-normal distribution was assessed using the Anderson−Darling goodness-of-fit test. If the distribution did not pass the goodness-of-fit test at $p = 0.05$, separate distributions were constructed for vertebrates and nonvertebrates and the

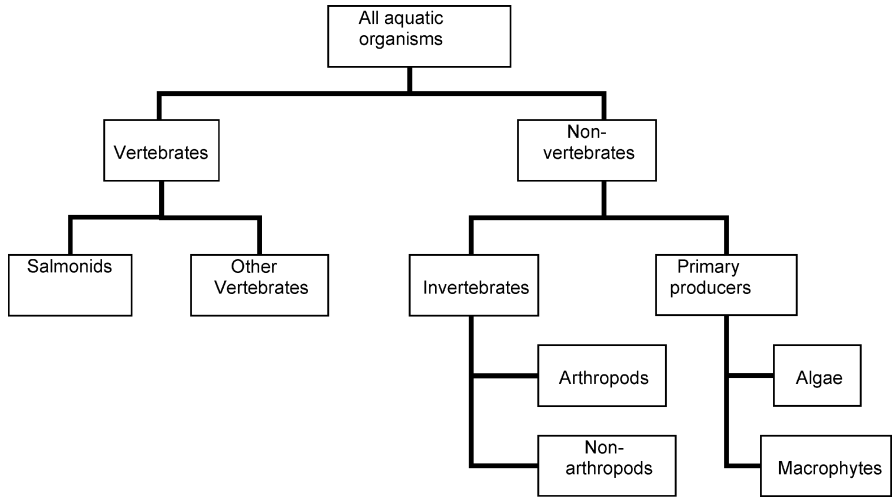

FIGURE 1. Diagram illustrating how data were processed for the generation of SSDs. At each level, the fit of the distribution to a log-normal model is assessed. If no fit is found, then the data are split as indicated in the next level down. Vertebrates were divided into "salmonids" and "other vertebrates" to reflect the enhanced protection given to salmonids in the European Freshwater Fish Directive (*39*).

most sensitive distribution was used. If these distributions were not described by a log-normal model, then the data set was partitioned further using the scheme illustrated in Figure 1 until either there was an adequate fit to the log-normal model or there were insufficient data to generate an SSD. Median HC5 values (50% confidence, hereafter denoted HC5 values), the lower-limit of the HC5 (95% confidence, denoted LLHC5 values) and the median HC50 value (50% confidence, hereafter denoted HC50 values) were calculated for each SSD. The $E_TX^{2.0}$ software (*18*) was used to construct SSDs, to estimate HC5, LLHC5, and HC50 values, and to perform the Anderson−Darling goodness-of-fit tests. Median HC1 values (hereafter denoted HC1 values) were estimated using the Pesticide Risk Assessment Tool (www.webfram.com) as the $E_TX^{2.0}$ program does not provide them. The HC50:HC5 ratio was used as a measure of interspecific variation in sensitivity; the greater the ratio, the shallower the slope of the SSD and hence the greater the interspecific variation. HC50:HC5 ratios and HC5 estimates were log-transformed and compared across toxic modes of action using analysis of variance.

The compiled database was used to investigate taxonomic variation in sensitivity to fungicides, focusing on the comparison among fish, invertebrates, and primary producers. Two approaches were used: the first was to assess whether there was any statistically significant difference in the average sensitivity (i.e., median EC50 value) of fish, invertebrates, and primary producers; the second was to generate separate SSDs for each taxonomic grouping and compare the resulting HC5 values. Median EC50 and HC5 values were compared within toxic modes of action; data being log transformed and analyzed using paired *t* tests. HC5 values were converted to molarity before being compared across compounds.

