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A critical examination of the protection level for primary producers in the first tier of the aquatic risk assessment for plant protection products

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Abstract

Background The aim of environmental risk assessment (ERA) for pesticides is to protect ecosystems by ensuring that specific protection goals (SPGs) are met. The ERA follows a prospective tiered approach, starting with the most conservative and simple step in risk assessment (RA) (so-called tier 1) using the lowest available appropriate endpoint derived from ecotoxicological tests. In 2015, for the tier 1 RA of aquatic primary producers, the recommendation was changed from using the lowest of the 50% inhibition (EC50) values based on biomass (area under the curve—E_bC_{50}), increase in biomass (yield- E_yC_{50}) or growth rate (E_rC_{50}) to only using the growth rate inhibition endpoint (E_rC_{50}) because it is independent of the test design and thus more robust. This study examines the implications of this such on the level of conservatism provided by the tier 1 RA and evaluates whether it ensures a suitable minimum protection level.

Results Our analysis shows that replacing the lowest endpoint with the growth rate inhibition endpoint while maintaining the assessment factor (AF) of 10 significantly reduces the conservatism in the tier 1 RA. Comparing protection levels achieved with different endpoints reveals that the current assessment is less protective. To maintain the previous level of protection, and since the protection goals have not changed, we recommend to multiply the default AF of 10 by an extra factor of minimum 2.4 in the tier 1 RA based on E_rC_{50}. Independently of the endpoint selected in tier 1 RA, several issues in the general RA of pesticides contribute to uncertainties when assessing the protection levels, e.g., lack of appropriate comparison of the higher tier experimental studies (i.e., best achievable approximation of field situation, so-called surrogate reference tier) with field conditions or the regulatory framework's failure to consider realistic conditions in agricultural landscapes with multiple stressors and pesticide mixtures.

Conclusions We advise to consider adjusting the risk assessment in order to reach at least the previous protection level for aquatic primary producers. Indeed continuing using an endpoint with a higher value and without adjustment of the assessment factor is likely to jeopardize the need of halting biodiversity loss in surface waters.

Keywords Micro-/mesocosm study, Tier 1 standard test, Tiered approach, Regulatory acceptable concentration (RAC), Endpoint, E_rC_{50}, $E_{b/y}C_{50}$, Calibration, Algae, Macrophytes

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Background

The EU regulation (EC) No. 1107/2009 concerning the placing of plant protection products (PPPs) on the market [1] states that a PPP "shall have no unacceptable effects on the environment, having particular regard to… its impact on non-target species… its impact on biodiversity and the ecosystem".

The environmental risk assessment (ERA) for active substance approval and PPP authorization is based on a prospective and tiered approach. The tier 1 risk assessment (RA) relies on the data requirements outlined in Commission Regulation (EU) 283/2013 for the approval of active substances contained in PPPs [2] and Commission Regulation (EU) 284/2013 for the authorization of PPPs [3]). For example, the requirements for substances with an herbicidal mode of action (MoA) are to perform ecotoxicological tests on a green alga, an alga of a second taxonomic group, and usually on a macrophyte (mostly *Lemna* spp.) following established tests guidelines (e.g., OECD, US EPA). The toxicity endpoints derived from the data obtained in these tests via regression models (dose–response models) are the effect concentrations (EC_x) corresponding to e.g. 10, 20 and 50% of growth inhibition (i.e., EC_{10}, EC_{20}, EC_{50} values). For quantification of the growth inhibition, specific proxies are used to measure the biomass of the primary producer, e.g., cell counts for algae, number of fronds and at least one additional response variable (e.g., dry weight) for *Lemna* spp. But there is no specification provided on the type of estimates that should be used to calculate the EC_x values. For example, endpoints based on the response variable biomass could be calculated as (i) reduction in biomass, calculated from yield; (ii) the integral biomass "Area Under the Curve-AUC", or (iii) inhibition of the average specific growth rate with the inhibition of growth, expressed as the logarithmic increase in biomass during the exposure period, being the test endpoint. For simplicity, these are referred to as yield (E_yC_{50}), AUC or "biomass" (E_bC_{50}) and growth rate (E_rC_{50}) endpoints.

