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Predictive Value of Species Sensitivity Distributions for Effects of Herbicides in Freshwater Ecosystems

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ABSTRACT

In this article we present a review of the laboratory and field toxicity of herbicides to aquatic ecosystems. Single-species acute toxicity data and (micro)mesocosm data were collated for nine herbicides. These data were used to investigate the importance of test species selection in constructing species sensitivity distributions (SSDs), and in estimating hazardous concentrations (*i.e.*, HC5) protective for freshwater aquatic ecosystems. A lognormal model was fitted to toxicity data (acute EC50s and chronic NOECs) and the resulting distribution used to estimate lower (95% confidence), median (50% confidence), and upper (5% confidence), HC5 values. The taxonomic composition of the species assemblage used to construct the SSD does have a significant influence on the assessment of hazard and only sensitive primary producers should be included for the risk assessment of herbicides. No systematic difference in sensitivity between standard and non-standard test species was observed. Hazardous concentrations estimated using laboratory-derived acute and chronic toxicity data for sensitive freshwater primary producers were compared to the response of herbicide-stressed freshwater ecosystems using a similar exposure regime. The lower limit of the acute HC5 and the median value of the chronic HC5 were protective of adverse effects in aquatic micro/mesocosms even under a long-term exposure regime. The median HC5 estimate based on acute data was protective of adverse ecological effects in freshwater ecosystems when a pulsed or short-term exposure regime was used in the microcosm and mesocosm experiments. There was also concordance between the predictions from the effect model PERPEST and the concentrations at which clear effects started to emerge in laboratory and field studies. However, compared to the SSD concept, the PERPEST model is able to provide more information on ecological risks when a common

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toxicological mode of action is evaluated as it considers both recovery and indirect effects.

Key Words: ecological risk assessment, review, pesticides, herbicides, aquatic ecosystem.

INTRODUCTION

The ecological risk assessment of pesticides normally consists of a relatively worst-case first tier assessment, followed by more realistic higher tier assessments if unacceptable risks are indicated. The first tier assessment consists of calculating a Predicted Environmental Concentration (PEC) for the pesticide under a given application scenario (*e.g.*, FOCUS 2001), which is compared to a toxicity endpoint being an EC50 or NOEC. The ratio between the toxicity value and the PEC, the Toxicity Exposure Ratio (TER) is compared with a trigger value of 10 or 100 depending on the toxicity endpoint and standard test species used (EC 1997; USEPA 1998). Because many pesticides do not meet the first-tier trigger value and the trigger values used in the first tier assessment are usually conservative (Brock *et al.* 2000a,b), higher-tier effect assessments are often performed in order to refine the assessment of risk and reduce uncertainties. Campbell *et al.* (1999) identified four different types of approach that could be adopted for higher tier aquatic effects assessment: (i) detailed interrogation of first tier data, (ii) additional single-species studies, (iii) indoor multispecies tests, and (iv) field studies.

Whereas models are an acceptable approach for assessing the fate of pesticides (FOCUS 2001), the assessment of effects is primarily based on experimental data. Risk assessors are hesitant to use models to assess effects, partly due to the inherent complexity of ecosystems and partly due to the limited knowledge of processes driving these ecosystems. In addition, the lack of clear protection goals hampers the development of effect models, that is, the assessment endpoint of the model is difficult to define.

One model that is used in effects assessment is the Species Sensitivity Distributions (SSD). The SSD concept is used to reduce the uncertainty relating to differences in the sensitivity of standard test species and those expected to be exposed in nature and uses interspecific variation in sensitivity to toxicants to predict effects at the community level (Posthuma *et al.* 2002). The SSD is defined as a cumulative distribution function of the toxicity of a single compound or mixture to a set of species that constitutes an assemblage or community. In the USA and EU, the SSD concept has been used during the previous decade to set water quality criteria and estimate risks based on results of water quality monitoring programs (Stephan 2002; Van Straalen 2002; Knoben *et al.* 1998; Preston and Shackelford 2002). A small cut-off value in the left tail of the distribution must be chosen to estimate a concentration below which the fraction of species exposed above their NOEC/ECx level is considered acceptable. Usually a cut-off value of 5 or 10% is chosen and their corresponding concentrations are named HC5 and HC10 (Hazardous Concentration to 5 or 10% of the species).

The use of the SSD concept in ecological risk assessment is based on several assumptions (Versteeg *et al.* 1999; Forbes and Calow 2002), some of which are:

1. The sample of the species on which the SSD is based is a random selection of the community of concern, and is herewith representative for this community.
2. Interactions among species do not influence the sensitivity distribution.
3. Because functional endpoints are normally not incorporated in the SSD, community structure is the target of concern.
4. The laboratory sensitivity of a species approximates its field sensitivity.
5. The protection of the prescribed percentile of species ensures an "appropriate" protection of field ecosystems.

In order to test some of these assumptions we reviewed the laboratory and field toxicity of insecticides and herbicides. The results for insecticides are published elsewhere (Maltby *et al.* 2005) and here we report the results for herbicides. We performed this review in order to:

- assess which grouping of species is most appropriate for the risk assessment of herbicides;
- assess the relative sensitivity of standard test species belonging to primary producers relative to other primary producers;
- compare the acute and chronic toxicity of herbicides to primary producers in laboratory tests;
- test whether the HC5 based on acute EC50s or chronic NOECs is protective in case of a similar exposure in (semi-) field experiments; and
- compare laboratory and (semi-)field toxicity of herbicides.

MATERIALS AND METHODS

Selected Herbicides

Nine herbicides were selected on the basis of available laboratory and field toxicity data: atrazine, simazine, metribuzin, metamitron, linuron, diuron, diquat, 2,4-D, and pendimethalin. In general, the minimum data requirements for each herbicide were laboratory toxicity data (EC50 or NOECs) for six species of primary producers and one microcosm and/or mesocosm experiment from which a $NOEC_{ecosystem}$ (*i.e.*, the highest concentration tested that showed no consistent or significant effects on the most sensitive endpoint of the studied ecosystem) could be deduced. Six NOEC values, but only five EC50 values, were available for pendimethalin, whereas toxicity data were only available for four species of primary producers exposed to metamitron. However, both these compounds were included because we had access to the raw data and could therefore ensure quality. Six of the nine herbicides selected inhibit photosynthesis at PS II (atrazine, simazine, metribuzin, metamitron, linuron, and diuron), one simulates the growth hormone auxin (2,4-D), one has a PS I electron diversion mode of action (diquat) and pendimethalin inhibits cell division and cell elongation (Tomlin 2000).

Table 1. Data selection criteria used for short-term toxicity test, long-term toxicity test, and semi-field data.

Short-term toxicity test data
Endpoint: L(E)C50 mortality, immobilization (animals) or biomass, growth (plants) Test duration (days): Fish 2–21, invertebrates 1–7, macrophytes 2–28, algae 1–7
Long-term toxicity test data
Endpoint: NOEC or EC5 – 10: growth, feeding, reproduction, mortality or immobilization Test duration (days): Fish > 20, invertebrate >6, macrophytes >6, algae >2
(Micro)mesocosm experiments (after Brock et al. *2000a,b)*
• Test system represents a realistic freshwater community.
• Adequate description of the experimental set-up and the appropriate study design.
• Relevant exposure concentrations are specified and any solvents are applied to both treatment and control systems.
• Endpoints measured are relevant to the working mechanism(s) of the test substance.
• Effects are statistically significant and either show an unambiguous concentration-effect relationship, or are in agreement with a concentration-effect relationship from additional studies.

Laboratory Toxicity Data

Laboratory toxicity data were obtained from the database described in De Zwart (2002), the open literature, confidential industry reports, and own unpublished data. To guarantee that the quality of the data was sufficient, we only included data from industry when we had access to the raw data. Data included in the database of De Zwart also were evaluated using several quality criteria (De Zwart 2002). The selection criteria summarised in Table 1 were used to reduce variability in endpoint and test duration. The geometric mean was calculated when multiple values for an endpoint were available for a species. The HC5 and HC50 and their 95% confidence intervals were calculated using the ETX software (Van Vlaardingen *et al.* 2003). This excel add-in calculates hazardous concentrations assuming a lognormal distribution of the toxicity data using the methodology described by Aldenberg and Jaworska (2000). This software also includes the Anderson-Darling Test for goodness of fit on log-normality, which was evaluated at the 5% significance level.

Semi-Field Toxicity Data of Selected Herbicides

Data on the toxicity of selected herbicides under (semi-)field conditions was taken from Brock *et al.* (2000a) and updated using information from the open literature and industry. Brock *et al.* (2000a) contains a review of all experiments performed in microcosms and mesocosms and published between 1979 and 2000 that evaluated the effects of herbicides. For each concentration tested, Brock *et al.* (2000a) classified the effects on seven structural endpoints (*i.e.*, macrophytes, periphyton, phytoplankton, zooplankton, molluscs, macrocrustaceans, and insects combined, and fish and tadpoles combined) and one functional endpoint (community metabolism). Effects were assigned to five classes: Class 1, no effect; Class 2, slight effect usually on a single sampling date immediately after application only; Class 3, clear short-term effect (recovery within 8 weeks post last application); Class 4, clear effect duration unknown; Class 5, clear long-lasting effects (no recovery within 8 weeks post last

application). Because recovery is a process that can never be accounted for by using the SSD concept, for this article the Class 3 to 5 are combined to one new effect class 3, named "clear effects." Only experiments evaluating community metabolism and/or primary producers endpoints (*i.e.*, evaluating expected sensitive endpoints), were considered in this analysis. In the evaluated micro/mesocosm experiments the most sensitive endpoints usually concerned densities/biomass of algae and aquatic vascular plants and/or dissolved oxygen and pH values (endpoints indicative for effects on photosynthesis).