Multispecies data were collated from 21 studies with 12 fungicides. Each study was classified into one of four exposure categories (Table 2) and responses observed for the most sensitive end point, ascribed to one of five effect classes (*4*). The $NOEC_{eco}$ (i.e., lowest test concentration at which no or slight/transient effects (Class 1 and 2) were observed) and $LOEC_{eco}$ (i.e., lowest test concentration at which clear effects (Class 3) were observed) were determined for each compound and exposure scenario. Ecosystem threshold levels ($NOEC_{eco}$) were then compared to estimates of HC5, LLHC5, and HC1 values derived from single-species acute data for either all taxa or, where possible (i.e., data were log-normally distributed) and appropriate (i.e., critical multispecies studies did not include vertebrates), nonvertebrate taxa. The comparison

TABLE 2. Classification of Exposure Regimes in Micro/Mesocosm Experiments Based on Number of Applications and Dissipation Constant from Water−Sediment Studies (DT50)

exposure category	definition and criteria
1	short-term pulse exposure - dissipation DT50 < 1 d
2	short-term exposure - single application and dissipation DT50 > 1 d < 10 d
3	medium-term exposure a. single application; dissipation DT50 >10 ≤25 d b. repeated applications; dissipation DT50 > 1 d <10 d
4	long-term exposure a. single application; dissipation DT50 > 25 d b. more or less constant chronic exposure.

of HC5, LLHC5, or HC1 and $NOEC_{eco}$ values was extended to insecticides and herbicides using data sets described in Maltby et al. (*7*) and van den Brink et al. (*8*). $NOEC_{eco}$ values for each pesticide were plotted against HC5, LLHC5, or HC1 values and compared to the 1:1 HCp:$NOEC_{eco}$ ratio on a log−log plot. Pesticides falling below the 1:1 line indicate that HCp values from single-species acute toxicity tests were protective of ecological effects in multispecies semifield studies. Analysis of variance of log-transformed data was used to investigate the effect of exposure regime, as defined in Table 2, on the HCp:$NOEC_{eco}$ ratio of pesticides.

Results

Single-species toxicity data were compiled for 42 fungicides covering seven toxic modes of action (Table 1). The most common modes of action were binding to sulphydryl bonds resulting in multisite activity (17 compounds: 5 EBDC and 12 non-EBDC), inhibiting sterol biosynthesis (6 demethylase inhibitors), or disrupting energy production (12 compounds). The data set included compounds that disrupt energy production by inhibiting succinic dehydrogenase (flutolanil), inhibiting electron transfer from cytochrome *b* to cytochrome C_1 (Quinone outside inhibitors), uncoupling oxidative phosphorylation (binapacryl, DNOC, fluazinam, PCP) or inhibiting ATP synthase (organotin compounds). The median number of taxa per compound was 13.

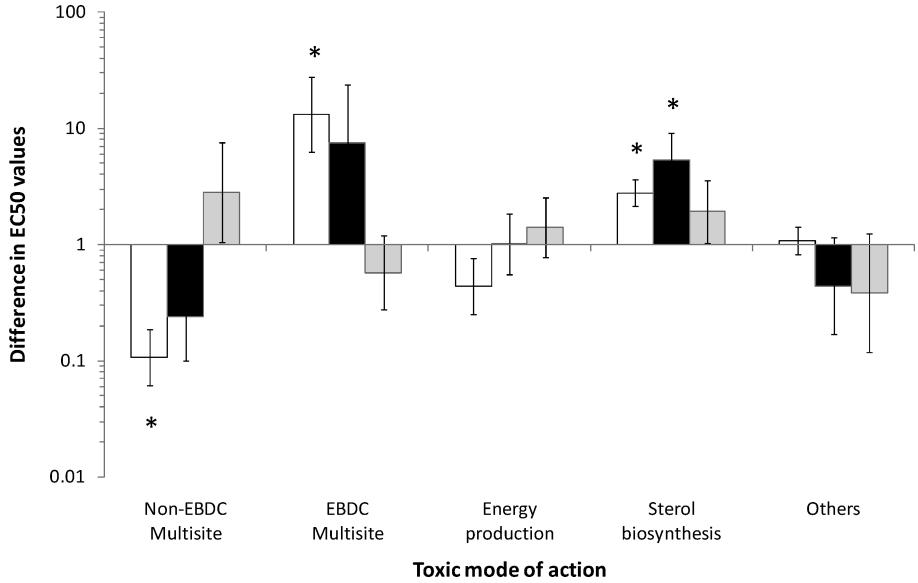

FIGURE 2. Mean (±SE) pairwise difference in median EC50 values (log transformed) for fungicides with different toxic modes of actions. Comparisons were made between the EC50 of fish and invertebrates (open bar), fish and primary producers (black bar), and invertebrates and primary producers (gray bar). Asterisk denotes significant pairwise differences.

