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Critical Review

DEVELOPMENT AND APPLICATION OF THE SSD APPROACH IN SCIENTIFIC CASE STUDIES FOR ECOLOGICAL RISK ASSESSMENT

Anastasia Del Signore,† A. Jan Hendriks,*† H.J. Rob Lenders,† Rob S.E.W. Leuven,† and A.M. Breure†‡
†Department of Environmental Science, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Nijmegen, The Netherlands
‡National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

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Abstract: Species sensitivity distributions (SSDs) are used in ecological risk assessment for extrapolation of the results of toxicity tests with single species to a toxicity threshold considered protective of ecosystem structure and functioning. The attention to and importance of the SSD approach has increased in scientific and regulatory communities since the 1990s. Discussion and criticism have been triggered on the concept of the approach as well as its technical aspects (e.g., distribution type, number of toxicity endpoints). Various questions remain unanswered, especially with regard to different endpoints, statistical methods, and protectiveness of threshold levels, for example. In the present literature review (covering the period 2002–2013), case studies are explored in which the SSD approach was applied, as well as how endpoint types, species choice, and data availability affect SSDs. How statistical methods may be used to construct reliable SSDs and whether the lower 5th percentile hazard concentrations (HC5s) from a generic SSD can be protective for a specific local community are also investigated. It is shown that estimated protective concentrations were determined by taxonomic groups rather than the statistical method used to construct the distribution. Based on comparisons between semifield and laboratory-based SSDs, the output from a laboratory SSD was protective of semifield communities in the majority of studies. *Environ Toxicol Chem* 2016;35:2149–2161. © 2016 SETAC

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INTRODUCTION

The world faces a major challenge in determining the consequences of the widespread occurrence of toxic chemicals in the environment. Ecological risk assessment deals with this challenge by combining estimations of the potential of chemicals to cause harm with the likelihood of that potential being realized [1]. An ecological risk assessment aims to find an exposure level or a toxicity threshold (e.g., predicted no-effect concentration [PNEC]), below which an ecosystem will not suffer unacceptable damage.

The problem, however, is how to assess such thresholds if only data on a few species are available [2]. Given the impossibility of testing the effects of all chemical compounds on all species, traditional approaches to risk assessment are based on observations of effects of individual chemicals on the survival, growth, and reproduction of a limited number of test species [3,4]. The results of such toxicity tests are then extrapolated by deterministic or probabilistic models to ecologically relevant levels including populations, communities, and ecosystems [1,5]. One of the approaches for extrapolation from single-species toxicity tests to ecosystems is the use of species sensitivity distributions (SSDs). These are cumulative plots of effect concentrations (e.g., median effect concentration [EC50] or no-observed-effect concentration [NOEC]) fitted to a statistical distribution. The lower 5th percentile of the estimated hazardous concentration based on NOECs, the HC5, is generally divided by an application factor ranging from 1 to 5 to determine a toxicity threshold considered protective of ecosystem structure and function [6]. The SSD approach has become a useful method in decision-making processes for the derivation of environmental quality criteria, benchmarks for screening assessments, and estimation of ecological risks [7], and is an accepted instrument in ecological risk assessment around the world [8,9]. In the United States, the SSD concept is being used as the basis for the screening benchmarks for contaminants in water for National Ambient Water Quality Criteria [10,11]. In Canada, SSDs are recommended in the Canadian Water Quality Guidelines for the Protection of Aquatic Life [11]. The use of SSDs has been approved in the guidelines of several regulations of the European Union, including the Registration, Evaluation, Authorization, and Restriction of Chemicals program [REACH]), registration of plant protection products [12], and the Water Framework Directive [13]. Application of SSDs is also recommended by public organizations in Australia and New Zealand [11].

The importance of SSDs in ecotoxicity assessment has grown steadily since the 1980s, as a result of their rising popularity in both the regulatory and scientific communities. However, in that same period intensive discussions have taken place on the basic assumptions, statistics, data limitations, and applications [3,14,15]. Although the discussions and criticisms were fruitful in terms of improvements in the SSD approach, various questions remain unanswered. For example, Forbes and Calow [16] argued that common test species might be more sensitive and that some endpoints might not be relevant for ecologically important population responses. Also, no consensus has been reached on statistical aspects for SSD construction such as minimum number of data points. Moreover, because of the exclusion of biotic and abiotic interactions in real ecosystems, the protectiveness of threshold levels (e.g., HC5) for actual ecosystems is questionable. To evaluate the overall

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* Address correspondence to A.J.Hendriks@science.ru.nl

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success of applying the SSD approach in scientific research, the present review focuses on the following research questions: 1) How are SSDs being applied in scientific research for risk assessment of (non) chemical stress on aquatic and terrestrial ecosystems?; 2) How does selection of various types of endpoints, species, and data availability affect SSDs?; 3) Which statistical methods should be used to construct a reliable SSD? and 4) Is an HC5, derived from a generic SSD, protective for a specific local community?

We sought to answer these questions by analyzing the available literature since 2002, because developments until that year have already been described by Posthuma et al. [14] and Forbes and Calow [16]. More specifically, we analyzed how statistical aspects (model choice, data points) were tackled when the SSD approach was applied in scientific research in different case studies. To that end, we analyzed case studies that employed SSDs for various research questions related to the structure and functioning of aquatic and soil ecosystems rather than studies solely performing risk assessments for the derivation of environmental quality criteria for different chemical compounds. We focused on single chemicals rather than mixtures because application of the SSD approach for mixtures involves many additional technical questions requiring separate and extensive examination (e.g., concerning the use of a concentration or response addition approach based on various modes of action of chemicals). We excluded studies applying the SSD approach in life cycle impact assessments because they do not focus on the SSD methodology per se.