COMPARISONS

In order to evaluate which toxicity data are the most appropriate for assessing the risk of herbicides to aquatic communities, SSDs were constructed for the nine herbicides using all available data, and for algae, macrophytes, primary producers, invertebrates, and vertebrates separately. The Anderson-Darling goodness of fit test was used to assess whether data were log-normally distributed.

The relative sensitivity of standard primary producer test species and non-standard primary producers was compared by constructing SSDs using data for all primary producers and indicating the position of the standard test species (*i.e.*, recommended by OECD, EU, USEPA, ASTM, and Environment Canada and listed in Maltby *et al.* (2002)). Data limitations meant that separate SSD curves could not be generated for standard test species and therefore an ANOVA was performed on log-transformed toxicity values to compare the relative sensitivity of standard test and non-standard species. For seven of the nine herbicides, it was possible to construct separate SSDs for acute and chronic data. The HC5 and HC50, plus their confidence intervals, were calculated for acute as well as chronic data using the ETX program and these values were used to derive acute to chronic ratios.

Laboratory toxicity data were compared to (semi-)field toxicity data in three ways: (i) by comparing laboratory derived HC5 values with ecological threshold values (effect classes 1 or 2) obtained from microcosm and mesocosm experiments; (ii) by comparing the complete laboratory SSD curves based on acute and chronic data with the total data set of classified effects from microcosm and mesocosm studies at corresponding concentrations; and (iii) by comparing laboratory SSDs with the predictions of community effects made by the ecological effect model PERPEST (Predicts the Ecological Risks of PESTicides; Van den Brink *et al.* 2002a).

The $NOEC_{ecosystem}$ was defined as the highest test concentration causing no effect (Class 1) in microcosm or mesocosm experiments. The $NOEC_{ecosystem}$ and the class 2-LOEC were determined (where possible) for four exposure regimes:

i. pulsed exposure (water is renewed 24 h after application),
ii. short-term exposure (single application of a herbicide with a field DT50 (Dissipation Time 50%) <10 d),
iii. medium-term exposure (single application of a herbicide with a DT50 between 10 and 25 d or a multiple application of a herbicide with a field DT50 <10 d)
iv. long-term exposure (a single application of a herbicide with a DT50 >25 d or a chronic application).

These NOEC and LOEC values were compared with the HC5 based on a similar exposure regime. This means that, in practice, the ecological threshold level (effect classes 1 and 2) based on a short-term exposure regime were compared with the HC5 based on acute EC50s and that the threshold level based on a long-term exposure was compared with the HC5 based on chronic NOECs. We also collated the DT50s from the available semi-field experiments for each compound to evaluate the acute nature of the exposure due to a single application.

The second comparison between laboratory and field data involved all data, that is, using the complete SSD curves and all classified effects for the four different exposure regimes. For this comparison the classified effects for a certain exposure regime are displayed together with the acute and chronic SSDs in a diagram. Although the y-axes are different for the classified effects (Class 1–3) and the SSDs (Potentially Affected Fraction), a qualitative relationship can be obtained by visual inspection, that is, whether the occurrence of slight effects (Class 2) correspond with the lower left tail of the SSD curve and clear effects (Class 3) correspond with the higher end of the curve.

For the third comparison, predictions of the toxicity of atrazine and 2,4-D to aquatic ecosystems were made by the expert model PERPEST (Van den Brink *et al.* 2002a), which simultaneously predicts the effects of a particular concentration of a pesticide on various (community) endpoints. In contrast to most effect models, PERPEST is based on empirical data extracted from the literature. It uses Case-Based Reasoning (CBR), a technique that solves new problems by using past experience. The database containing the "past experience" has been constructed by performing a review of freshwater model ecosystem studies with pesticides (Brock *et al.* 2000a,b). The PERPEST model searches for analogous situations in the database, based on relevant (toxicity) characteristics of the compound. This allows the model to use information on other pesticides when predicting effects of a particular pesticide. The PERPEST model results in a prediction showing the probability of classes of effects (no, slight, or clear effects) on the various grouped endpoints.

The effects of atrazine and 2,4-D were predicted using the parameter values and model options listed in the appendix, which were optimized using the controlled random search option (Van Nes and Van den Brink 2003). These predictions are not only based on the data presented in this article, but are also based on studies evaluating other herbicides with a similar mode of action to either atrazine or 2,4-D. These herbicides were chosen because they have a well-studied toxicological mode of action and both an acute and a chronic SSD was available. The predicted probabilities of no, slight or clear effects on functional and structural endpoints are compared with the acute and chronic SSDs for atrazine and 2,4-D.

RESULTS

Species Sensitivity Distributions

There were sufficient laboratory toxicity data to generate separate SSDs for algae, macrophytes, invertebrates, and vertebrates exposed to atrazine, diquat, or 2,4-D (Figure 1). For all other herbicides, only a comparison between primary producers and (in)vertebrates could be made. The ratio between the HC50s of the primary producers and (in)vertebrates for all herbicides except 2,4-D was on average 191

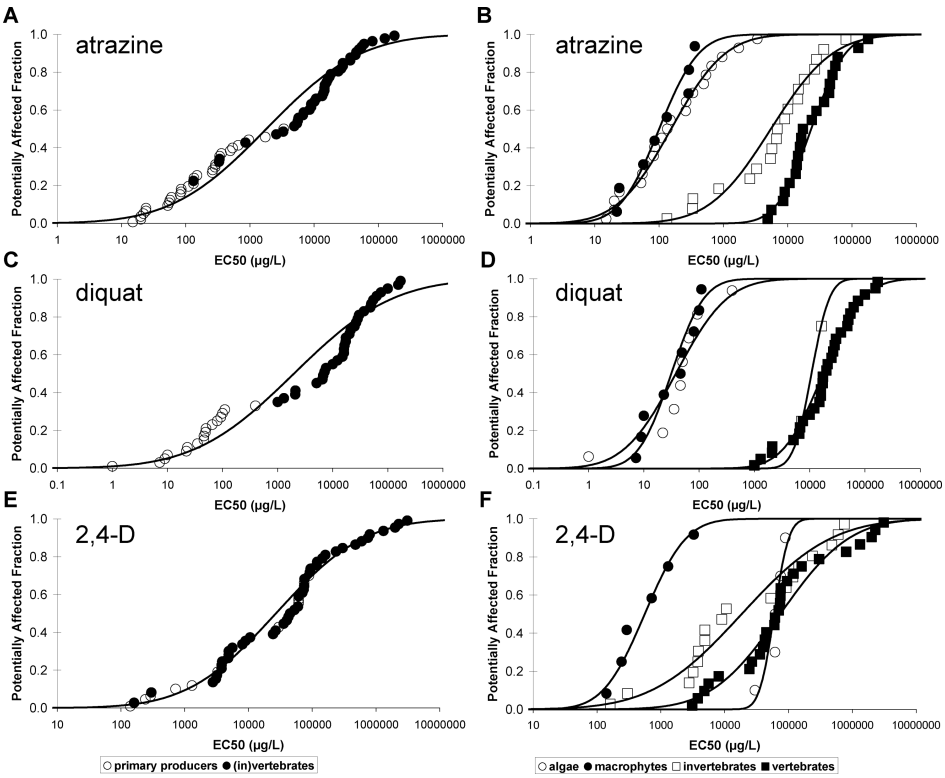

Figure 1. SSDs based on short term toxicity data for all aquatic species for atrazine (A), diquat (C) and 2,4-D (E). Figures (B), (D) and (F) provides the same data but itemized to the organism groups algae, macrophytes, invertebrates, and vertebrates.

(95% CI: 96–380). Figure 1A, 1C, and 1E present the SSD curves for atrazine, diquat, or 2,4-D generated using all available toxicity data, whereas Figure 1B, 1D, and 1F present the data separately for each taxonomic group. The complete toxicity datasets for these three compounds are polymodal and the SSD curves for atrazine and diquat do not fit a lognormal distribution, as confirmed by failure of the Anderson-Darling test at the 5% level. For atrazine and diquat, most primary producers are located on the left side of the curve indicating that they are more sensitive than animals. The SSD curve generated using all available toxicity data for 2,4-D passed the Anderson-Darling test for log-normality at the 5% level (Figure 1E). However, when taxonomically distinct SSDs were compared, it was clear that macrophytes were more sensitive to 2,4-D than other taxonomic groups (*i.e.*, algae, invertebrates, and vertebrates; Figure 1F). Therefore, toxicity data for algae and macrophytes can be grouped for the risk assessment of atrazine and diquat, but only macrophyte data should be used for the assessment of 2,4-D.