The data set contained single-species toxicity data for 424 taxa, of which 67% were invertebrates, 23% were vertebrates, and 10% were primary producers. The very limited single-species toxicity data available for nonpathogenic aquatic fungi (i.e., *19*−*21*) provide evidence of interspecific differences in sensitivity to fungicides, but unfortunately are unsuitable for use in this analysis. Appropriate data for primary producers were unavailable for 5 compounds (binapacryl, captafol, dichlone, HCBD, mercuric chloride). Data sets for flutalonil, maneb, captan were dominated (≥70%) by vertebrate taxa whereas those for mercuric chloride and fluoxystrobin were dominated by invertebrate taxa.

Based on EC50 values, fish were approximately 10 times more sensitive than invertebrates to non-EBDC fungicides with multisite activity ($t_{11} = 4.05$, $p = 0.002$), but 10 times less sensitive than invertebrates to EBDC fungicides ($t_4 = 3.5$, $p = 0.025$). Fish were also less sensitive than invertebrates and primary producers to fungicides that inhibit sterol biosynthesis ($t_5 > 3$, $p \leq 0.03$). No overall significant taxonomic differences were observed for fungicides that inhibit energy production ($t_{11} < 1.5$, $p > 0.05$) or the other modes of action considered ($t_6 < 0.75$, $p > 0.05$, Figure 2).

Toxicity data for 30 of the 42 fungicides were described by an SSD comprising all taxonomic groups (i.e., overall SSD). Nine of the remaining 12 compounds were described either by a nonvertebrate SSD (folpet, metiram, and triphenyltin acetate), a vertebrate SSD (PCP, propiconazole, and ziram), a salmonid SSD (captan), an invertebrate SSD (cyprodinil), or an arthropod SSD (tributyltin oxide). It was not possible to generate SSDs for three compounds (benzalkonium chloride, binapacryl, and dichlone). For overall SSDs, the HC50:HC5 ratio ranged from 3.6 (fenbuconazole) to 106 (captafol), but a significant effect of mode of action on interspecific variation in sensitivity was not observed ($F_{4,25} = 0.69$, $p > 0.05$). There was a significant difference in average HC5 values across toxic modes of action ($F_{4,25} = 3.06$, $p =$

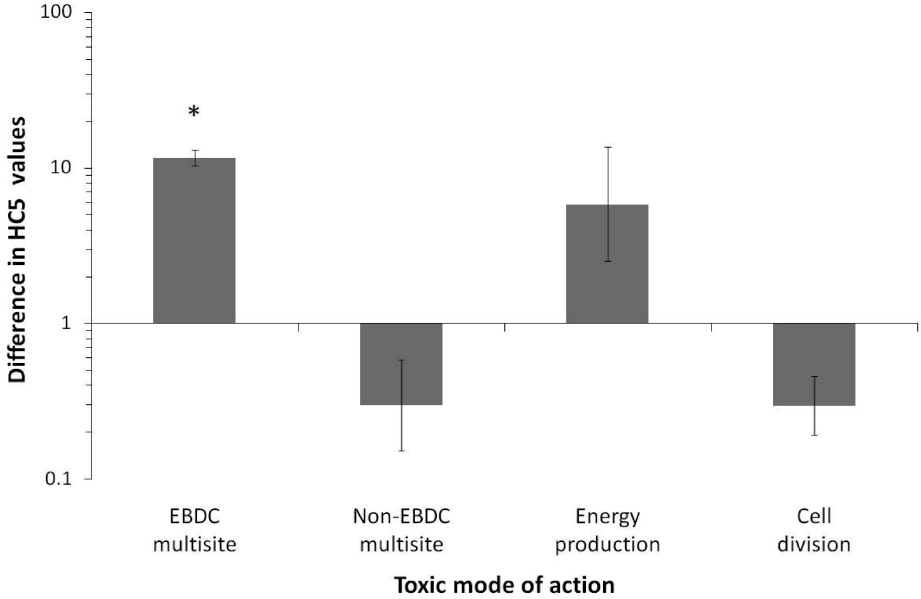

FIGURE 3. Mean (±SE) pairwise difference in HC5 values (log transformed) derived from vertebrate or nonvertebrate SSDs for fungicides with different toxic modes of actions. Asterisk denotes a significant pairwise difference within a mode of action.

