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Confirming the Species-Sensitivity Distribution Concept for Endosulfan Using Laboratory, Mesocosm, and Field Data

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Abstract. In Australia, water-quality trigger values for toxicants are derived using protective concentration values based on species-sensitivity distribution (SSD) curves. SSD curves are generally derived from laboratory data with an emphasis on using local or site-specific data. In this study, Australian and non-Australian laboratory-species based SSD curves were compared and the concept of species protection confirmed by comparison of laboratory-based SSD curves with local mesocosm experiments and field monitoring data. Acute LC50 data for the organochlorine pesticide endosulfan were used for these comparisons; SSD curves were fitted using the Burr type III distribution. SSD curves indicated that the sensitivities of Australian fish and arthropods were not significantly different from those of corresponding non-Australian taxa. Arthropod taxa in the mesocosm were less sensitive than taxa in laboratory tests, which suggests that laboratory-generated single-species data may be used to predict concentrations protective of semifield (mesocosm) systems. SSDs based on laboratory data were also protective of field populations.

The species-sensitivity distribution (SSD) concept is frequently used in ecologic risk assessments for the formulation of water-quality guidelines. The aim of SSDs is to determine the concentration of a toxicant that is protective of most species (usually 95%) in the environment. SSDs are constructed by fitting a cumulative distribution function to a plot of species toxicity data against rank-assigned percentiles (Kooijman 1987; Van Straalen and Denneman 1989; Wheeler *et al.* 2002). From the cumulative distribution, the PC95 (protective concentration 95%, i.e., concentration that is protective of 95% of species) value is extrapolated. The PC95 is often referred to as "HC5" (hazardous concentration 5%) in Europe (Van Straalen and Van Leeuwen 2002) and "FAV" (final acute value) or "FCV" (final chronic value) in North America (Suter 2002), although the latter two methods use different species-selection criteria, data collection methods, and fitted distributions.

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SSD curves are generally constructed using laboratory-derived toxicity data, which leaves the approach open to question whether standards based on such data provide appropriate protection of organisms in the field (Solomon *et al.* 1996; Versteeg *et al.* 1999). Van den Brink *et al.* (2002) and Schroer *et al.* (2004) showed that SSD curves and resulting PC95 values derived from laboratory data were similar to those derived from semifield (mesocosm) data, which supports the adequacy of the SSD concept for predicting safe environmental concentrations. Our study added further observations to evaluate the use of the SSD approach.

In Australia, SSD curves and PC95 values have been used to derive water-quality guidelines for toxicants with an emphasis on using Australian or site-specific data where available (*Australian 2000*, Chapman *et al.* 2001). The use of non-Australian toxicity data to address local problems has been frequently explored, particularly in Australia where the transhemisphere application of toxicity has long been questioned (Johnston *et al.* 1990; Sunderam *et al.* 1992; Davies *et al.* 1994). However, Australian and non-Australian toxicity data have not been compared in an SSD context, largely because of the paucity of Australian data for most toxicants. The organochlorine insecticide endosulfan is an exception. Endosulfan has been heavily used by agricultural industries in Australia, and the environmental impacts of its use have been the focus of considerable research effort (e.g., Sunderam *et al.* 1992; Leonard *et al.* 2000, 2001; Hose *et al.* 2002, 2003a). Therefore, we used endosulfan in this study as an example substance to compare Australian laboratory and field data with each other and with data gathered on other continents. These comparisons had three aims:

1. We compared the sensitivity of Australian and non-Australian taxa to endosulfan by comparing SSD curves and PC95 values for each group. This was done to assess whether non-Australian data can be used for setting water-quality guidelines for a specific region such as Australia.
2. We compared species-sensitivity distributions based on data from laboratory and semifield experiments to assess whether laboratory sensitivity is representative of field sensitivity.
3. We assessed and compared the suitability of SSD curves

and PC95 values derived from laboratory studies to predict concentrations protective of communities in the field.

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3-oxide) is a broad-spectrum organochlorine pesticide widely used in agriculture for the control of invertebrate pests. Endosulfan has a specific neurotoxic mode of action and works by attacking the gamma aminobutyric acid receptor complex in the central nervous system (Hassall 1990). Endosulfan is from the cyclodiene group of chlorinated hydrocarbons, but it is slightly more reactive, more readily metabolized, and less prone to bioaccumulation compared with other cyclodienes such as aldrin and dieldrin (Guerin and Kennedy 1992). It is a stereoisomer mixture with alpha (α) and beta (β) isomers in the ratio 7:3 (Hayes and Laws 1991). Endosulfan sulfate is a principal breakdown product and is as toxic to biota and is more persistent than the parent compound (e.g., Leonard *et al.* 2001).