This difference in sensitivity between animals and plants was clearly demonstrated when separate SSDs were generated for primary producers and (in)vertebrates for all herbicides (Figure 2). On average, the difference between the HC5 and HC50

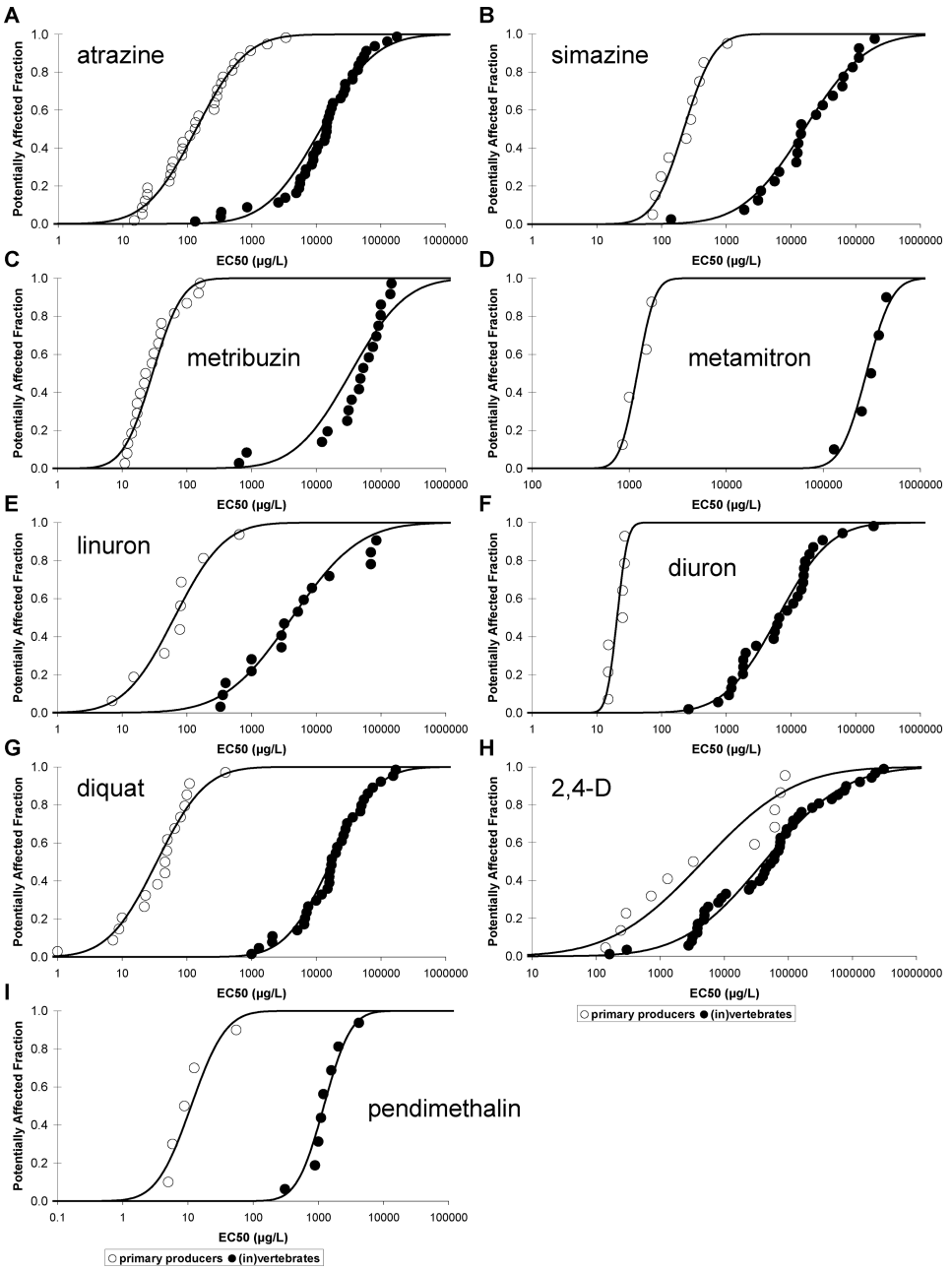

Figure 2. SSDs based on short-term toxicity data for all aquatic species belonging to primary producers (○) and (in)vertebrates (●) for atrazine (A), simazine (B), metribuzin (C), metamitron (D), linuron (E), diuron (F), diquat (G), 2,4-D (H), and pendimethalin (I).

of primary producers and (in)vertebrates was 1.9 and 2.1 orders of magnitude, respectively. This taxonomic difference was smallest for 2,4-D (Figure 2H) and largest for metribuzin and diquat (Figure 2C and 2G).

Figure 3 shows the SSD curves based on acute toxicity values for primary producers for eight of the nine herbicides evaluated (*i.e.*, atrazine, simazine, metribuzin, metamitron, linuron, diuron, diquat and 2,4-D), and highlights the position of the standard test species on each curve. There was no consistent pattern in the relative sensitivity of standard test species across compounds. For instance, *Lemna minor* was the most sensitive species to linuron (Figure 3E), but the least sensitive to metamitron (Figure 3D). Similarly, *Chlorella* was sensitive to metribuzin (Figure 3C), but not to diquat (Figure 3G). The ANOVA also did not yield any significant outcome.

The HC5 and HC50 values based on acute EC50 and chronic NOEC values of primary producers are summarized for all nine herbicides in Table 2. In the case of 2,4-D, only data for submerged macrophytes was used. There were insufficient chronic toxicity data to construct SSDs for metamitron and diquat, and the atrazine chronic SSD and diuron acute SSDs did not pass the Anderson-Darling test for log-normality at the 5% level. However, the primary producer SSDs for atrazine and diuron, presented in Figure 4A and 4E, do not show clear misfits in the lower tail, giving confidence in the HC5 values. Figure 4 shows that for five of the seven herbicides for which acute and chronic SSDs could be constructed, the acute and chronic SSDs run more or less parallel, indicating that the acute to chronic ratio is constant over the whole range of toxicity. For these five herbicides (*i.e.*, atrazine, simazine, metribuzin, linuron, and 2,4-D), ratios between HC5 and HC50 values based on acute EC50 values and chronic NOEC values range between 3.7 and 14, with a mean of 7.8 (Table 2). The acute SSD for diuron is much steeper than the chronic SSD (Figure 4E), resulting in a large acute-to-chronic ratio between the HC5 values (36), a smaller ratio between the HC50 values (4.3), and even a ratio below one between the HC95 values (0.5).

The overall geometric mean of the acute-chronic ratio (ACR) estimated using the HC5 values was 8.9 (95% CI: 5.0–16). The 95% value of the distribution of the ACR was 35 (data passed Anderson-Darling Test for log-normality, 95% CI: 9.4–66). Using the HC50 values these values are lower, the geometric mean of the ACR is 5.1 (3.3–7.8), and the 95% of the ACR distribution was 14 (5.3–23).

There was a significant ($p = .042$) relation between the orders of magnitude difference between the lower and upper limits of the HC5 and the number of data points. This relation is more significant when only chronic HC5 estimations are included ($p = .020$).

Semi-Field Experiments

The results of the literature review of the effects of the nine selected herbicides on the ecology of semi-field experiments are summarized in Tables 3 and 4. Table 3 provides information on the test system, application regime, geographical location of the study, and overall field DT50 in water as observed in semi-field experiments, whereas Table 4 summarizes the effects data by listing the effect concentrations for the most sensitive structural and functional endpoint. For instance, Johnson (1986, No 7 in Table 3) recorded slight effects of a single application of 10 μg/L atrazine