METHODS

To capture relevant scientific publications, the database Web of Science was searched from January 2002 through December 2013 for primary studies on SSDs using the key phrase "species sensitivity distribution". This effort resulted in 317 publications from which we selected relevant studies for review. Of these, 150 publications were excluded as not useful for our objectives because SSDs were only mentioned rather than actually derived or discussed. Publications related to mixture toxicity, life cycle assessment, or simply generation of toxicity data were also excluded. The remaining papers were assessed and allocated into separate groups according to their focus, which helped in sorting the information regarding our research questions (Table 1). One group of papers included publications in which the SSD approach was applied for deriving environmental quality criteria, risk limits, benchmarks for chemicals, ecological risks, or other case studies (related to research question 1). To gain an impression of how the SSD approach has been developed and applied in scientific research, we focused in detail on case studies with purposes other than merely deriving environmental quality criteria or assessing ecological risks. The main findings from these case studies are discussed. Because such case studies do not concern derivation of quality standards or risk estimation for a specific chemical, guidance requirements on how to construct the SSD were usually not followed (e.g., concerning technical aspects such as number of data points, so-called default log-normal or log-logistic distribution). To determine how these technical aspects have been dealt with, we reviewed every study in detail and determined the distribution assumed, the endpoints observed, and the sample size used. Another group of publications included discussions on statistical methods and underlying data for the construction of SSDs (related to research questions 2 and 3). In a third group, we allocated publications on the ecological relevance and validation of the SSDs (related to research question 4).

Table 1. Number of scientific publications found by the key phrase species sensitivity distribution (SSD) in the database Web of Science (2002–2013) and analyzed in the present review

Criteria	Results
Total publications	317
Excluded from review	150
General discussion on SSDs, applications for sediment, soil, groundwater quality criteria setting	26
Case study focus on environmental quality criteria	44
Case study focus on risk assessment	46
Other case studies	26
Statistical methods	13
Ecological relevance and validation of the SSDs	12

RESULTS AND DISCUSSION

Application of the SSD approach in scientific research for risk assessment

The majority of the publications found can be grouped by their common focus. Eighteen publications were related to discussions on how to improve the SSD approach for setting quality criteria for sediment, soil, and groundwater, which is not discussed in detail in the present review. The SSD approach has also been applied in scientific research in various case studies. We identified numerous publications (Table 1) that applied the SSD approach for deriving environmental quality criteria (Supplemental Data, Table S1) and ecological risks for various chemicals (Supplemental Data, Table S2) or in case studies with other goals, so called nonstandard case studies (e.g., comparing sensitivity among taxonomic groups or species from different geographic areas; Supplemental Data, Table S3).

In the following sections, we explore the application of the SSD approach in so-called nonstandard case studies and summarize their main findings, focusing on research question 1. Table 2 summarizes the influences of the topics considered in these case studies on the derived SSDs and HC5s. Figure 1 shows the overall topics covered by the case studies discussed in the following sections.

Most of the environmental quality criteria derivation studies focused on freshwater ecosystems, followed by soil and marine ecosystems. Less attention was given to sediment environmental quality criteria. Zinc, copper, and cadmium were the most studied chemicals, followed by diverse pesticides (Supplemental Data, Table S1). Geographically, most of the studies were performed in countries such as China and Japan, followed by the United States and The Netherlands (Supplemental Data, Table S2). Other studies focused on several countries in Europe, Australia, and South Africa. The geographical distribution of research may be related to the general distribution of scientists in the field of ecotoxicology [17], and attention to a limited number of chemical compounds is obviously related to the general lack of toxicity data [2]. In several studies the ecological risks derived were used to prioritize different chemicals or sites of concern, indicating the major threats to a specific group of species at a certain location [18–21].

Comparison of species from different geographic areas

The construction of SSDs most often relies on toxicity data for species in temperate regions. Several studies have shown that the sensitivity of species to various chemical compounds may or may not differ when climate zones or regions are

Table 2. Different aspects important to consider for the species sensitivity distribution (SSD) approach based on an overview of the studies explored in the present review[a]

Aspect to consider	SSD curve	HC5/HC50	Remark	Example
Species group selection	+++	+++	Curve can be multimodal if sensitive taxonomic groups are included.	Including less sensitive group will increase the HC5. Variation in sensitivity within a taxon is lower (thus, SSD curve is steeper) than among taxa.
Habitat type	+	+	Mainly similar sensitivity, but marine species were found more often with higher sensitivity than freshwater species.	Macroinvertebrates from static and lotic habitats did not differ in their sensitivity to pesticides whereas sensitivity of species from freshwater and marine habitats may vary depending on a stressor.
Geographic location	+	+	Mainly similar sensitivity	No significant difference in sensitivity of Australian and non-Australian arthropods and fish to endosulfan. Amazonian invertebrates significantly less sensitive than their temperate counterparts to carbendazim.
Mode of action of chemicals	+++	+++	Importance of mode of action varies per taxonomic group	Sensitivity of plants to a specific mode of action of a herbicide is higher than that of other taxonomic groups.
No. of data points	++	+++	Overall lack of toxicity data for SSD curves	Uncertainty in HC*x* decreases when number of data points are >4.
Distribution type	+	++	Depends on number of data points. Goodness-of-fit tests should be performed	For acute toxicity data on Cu and Zn for cladoceran species, Weibull, uniform, and beta distributions gave underestimation, and log-logistic and triangular distribution gave overestimation in the lower tail compared with a log-normal distribution.
Endpoint (NOEC vs LC50)	+	+++	Any endpoint can be used. NOECs are required by regulations to derive HC5 and EQCs.	Uncertainty in acute-to-chronic ratios depends on species and mode of action of chemical. SSD position relative to *x*-axis will vary depending on input data.

[a]The influence on the SSD curve and the hazard concentration (HC)x is indicated by +++ (very important), ++ (important), and + (less important). NOEC = no-observed-effect concentration; LC50 = median lethal concentration; EQC = environmental quality criteria.

compared. By comparing SSD curves and HC5 values, Hose and Van den Brink [22] found no significant difference in sensitivity of Australian and non-Australian arthropods and fish to endosulfan. Similarly, no significant differences were found in the sensitivity distributions of tropical and temperate vertebrates, arthropods, and non-arthropod invertebrates for 16 pesticide compounds [23].

Rico et al. [24,25] compared the tropical and temperate fish and invertebrate SSDs of malathion and carbendazim, and parathion-methyl, respectively. They found that Amazonian species were equally sensitive as their temperate counterpart to malathion and parathion-methyl. However, Amazonian fish appeared to be slightly less sensitive for carbendazim than temperate fish. Amazonian invertebrates, however, were significantly less sensitive for this substance than temperate species [25].

Olsen et al. [26] found no difference in sensitivity of Arctic and temperate marine species from 7 taxonomic classes to 2-methyl naphthalene, either at the species level or the community level. De Hoop et al. [27] showed that HC50s of polar and temperate marine species for petroleum and petroleum components differed by less than a factor 3. However, differences in sensitivity to naphthalene of arthropods, chordates, and echinoderms were significant.