Materials and Methods

Toxicity Data

Toxicity data for endosulfan were obtained primarily from *Water Quality Guideline Database for Toxicants* (*Australian 2000*) and supplemented with data from the AQUIRE database (United States Environmental Protection Agency 1998); the Australasian Ecotoxicology database (Warne *et al.* 1998), and data from Hose *et al.* (2003b). Where multiple data points were available for the same end point for a particular taxon, the geometric mean of those values was used. A summary of the data for each taxon is given in the Appendix. Van den Brink *et al.* (2002) and Maltby *et al.* (2003) noted the importance of considering the SSDs for taxonomic groups separately when a specific mode of action for the toxicant is known, particularly when comparing geographic regions and laboratory- and field-derived SSDs. In light of this, we first created SSDs using data from specific groups rather than for all aquatic organisms.

Toxicity data were limited to acute LC50 and EC50 values from studies with exposure periods between 48 and 96 hours. EC50 data were used in the absence of LC50 data when the end point was a surrogate for mortality (such as immobility). Test organisms were categorized as being either arthropod, nonarthropod invertebrate, fish, or amphibian, and each group was analyzed separately including Australian and/or non-Australian species. Toxicity data were considered "Australian" if test organisms occurred in natural ecosystems of Australia and if tests were conducted under local conditions (such as local river water). Under this definition, data for introduced species (in this case, European carp *Cyprinus carpio*, mosquito fish *Gambusia holbrooki*, and rainbow trout *Oncorhynchus mykiss*) could be included when tests were conducted in Australia using local water and appropriate temperature conditions. Data from tests on these species conducted elsewhere were considered non-Australian. By classifying species as being Australian or non-Australian, a direct comparison between SSD curves obtained from Australian species and non-Australian species could be made (see aim no. 1 previously mentioned).

The use of acute data allowed direct comparison with EC50 values based on published mesocosm data (Hose *et al.* 2003a; see section on "Mesocosm data"). In this way, a full-curve comparison could be made between SSDs based on laboratory and semifield data for arthropods (see aim no. 2).

Chronic or no observed effect concentration (NOEC) values could not be used to calculate safe ecologic thresholds because insufficient data were available. Although SSDs are preferably based on chronic NOEC data (ANZECC and *Australian 2000*), there is no theoretical reason why SSDs cannot be based on acute-effects data (e.g., EC50 values, Van den Brink *et al.* 2002; Maltby *et al.* 2003). If acute-effects data are used, safety factors are applied to convert the resulting acute PC95 values to chronic no-effect concentrations. To compare laboratory-based PC95 values with effects observed in the field (see "Field data" section), a safety factor was applied (*Australian 2000*). The safety factor was calculated by taking the geometric mean of acute-to-chronic ratios (ACR) obtained from the literature. Acute (LC50) and chronic (NOEC) data used in such ratios do not have to be for the same end point, but they must be for the same species and have been reported in the same article or at least the work done in the same laboratory (Warne 2001).

Mesocosm Data

To construct an SSD based on field-derived EC50 values, mesocosm data were obtained from a study conducted in artificial stream mesocosms on the banks of the Namoi River near Gunnedah, New South Wales, Australia. The stream mesocosms (hereafter referred to as "mesocosms") have been described in detail by Hose *et al.* (2002). Detailed descriptions of the experiment—including dosing methods, chemical analysis, dose calculations and benthic macroinvertebrate sampling—can be found in in Hose *et al.* (2003a).

For the mesocosm experiment endosulfan was applied to the mesocosms for 48 hours. Thirteen mesocosms were used. Four mesocosms were used as controls, and the remainder were allocated to three endosulfan treatments each with three replicates. Actual endosulfan concentrations were measured throughout the experiment and used to determine average ($\pm$ SD) treatment concentrations (ATC) of 1.0 $\pm$ 0.1, 6.7 $\pm$ 0.9, and 30.7 $\pm$ 0.5 μg/L for the three treatments (Hose *et al.* 2003a).

EC50 values for mesocosm taxa were obtained using the approach of Van Wijngaarden *et al.* (1996). We referred to these as EC50 values (rather than LC50 values) because of uncertainty whether changes in population abundance were caused by mortality or an avoidance response. We fitted a three-parameter logistic regression model to data on abundances of macroinvertebrates collected at 144 hours after dosing (i.e., 96 hours after the 48-hour exposure period had ended). Recruitment of macroinvertebrates to the streams between the exposure period and sampling was considered negligible. To calculate the EC50 values, macroinvertebrate abundances were assumed to be quasi-Poisson distributed and to depend on the exposure concentration in the following manner:

$$\text{Expected number} = \frac{c}{1+e^{-b[\ln(\text{ATC})-a]}} \quad (1)$$

The model resulted in a sigmoid concentration-response curve for ln(ATC), with the parameters c = expected number in the control mesocosms, a = log of the concentration (ln[ATC]) at which point expected numbers will have decreased by 50%, and b = slope parameter. The value e^a is denoted by the mesocosm EC50. The mesocosm EC50 is defined as the ATC at which expected numbers have decreased by 50%. Regression models were fitted using CETIS software (Tidepool Scientific, McKinleyville, CA).