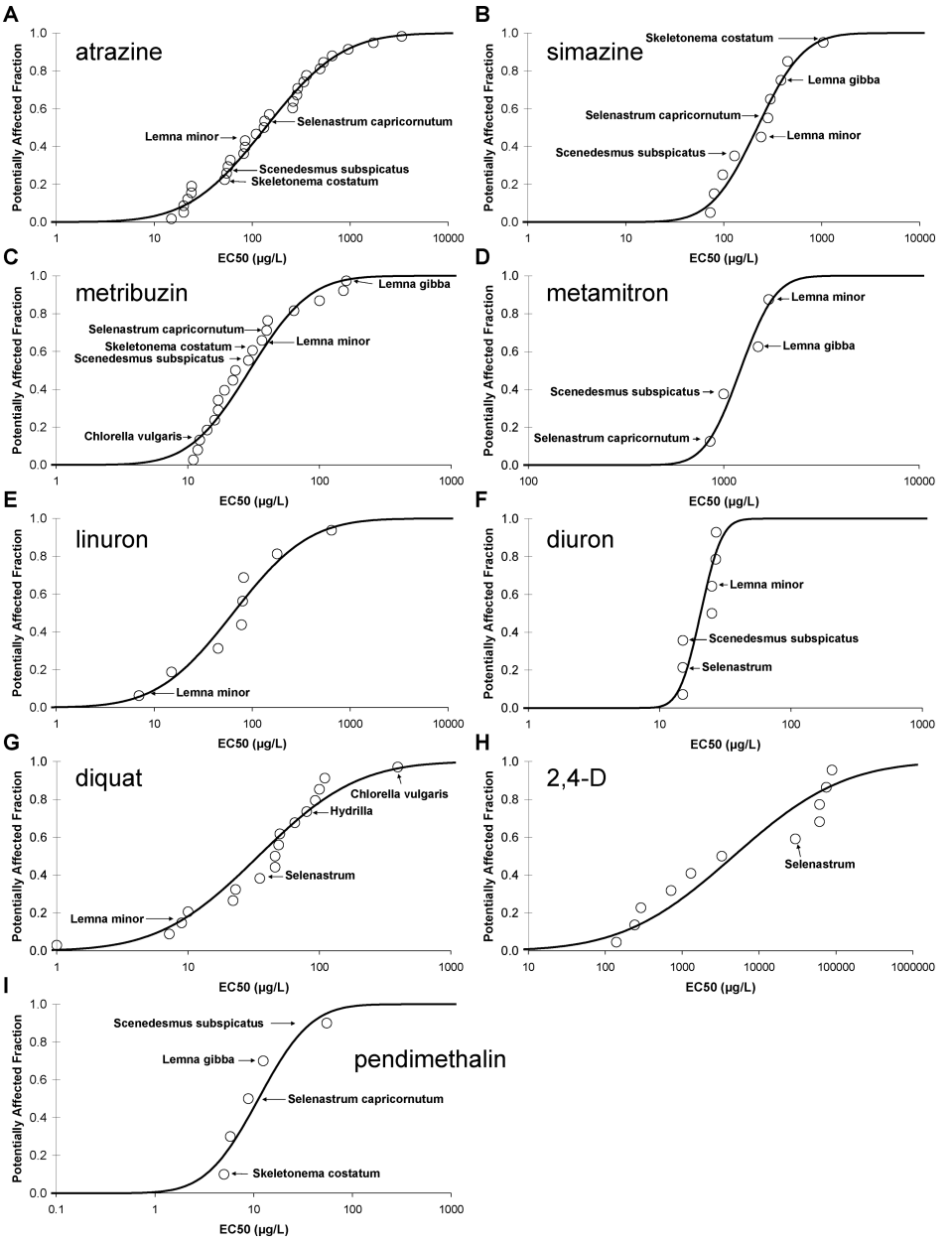

Figure 3. SSDs based on short-term toxicity data for all aquatic species belonging to primary producers for atrazine (A), simazine (B), metribuzin (C), metamitron (D), linuron (E), diuron (F), diquat (G), 2,4-D (H), and pendimethalin (I). The placement of the standard test species are indicated by arrows and their name.

Table 2. Number of acute EC50 and chronic NOEC data points belonging to primary producers (in case of 2,4-D to submerged macrophytes) and associated HC5 and HC50 values plus confidence intervals for the selected herbicides. Also the acute to chronic ratios are calculated for each compound using the median HC5 and HC50 values.

	Laboratory exposure	No. of data points	HC5 (μg/L)	HC50 (μg/L)	Acute–Chronic ratio HC5	Acute–Chronic ratio HC50
Atrazine	Acute	29	13 (5.8–24)	137 (88–214)	4.4	7.6
	Chronic*	17	3.0 (1.3–5.3)	18 (11–28)		
Simazine	Acute	10	52 (18–92)	221 (134–362)	8.1	5.5
	Chronic	10	6.4 (1.7–13)	40 (21–75)		
Metribuzin	Acute	19	7.4 (4.0–11)	29 (21–41)	5.3	3.7
	Chronic	6	1.4 (0.20–3.3)	8.0 (3.5–18)		
Metramitron	Acute	4	667 (226–952)	1214 (826–1784)	n.e.d.	n.e.d.
	Chronic	2	n.e.d.	n.e.d.		
Linuron	Acute	8	5.8 (0.74–17)	64 (25–163)	12	9.0
	Chronic	11	0.50 (0.086–1.4)	7.1 (3.0–17)		
Diuron	Acute*	7	12 (7.6–16)	21 (17–25)	36	4.3
	Chronic	9	0.34 (0.044–1.0)	4.8 (1.8–13)		
Diquat	Acute	17	3.5 (1.2–7.3)	34 (19–61)	n.e.d.	n.e.d.
	Chronic	0	n.e.d.	n.e.d.		
2,4-D	Acute	6	71 (7.1–199)	558 (212–1472)	14	8.5
	Chronic	8	5.1 (0.57–16)	66 (24–180)		
Pendimethalin	Acute	5	2.0 (0.20–5.1)	11 (4.5–28)	3.9	1.7
	Chronic	6	0.51 (0.030–1.8)	6.4 (1.9–21)		

*Data did not pass the Anderson-Darling test on log-normality at the 5% level. n.e.d.: not enough data.

on community metabolism (Table 4), but no effects on the structural endpoints. At 100 μg/L, clear effects on community metabolism were detected, but there were still no detectable effects on structure (Table 4). At the highest test concentration of 1000 μg/L, clear effects on both endpoint types were recorded.

The number of semi-field experiments performed for an individual herbicide varied from one (*i.e.*, metribuzin, metamitron, diuron, and pendimethalin) to 22 (*i.e.*, atrazine) (Table 3). Although many of these studies have been performed in different types of systems (*i.e.*, stagnant vs. flow-through, laboratory vs. field), in different parts of the world (*i.e.*, USA, Canada, Europe), with exposures to different application scenarios (*i.e.*, single, repeated, constant), there is a surprising degree of agreement in threshold effect concentration. Slight effects of atrazine on sensitive endpoints start to emerge at a concentration between 2 and 10 μg/L and there is no apparent difference in effects between a single and a repeated application due to the persistence of the compound in water and, consequently, the long-term exposure regime in "closed" test systems. Omitting the study by Jurgensen and Hoagland (1990) because of the very short, pulsed exposure used, the $NOEC_{ecosystem}$ (effect class 1) for both functional and structural endpoints ranges from <2 to 20 μg/L. The

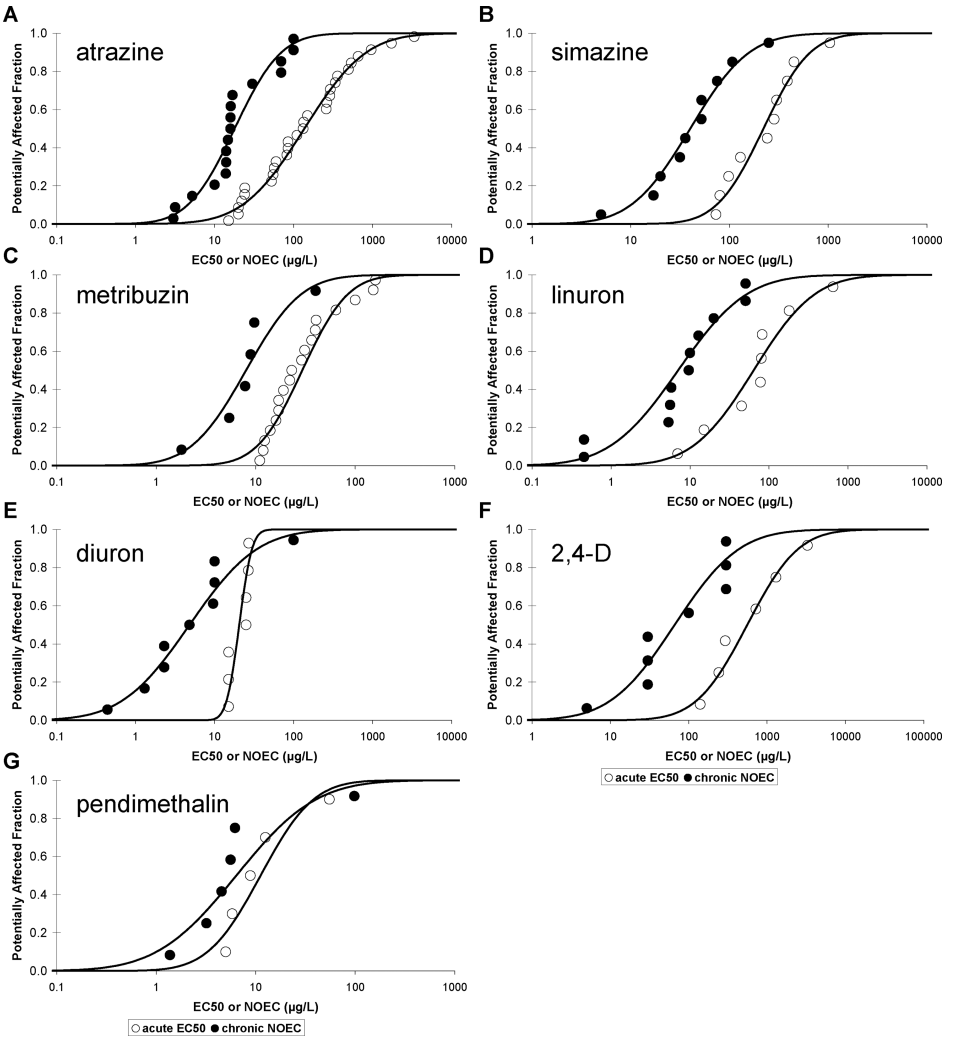

Figure 4. SSDs based on short-(○) and long-term (●) toxicity data for all aquatic species belonging to primary producers for atrazine (A), simazine (B), metribuzin (C), linuron (D), diuron (E), 2,4-D (F), and pendimethalin (G). For 2,4-D only data of submerged macrophytes are used.

5th centile (NOEC_5%) of the NOECs (lowest class 1 observation of structural and functional endpoints) of the studies no. (6), (9), (13), (15), (16), (17), and (18) (see Table 3) is 2.8 μg/L with a 95% confidence interval of 1.1 to 4.5 μg/L (ETX software, Van Vlaardingen *et al.* 2003). Other studies could not be included because their lowest concentration tested resulted in an effect. The median value (NOEC_50%) is 7.8 μg/L (5.1–12). These values change only slightly when Class 2 effects (*i.e.*, slight effects) are also considered acceptable (5th centile 2.2, 1.0–3.5; 50th centile 6.7, 4.5–9.8). For further risk assessment the $NOEC_{ecosystem}$ for long-term exposure of atrazine was set at the lowest levels reported for class 1 effects in Table 4, that is, 5 μg/L,

Table 3. Overview and description of the ecosystem studies that simulated a community of a freshwater ecosystem and that studied the ecological impact of a single or chronic application of the selected herbicides.