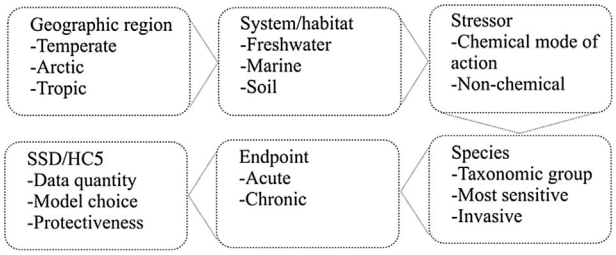

Figure 1. Overall topics covered by case studies discussed in the following sections. SSD = species sensitivity distribution; HC5 = lower 5th percentile hazard concentration.

Iwasaki et al. [28] compared SSDs based on intercontinental field observations of macroinvertebrate species in relation to several metal concentration gradients based on laboratory-derived toxicity values. The estimated HC values for copper, zinc, and cadmium overlapped closely with laboratory-derived values available from water quality criteria in the United States, United Kingdom, and European Union. This overlap not only increases confidence in the application of existing metal standards, but also illustrates that standard values might be widely applied geographically [28]. By contrast, Chapman et al. [29] found no universal and predictable patterns of acute sensitivity of marine invertebrates from different regions to 4 metals.

Kefford et al. [30] investigated whether related taxa of freshwater macroinvertebrates from South Africa and Australia have similar sensitivities to salinity. They concluded that the broad similarity in sensitivity within most taxa at the order level suggests that, in the absence of other information, one may assume similar salinity sensitivity in different geographic locations within families and orders. Likewise, Van Dam et al. [31] found no significant difference between acute and chronic toxicity of the herbicide tebuthiuron between northern hemisphere temperate and Australian tropical aquatic species (fish and green algae). These studies show that the sensitivity of organisms to toxicants is independent of their geographic origin and that there is no consistent geographical pattern in species sensitivity.

Comparison of rare and endangered species

Kefford et al. [32] found that locally rare macroinvertebrates tended to be more tolerant than locally common ones. The

authors argued that the relationship between rarity and salt tolerance may be because rare species belong to particular taxa, such as Coleoptera and Odonata, that lean toward salinity tolerance [33]. The authors hypothesized that, under the assumption that rare and common species are K- and r-selected, respectively, the inability of K-selected species to recover rapidly from disturbances should constitute a strong selection pressure to develop resistance to environmental extremes. Raimondo et al. [34] compared the sensitivity of endangered species with surrogate species commonly used in toxicity tests for 68 chemicals. The authors concluded that SSDs for standard species can be used for protections of endangered species for which toxicity data are not available.

Comparison of freshwater and marine species

Most available ecotoxicological data apply to aquatic species from freshwater habitats. Data on marine and estuarine species are scarce, increasing uncertainty in salt water risk assessment in comparison with freshwater [35]. A strategy to assess the risk of chemicals for these saltwater environments is to apply safety factors to the risk level calculated based on freshwater organisms. However, the sensitivity to chemical compounds of species from different habitats may differ. Therefore, an important question to consider in risk assessment is whether protection levels derived from species living in 1 type of habitat would be protective also for species typical for other types of habitats. For example, Hose [36] found significant differences in the sensitivity of aquatic taxa living in surface water habitats compared with taxa from groundwater habitats for atrazine and chlorpyrifos, whereas Maltby et al. [23] showed similar sensitivities among arthropod species to 8 pesticides in lentic and lotic habitat types.

Thus, driven by the relevance for regulation, several authors have investigated potential differences in the sensitivity of species from marine and freshwater habitats. For example, Maltby et al. [23] found no significant difference in median HC5 values for freshwater or marine taxa for 10 chemical compounds. However, the median HC5 for marine arthropods was smaller than for freshwater arthropods. The sensitivity distributions for freshwater and marine arthropods were significantly different for permethrin and chlorpyrifos, although this difference was not significant when the analysis was restricted to crustaceans alone [23].

Bollmohr et al. [37] showed that marine organisms (arthropods and fish) were more sensitive (by a factor between 1.5 and 2.8 based on HC5s) to the pesticides cypermethrin, endosulfan, chlorpyrifos, and fenvalerate than freshwater organisms. Wheeler et al. [35] compared freshwater and marine data sets based on HC5 and regression parameter values (slopes and intercepts). Although the overall sensitivity between freshwater species and marine species was not significantly different, freshwater species exhibited slightly greater sensitivity to ammonia and metals than marine species (crustacea and fish) [35]. In contrast, for pesticide and narcotic compounds, marine species tended to be more sensitive than freshwater species. The HC5 values for the antifouling biocide tributyltin for marine fish, invertebrates, and algae were significantly lower (by a factor of ~8) than that for freshwater species, indicating that marine species might be more susceptible to tributyltin than their freshwater counterparts [38]. Zhang et al. [39] found that freshwater primary producers, however, were more sensitive to other antifouling paint than their marine counterparts, by a factor of approximately 9. Verbruggen et al. [40] found no significant differences for freshwater sediment and marine sediment species in sensitivity to total petroleum hydrocarbons.

Considering all available toxicity data for various chemical compounds, De Zwart [41] concluded that approximately one-third of the marine fish, invertebrate, and algal species were more sensitive by a factor 2 or more than their freshwater counterparts. Biological and physicochemical factors may contribute to differences in freshwater and marine species sensitivities including chemical differences in each medium, especially bioavailability, but also methodological differences in toxicity tests. However, the results of available studies do not indicate systematic or consistent differences in the sensitivity of marine versus freshwater taxa. Thus, protection levels (e.g., HC5 derived from freshwater species only) may still be uncertain for marine species. Further research is needed to investigate the protectiveness of the threshold levels derived for species from 1 type of habitat compared with species from other types.

Comparison of species sensitivity to nonchemical stress

Although the application of SSDs in ecological risk assessment primarily aims at protecting ecosystems from toxic chemical stress, other, nonchemical, stressors can affect ecosystems too. As for chemical compounds, data for nonchemical stressors may be derived in the laboratory for various endpoints. Smit et al. [42], for instance, developed SSDs for suspended clays, burial by sediment, and change in sediment grain size for marine species and estimated potentially affected fractions to communicate potential risks related to drilling of oil and gas wells in the North Sea. De Vries et al. [43] showed that the SSD approach is also suitable to estimate the risk of thermal effects, especially based on site-relevant species for location-specific assessment. Empirical occurrence data from field studies can also be applied to derive SSDs for nonchemical stress, as demonstrated for temperature, salinity, and nutrients [44,45] to quantify the fraction of species potentially lost because of these stressors.