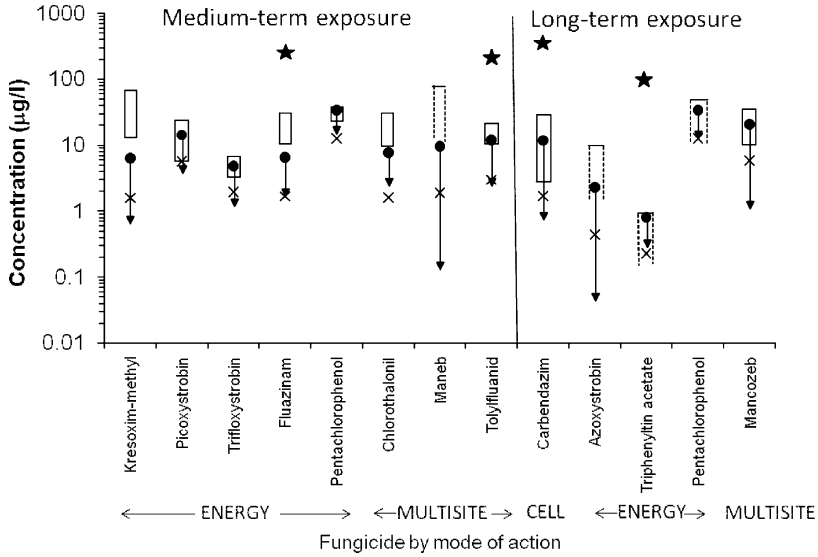

FIGURE 4. Comparison between median HC5 (dot) and HC1 (cross) values derived from single-species acute toxicity tests and threshold concentrations determined from multispecies studies for 12 fungicides. Arrows indicate lower limit HC5 and multispecies data presented as the range between the $NOEC_{eco}$ (Class 1−2) and the $LOEC_{eco}$ (Class 3) (rectangle). Stars denote NOEC values for decomposition and dotted lines indicate that the $NOEC_{eco}$ is unknown. Modes of action were inhibition of cell division (CELL), inhibition of energy production (ENERGY) or multisite activity (MULTISITE). Dotted vertical lines separate exposure categories (see Table 2 for definitions).

0.035); the potency of non-EBDC multisite fungicides being significantly greater than that for fungicides that inhibit sterol biosynthesis.

Separate vertebrate and nonvertebrate SSDs were constructed for 14 fungicides and the mean difference in HC5 values varied across modes of action ($F_{3,10} = 5.93$, $p = 0.014$, Figure 3). On average, vertebrate HC5 values for EBDC fungicides were 10 times greater than nonvertebrate HC5 values ($t_3 = 21.56$, $p = 0.02$), however there was no significant difference between vertebrate and nonvertebrate HC5 values for non-EBDC multisite fungicides ($t_7 = 1.79$, $p > 0.05$). Although the vertebrate HC5 was consistently lower for fungicides that inhibit cell division and consistently larger for fungicides that inhibit energy production, these differences were not statistically different, possibly due to the small sample size ($t_2 < 2.8$, $p > 0.05$).

HC5 estimates were compared to ecosystem thresholds derived from 21 multispecies studies for 12 fungicides. The HC5 was lower than the $LOEC_{eco}$ for all compounds and lower than the $NOEC_{eco}$ for 3 of the 9 fungicides for which a $NOEC_{eco}$ could be calculated (Figure 4). The HC1 and the LLHC5 estimates were also less than the $NOEC_{eco}$ for all 9 fungicides for which it could be calculated. Decomposition, a process in which aquatic fungi play an important role and hence potentially sensitive to fungicides, was measured in 4 of the multispecies studies (carbendazim, fluazinam, triphenyltin acetate, tolyfluanid), but in no case was it the most sensitive end point. In fact, in all cases it was an order of magnitude greater than the $LOEC_{eco}$ (Figure 4).