Field Data

To evaluate the protective nature of water-quality guidelines derived from laboratory toxicity data, we compared these guidelines with field observations (see aim no. 3). Data on macroinvertebrate abundance in the Namoi River during the 1995 to 1996 and 1997 to 1998 pesticide

spray seasons were taken from Leonard *et al.* (2000). Detailed methodologies for data collection and analysis are given therein.

Endosulfan concentrations were measured using solvent-filled passive samplers deployed in impacted and reference sites in the Namoi River during the summer pesticide spray seasons in 1995 to 1996 and 1997 to 1998 (Leonard *et al.* 2000). Reference sites were on the Namoi River and its main tributary upstream of the cotton-growing region (Leonard *et al.* 2000). Passive samplers were deployed in the river approximately monthly throughout the pesticide spray season. Concentrations of endosulfan measured in the passive samplers were converted to average daily concentrations in the river water using concentration factors reported by Leonard *et al.* (2002). Concentration factors for endosulfan isomers (α and β) and endosulfan sulfate (the principal toxic metabolite) were averaged. PC95 values derived from acute toxicity data were compared with the concentrations recorded at reference sites. In doing so, we assumed that no impact occurred at those reference sites.

Data Analysis

SSDs were fitted using the BurrliOZ program (CSIRO v I.O.14; Perth, Australia) (Campbell *et al.* 2000). BurrliOZ fits the Burr type III distribution (Shao 2000), which is a flexible three-parameter distribution that provides good approximations to many commonly used distributions such as the log-normal, log-logistic, and Weibull.

The three parameters of the Burr type III distribution—*b*, *c*, and *k*—are estimated by maximum likelihood using the Nelder-Mead simplex algorithm, a derivative-free optimization technique. The Burr type III distribution tends toward one of a set of limiting distributions as its parameters approach limiting values (Shao 2000). For example, as *k* becomes very large, the Burr type III distribution tends toward the reciprocal Weibull distribution. If *c* is estimated to be very large, the Burr type III distribution tends toward the reciprocal Pareto distribution. In the BurrliOZ software, if *k* is estimated >100 in a fit of the Burr distribution, then the parameter estimation is repeated, and a reciprocal Weibull is fitted. Similarly, if *c* is estimated >80, then the reciprocal Pareto distribution is fitted.

From the fitted SSDs, we calculated the PC95 and PC50 values. The BurrliOZ software calculates confidence intervals (CIs) for PC values using a bootstrap technique (Campbell *et al.* 2000). As a result, CIs may vary with subsequent reruns. We estimated 95% CIs by calculating the 2.5% and 97.5% intervals for the PC95 and PC50 values. Each interval was estimated 10 times using 1000 permutations. The geometric mean of those 10 calculations was used as the best estimate of the lower and upper boundaries of the 95% CIs. Nonoverlapping 95% CIs were used as the criterion to determine significant differences among PC95 and PC50 values.

The BurrliOZ program does not provide r^2 values to indicate how well the distributions fit the data. In this study, r^2 values were derived using nonlinear regression (SPSS v10.0: Chicago, IL) with the distribution parameters from BurrliOZ as the starting values. Because SPSS software uses a different algorithm for the maximum likelihood estimates compared with BurrliOZ, the resulting parameter estimates differ slightly, but the r^2 values are likely to be indicative of the fit provided by BurrliOZ.

Results

Comparison of Australian and non-Australian Taxa

Eighty-eight 48- to 96-hour LC50 values were found for non-Australian taxa. Twenty-five values were for arthropod taxa, 14 were for nonarthropod invertebrates, 46 were for fish taxa, and 3 were for amphibians. Nine and 8 LC50 values were obtained for Australian arthropod and fish taxa, respectively. Only 2 LC50 values were found for Australian nonarthropod invertebrates, so this group could not be compared with non-Australian taxa. There were insufficient amphibian data with which to make comparisons.

Burr type III distributions were fitted to all non-Australian datasets. Reciprocal Weibull and Pareto distributions were fitted to data for Australian arthropods and fish, respectively. When data for all arthropods were combined, the *k* parameter of the Burr type III distribution was estimated >100, so a reciprocal Weibull distribution was fitted. Burr type III distributions were fitted for the data sets containing all fish species, all nonarthropod, and all arthropod and fish species data combined. Parameters for the distributions are given in Table 1. For all distributions, r^2 values were >0.9 (Table 1).

The PC95 and PC50 values for Australian and non-Australian fish and arthropods were not significantly different (Fig. 1 and Table 1). The PC50 and PC95 values of the Australian and non-Australian arthropods only differed by a factor of approximately 2, and those calculated for Australian and non-Australian fish differed by a factor of approximately 3 and 5, respectively (Table 1). It must be noted that the most sensitive fish classified as an Australian fish is the introduced species *Cyprinus carpio*.