Other studies on nonchemical stress focused on comparison of sensitivities of native and non-native species. Leuven et al. [46] applied the SSD approach for a location-specific assessment of fish diversity in relation to river temperature conditions. Their study focused on comparison of tolerance levels to water temperature of native and non-native fish species in the Rhine River. They concluded that temperature tolerance of non-native fish species was consistently higher than that of the native species, but the differences were not statistically significant. Furthermore, no significant differences between native and non-native fish species in the Rhine River were found in tolerance to low oxygen concentrations [47], indicating that such conditions do not facilitate the spread of invasive fish species. The maximum temperature tolerance of mollusks was significantly higher for non-native than for native species, but their mean maximum salinity tolerance did not significantly differ [44].

These case studies show that the SSD approach can be applied to quantify the impact of nonchemical stressors such as temperature, hypoxia, pH, and changes in sediment particle size. Applying the SSD approach to quantify the impact of nonchemical stress in a similar way to ecotoxicological assessments may allow for comparison of different types of impacts on ecosystems and prioritization of the results for better ecological management.

Determining the sensitivity of different taxonomic groups

Following research question 2, we analyzed the available studies related to different species used in SSDs to investigate

how different species may influence the construction of SSDs. In the present review we analyze the findings from 26 publications discussing variation among different taxonomic groups, different endpoints, and effect levels.

The SSD approach presumes that the sensitivity of a community depends on the sensitivity of the individual species of which it is composed, taking into account that some species are more susceptible to stress than others [16]. Moreover, in risk assessment laboratory tests, species act as surrogates for taxa in the target ecosystems, assuming that their sensitivities are equal [14]. Therefore, taxonomic groups that are included in an SSD should be carefully selected because the taxonomic composition of the species assemblage may have a significant influence on the assessment of hazard [48,49]. In practice however, nonrandom selection of laboratory test species causes a particular taxonomic group to be over-represented [14,16].

Interspecific variation in the sensitivity of different taxonomic groups has been widely studied [50,51] and can be partly attributed to the specific toxic mode of action of a chemical. For example, in the case of insecticides, aquatic arthropods (crustaceans and insects) are obviously the most sensitive [23], whereas algae and macrophytes tend to be more sensitive to herbicides [52]. In addition, within closely related taxonomic groups, some groups of species may be more sensitive than others. Within arthropods, for example, insects appear to be more sensitive than microcrustaceans to novel types of insecticides as neonicotinoids [53,54].

The sensitivity of different taxonomic groups of species within 1 taxonomic group may vary widely [50]. For example, in their comparison of relative sensitivity of broad taxonomic groups to acute toxicity of a wide range of chemical compounds, Raimondo et al. [34] showed that crustaceans were generally the most sensitive taxa to all mode of actions of 68 chemicals tested compared with mollusks, fish, amphibians, and insects. Similarly, Crane et al. [55] found that crustaceans were among the most sensitive species to chlorpyrifos, followed by insects and fish, whereas flatworms, snails, and rotifers were the least sensitive in the distribution. The water flea *Ceriodaphnia dubia* was the most sensitive species among all species for which toxicity data were available for chlorpyrifos [55]. Wong et al. [56], in comparing the sensitivities of cladocerans and copepods to the metals Cd, Cu, Pb, Ni, and Zn, indicated that cladocerans were consistently more sensitive than copepods to Cd and Cu.

There was no significant difference in sensitivity of arthropods and fish to the organochlorine pesticide lindane, but both groups were significantly more sensitive than non-arthropod invertebrates [23]. Weltje et al. [57] compared the relative sensitivity of amphibians and fish to 55 chemicals based on acute and chronic toxicity data. Their results indicate that fish were more sensitive than amphibians in acute and chronic tests. In the study of Framptom et al. [58] with soil organisms, the earthworm *Eisenia fetida* was the least sensitive to insecticides, whereas the collembolan *Folsomia candida* was among the most sensitive species for a broad range of toxic modes of action (biocide, fungicide, herbicide, and insecticide).

Analysis of species sensitivity to the fungicide triphenyltin acetate indicated that there were no significant differences in sensitivity among aquatic primary producers, invertebrates, and vertebrates [59]. The authors concluded that every aquatic community can be expected to include taxa sensitive to this fungicide. Also, in case of chronic exposure to ionic radiation, no statistical differences were revealed between sensitivity of species from terrestrial, marine, and freshwater ecosystems [60].

In a study on species sensitivity within 1 taxonomic group, Bossuyt et al. [61] concluded that generic cladoceran SSDs for Zn and Cu were not significantly different from the SSD based on the cladoceran species representative of a specific location. The authors suggested that the community sensitivity of different cladoceran populations is similar among aquatic systems and independent of the species composition. Hence, the generic SSD can be used for a range of aquatic systems. Concerning the sensitivity of different fish species, several authors concurred that, while no 1 species is consistently the most sensitive, rainbow trout and other salmonids are generally more sensitive to a range of chemicals than standard test species used in toxicity tests (e.g., fathead minnow, sheepshead minnow, catfish, and bluegill) [34,62,63]. However, Van den Brink [52] demonstrated no difference in sensitivity between standard aquatic plant species and other primary producers.

Over the range of chemicals with specific modes of action, crustaceans (cladocerans) appeared to be among the most sensitive taxa. However, Maltby et al. [23] concluded that, depending on the group of chemicals, different taxonomic groups are more or less sensitive. They suggested that differences in the sensitivity of taxonomic groups are most likely for toxicants with a specific toxic mode of action, such as insecticides or herbicides, yet Hendriks et al. [64] have argued that the mode of action varies among groups of organisms. Even well-studied toxicants such as organophosphate insecticides may not be homogeneous in terms of their mode of action [64].