Combining information for 10 insecticides, 9 herbicides, and 9 fungicides, it was apparent that the HC5 value was no more than a factor of 10 greater than the $NOEC_{eco}$ and often much lower than the $NOEC_{eco}$, especially for insecticides (Figure 5a). In addition, with the exception of 1 (diuron) or 2 (diuron and 2,4-D) herbicides the LLHC5 and HC1 values were equal to or less that the $NOEC_{eco}$ (Figure 5b and c). HC5 values for 3 of the 13 insecticides (carbofuran, diflubenzuron, methoxychlor) were more than an order of magnitude less than the $NOEC_{eco}$. This increased to 5 insecticides for the HC1 and 7 insecticides and 1 fungicide (kresoxim-methyl) for the LLHC5. The ratio between the HCp and $NOEC_{eco}$ was dependent on exposure regime, as defined in Table 2 ($F_{2,27} > 5.7$, $p < 0.01$). Ratios were significantly lower when HCp estimates were compared to $NOEC_{eco}$ values from long-term exposure studies than medium-term or short-term exposure studies (Figure 6).

Discussion

The first aim of this study was to investigate taxonomic differences in sensitivity to fungicides with different toxic modes of action. Of the 42 fungicides studied, 17 exhibited multisite activity and 5 of these were EBDC fungicides. Comparing EC50 values, fish were less sensitive than invertebrates to EBDC fungicides and less sensitive than invertebrates or primary producers to sterol biosynthesis inhibitors. However, fish were more sensitive than invertebrates to non-EBDC fungicides with multisite activity, in concordance with previously studies with chlorothalonil (*22, 23*) and thiram (*24*). Separate SSDs were generated using vertebrate or nonvertebrate toxicity data for 14 fungicides. This analysis confirmed the lower sensitivity of fish to EBDC fungicides (nonvertebrate HC5 values being significantly less than vertebrate HC5 values), but did not detect significant taxonomic differences in HC5 estimates within other modes of action.

It was possible to generate a single SSD, using data for all taxonomic groups, for 30 of the 39 fungicides for which an SSD could be produced, indicating that most of the fungicides considered act as general biocides. In fact, 17 of the 42 compounds used in this study are listed in Commission Regulation 2032/2003 of the EU Biocides Directive (*25*). The consequence of this for aquatic risk assessment is that the default approach should be to generate fungicide SSDs using data from all major taxonomic groups (vertebrates, invertebrates, and primary producers). The inclusion of vertebrate data is particularly important for non-EBDC fungicides with multisite activity as fish appear to be particularly sensitive to these compounds. This is in stark contrast to the approach proposed for herbicides and insecticides where SSDs are generated using the most sensitive taxonomic groups, arthropods for insecticides (*7*) and primary producers for herbicides (*8*).

The second aim of this study was to evaluate the predictive values of the SSD approach for assessing the ecological risk