The PC95 value of the non-Australian, nonarthropod invertebrates was not significantly different from the that of the nonAustralian fish and arthropods (Fig. 2, Table 1), but it was almost two orders of magnitude greater than PC95 values for the other taxonomic groups (Table 1). However, significant differences were evident among the PC50 values (Table 1).

When Australian and non-Australian data were pooled, no significant differences between the PC50 or PC95 values of all arthropods and all fish could be observed (Fig. 2, Table 1), which implies that data for arthropods and fish can be combined into one distribution. So the PC95 value that can be used for the general risk assessment of endosulfan in freshwater ecosystems is the one calculated for all arthropods and fish together (0.24 μg/L; CI 0.16 to 0.41).

Comparison of Laboratory and Mesocosm Data

Australian and non-Australian laboratory-derived 48- to 96-hour LC50 values for arthropods were pooled for comparison with 48-hour EC50 values from the mesocosms. LC50 values from laboratory studies were available for 34 taxa (Table 1). EC50 values were determined for 8 insect (arthropod) taxa (Table 2) from the 61 taxa recorded in the mesocosms (Hose *et al.* 2003). A Burr type III distribution was fitted to the all-arthropod (laboratory) data, and a reciprocal Pareto distribution was fitted to the mesocosm arthropod data (Table 1). The latter is not surprising because the highest treatment level of the mesocosm experiment was 30.7 μg/L, so field EC50 values >30.7 μg/L could not be expected. As a consequence, the resulting SSD curves were distinctly different (Fig. 3). The curve for all arthropods was very wide (5 orders of magnitude), whereas the curve for the mesocosm EC50 values was quite steep, partly because of the upper limit of 30.7 μg/L. Although

Table 1. Distribution types and parameters PC50 and PC95 values for species sensitivity distribution curves fitted to 48- to 96-hour LC50 values from endosulfan exposure. All data are from laboratory studies unless specified

Taxa Group	n	Distribution	r^2	PC50 50% (μg/L)	PC50 95% CI (μg/L)	PC95 50% (μg/L)	PC950 95% CI (μg/L)	Parameters
Australian arthropods	9	Reciprocal Weibull	0.91	5.01	1.5–27.5	0.31	0.23–0.91	$\alpha = 1.62$, $\beta = 0.53$
Australian fish	8	Reciprocal Pareto	0.92	1.21	0.7–2.2	0.084	0.0085–1.0	$x_0 = 2.7$, $\theta = 0.86$
Non-Australian arthropods	25	Burr Type III	0.99	9.16	2.9–53.2	0.17	0.055–0.72	$b = 0.001$, $c = 0.379$, $k = 25.7$
Non-Australian fish	46	Burr Type III	1.00	3.1	2.2–4.7	0.47	0.28–0.89	$b = 0.76$, $c = 0.99$, $k = 3.1$
Non-Australian, non-arthropod invertebrates	14	Burr Type III	0.99	3885	1318–10437	15	0.0–616	$b = 20339$, $c = 1.95$, $k = 0.212$
All arthropods	34	Reciprocal Weibull	0.99	7.70	3.1–22.3	0.19	0.10–0.59	$\alpha = 1.56$, $\beta = 0.397$
All fish	54	Burr Type III	0.99	2.64	1.9–3.9	0.31	0.17–0.64	$b = 1.351$, $c = 1.049$, $k = 1.722$
All non-arthropod invertebrates	16	Burr Type III	0.98	2560	909–8627	28	0.0–564	$b = 7847.8$, $c = 1.23$, $k = 0.432$
All arthropods and fish	88	Burr Type III	0.99	3.59	2.41–5.8	0.24	0.16–0.41	$b = 0.007$, $c = 0.563$, $k = 22.87$
Insects (LC50 < 30.7 μg/L)	8	Reciprocal Weibull	0.92	2.03	1.2–4.0	0.47	0.40–0.81	$\alpha = 1.41$, $\beta = 0.99$
Mesocosm arthropods (all insects)	8	Reciprocal Pareto	0.97	15.28	9.3–22.7	1.57	0.38–10	$x_0 = 30.3$, $\theta = 1.01$

CI = confidence interval.