Overall, assessment of the differences between species is important for the SSD approach because selection of species will influence the derivation of protection levels protective or not to the whole community [16]. According to several authors [23,52], only the most sensitive taxonomic groups should be used for risk assessment based on SSDs for substances with a specific mode of action when clear gaps exist between the sensitivities of different taxonomic groups (e.g., only primary producers for herbicides; arthropods in case of insecticides). The HC5 or potentially affected fraction values are then related to effects on the most sensitive group of organisms. The motivation for such an approach is regulation driven, because it is important for risk assessors to be cautious and conservative. This approach, however, may result in protection for 95% of the most sensitive species of a selected taxon rather than the whole community. Including less sensitive groups might increase the HC5 [58,65]. The variation in sensitivity of different taxonomic groups and species within 1 taxonomic group is reflected in the SSD curve. Including the data for only 1 taxonomic group with little variation in sensitivity among species will result in a steep SSD curve. Increased variation from additional taxonomic groups will be indicated by a gentler SSD curve. Thus, it is important to specify the most sensitive species in the ecosystem to be protected. To do so, for example, the rapid toxicity testing [30] as described in the previous section (*Comparison of species sensitivity to nonchemical stress*) could be used. Deriving HC5 from an SSD based on the single most sensitive taxonomic group may be, however, overconservative for the entire community.

Test endpoints and extrapolation from acute to chronic data

The endpoints of toxicity tests used in SSDs usually represent the most sensitive endpoints that are toxicologically

and ecologically relevant [16]. For animals, acute toxicity data usually cover mortality and immobility, whereas chronic toxicity mostly addresses reproduction and growth. For plants, biomass and growth rates are often measured. These standard endpoints are required by international guidelines for assessment of ecological effects from chemicals [66].

While lethality is undoubtedly a response with important ecological consequences, toxic substances may cause other ecologically important effects [67]. These may include reduced feeding, impairment of reproduction and growth, and behavioral changes. The advantage of using acute EC50 data is the possibility of describing distinct exposure effects of population responses using endpoints such as EC50 on growth rate, rather than no-observed-response endpoints. The disadvantage of using acute EC50 data is that they do not capture chronic and delayed toxic effects [68]. However, the chronic NOECs in their turn have been criticized by several authors, for being a fundamentally invalid interpretation of hypothesis testing, because they depend on the experimental design [69]. It has been suggested that these values no longer be produced and used in scientific studies [69–71].

The relevance of using different endpoints for construction of SSDs and their relation to population change has been pointed out earlier [16]. However, no study has been performed examining the meaning of HC5 derived from SSDs based on different endpoints, such as, for example, 50% reduction in feeding or 50% reduction in growth. Nevertheless, several authors have investigated the differences between effect levels (i.e., acute EC50 vs chronic NOEC). Studies on effect levels have been performed to analyze the adequacy of acute-to-chronic ratios (ACRs), which are commonly used when insufficient chronic data are available to perform long-term exposure assessments [72]. In general, the ACR varies depending on the taxonomic group and chemical concerned [73]. The ACR for anuran species and 6 pesticides was between 0.9 and 26, with an average of 7.2. Average ACRs for various chemicals have been reported to be 10.5 for fish, 7.0 for daphnids, and 5.4 for algae [22]. Dom et al. [74] illustrated that even for certain simple organic compounds with a designated mode of action (i.e., narcotic toxicity), unexpected differences in acute and chronic toxicity can be observed. For example, the ACRs for methanol and ethanol were shown to be species dependent, and varied from 10 to 1000. The authors stressed that in risk assessment procedures more attention should be given to acute-to-chronic extrapolations. Raimondo et al. [75] found that invertebrate ACRs were more variable than fish ACRs and therefore some species may be at an increased risk of underestimated chronic toxicity when mean or median ACRs are used. Dom et al. [75] showed that fixed ACRs do not account for the interchemical and interspecies differences. This diversity in ACRs can be explained taking into account not only physicochemistry but also toxicokinetics and toxicodynamics, which are not necessarily the same in acute and chronic exposure [76,77].

Overall, the use of NOEC and median lethal concentration (LC50) endpoints is driven by regulatory requirements allowing for standardization and comparison among species and chemicals. In principle, each endpoint could form the basis of an SSD including responses at the cellular biomarker and genome levels [78,79]. As the difference between endpoints can be of certain magnitude, SSDs based on different endpoints will have different positions relative to the x axis [80]. The results from such SSDs must be interpreted with caution considering their ecological relevance for population responses. However, few data are usually available, especially for specific modes of action, and presently SSDs are largely limited to mortality [81]. Extrapolation from LC50 to NOEC endpoints using ACRs can be performed. However, high awareness of diversity and irregularities in acute-to-chronic extrapolations is required.

Quantity of the underlying toxicity data

The criteria for minimum sample size for an SSD and an HCx are often an arbitrary policy decision [82]. For example, the US Environmental Protection Agency requires at least 8 species, the European Union between 5 and 8, and Australia and New Zealand 5 species [11]. The minimum number of data points as input for SSD has also been discussed in the scientific literature. Wheeler et al. [83] estimated that a minimum of 10 to 15 data points per toxicant are needed to derive a reliable estimate of a particular endpoint (e.g., HC5). Smaller data sets give greater uncertainty in the model output (e.g., HC50 values), which can be significantly reduced if the sample size includes at least 4 data points [64,84,85]. Overall, low data numbers imply wider confidence intervals [67,82], which also influences the test for normality of the data. For scientific case studies, justification for a certain number of data points should be made in view of a specific problem definition [82,86].

The discussions on the data quantity for the SSDs are triggered by a general lack of toxicity data in risk assessment of chemicals. Therefore, solutions are sought by combining already available data with ecotoxicological modeling [4], for example, interspecies correlation estimations (ICEs) and quantitative structure–activity relationships (QSARs) [87–90]. The ICEs allow the prediction of acute toxicity values for a wide variety of species based on a single acute toxicity value that can be used to develop the SSD and HC5 [87]. Recent research has shown that ICE can be used to postulate SSDs by providing toxicity estimates for a diversity of species. Raimondo et al. [91], Awkerman et al. [89], and Raimondo et al. [92] have validated ICE models for both aquatic and wildlife species. They showed that HCx values derived from SSDs using toxicity values derived from ICE were similar to hazard levels derived from SSDs of measured data for aquatic organisms and wildlife. For acute Zn toxicity, the ICE-based HC5 was approximately twice as high as the measured HC5 although not significantly different [93]. Dyer et al. [87] also have shown that in general, the ICE-based SSDs had HC5 values within 1 order of magnitude of the measured HC5 values based on 3 surrogate species (*Pimephales promelas, Onchorynchus mykiss, Daphnia magna*) and chemicals with diverse modes of action (dodecyl linear alkylbenzenesulfonate [LAS], nonylphenol, fenvalerate, atrazine, and copper). Thus, the application of ICEs was recommended as a valid approach for generating SSDs and hazard concentrations for chemicals with limited toxicity data [88,94].