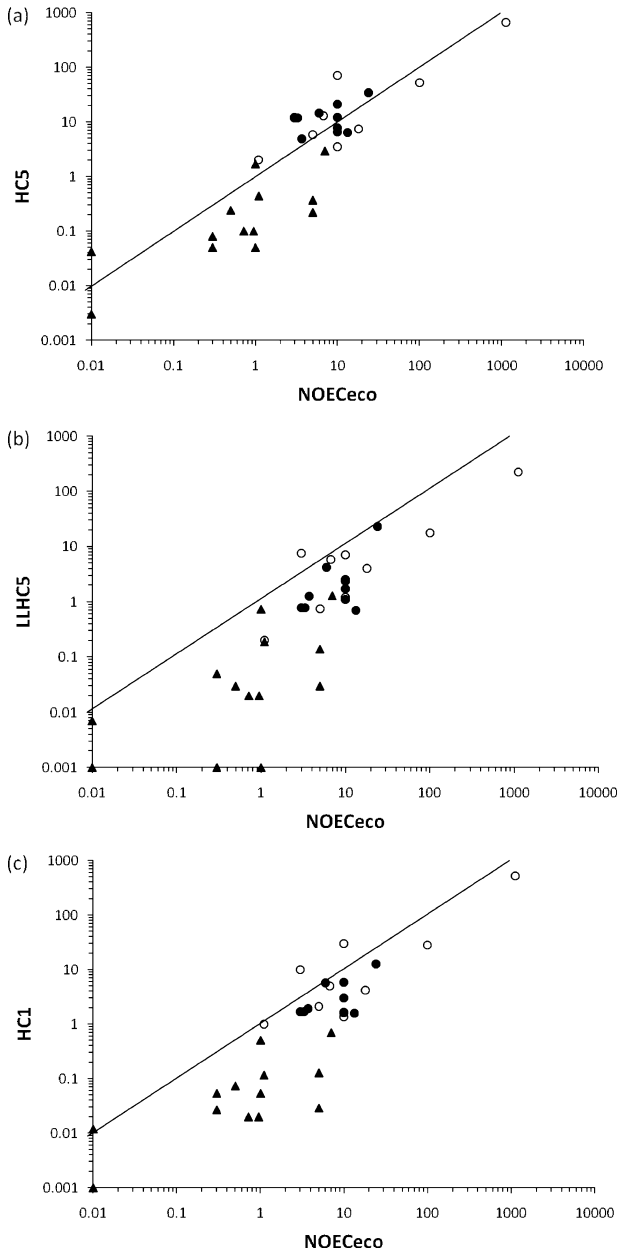

FIGURE 5. Plot of (a) HC5 estimate, (b) lower limit HC5 (LLHC5) estimate, and (c) HC1 estimate from SSDs based on single-species acute toxicity data against $NOEC_{eco}$ derived from 32 multispecies studies with 9 fungicides (●), 11 insecticides (▲), and 9 herbicides (○). Diagonal lines represent 1:1 ratio.

of fungicides to freshwater ecosystems. This was addressed by comparing hazardous concentrations (HCp) estimated from SSDs with ecological threshold concentrations derived from multispecies semifield studies (i.e., micro/mesocosm studies). HC1, HC5, and LLHC5 values were estimated from SSDs generated using nonvertebrate toxicity data where possible (i.e., fits log-normal model) and appropriate (i.e., no fish were evaluated in the multispecies study) and the $NOEC_{eco}$ based on Class 1 or 2 effects (sensu (*4*)) was used as the threshold value. This comparison was possible for 12 fungicides covering 3 toxic modes of action and 2 exposure regimes. In all cases, the HC5 was less than the $LOEC_{eco}$ and the LLHC5 and HC1 were less than the $NOEC_{eco}$, where known. Therefore, the LLHC5 or the HC1 values derived from single-species acute toxicity studies were protective of adverse ecological effects of fungicides, even under repeated or long-term exposure regimes.

The third and final aim was to determine the most appropriate HCp estimates for setting threshold values for different exposure regimes. This analysis was broadened to encompass insecticides and herbicides giving a total of 30 pesticides and 32 studies. For over half these pesticides (i.e., 62%), the HC5 value from the most appropriate SSD was protective of ecological effects in semifield studies whereas the HC1 and LLHC5 were protective for 94% and 97% of the pesticides, respectively. However, in many cases, especially for insecticides, basing the threshold level on the HC1 or LLHC5 is extremely precautionary. Refining this analysis by calculating the 95% confidence interval for the mean HCp to $NOEC_{eco}$ ratio for each exposure regime leads to the following proposed threshold values, derived from SSDs based on the most sensitive taxonomic group: (i) the HC5 can be used to protect against adverse ecological effects arising from short-term exposure; (ii) the HC5 divided by 1.5 can be used to protect against adverse ecological effects arising from medium-term exposure; (iii) either the HC1 or the HC5 divided by 3 can be used to protect against adverse ecological effects arising from long-term exposure to pesticides.

A limitation of this study is that, although the focus is on fungicides, no appropriate toxicity data for fungi were available for use in the analysis. Aquatic fungi play a key role in the ecology of many freshwater systems and in particular in decomposition and nutrient cycling processes (*2*). Leaf litter decomposition in streams is driven by aquatic hyphomycetes (*26*), which are presumed to belong mainly to the Ascomycota, with a small percentage related to the Basidiomycota (*27*). There are 290 known species worldwide (*27*) and individual leaves may be colonized by a mosaic of 10−20 fungal species (*28*). Recent studies have suggested that the composition of fungal assemblages (i.e., species identity) rather than species richness, is important in determining leaf decomposition rates (*29*).