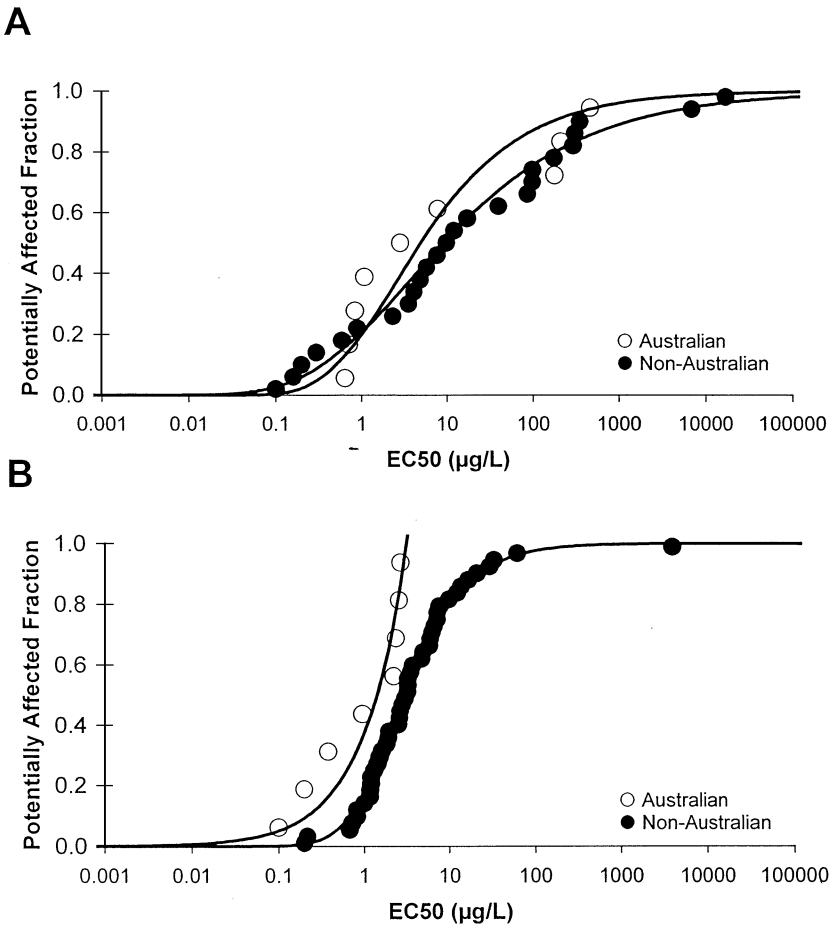

Fig. 1. SSD curves for 48- to 96-hour LC50 data for endosulfan. (**A**) Australian and non-Australian arthropods. (**B**) Australian and non-Australian fish. SSD = species-sensitivity distribution

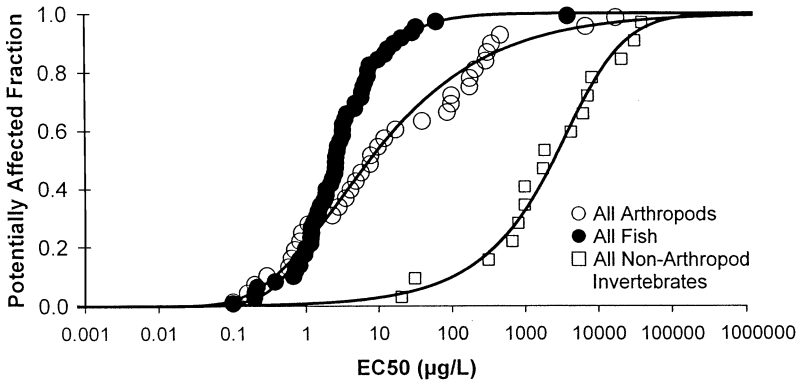

Fig. 2. SSD curves for 48- to 96-hour LC50 data for endosulfan for all arthropods, fish, and nonarthropod invertebrates. SSD = species-sensitivity distribution

Table 2. Forty-eight–hour EC50 values for mesocosm arthropod taxa

Taxa	Mesocosm EC50	Laboratory LC50
Micronecta sp.	4.1	
Cloeon sp.	23.0	
Ulmerophlebia sp.	10.0	
Tasmanocoenis sp.	30.3	
Jappa kutera	2.1	1.0[a]
Austrophleboides sp.	10.2	
Chironomidae	19.5	
Atalophlebia spp.	22.1	12.3[b]

[a] Data from Leonard *et al.* (1999).
[b] Data from Hose *et al.* (2003b).

the PC50 values of the all-arthropods (PC50 = 7.7 µg/L) and mesocosm insects (PC50 = 15 µg/L) were similar, their PC95 values differed by almost one order of magnitude (Table 1).

It is difficult to draw an exact parallel between the laboratory and mesocosm data because the mesocosm data consisted only of insect taxa and had an upper limit of 30.7 µg/L. To overcome these problems, we also constructed an SSD containing laboratory data for insect taxa with an upper limit of 30.7 µg/L. Comparison of the PC95 value from this analysis with the mesocosm PC95 value showed a smaller difference of a factor of approximately three (Table 1). Also, the CI of the PC95 values overlapped, thus indicating that the differences were not significant. When comparing the full curve, however, the mesocosm arthropod populations were indicated to be less sensitive to endosulfan than was expected from the laboratory data (Fig. 3). This was further confirmed by comparing laboratory LC50 and mesocosm EC50 values for common taxa (Table 2).

Field Concentration Data

Leonard *et al.* (2000) showed a significant difference in the macroinvertebrate assemblages in reference and impacted sites on the Namoi River during the 1995 to 1996 and 1997 to 1998 pesticide spray seasons. Hose *et al.* (2003) showed significantly lower abundances (up to >95% decrease) of the mayfly *Atalophlebia* spp. at impacted compared with reference sites. The average concentrations of endosulfan measured at reference sites during the summer pesticide spray season were 0.029 and 0.038 µg/L in the 1995 to 1996 season and ranged from 0.001 to 0.020 µg/L in the 1997 to 1998 season. We considered these to be equivalent to NOEC values.