The previous guidance document recommendation for regulatory decisions about PPP and aquatic primary producers was that the lowest endpoint value among yield (E_yC_{50}), AUC or "biomass" (E_bC_{50}) and growth rate (E_rC_{50}) should be chosen [4]. The current recommendation, however, is to always use the endpoint growth rate E_rC_{50}. To proceed with risk calculation, the endpoint value is divided by a default assessment factor (AF) of 10, in line with the so-called Uniform Principles for assessment of active substances in PPP [5]; this is irrespective of its relationships to other toxicity data determined in the test or in other tests. The AFs are used to account for uncertainties in the risk assessment process (and thus can vary between tiers); e.g., for tier 1, uncertainties cover for extrapolations from laboratory to field conditions and from single individuals to populations. This leads to the derivation of the regulatory acceptable concentration (RAC), which is the maximum surface water concentration resulting from pesticide application that can be tolerated, as it should have no unacceptable impacts on surface water communities. This shift to the use the endpoint growth rate E_rC_{50} is from the guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (so-called aquatic guidance document AGD [6] also referred to as SANTE-2015-00080) and also in line with OECD Guidelines (e.g., OECD TG 201). This shift has been justified since the growth rate E_rC_{50} endpoint is a more robust estimate compared to the AUC/"biomass" or yield (E_bC_{50} or E_yC_{50}) endpoints; indeed, the latter are more dependent on the growth rate of the test species, test duration, and other test design elements [7]. These aspects were raised in [6] in a section for algae as "direct use of the biomass concentration without logarithmic transformation cannot be applied to an analysis of results from a system in exponential growth" (see [8]), and in a section for macrophytes as "it [growth rate] is more robust considering varying test conditions. It should be calculated (as E_rC_{50}) on the basis of the most sensitive endpoint."

This shift in the decision on which endpoint to select for the risk assessment may have significant implications as it may result in the derivation of a different RAC compared to before and, consequently, to a shift in the level of conservativeness of regulatory decisions. It is therefore important to evaluate the impact of selecting E_rC_{50} values or E_bC_{50}/E_yC_{50} values for the risk assessment, in order to determine the level of conservatism of the tier 1 when comparing past and current practices. Since the consequences of the changes in the guidance were not systematically assessed, we performed this analysis to assess if the shift in the guidance on which endpoint to select has direct implications for decision-making.

The ultimate aim of the aquatic ERA is to ensure an adequate protection of populations and communities in surface waters. Therefore, decisions taken at every step of the tiered approach are deemed to correctly indicate whether the specific protection goals (SPGs) are met or not. Ensuring that the level of protection set in a specific tier is achieved should be ideally done by checking if the RAC values derived at that tier for each pesticide have really only negligible effects on populations in the field (i.e., the reference tier). However, this is not directly applicable, since the prospective approach used in the PPP regulation aims at predicting the environmental risks of a PPP before it is placed on the market. A so-called surrogate reference tier—representing the best achievable approximation to the field situation—is thus used to link the ERA with the SPGs [9].

For aquatic ERA, the surrogate reference tier typically refers to experimental model ecosystems (i.e., micro-/mesocosm studies) performed with so far as possible natural assemblages of organisms, especially for phytoplankton and invertebrates communities. These experiments are used in higher steps of the risk assessment schemes (i.e., for aquatic organisms, tier 3) and are deemed to provide a more realistic representation of ecosystem responses to applied plant protection products. However, the results of such experiments are also subject to uncertainties, addressed using an AF which is primarily intended to account for variations between field situations.

To ensure the maintenance of the protection level provided across the tiered system, it is crucial to compare RAC values from different tiers. Assuming that the Tier-3 RACs derived from aquatic micro- and mesocosm experiments are not under or over-protective, they can be used to evaluate the adequacy of lower tier RAC values (Tier-1 RACs). The change of endpoint used in the aquatic risk assessment of primary producers for deriving Tier-1 RACs between past and current practices makes such comparison essential. Indeed, lower tier risk assessment methods according to Regulation (EC) No. 1107/2009 should be calibrated with the reference tier to meet SPGs [9].