The QSARs can be used in the absence of experimental test data to predict the aquatic toxicity of untested chemicals based on their structural similarity to substances for which aquatic studies are available [95]. Wu et al. [96] developed a set of predictive QSAR relationships, based on metal characteristics, and successfully predicted acute toxicities of each species for 5 phyla and 8 families. However, Dom et al. [74] showed that chronic QSARs of chlorinated anilines did not account for interchemical and interspecies differences. Although the potential application of ICE to increase the number of data for SSDs and the use of QSARs for effects assessments based

on chemical structure alone have been demonstrated, this approach needs further evaluation for different species and chemicals.

Toxicity data used in case studies

In the case studies discussed in the previous sections, the number of data points used to construct SSDs and derive HC5 varied greatly, with a minimum of 4 data points per set (Supplemental Data, Table S3). The acute mortality endpoints (LC50s) were most frequently used for the SSD construction in case studies compared with other endpoints (NOEC and lowest-observed-effect concentration [LOEC]) because of overall lack of sublethal effect data. Most of the studies did not follow any regulatory requirements for a minimum data set (fish, invertebrates, algae). Buckler et al. [63] stated that although minimum data sets provide satisfactory prediction of toxicity values, it is obviously desirable to have high-quality data for as many species as possible. In the majority of the case studies the toxicity data were extracted from large databases [97].

Statistical methods to fit the SSD curves

It has been argued that with increasing use of SSDs in ecological risk assessment, it is important that the scientific community agree on appropriate methods for their derivation [98]. Focusing on our research question 3, we investigated which statistical distributions and methods are recommended for fitting the SSD curves [99–106]. However, Aldenberg and Jaworska [107] stated that there is no theoretical justification for any distribution from the point of toxicity. Moreover, one cannot statistically decide between different distributions at small sample size. In any case, it is important to choose an appropriate method because different approaches may generate different HC5 values. For example, the standard bootstrap method fitting a model distribution to a Cd data set generated an HC5 16 times lower than the HC5 produced by the conventional log-logistic method [83].

The choice of the distribution function to fit the data may be based on goodness-of-fit tests. However, the choice of distribution types is constrained to a few standard distributions for which goodness-of-fit tests are available [16]. However, if one needs to select the best fit distribution type, (e.g., normal or logistic), which are used for regulatory purposes, several tests can help. The most common procedures to check the normality assumption for the data are the Shapiro–Wilk, Kolmogorov–Smirnov, Anderson–Darling, and Lilliefors tests. Shapiro–Wilk was shown to be the most powerful normality test, followed by Anderson–Darling, Lilliefors, and Kolmogorov–Smirnov [108]. However, the power for all tests to detect deviation from normality is low for small data sets [109]. Shapiro–Wilk can be used for a sample size between 3 and 5000, but for sample sizes of 30 or less, the power at 5% significance level is less than 40% [108].

Despite violations of statistical guidelines, unimodal models still provide reasonable estimates of the HC5 [82]. Above the 5th percentile (HC5), the log-normal, log-logistic, and log-triangular parametric distributions are similar [82]. Differences between such parametric representations are generally reflected in the tails [110]. However, the uncertainty around HC5 will be strongly dependent on the number of test results taken into account.

Considering the limitations of goodness-of-fit tests, some authors argue that there is no reason to assume an underlying distribution for species sensitivities [3] because an alternative resampling (nonparametric bootstrap) method can be used that does not rely on any assumed distribution [35]. It has been shown that the nonparametric bootstrap approach can fit data better than a parametric approach [107]. However, this requires at least 20 data points to obtain the HC5 and associated confidence intervals [35]. As a compromise between the power of resampling and fitting an underlying distribution, a bootstrap regression method, based on a log-logistic regression model, was described by Grist et al. [98]. This hybrid technique allows for the use of smaller data sets and the calculation of confidence intervals. Comparing the SSDs derived by 4 methods (i.e., based on log-normal, log-logistic, bootstrap, and bootstrap regression models) for 15 chemicals with different modes of action, Wheeler et al. [35] showed that differences in the HC5 values were within a factor of 2. Wang et al. [107] also showed that the HC5 estimated from SSDs based on the same parametric and nonparametric models coincided well with each other, with the standard deviations mostly within a factor of 2. Thus, the estimates of the HC5 are not highly influenced by the selection of 1 of these models.

Other authors have investigated different models for SSD construction. Van Straalen [110] explored the possibility of introducing a true no-effect principle in the SSD framework by considering models with a finite lower threshold using the data set for 21 species for Zn. Four distributions analyzed in their study (the uniform, triangular, exponential, and Weibull) tended to underestimate the data in the low concentration range. The estimates of an HC0 obtained using these threshold models varied within a range that included the HC5 estimates from the infinite tail logistic and normal models. Van Sprang et al. [106] showed that nonthreshold distribution models (logistic, inverse Gaussian, extreme value, Weibull, gamma, Pearson VI, and normal distributions) tended to overestimate toxicity for Zn in the lower tail, whereas threshold models such as Pareto, beta, and triangular produced higher, less conservative thresholds. Chen [111] proposed a distribution-free method for calculating HC5 based on asymmetric loss function. This method yields conservative HC5 values but requires a relatively large data set (at least 19 data points).

Hickey et al. [103] analyzed several models from a Bayesian perspective. This Bayesian approach can include all information to determine HCx values and allow expert opinions to be introduced for taxonomic groups with little or no data. Overall, the uncertainty in SSD estimation can be reduced by applying a Bayesian approach that incorporates expert knowledge [101]. Bayesian statistics treat data as fixed and allows one to use data to update prior distributions on the unknown parameters to obtain posterior distributions. Hickey et al. [103] compared these new models with a Kaplan–Meier and a log-normal distribution using data on the salinity sensitivity of freshwater macroinvertebrates from Australia. The maximum likelihood Kaplan–Meier survival function estimator allows censored data (endpoints from interval concentrations and exceeding the reported concentrations) to be included in the model. The log-normal analysis yielded an SSD that overestimated the hazard to species relative to the Kaplan–Meier survival function and Bayesian analyses. Similarly, Dowse et al. [99] analyzed the influence of quality of the toxicity data and the statistical models Kaplan–Meier survival function, Bayesian statistical models based on the log-normal assumption, and Burr type III distribution on the derivation of HC5. The Burr III distribution is a flexible 3-parameter distribution that can provide good approximations to many commonly used distributions such as

the log-normal, log-logistic, and Weibull [112]. Dowse et al. [99] included modeled data from concentration–response curves generated from toxicity testing (uncensored data) and censored data on the salinity sensitivity of freshwater macroinvertebrates. The most conservative protective concentrations were estimated with Burr type III and uncensored data (28 data points). There was an increase in HC5 values when censored data were included in the SSDs. The overall conclusion of their study was, however, that the protective concentrations estimated were determined by taxonomic groups rather than model type.