No.	Test system	Water regime	Dose	Location	Reference(s)
Atrazine (field DT50 water = 90 days; Cunningham *et al.* 1984)					
1	Natural stream	Flow-through	Pulse (24 h)	Nebraska, USA	Jurgensen and Hoagland (1990)
2	Exp. streams	Recirculating	Single	Texas, USA	Moorhead and Kosinski (1986); Kosinski (1984); Kosinski and Merkle (1984)
3	Exp. streams, lab.	Recirculating	Single	Vermont, USA	Gruessner and Watzin (1996)
4	Exp. ponds	Stagnant	Single	Montana, USA	Fairchild *et al.* (1994)
5	Mesocosms	Stagnant	Single	France	Seguin *et al.* (2001)
6	Microcosms, lab.	Stagnant	Single	Georgia, USA	Brockway *et al.* (1984)
7	Microcosms, lab.	Stagnant	Single	Missouri, USA	Johnson (1986)
8	Microcosms, lab.	Stagnant	Single	Oregon, USA	Stay *et al.* (1985)
9	Microcosms, lab.	Stagnant	Single	Oregon, USA	Stay *et al.* (1989)
10	Enclosures in lake	Stagnant	Repeated	Canada	Hamilton *et al.* (1987)
11	Enclosures in lake	Stagnant	Eepeated	Canada	Herman *et al.* (1986); Hamilton *et al.* (1988); Hamilton *et al.* (1989)
12	Exp. ponds	Stagnant	Repeated	Kansas, USA	DeNoyelles *et al.* (1982, 1989, 1994); Dewey (1986); Kettle *et al.* (1987)
13	Exp. streams, lab.	Flow-through	Constant	Georgia, USA	Brockway *et al.* (1984)
14	Exp. swamp	Flow-through	Constant	Minnesota, USA	Detenbeck *et al.* (1996)
15	Microcosms, lab.	Flow-through	Constant	Virginia, USA	Pratt *et al.* (1988)
16	Enclosures in pond	Stagnant	Constant	Germany	Jüttner *et al.* (1995)
17	Microcosms, lab.	Stagnant	Constant	Netherlands	Van den Brink *et al.* (1995)
18	Exp. streams	Flow-through	Constant	Sweden	Nyström *et al.* (2000)
19	Exp. streams	Flow-through	Constant	Spain	Muñoz *et al.* (2001)
20	Exp. streams, lab.	Flow-through	Constant	Georgia, USA	Hamala and Kollig (1985)
21	Exp. streams	Recirculating	Constant	Texas, USA	Kosinski (1984); Kosinski and Merkle (1984)
22	Exp. streams	Recirculating	Constant	Ohio, USA	Krieger *et al.* (1988)
Simazine (field DT50 water = 20 days; Jenkins and Buikema 1990; Goldsborough and Robinson 1985)					
23	Enclosures in swamp	Stagnant	Single	Manitoba, Canada	Goldsborough and Robinson (1983, 1986)

(Continued on next page)

Table 3. Overview and description of the ecosystem studies that simulated a community of a freshwater ecosystem and that studied the ecological impact of a single or chronic application of the selected herbicides. *(Continued)*

No	Test system	Water regime	Dose	Location	Reference(s)
24	Enclosures in pond	Stagnant	Single	Manitoba, Canada	Goldsborough and Robinson (1985)
25	Enclosures in swamp	Stagnant	Single	Manitoba, Canada	Gurney and Robinson (1989)
26	Microcosms in pond	Stagnant	Single	Virginia, USA	Jenkins and Buikema (1990)
27	Microcosms, lab.	Stagnant	Single	Unknown	Bryfogle and McDiffett (1979)
Metribuzin (field DT50 water = 7.1 days; Brock *et al.* 2004)					
28	Enclosures in mesocosms	Stagnant	Single	Netherlands	Brock *et al.* (2004)
Metamitron (field DT50 water = 1.9 days; Brock *et al.* 2004)					
29	Enclosures in mesocosms	Stagnant	Single	Netherlands	Brock *et al.* (2004)
Linuron (field DT50 water = 7.2–11.8 days; Crum *et al.* 1998)					
30	Enclosures in pond	Stagnant	Single	Kent, UK	Stephenson and Kane (1984)
31	Mesocosms	Stagnant	3 Pulses of a week	Netherlands	Kersting and Van Wijngaarden (1999); Van Geest *et al.* (1999)
32	Microcosms, lab.	Stagnant	Constant	Netherlands	Van den Brink *et al.* (1997); Cuppen *et al.* (1997)
Diuron (field DT50 water = 22 days; Hartgers *et al.* 1998)					
33	Microcosms, lab.	Stagnant	Single	Minnesota, USA	Flum and Shannon (1987)
Diquat (field DT50 water in presence of sediments = <1 day; Ritter *et al.* 2000)					
34	Microcosms, lab.	Flow-through	Pulse (24 h)	Bristol, UK	Paterson and Wright (1987)
35	Microcosms, lab.	Stagnant	Single	Germany	Draxl *et al.* (1991)
36	Microcosms, lab.	Stagnant	Single	Pennsylvania, USA	Barreiro-Lozano and Pratt (1994)
37	Microcosms, lab.	Stagnant	Single	Pennsylvania, USA	Pratt *et al.* (1990)
38	Microcosms, lab.	Stagnant	Constant	Germany	Draxl *et al.* (1991)
2,4-D (field DT50 water = 14–20 days ; Boyle 1980))					
39	Exp. ponds	Stagnant	Single	Not mentioned	Boyle (1980)
40	Enclosures in pond	Stagnant	Single	Canada	Forsyth *et al.* (1997)
41	Lake	Stagnant	Single	Kentucky, USA	Kobriae and White (1996)
42	Exp. ponds	Stagnant	Single	Canada	Scott *et al.* (1981); Stephenson and Mackie 1986; Sherry 1994
Pendimethalin (field DT50 water = 1.5 days; Ebke *et al.* 2001)					
43	Enclosures in pond	Stagnant	Single	Münster, Germany	Ebke *et al.* (2001)

Table 4. Classification of the most sensitive endpoints in model ecosystem studies that studied the ecological impact of a single or chronic application of the selected herbicides (see Table 3 for a description of the studies). Concentrations are given in μg/L.

	Effects on structure			Effects on function		
No.	No effect (Class 1)	Slight effect (Class 2)	Clear effect (Class 3)	No effect (Class 1)	Slight effect (Class 2)	Clear effect (Class 3)
Atrazine						
1	100	—	—	—	—	—
2	—	—	—	—	—	100
3	—	5	—	—	—	—
4	—	—	50	—	—	50
5	—	2	30	—	—	—
6	—	—	—	5	50	100
7	100	—	1000	—	10	100
8	—	—	—	—	—	60
9	—	—	—	20	—	100
10	—	—	80	—	—	—
11	—	—	155	—	—	155
12	—	—	20	—	—	20
13	—	—	—	5	—	50
14	75	—	—	—	—	15
15	110	—	337	10	—	32
16	25	—	75	5	10	75
17	5	—	—	5	—	—
18	14	25	80	—	—	—
19	—	—	14	—	—	14
20	—	—	100	—	—	100
21	—	—	10	—	—	10
22	—	—	24	—	—	—
Simazine						
23	—	100	1000	—	—	100
24	—	—		—	100	1000
25	—	—	2000	—	—	2000
26	100	—	500	100	—	500
27	50	—	150	50	—	150
Metribuzin						
28	6	18	56	6	18	56
Metamitron						
29	4480	—	—	280	1120	4480
Linuron						
30	—	—	1000	—	—	1000
31	5	—	15	0.5	5	15
32	0.5	—	5	0.5	—	5
Diuron						
33	—	—	—	3	—	30
Diquat						
34	10	—	50	—	—	—

(Continued on next page)

Table 4. Classification of the most sensitive endpoints in model ecosystem studies that studied the ecological impact of a single or chronic application of the selected herbicides (see Table 3 for a description of the studies). Concentrations are given in μg/L. (Continued)

	Effects on structure			Effects on function		
No	No effect (Class 1)	Slight effect (Class 2)	Clear effect (Class 3)	No effect (Class 1)	Slight effect (Class 2)	Clear effect (Class 3)
35	—	—	300	—	—	300
36	—	—	3500	—	—	3500
37	—	—	850	850	—	—
38	—	—	1000	—	—	1000
2,4-D						
39	—	—	500	500	—	—
40	10	—	100	—	—	—
41	—	—	2000	—	—	2000
42	—	—	1000	1000	—	—
Pendimethalin						
43	0.23	1.1	4.9	150	—	—

although slight effects cannot be excluded at this concentration (Gruessner and Watzin 1996; Seguin *et al.* 2001). In most cases the functional endpoint proved to be more sensitive compared to structural ones (study (7), (14), (15) and (16)). The study of Jurgensen and Hoagland (1990) can be considered the only atrazine study available that investigated a short-term exposure regime. The $NOEC_{ecosystem}$ (effect class 1) for short-term exposure to atrazine is 100 μg/L.