A safety factor of 11.6 was calculated from ACRs for fish (3, Macek *et al.* 1976) and the water flea *Moinodaphnia maclaeyi* (10.75) (Sunderam 1990) and *Ceriodaphnia* cf *dubia* (48.6, Sunderam 1990). When applying this safety factor, the PC95 value for chronic effects became 0.021 µg/L. The concentrations of 0.001 and 0.038 µg/L (i.e., the range of concentrations measured at reference sites) corresponded, respectively, with the PC100 and PC92 value of the SSD based on chronic NOECs for all arthropods and fish.

Average concentrations at impacted sites ranged between 0.069 and 0.352 µg/L in the 1995 to 1996 season and between 0.052 and 0.137 µg/L in the 1997 to 1998 season. Because of the significant changes in the macroinvertebrate assemblages at these impacted sites (Leonard *et al.* 2000), we considered these concentrations to be equivalent to LOEC values. The concentrations 0.052 and 0.352 µg/L corresponded, respectively, with the PC88 and PC53 values of the SSD for all arthropods and fish.

Discussion

Comparison of Australian and Non-Australian Taxa

This study is part of a growing body of literature showing that the sensitivity of organisms to toxicants is independent of their geographic origin. Several studies have shown similar sensitivities between Australian and non-Australian organisms exposed to endosulfan (fish only, Sunderam *et al.* 1992), metals (Markich and Camilleri 1997), and organic chemicals (Johnston *et al.* 1990), but the literature is equivocal (see Davies *et al.* 1994; Rose *et al.* 1997). From a global perspective, Maltby *et al.* (2003) and Dyer *et al.* (1997) showed similar sensitivities among North American and European taxa with different geographic distributions.

The difference in the sensitivities of different taxonomic groups might be expected for toxicants, such as endosulfan, that have a specific toxic mode of action (Van den Brink *et al.* 2002; Maltby *et al.* 2003). Van den Brink *et al.* (2002) and Maltby *et al.* (2003) both identified the need to derive SSDs for

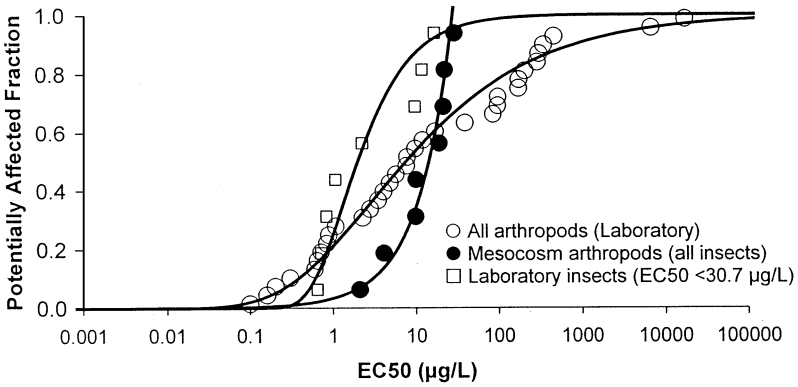

Fig. 3. SSD curves for 48- to 96-hour LC50 data for endosulfan based on laboratory and mesocosm arthropod data. SSD = species-sensitivity distribution

taxonomic groups separately for toxicants that have specific modes of action. Maltby *et al.* (2003) showed that for numerous pyrethroid and organophosphate insecticides, there was a significant difference in the sensitivity of vertebrate (predominantly fish) and arthropod groups. However, for the organochlorine pesticide lindane, there was no significant difference in sensitivity of arthropods and fish; however, both groups were significantly more sensitive than nonarthropod invertebrates (Maltby *et al.* 2003). The similar response observed in our study for endosulfan implies that there may be a pattern of toxicity characteristic of organochlorines and that for the derivation of SSDs, fish and arthropod data may be pooled but nonarthropod data should not.

Validation of the SSD Approach

Van den Brink *et al.* (2002) and Schroer *et al.* (2004) confirmed the validity of the SSD approach for two insecticides by showing similar SSD curves for arthropods under laboratory and semifield conditions. Our study also showed this and added further confirming observations of the SSD approach to the scientific literature. By discussing the assumptions of the SSDs (Versteeg *et al.* 1999) and how these were met for our data, we were able to show confirmation of the SSD approach.

The SSD approach assumes that the sensitivity of organisms in the laboratory approximates their sensitivity in the field (Versteeg *et al.* 1999). Laboratory tests often ignore natural environmental factors (such as light, temperature, habitat suitability, shelter, etc.) that may influence toxicity in the field (Cairns 1983; Geisy 1985). The findings of this study suggest that the exclusion of these factors in laboratory tests does not significantly affect the outcome of risk assessments. The PC95 value derived from laboratory data was less than that derived from the mesocosm data (Table 1) and was thus protective of those populations.