Van der Hoeven [2] argued that much energy is put into choosing the best distribution for the data and refining the estimates of the confidence intervals. However, the most serious problem with HCx estimation methods is the assumption that the species for which data are available are a random sample from all species in the ecosystem. A priori, this assumption may be false. Often some taxonomic groups are over-represented, for instance fish, whereas insects are almost always under-represented [16]. Related to this issue, Duboudin et al. [73] showed that the choice of data (i.e., intraspecies variation and proportions between taxonomic groups) had more effect on the value of the HC5 than the statistical method used to construct the distribution. Similarly, Hickey et al. [103] demonstrated that a weighting factor to account for the richness (or importance) of taxonomic groups using the Bayesian model influenced the calculated hazard estimates. These Bayesian methods presented by Duboudin et al. [73], Hickey et al. [103], and Grist et al. [100] can be used to control for the contribution of data from different taxonomic groups.

Although the advantages and disadvantages of applying different statistical models to construct SSDs and derive hazardous thresholds have been described in the literature, no specific model has been identified as a "default" or "the best fit". However, a clear overall guidance suggesting different options in specific cases and clear procedures for calculations would be a great benefit for the entire scientific and regulatory communities using the SSD approach. Overall, results of several studies showed that estimates of the HC5 do not strongly depend on the selection of a distribution model.

Model choice in case studies

Although various statistical methods have recently been applied to construct SSDs, log-normal distributions were the most frequently used (Supplemental Data, Table S3). Several studies used a bootstrap approach to estimate a hazardous concentration without assuming any distribution. Some users of the SSD approach simply follow the method provided by Aldenberg and Jaworska [107], assuming a distribution apparently without testing the fit. Others choose a log-logistic model because it often provides the best overall fit to toxicity data sets, yielding a more conservative HC5 [34,113]. When the data were checked for the best fit to a distribution type, the Anderson–Darling test was most commonly used (Supplemental Data, Table S3) because it places more emphasis on tail values [114].

Validation of the SSD predictions

The ability of the SSDs to predict effects in the field is of prime concern. Conceptual discussions on the SSD approach and its validity mainly focus on the following questions: 1) Are standards, derived with SSDs, sufficiently protective? and 2) Can any extrapolation of laboratory test data to estimate ecological impacts in the field be valid? [14]. However, validation of estimated impacts based on laboratory test data to the real impacts in the field is rarely performed. In the following section we focus on the studies related to our research question 4, to investigate whether thresholds derived from SSDs (HC5) are protective of real ecosystems.

Posthuma and de Zwart [115] investigated the relationship between the predicted risks based on the SSD approach and observed impacts on fish communities in Ohio rivers. Their validation study was based on a large data set and confirmed that chemical impacts estimated by the SSDs, albeit often small, were related to degradation of fish diversity. Kefford et al. [116] tested macroinvertebrates collected from the Murray–Darling River Basin in Australia for salinity tolerance in the laboratory and compared SSDs with the loss of riverine macroinvertebrate species as a result of increasing salinity. The SSD approach predicted the decline of species with increasing salinity accurately, confirming that SSDs can be used to indicate the fraction of species affected in the field. Because a lack of monitoring data usually restricts such field validation studies, most of the studies aiming at investigating the protectiveness of individual species based on SSDs (e.g., a level of 95% of species to protect ecosystem structure and functioning) rely on microcosm and mesocosm experiments. Microcosms and mesocosms are seminatural model ecosystems used for risk assessment and are known as higher tier risk assessment testing systems. In these tests, artificial ponds or streams or enclosed parts of natural waters are sprayed with the compound under investigation at different concentrations to identify concentration–effect relationships at the population and community level.

In recent years, a number of case studies have been conducted illustrating that HC5s derived from SSDs appear to be protective for (seminatural) ecosystems [117]. Summaries of the studies are provided in Table 3. Based on comparisons between endpoints derived from semifield studies and laboratory-based SSDs, the output from an SSD as HC5 is a factor of 1.4 to 75 lower than the NOEC based on the lowest endpoints measured in the field. In a few cases, the HC5 calculated from toxicity data for semifields was a factor of 1.1 to 4 lower than the HC5 calculated from laboratory data.

The majority of the mesocosm studies with invertebrates exposed to several chemicals, mainly pesticides, showed that HC5 levels derived from SSDs can be protective for real-world ecosystems based on such studies. Versteeg et al. [118] discussed the likeliness of lower sensitivities of organisms in mesocosm studies than in laboratory studies. They argued that the lack of random species selection and the development of toxicity tests with sensitive taxa for use in laboratory tests may be 1 explanation. Furthermore, differences in water quality and availability of habitat or shelter in laboratory and semifield studies are likely to favor greater sensitivity under laboratory conditions [118]. Moreover, the SSD approach ignores ecological relationships between species, assuming that such interactions do not influence the sensitivity of ecosystems. In field enclosures, population and community effects were determined by 1) the inherent sensitivity of the species, and 2) the ecological relationships between the species [119,120]. Hence, knowledge of ecological interactions should be more accurately incorporated into effect assessments. Additional confirmation of the protectiveness of HC5s from a generic laboratory-based SSD for local communities based on aquatic vertebrates, soil invertebrates, and other types of chemicals would strengthen the use of the SSD approach in risk assessment [121].