Unfortunately, far less information was available for all other herbicides (Table 4). Effects of a single application of simazine on both structural and functional endpoints start to emerge at a concentration of 100 μg/L with 50 μg/L taken as a $NOEC_{ecosystem}$ (Bryfogle and McDiffet 1979). Brock *et al.* (2004) report a $NOEC_{ecosystem}$ values of 6 μg/L for a single application of metribuzin. They also report a $NOEC_{ecosystem}$ of 280 μg/L for a single application of metamitron in an outdoor semi-field experiment, based on community metabolism endpoints. A $NOEC_{ecosystem}$ of 0.5 μg/L, based on community metabolism endpoints, has been recorded for both repeated (3 pulses of 7 days) and continuous applications of linuron. Although clear effects were recorded for constant exposure of 5 μg/L, only slight effects were found at this concentration at the repeated pulse application (Table 4). A single application of diuron showed no effects at 3 μg/L in a study by Flum and Shannon (1987). For diuron, only data for effects on community metabolism could be found. From a risk assessment perspective, however, this information is still valid because community metabolism is generally more sensitive than structural endpoints to herbicides (see atrazine cases listed earlier and studies (23), (29), and (31)). In four cases (studies (37), (39), (42), and (43)) structural endpoints were more sensitive than community metabolism. For a 24 h pulse exposure to diquat a $NOEC_{ecosystem}$ of 10 μg/L was recorded for a single application (Paterson and Wright 1987). A single application

of pendimethalin and 2,4-D resulted in $NOEC_{ecosystem}$ values of 0.23 μg/L (Ebke *et al.* 2001) and 10 μg/L (Forsyth *et al.* 1997), respectively.

Comparison Between SSD and Semi-Field Data

Table 5 shows the HC5 values, plus confidence interval, the associated $NOEC_{ecosystem}$ (= effect class 1) and the effect class 2 and effect class 3 $LOEC_{ecosystem}$ values. For this analysis, class 1 and class 2 effects were considered as acceptable concentrations in surface waters. Because recovery is a process that cannot be assessed with the SSD concept we could not include the acceptability of short-term effects into our assessment, and all clear effect classes (3, 4, and 5) were combined into one overall "clear effect" class 3. Although at class 2 slight and transient effects were recorded, we considered this LOEC acceptable because the extent of the effects is very small and their duration very short (*i.e.*, only recorded on one sampling date). In cases where it was uncertain whether effects should be classified as class 1 or class 2, they are always classified as class 2. In Table 5, the ecological threshold concentrations (effect classes 1 or 2) observed in test systems with different exposure regimes are compared with the HC5 based on acute EC50s, and HC5 based on chronic NOECs. For this comparison a distinction is made between pulsed (atrazine, diquat), short-term (metamitron, metribuzin, pendimethalin), medium-term (linuron, 2,4-D, diuron, simazine), and long-term (linuron, atrazine) exposure.

The median value of the chronic HC5 is lower than the class 2-$LOEC_{ecosystem}$ for all nine herbicides, irrespective of the exposure regime, and in most cases, also lower or equal to the class 1-$NOEC_{ecosystem}$ (Table 5). Also the lower limit value of the acute HC5 is in all cases lower than, or near to, the class 1-NOEC or the class 2-LOEC, irrespective of the exposure regime (Table 5). When focussing on the exposure categories "24 h pulsed exposure regime" and the "short-term exposure regime" (see Table 5) the median value of the acute HC5 is in 4 of the 5 cases lower than the class 1-NOEC or class 2-LOEC. Only in the case of pendimethalin the median HC5 value is slightly higher than the class 2-LOEC. In the mesocosm experiment with pedimethalin, however, up to 4.9 μg/L treatment level only few species were found to be affected, whereas effects on functional endpoints were absent at all treatment levels (Table 4).

Figure 5 shows the classified semi-field effects for all herbicides together with the acute SSD and, if available, chronic SSD generated using laboratory toxicity data. Semi-field effects are derived from single application studies, except for atrazine and linuron where they are derived from both single and chronic application studies. Also, the resulting exposure regime is provided (*i.e.*, pulsed, short-, medium-, and long-term). For atrazine and linuron, the shift from no effect to slight and clear effects corresponds to the lower tail of the chronic SSD (Figure 5A and E), whereas for most other herbicides, this shift corresponds with the lower tail of the acute SSD. Only for 2,4-D do the effects start to emerge in between the lower tails of the two SSD curves (Figure 5H). If both structural and functional endpoints are measured, community metabolism is generally more sensitive (atrazine, metamitron, linuron, diquat) or equally sensitive (atrazine, simazine, metribuzin, linuron) than structural endpoints, although an exception exists in the form of pendimethalin (Table 4).

Table 5. HC5 values plus confidence intervals based on acute EC50s and chronic NOECs of the different herbicides. Effects observed in semi-field experiments are categorized in different exposure regimes.

Exposure/substance	Study no.	Field DT50 water	HC5 acute	HC5 chronic	Class 1	Class 2	Class 3	Remarks
Pulse exposure								
Atrazine	1	90 d	13 (5.8–24)	3.0 (1.3–5.3)	100	—	—	24 h pulse, Phot. inhibitor
Diquat	34	<1 d	3.5 (1.2–7.3)	—	10	—	50	24 h pulse, Phot. inhibitor
Short-term exposure (single application, field DT50 <10d)								
Metramitron	29	1.9 d	667 (226–952)	—	280	1120	4480	Phot. inhibitor
Metribuzin	28	7.1 d	7.4 (4.0–11)	1.4 (0.2–3.3)	6	18	56	Phot. inhibitor
Pendimethalin	43	1.5 d	2.0 (0.20–5.1)	0.51 (0.030–1.8)	0.23	1.1	4.9	Selective herbicide, adsorbed by leaves and roots
Medium-term exposure (single application, field DT50 >10d and <25 d; multiple application, field DT50 < 10 d)								
Linuron	31	7–12 d	5.8 (0.74–17)	0.50 (0.086–1.4)	0.5	5	15	3 times 7 d pulses, Phot. inhibitor
2,4-D	40	14–20 d	71 (7.1–199)	5.1 (0.57–16)	10	—	100	Auxin simulator
Diuron	33	21d	12 (7.6–16)	0.34 (0.044–1.0)	3	—	30	Phot. inhibitor
Simazine	23–27	20 d	52 (18–92)	6.4 (1.7–13)	50	100	100	Phot. inhibitor
Long-term exposure (single application, field DT50 >25d; chronic application)								
Atrazine	2–22	90 d	13 (5.8–24)	3.0 (1.3–5.3)	5	2–10	10	Phot. inhibitor
Linuron	32	7–12 d	5.8 (0.74–17)	0.5 (0.086–1.4)	0.5	—	5	Phot. Inhibitor

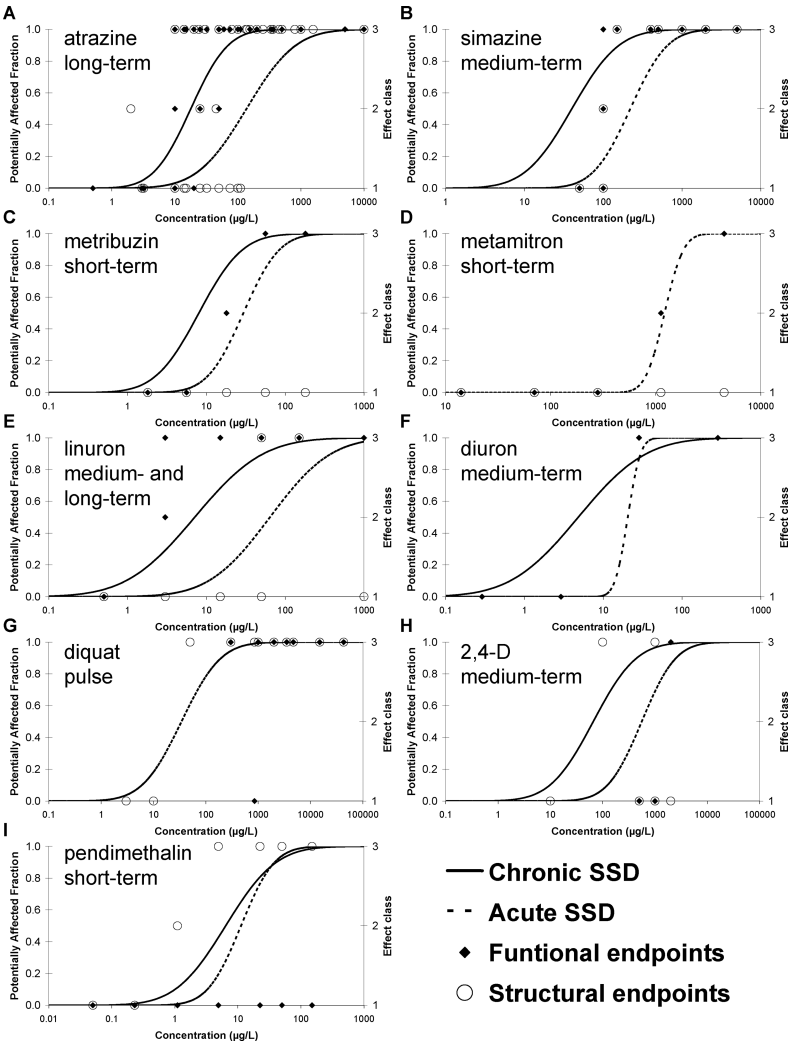

Figure 5. Classified effects observed in (semi) field studies for the different pesticides. Panel A shows the effects of a long-term exposure to atrazine, panel B the effects of a medium-term exposure to simazine, panel C and D the effects of a short-term exposure to metribuzin and metamitron, respectively, panel E the effects of a medium- and long-term exposure to linuron, panel F the effects of a medium-term exposure to diuron, panel G the effects of a pulsed exposure to diquat, panel H the effects of a medium-term exposure to 2,4-D and panel I the effects of a short-term exposure to pendimethalin (Table 5). The effects are classified into a functional (community metabolism) endpoint (⬥) and structural endpoints (phytoplankton, macrophytes, and periphyton) (○). The effects are also classified according to magnitude. 1 = no effect, 2 = slight effect, 3 = clear effect. For an extensive description of the effect classes we refer to Brock *et al.* (2000a). The straight line represents the chronic SSD, whereas the acute SSD is represented by a discontinuous line.