There are very few studies on the sensitivity of aquatic hyphomycetes to pesticides (*30*). Chandrashekar and Kaveriappa (*19, 20*) investigated the fungicides mancozeb and carbendazim and found no evidence of inhibition of growth or sporulation up to 5 mg/L and no inhibition of germination up to 1 mg/L for either compound. Similarly, Roussel, Chauvet, and Bonzom (*31*) found no effect of copper concentration (up to 75 μg/L) on mycelia biomass or sporulation rates. In contrast, Bärlocher and Premdas (*32*) reported a linear negative relationship between conidia production and pentachlorophenol concentration (0.0001 to 10 mg/L), with the main effect occurring at concentrations greater than 0.1 mg/L. More studies have investigated the effects of fungicides on soil microbial assemblages. Several fungicides have been reported to reduce fungal enzyme activity and abundance, but the effects are often transitory (*33, 34*) and, with the exception of a recent study on azoxystrobin (*35*), are not reflected in changes in community functioning (e.g., decomposition) or structure (*34, 36, 37*).

There is a limited body of evidence suggesting that fungicides may have effects on nontarget soil and aquatic fungi and that fungal species may differ in their relative sensitivities. Although none of the semifield studies considered in this analysis investigated effects on fungal assemblages, leaf decomposition was measured for 4 fungicides (carbendazim, fluazinam, tolyfluanid, and triphenyltin acaetate). In all cases, the LOEC for leaf decomposition was at least an order of magnitude greater than the $LOEC_{eco}$, based on the most sensitive end point. Moreover, the $NOEC_{eco}$ for mancozeb and PCP were at least 4 times lower than concentrations reported to affect aquatic hyphomycetes (*20, 32*). Consequently, there is no evidence to suggest that threshold values for fungicides based on HCp estimates derived from SSDs generated using acute toxicity data for

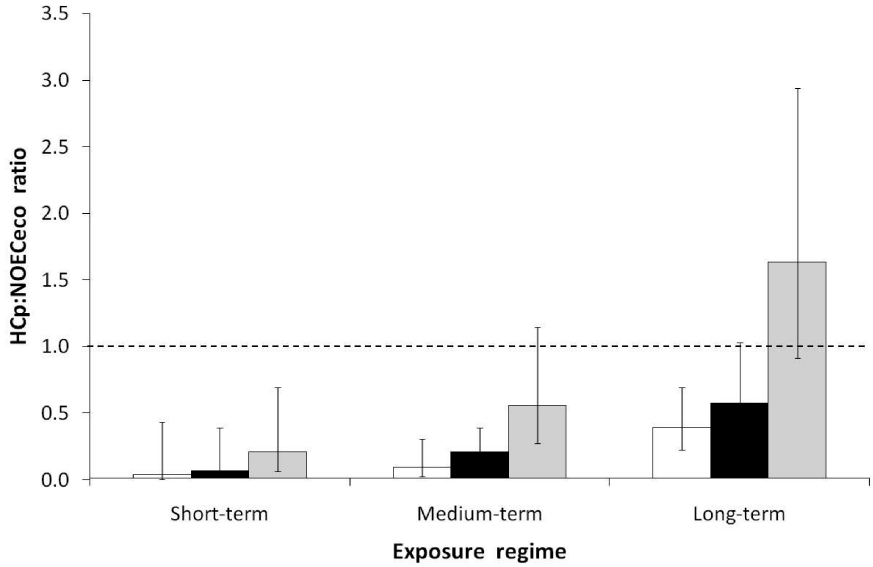

FIGURE 6. Mean (95% CI) ratio between the LLHC5 and NOECeco (white bars), HC1 and NOECeco (black bars), or HC5 and NOECeco (gray bars) derived from 28 multispecies studies with 26 pesticides categorized by exposure regime (Table 2). The 1:1 HCp:NOECeco ratio is denoted by the dotted line, values below this line indicating that the HCp estimate is protective of ecological effects in multispecies studies.

nonfungal species pose a risk to the abundance or functioning of aquatic hyphomycetes. However, this conclusion is based on a limited number of studies, and further research on the effects of fungicides on nontarget fungi and the ecological processes they drive should be conducted (*2*).

Acknowledgments

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Supporting Information Available

Median EC50 values for fish, invertebrates, and primary producers and median (50% confidence) HC5 and HC50 values for fungicides used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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