Versteeg *et al.* (1999) provided a detailed discussion of why organisms in laboratory studies are likely to be more sensitive than those in mesocosm studies. In particular, Versteeg *et al.* (1999) cited the lack of random species selection and the development of toxicity tests with sensitive taxa for use in laboratory tests. Differences in water quality and availability of habitat or shelter between laboratory and semifield studies are also likely to favor greater sensitivity under laboratory conditions (Versteeg *et al.* 1999).

The PC95 value derived from laboratory data was also protective of field populations. The laboratory-derived value was generally below the concentrations of endosulfan measured at reference sites in the field when the safety factor of 11.6 was applied. Based on the SSD for fish and arthropod taxa combined, the average daily concentrations recorded at reference sites are protective of 92% to 100% of taxa. The laboratory-derived PC95 value was also protective of secondary effects (in the form of algal blooms) that were observed in the mesocosms dosed with endosulfan at ≥6.9 µg/L (NOEC 1.0 µg/L) (Hose *et al.* 2003a).

In comparing laboratory-derived SSDs with field-monitoring data, we ignored the presence of other stressors at field sites and attributed any change at impacted field sites to endosulfan alone. In the field, endosulfan rarely occurs alone and is often part of a cocktail of agricultural chemicals (e.g., Muschal 1998; Leonard *et al.* 2000). Based on the SSD for fish and arthropod taxa, the concentrations recorded at impacted sites are only protective of between 50% and 86% of taxa, which might be expected to result in mild to severe impacts on the macroinvertebrate community. However, because field concentrations are based on average daily concentrations from passive samplers, they may underestimate peak exposures, which are a likely cause of the observed biologic effects (e.g., Schulz and Liess 1999). The importance of this is that concentrations linked to effects in the field are underestimated, and thus the extent to which laboratory based SSDs are protective of field situations is in reality greater than that previously indicated.

A further assumption of the SSD approach is that the selection of species data used to derive an SSD curve should be random (Versteeg *et al.* 1999). This will rarely be the case for any toxicant because the majority of toxicity data have been derived using a suite of standard test organisms (Forbes and Calow 2002). Although endosulfan toxicity data are available for a range of nonstandard test species, the selection is not random because choice of test organism is constrained to those organisms that are abundant in the field, easily transportable, and amenable to toxicity testing. Rare or cryptic taxa are unlikely to ever be collected for toxicity testing, so the selection of species data for SSD curves is unlikely to ever be random. Despite this, the SSD based on a haphazard collection of laboratory taxa was protective of a functioning assemblage of species in the field.

The SSD approach assumes that the distribution of the data is well modelled (Versteeg *et al.* 1999). In this regard, the Burr

type III distribution is superior to previous approaches of fitting a number of specific models (e.g., Wheeler *et al.* 2002). The Burr type III distribution can provide good approximations to many commonly used distributions such as the log-normal and Weibull (Shao 2000). Newman *et al.* (2000) showed that the log-normal distribution, although suitable for many compounds and routinely used for determining environmental quality criteria, was not suitable for endosulfan toxicity data. Consistent with this, the Burr type III distribution was most commonly fitted to our data. The flexibility of the Burr type III distribution ensures that the best model fit is found; however, as with any model approach, fitted models and resulting median PC95 values are likely to be unstable or less accurate when data sets are small.

It is evident from this study that PC50 values are more stable (have smaller 95% CIs) than PC95 values, which may make PC50 values more suitable for comparing the relative sensitivities of SSD curves. Large CIs for PC95 values made comparing curves on this basis difficult. For example, the CIs for nonarthropod invertebrates range from 0 to approximately 600 μg/L, meaning that any group with a smaller PC95 value could not be significantly different. This problem is probably a consequence of the nature and paucity of data. For the nonarthropod data, there was a gap of one order of magnitude in the toxicity data between the second- (31 μg/L) and third-ranked (316 μg/L) toxicity values (Fig 2). As a result, this lower region of the SSD curve is likely to be poorly estimated, thus contributing to the large CIs. Other data sets with a more contiguous range of toxicity values, particularly at the lower end, will lead to more reliable PC95 values with smaller 95% CIs.

The small number of data available, particularly for Australian and mesocosm studies, is a limitation to this study. The Organisation for Economic Cooperation and Development (1992) and the Australian water quality guidelines (Australian 2000) require 5 data points to develop an SSD, and the European guidance document (Campbell *et al.* 1999) recommends 8. Wheeler *et al.* (2002) suggested that at least 10 are needed, and Newman *et al.* (2000) reported that data for 35 freshwater taxa are necessary for deriving optimal HC5 values for endosulfan (using a resampling approach). It is likely that the small number of mesocosm data points is partly responsible for the distinctly different (reciprocal Pareto) distribution of the mesocosm data compared with the laboratory arthropod data. This is also partly due to only having data from a single study with a limited exposure range.