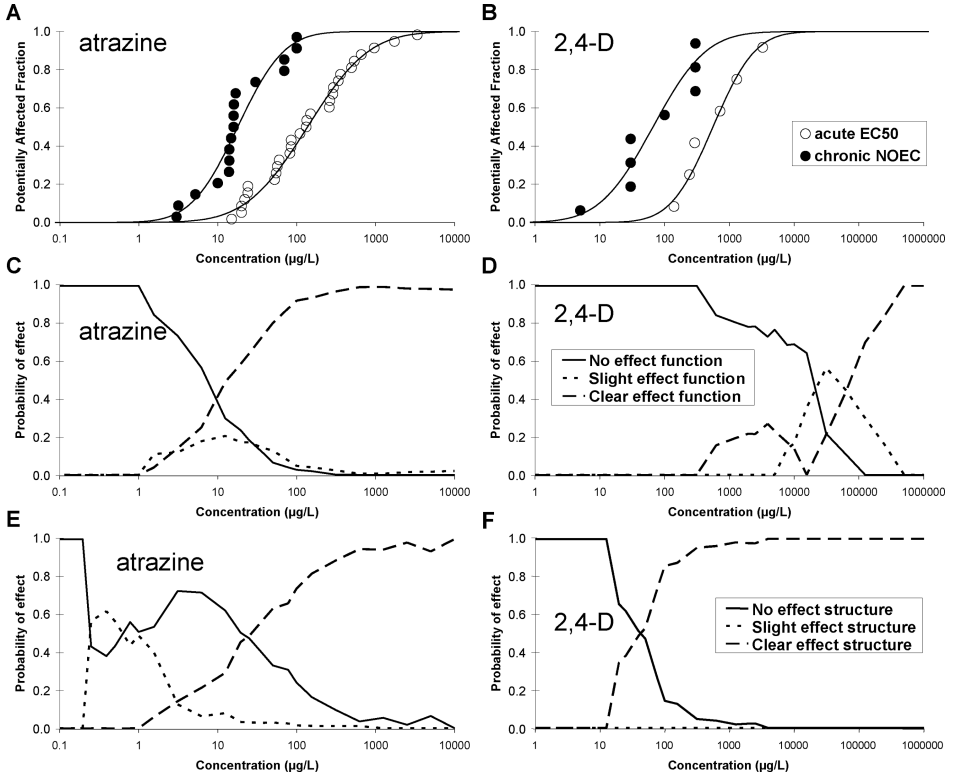

Figure 6. Relation between the probability of no, slight, and clear effects on functional and structural endpoints as predicted by the expert model PERPEST for atrazine and 2,4-D and their SSDs based on acute and chronic toxicity values. Figures A, C, and E displays the laboratory toxicity of atrazine and indicated effects on functional and structural endpoints, respectively. Figures B, D, and F shows the same information for 2,4-D.

Comparison Using SSD and the PERPEST Model

The SSDs of atrazine and 2,4-D (Figure 6A and 6B) were compared to the effects on functional (Figure 6C and 6D) and structural (Figure 6E and 6F) endpoints predicted using the PERPEST model (Van den Brink *et al.* 2002a). For atrazine, the probability of clear effects on function and structure are very similar (*i.e.*, clear effects predicted at concentrations >1 μg/L) and correspond well with the chronic SSD, reflecting the persistence of this herbicide. The model also predicts the occurrence of slight effects on structural endpoints at very low concentrations of atrazine (*i.e.*, Figure 6E). This prediction is heavily influenced by the findings of Seguin *et al.* (2001), who reported slight effects on phytoplankton at a concentration of 2 μg/L (Table 4). For 2,4-D effects on structural endpoints (macrophytes) are reported at lower concentrations compared to functional ones (Figure 6D and 6F). The large influence of one data point also explains the non-monotonic shape of the chance of a clear effect on structural endpoints. Kobriae and White (1996) reported clear effects on community metabolism at a concentration of 2000 μg/L, and slight effects

at 40000 μg/L 2,4-D. It must be noted that the latter observation was done in a plankton-dominated microcosm, which is less relevant for the risk assessment of 2,4-D.

DISCUSSION AND CONCLUSIONS

The hesitation of risk assessors to use models to estimate the effects of pesticides explains why a relatively simple concept such as the SSD is disputed in the arena of ecological risk assessment (see, *e.g.*, Posthuma *et al.* 2002 for an overview). This is not without reason, as the results of an SSD largely depend on the way the toxicity data are processed (Duboudin *et al.* 2004a). In this article we, therefore, made an empirical comparison between expected sensitive and non-sensitive species, standard and non-standard test species, acute and chronic toxicity, and the laboratory and field toxicity of herbicides. In all these comparisons SSD is used to describe sensitivity at the community level. The same comparisons have also been performed for insecticides as described by Maltby *et al.* (2005).

Maltby *et al.* (2005) reviewed the usefulness of the SSD concept for the risk assessment of insecticides and concluded that all 16 insecticides investigated in their study were more toxic to arthropods than vertebrates or non-arthropod invertebrates. The magnitude of difference between median HC5 values derived from vertebrate or arthropod SSDs ranged from a factor of 4 to a factor of 4×10^5. This is of course a result of the toxicological mode of action of these insecticides, that is, they are designed to kill arthropods. The specific mode of action of insecticides explains why SSDs constructed using all available toxicity data do not conform to the log-normal distribution (*e.g.*, methyl-parathion Scheringer *et al.* 2002) and why it is necessary to analyze arthropods separately from the other (in)vertebrates when constructing SSDs for insecticides (Maltby *et al.* 2005).

In this article we build on this knowledge by assessing whether the grouping of species based on the toxicological mode of action is also appropriate when using SSDs for the ecological risk assessment of herbicides. From our study it is obvious that primary producers are by far the most sensitive taxonomic group to photosynthesis-inhibiting herbicides and pendimethalin (inhibits cell division and elongation). In the case of 2,4-D, submerged macrophytes proved to be more sensitive than algae, although some invertebrate species were as sensitive as macrophytes. The difference in sensitive taxonomic groups between herbicides is related to their toxicological mode of action. As 2,4-D is an auxin-simulator, submerged aquatic vascular plants are more vulnerable that unicellular algae (Belgers *et al. submitted*). All the other herbicides evaluated in this article have a toxicological mode of action related either to disruption or inhibition of photosynthesis or to cell development and therefore algae and vascular plants are equally vulnerable.

Because of the large ratio between the HC50s of the primary producers and other aquatic organisms, Brock *et al.* (2004) also grouped the toxicity data into primary producers and (in)vertebrates when applying the SSD concept for the risk assessment of the herbicides metamitron and metribuzin. They found a difference of a factor of 339 between the HC5 based on acute toxicity values for these two groups, whereas we found a mean difference of 191 for all herbicides except 2,4-D.

The second aim of this article was to compare the sensitivity of primary producers identified as standard test species with those not identified as such. Standard test species are selected on the basis of their representativeness for other species, robustness to survive difficult circumstances, and rearing in the laboratory. It could be argued that because of this they are less sensitive compared to non-standard test species. The data presented in this article are limited, but do not show any systematic difference in sensitivity between standard test species and other primary producers. These findings are in accordance with the work performed by Fairchild and colleagues (Fairchild *et al.* 1997, 1998), who concluded that no single species was consistently the most sensitive, and that a suite of aquatic plant test species may be needed to perform accurate risk assessments of herbicides.

Although effect assessments are often carried out under a chronic exposure regime, acute toxicity data are normally more available than chronic data due to experimental and financial constraints. Methods have therefore been developed to predict chronic toxicity data from acute values, with the Acute to Chronic Ratio (ACR) being the most widely used (Kanega 1982). In this method the ratio between known acute and chronic toxicity data are determined and extrapolated to other species and/or other substances (De Zwart 2002). Duboudin *et al.* (2004b) presented the elegant acute to chronic transformation (ACT) methodology, which has the advantage over the ACR that it is based on SSDs rather than single values. It can, therefore, account for differences in standard deviation around the mean acute and chronic toxicity (HC50) of the compounds. Unfortunately, Duboudin *et al.* (2004b) grouped sensitive and insensitive invertebrates, which hampers the use of their predictions for risk assessment. They found a ratio of 29 between the acute and chronic SSDs that group together 3 taxonomic groups (vertebrates, invertebrates and algae) for 11 compounds, including metals and pesticides. However, given the specific mode of action of many pesticides, this may not be the appropriate ratio for the risk assessment of pesticides to sensitive organisms.