Table 3. Overview of the studies in which results from seminatural systems were compared with the outcome from species sensitivity distributions (SSDs) based on laboratory toxicity data

Description of seminatural system	Stressors tested	Species studies (endpoints measured)	HC5 or lowest NOEC	Input data for laboratory-SSD	HC5 from laboratory based SSD	Reference
Enclosure in a bay	Tributyltin (TBT) Linear alkylbenzene sulfonates (LAS)	Phytoplankton (photosynthesis, biomass, PICT), Bacteria, mollusk larvae and adult (mortality, abundance, shell growth)	NOEC (*Mytilus edulis* postlarval shell growth) TBT: 0.3 ng/L LAS: 0.052 mg /L	NOECs Marine phytoplankton, bacteria, arthropods, mollusks, fish	PNEC TBT: 0.03 ng/L LAS: 2.1 μg /L	[122]
Field soil plots	Zinc	Nematode abundance and species composition	NOEC 56 mg total Zn/kg dry soil	Soil microbial community (according to Dutch soil protection system)	HC5 61 mg total Zn/kg	[123]
Artificial stream on the banks of a river	Endosulfan	Macroinvertebrate abundances (per 8 arthropod taxa)	HC5 1.57 μg/L	LC50 Arthropods, non-arthropods, invertebrates, amphibians, fish	HC5 0.19 μg/L (arthropods only) HC5 0.021 μg/L (all data with different safety factors)	[22]
Micro- and mesocosm of different designs	Atrazine, simazine, metribuzin, metamitron, linuron, diuron, diquat, 2,4-D, pendimethalin	Densities/biomass of algae and aquatic vascular plants and/or dissolved oxygen and pH values	NOEC (1 example, atrazine) 5 μg/L	EC50 and NOECs Freshwater primary producers	Chronic HC5 for 1 example, atrazine 3 μg/L	[52]
Enclosures in experimental ditches	Gamma-cyhalothrin	Macroinvertebrates, zooplankton, phytoplankton, macrophytes	NOEC (macroinvertebrate community dynamics) 5 ng/l	LC50 Insects and crustaceans	HC5 2.12 ng/l	[124]
Divers mesocosms designs	Imidacloprid (I) Fipronil (Fi) Fenitrothion (F)	Various invertebrates	NOEC (no effect entire community) I: 0.6–1.6 μg/L Fi: 0.15 μg/L F: 1.1 μg/L	LC50 Arthropods	HC5 I: 0.43 μg/L Fi: 0.084 μg/L F: 0.44 μg/L	[125]
Enclosures in experimental ditches	Metamitron (Mm) Metribuzin (Mb)	Phytoplankton, Zooplankton (species composition) Periphyton (Chlorophyll-a) Macrophytes (different endpoints) Macroinvertebrates	Mm: Chronic NOEC (phytoplankton) 5.6 μg/L 391.88Mb: NOEC (community metabolism) 280 μg/L	NOEC and LC50 Algae, macrophytes	Mm: 1.39 μg/L (chronic HC5) 7.38 μg/L (acute HC5) Mb: 667 μg/L (acute HC5)	[126]
Indoor glass cylinder microcosms	Fluazinam	Phytoplankton, zooplankton, macroinvertebrates, macrophytes	NOEC (no effect entire community) 2 μg/L	LC10 and LC50 invertebrates	HC5 0.6 μg/L (LC10) 3.9 μg/L (LC50)	[127]
Enclosures in experimental ditches	Triphenyltin acetate	Macroinvertebrates, zooplankton, phytoplankton, macrophytes	HC5 0.3–0.6 μg/L	EC50 Macroinvertebrates, zooplankton, algae, macrophytes	HC5 1.3 μg /L	[59]

HC5 = lower 5th percentile hazard concentration; NOEC = no-observed-effect concentration; PNEC = predicted no-effect concentration; LC50 = median lethal concentration; EC50 = median effective concentration.

CONCLUSIONS

Out of 10 studies comparing species sensitivities with diverse chemical compounds in different geographic locations (arctic, temperate, tropic), only 2 cases indicated significant differences between species. Nevertheless, caution should be used when one is assessing ecological risk in regions for which no toxicity data for local species are available; differences in taxonomic composition and the possible consequences for HC5 values should be considered (Table 2).

A literature comparison indicates that marine species may be a factor 1.5 to 9 more sensitive than freshwater species, up to a factor 9 less sensitive than freshwater species, or not significantly different from freshwater species. Thus, protection levels (e.g., HC5 derived from freshwater species only) may still be uncertain for marine species. Further research would be needed to investigate the protectiveness of the threshold levels derived for species from 1 type of habitat for species from different types of habitats.

The ACR was shown to be taxon dependent. The HC50 values for SSDs on acute LC50s were 2 to 1000 times higher than those for chronic NOECs. Average ACRs for various chemicals have been reported to be 10.5 for fish, 7.2 for anuran species, 7.0 for daphnids, and 5.4 for algae. Variation in invertebrate ACRs was higher than in fish ACRs. Therefore, some species may be at an increased risk of underestimated chronic toxicity when mean or median ACRs are used. Extreme caution in risk assessment procedures should be used for ACRs because of their diversity and irregularities.

Attempts to discover the most sensitive species or taxon (e.g., primary producers for herbicides, arthropods in case of insecticides) by deriving HC5s from the SSD approach should be carried out with caution. This approach may result in protection of 95% of the most sensitive species of a selected taxon and may be over conservative for the entire community (Table 2).

Smaller sets of toxicity data give greater uncertainty in the SSD output, which can be significantly reduced if the sample size includes at least 4 data points.

By using ICE models to derive SSDs, several authors have shown that ICE model-based HC5s were within an order of magnitude of the measured HC5 values for chemicals of diverse modes of action.

From a vast number of studies investigating the influence of different methods to fit the toxicity data into SSDs and derive HC5 values, a general conclusion can be drawn that taxonomic groups (intraspecies variation and proportions between taxonomic groups) had the greatest effect on estimated protective concentrations, rather than the statistical method used to construct the distribution.

Based on comparisons between endpoints derived from semifield studies and laboratory-based SSDs, generally the output from an SSD as HC5 is a factor of 1.4 to 75 lower than the NOEC based on the most sensitive endpoints measured in the field. However, in a few cases, the HC5 calculated from semifield toxicity data was a factor of 1.1 to 4 lower than the HC5 calculated from laboratory data.

The horizon of application of the SSD approach has been widened in recent scientific research, and novel applications have involved nonchemical stressors and the derivation of effect levels from field monitoring data.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3474.

Data availability—The data are available publicly and have been extracted from the referenced peer-reviewed publications listed.

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