Conclusion

This study has confirmed the validity of the SSD approach for endosulfan by showing that PC95 values derived from laboratory studies were protective of populations in mesocosms and in the field. It is also possible to include data for organisms from different geographic regions in constructing SSDs. Separate SSDs should be constructed for different taxonomic groups, although fish and arthropod taxa showed similar sensitivity to endosulfan and thus can be combined.

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Appendix 1. Summary of 48- to 96-hour acute toxicity values (geometric mean) for exposure of Australian and Non-Australian taxa to endosulfan

Taxa	Australian Arthropods	Non-Australian Arthropods	Australian Fish	Non-Australian Fish	Australian nonarthropod Invertebrates	Non-Australian nonarthropod Invertebrates
Atalophlebia australis	0.6					
Caridinides sp.	2.8					
Ceriodaphnia dubia	182.2					
Cheumatopsyche	0.8					
Daphnia carinata	478.0					
Jappa kutera	1.1					
Moinodaphnia macleayi	215.0					
Notonecta sp.	0.7					
Paratya australiensis	7.9					
Aedes aegypti		316.2				
Alonella sp.		0.2				
Barytelphusa guerini		17780.0				
Caridina weberi		7.8				
Chironomus riparius		100.0				
Cypria sp.		0.9				
Daphnia carinata		180.0				
Daphnia longispina		0.3				
Daphnia magna		300.9				
Diaptomus sp.		0.6				
Enallagma sp.		17.5				
Eretes sticticus		10.0				
Eucyclops sp.		0.1				
Gammarus lacustris		5.8				
Ischnura sp.		87.7				
Macrobrachium dayanum		4.1				
Macrobrachium lamarrei		3.5				
Macrobrachium rosenbergii		4.9				
Oziotelphusa senex senex		7060.0				
Paratelphusa jacquemontii		0.2				
Potamonautes sp.		360.0				
Procambarus clarki		100.8				
Pteronarcys californica		2.3				
Sigara alternata		12.3				
Spicodiaptomus chilospinus		40.0				
Bidyanus bidyanus			2.3			
Cyprinus carpio			0.1	7.3		
Gambusia holbrooki			2.7	3.2		
Hypseleotris galii			2.2			
Macquaria ambigua			0.4			
Melanotaenia duboulayi			2.6			
Nematolosa erebi			0.2			
Oncorhynchus mykiss			1.0	0.8		
Anabas testudineus				1.2		
Anguilla anguilla				33.7		
Anguilla japonica				14.0		
Barbus conchonius				21.4		
Barbus javanicus				7.7		
Barbus sophore				1.0		
Barbus stigma				1.9		
Carassius auratus				0.7		
Catla catla				12.6		
Catostomus commersoni				3.2		
Channa gachua				7.3		
Channa punctatus				4.9		
Channa striata				4000.0		
Cirrhinus mrigala				2.5		
Clarias batrachus				6.7		
Ctenopharyngodon idella				3.0		
Gambusia patruelis				63.0		
Gasterosteus aculeatus				6.0		

Appendix 1. Continued

Taxa	Australian Arthropods	Non-Australian Arthropods	Australian Fish	Non-Australian Fish	Australian nonarthropod Invertebrates	Non-Australian nonarthropod Invertebrates
Gymnocorymbus ternetzi				1.6		
Heteropneustes fossilis				10.1		
Hypophthalmichthys molitrix				1.2		
Ictalurus punctatus				1.5		
Labeo rohita				1.2		
Lepidocephalus thermalis				30.0		
Lepomis macrochirus				3.2		
Macrognathus aculeatus				3.5		
Misgurnus anguillicaudatus				1.2		
Morone saxatilis				0.2		
Mystus cavasius				1.9		
Mystus vittatus				0.7		
Nuria danrica				17.0		
Oreochromis aureus				2.8		
Oryzias latipes				4.8		
Pimephales promelas				1.3		
Poecilia reticulata				3.7		
Rasbora sp.				0.2		
Salmo trutta				1.8		
Salvelinus fontinalis				2.6		
Tilapia				5.9		
Tilapia aurea				2.6		
Tilapia mossambica				6.3		
Tilapia nilotica				1.4		
Tilapia zillii				0.8		
Hydra viridissima					670.0	
Hydra vulgaris					810.0	
Aplexa hypnorum						1890.0
Bellamya dissimilis						1800.0
Cipangopaludina malleata						8500.0
Indoplanorbis exustus						21000.0
Lamellidens corrianus						31.0
Lamellidens marginalis						20.5
Lymnaea natalensis						4370.0
Lymnaea stagnalis						1000.0
Melanopsis dufouri						39891.8
Physa fontinalis						316.2
Physella acuta						6400.0
Planorbis corneus						1000.0
Semisulcospira libertina						7400.0
Tubifex tubifex						31622.8