In this article we also compared the acute and chronic toxicity of herbicides toward primary producers using ACR. For this we adopted an empirical approach based on HC5 and HC50 values, as these values are most relevant to risk assessment. We calculated HC5 and HC50 values based on both acute EC50 and chronic NOEC values. The geometric mean of the acute–chronic ratio (ACR) estimated using the HC50 values was 8.9 (95% CI: 5.0–16) and the 95% of the ACR distribution was 35. This indicates that the ACR of 10, which is often used in risk assessment, corresponds with the 50% of the ACR distribution of the median toxicity (HC50). If a worst case strategy is to be adopted a factor of 35 would be more appropriate, which is in accordance with the results of Duboudin *et al.* (2004b) as discussed earlier. For the acute and chronic toxicity comparison it would have been better to choose comparable data points, that is, acute EC50 and NOEC values of the same species evaluated in the same laboratory. For this assessment the data presented here was too sparse, but one has to bear in min that when using SSD other factors, for instance species identity, are potentially included in the ACR.

The fourth aim of this article was to test whether the HC5 based on acute EC50s or chronic NOECs was protective of assemblages subjected to a similar exposure regime in (semi-) field experiments. That is, to evaluate the protective power of threshold values established by the SSD concept. There is an extensive literature on the

comparison between laboratory and field sensitivity of species and the general conclusion is that the laboratory sensitivity of a species is indicative of its field sensitivity under the same exposure conditions (Van den Brink *et al.* 2002b; Schroer *et al.* 2004; Hose and Van den Brink 2004). Few studies have compared the SSDs of laboratory- and field-exposed assemblages of species. Maltby *et al.* (2005) performed this comparison for insecticides and Selck *et al.* (2002) performed it for TriButylTin (TBT) and Linear Alkylbenzene Sulphonate (LAS). They found that the lower 95% limit of the acute HC5 values based on both log-logistic and log-normal distributions were consistently lower than the NOEC values extracted from semi-field experiments. Note that the field DT50 value in water is relatively low for most insecticides evaluated by Maltby *et al.* (2005). Brock *et al.* (2004) made a comparison between the laboratory and field toxicity of metamitron and metribuzin and also concluded that HC5 values based on acute laboratory toxicity tests may be used to derive maximum permissible concentrations in a cost-effective way. It is, however, important to note that the field DT50 values after a single application were relatively low for these herbicides. Metribuzin had a DT50 in the water compartment of between 6.0–9.4 days and metramitron of between 1.1–3.2 days. So for these herbicides a single application resulted in a short-term exposure regime according to our classification presented in Table 5. From the data presented in Table 5 it also appears that the median acute HC5 is not protective of adverse effects in micro/mesocosms in case of a medium to long-term exposure regime to a herbicide. In this article we compared the laboratory and field toxicity of several herbicides and found that the median acute HC5 is protective of adverse effects in semi-field tests characterised by a 24-h pulse and a short-term exposure regime (see Table 5). When the lower limit of the acute HC5 is used, protection of the aquatic ecosystem is almost always ensured, even in case of a median and long-term exposure regime (Table 5). This is also the case when using the median value of the chronic HC5. These conclusions are consistent with previous comparisons performed by Maltby *et al.* (2005), Schroer *et al.* (2004), Selck *et al.* (2002), Versteeg *et al.* (1999), Van den Brink *et al.* (2002b) and Hose and Van den Brink (2004).

In this article we used between 4 and 29 toxicity values to construct a SSD. The adequate number of data points needed to construct an SSD depends on the method used. Generally distribution-free methods need more data points (30 or more) than distribution-based methods (Newman *et al.* 2000). Crane *et al.* (2003) stated that the quantity of toxicity data had little influence on the species sensitivity distribution for chlorpyrifos when fitted to all available toxicity data, when n was greater than 10 species. Unfortunately this exercise was not repeated for the sensitive taxonomic group (arthropods in the study of Crane *et al.* 2003). It might be expected that arthropod data alone will fit a log-normal distribution better because non-arthropods have a low susceptibility to chlorpyrifos and inclusion of these data can lead to the combination of two different tolerance distributions (Maltby *et al.* 2005). The HARAP workshop (Higher Tier Aquatic Risk Assessment for Pesticides, Campbell *et al.* 1999) recommended the inclusion of eight relevant species and five vertebrate fish species when SSDs are used in the admission procedure of pesticides. For example, for insecticides, arthropods might be considered relevant (Maltby *et al.* 2005) whereas, for herbicides, relevant organisms for SSD construction belong to primary producers (this article). This number is not only based on statistical examination but also on practical, ethical, and financial arguments and expert knowledge. In this article we

included herbicides for which six or more toxicity data were available, except in the case of metamitron and pendimethalin for which four and five data points were available, respectively. The significance of the relation between the spread of the HC5 and the number of data points indicates that inclusion of more data reduces the uncertainty of the HC5 estimation. This advocates the use of the lower confidence bound of the HC5 to be used as the regulatory endpoint, so an incentive to include more data into the risk assessment is provided. On the other hand, the use of the lower confidence bound could also lead to assessments that are equally worst-case as the first tier so no incentive is left to use the SSD concept in the tiered risk assessment.

Within the risk assessment of pesticides, small effects are considered acceptable if recovery takes place within an acceptable time window (Campbell *et al.* 1999). Because this approach is more liberal than the $NOEC_{ecosystem}$ it can be expected that the HC5 is a good representative for this concentration, although this is only partially supported by the laboratory–field comparison presented in this article. On the other hand, there is no empirical evidence presented here indicating unacceptable effects at the HC5. Figure 5 shows that for atrazine and linuron clear effects start to emerge above the HC5 of the chronic SSD, which is a result of their persistence and chronic exposure used in the semi-field experiments (Tables 3 and 5). For all other chemicals clear effects start to be recorded at concentrations higher than the HC5 of the acute SSD, reflecting the exposure regimes used in the semi-field experiments. These results indicate that if the exposure regimes match, sensitivity of sensitive species as estimated in the laboratory and described by SSD can be used for predicting direct effects in the field. Concerning the use of SSD, the Technical Guidance Document on Risk Assessment (TGD; EC 2003) specifies that SSDs should only be constructed from no observed effect concentrations (NOECs) from long-term/chronic studies, by using the most sensitive endpoint for each species, or the geometric mean of multiple endpoints. Our findings illustrate that SSDs generated using acute EC50s can be used in case of a short-term exposure regime.

The last objective was to compare the SSDs and the predictions provided by PERPEST for atrazine and 2,4-D. This comparison shows a good relation between the lower tail of the SSD curve and the probability of clear effects on community structure. For both chemicals the lower probabilities on clear effects correspond with the lower tail of the chronic SSD, reflecting the persistence of the chemicals. Above the threshold of effects, the results of the PERPEST model have more relevance to the "real field" because they also integrate indirect effects. PERPEST can also provide more detail; in this article the results of PERPEST are summarized in effects on functional and structural endpoints but the model provides predictions for one functional and seven structural endpoints and can also distinguish between short-term and long-term effects (Van den Brink *et al.* 2002a). Because predictions made by PERPEST are based on published microcosm and mesocosm experiments, its validity is limited to well-studied toxicological mode of actions like acetylchlolinesterase inhibition, auxine simulation, photosynthesis inhibition, and synthetic pyrethroid action.

From the results presented in this article we conclude that only sensitive species reflecting the toxicological mode of action should be included in the species sensitivity distributions used for the ecological risk assessment of herbicides. In case of most herbicides data on primary producers should be included, but in some special cases

like 2,4-D only data on submerged macrophytes should be included. We found no systematic difference in sensitivity between standard test species and other species belonging to the primary producers toward herbicides. The geometric mean ACR for all compounds was 8.9 at the median HC5 level indicating that using an ACR of 10 for risk assessment is acceptable, but is less suitable when a worst case approach is adopted. The lower limit of the acute HC5 and the median value of the chronic HC5 were protective of adverse effects in aquatic micro/mesocosms even under a long-term exposure regime. A detailed comparison between effects observed in the laboratory and (semi-)field also showed a great concordance in the concentrations at which the effects start to emerge, but the rate of recovery and occurrence of indirect effects is not taken into account when using laboratory data. Recovery and indirect effects are considered when using the PERPEST model to make predictions of pesticide effects.

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Appendix . Optimized parameter values used by the PERPEST model to predict the effects of atrazine and 2,4-D over a concentration range (see Figure 6 for output and www.perpest.alterra.nl or www.perpest.wur.nl for a free download of the model).

Parameter	Atrazine	2,4-D
Weight using		
Toxic units	10	10
Molecule group	9.49	6.03
Substance	4.24	2.51
Select using		
Nearby toxic unit	8.25	6.37
Mode of action	Yes	Yes
Standardization method	Normalize	Normalize
Dissimilarity measure	Euclidean distance	Euclidean distance
Prediction method	Inverse distance	Inverse distance
# of nearest points	93	88
Distance power	-4.98	-4.24
Min. distance (or NAN)	3.06	2.50
Max. distance (or NAN)	6.55	4.48
Critical dissimilarity (%)	100	100