Such comparisons have been previously undertaken for some insecticides [10, 11], fungicides [12] and herbicides [13–15]. In their work on aquatic primary producers, van Wijngaarden and Arts [13] presented some results showing that the protection level is maintained if the growth rate endpoint E_rC_{50} is taken as the regulatory endpoint with an AF of 10; thus they did not identify a need to correct or adjust this practice. This is not in line with our previous results [16, 17]. With the current work, we further contribute to elucidate the protection level for aquatic primary producer exposed to pesticides, by expanding the dataset and by exploring the level of protection achievable in the EU when using in the risk assessment the E_rC_{50} and the default AF 10 as compared to other options.

The first aim of this work is to determine how using always the growth rate endpoint (E_rC_{50}) instead of the lowest available endpoint in the aquatic RA shifts the level of conservatism of the tier 1 RA for primary producers, and whether any particular species group is affected. The second aim is to assess the protection levels achieved when based on the RACs derived from the E_rC_{50} endpoints and from the lowest E_xC_{50} endpoints by comparing them to the surrogate reference tier. The last aim is to assess whether the level of conservatism achieved by the current tier 1 risk assessment is acceptable for achieving the protection level intended by the SPGs and thus for adequately protecting aquatic communities in the field.

Methods

Endpoints from standard toxicity tests (tier 1 data)

Data collection

Ecotoxicological data from active substance (a.s.) approvals were selected in December 2021 using the COM Pesticide Data base (https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en) and the most recent publicly available information from the European Food Safety Authority, the list of endpoints of the EFSA conclusions of the respective substances (so-called EFSA LoEPs; see Additional file 1: Table S1). The focus was exclusively on a.s. with herbicidal and fungicidal MoA, that can affect non-target algae and aquatic macrophytes (in total 590 entries).

The collected data set comprises the approval status and the pesticide class of each a.s., the tested species and its affiliation to the respective organisms' group (algae or macrophytes).

The E_xC_{50} values from standard toxicity tests relevant for the tier 1 aquatic RA for primary producers were collected from the EFSA LoEPs. The endpoints corresponded to yield (E_yC_{50}), AUC (area under the growth curve, E_bC_{50}) and growth rate (E_rC_{50}).

Criteria for selection of data

An E_rC_{50} value as well as an E_bC_{50} and/or E_yC_{50} value ($E_{b/y}C_{50}$) should be reported per toxicity test to calculate the ratios of E_rC_{50}/E_bC_{50} and/ or E_rC_{50}/E_yC_{50}.

E_xC_{50} values were selected only when they were not above the highest concentration tested in the toxicity test. Only non-censored endpoints were considered.

If more than one response variable was reported for a toxicity test (e.g., frond numbers and total dry mass for *Lemna* sp.), the endpoint with the lowest E_xC_{50} values was selected. Note that the lowest E_rC_{50} value and the lowest $E_{b/y}C_{50}$ value were usually derived from the same response variable.

Only endpoints from toxicity tests with the a.s. were selected (i.e., no endpoints from toxicity tests with formulations were used).

The lowest calculated E_xC_{50} values from independent toxicity tests (i.e., other tests than the test delivering the endpoint for risk assessment but also listed in the EFSA LoEPs) performed with the same a.s. were considered.

Statistical analysis

For algae, the statistical analysis is performed on the best represented test species, i.e., *Anabaena flos-aquae* (freshwater cyanobacteria), *Desmodesmus subspicatus* (freshwater green algae), *Navicula pelliculosa* (freshwater diatom), *Raphidocelis subcapitata* (freshwater green algae; formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*) and *Skeletonema costatum* (marine diatom).

For macrophytes, the statistical analysis is also performed on the best represented test species, i.e., *Lemna gibba* and *Lemna minor* (duckweed species).

For the statistical analysis of differences between approved and not approved a.s., data points of a.s. with the status "pending" were excluded (not enough data points available; see asterisk * in Table 1).

For the statistical analysis of differences between a.s. with herbicidal and fungicidal MoA, data points of a.s. classified as both herbicide and fungicide (HB, FU) were excluded since they could not be assigned to one of the two groups (see asterisks ** in Table 1).

Table 1 Available dataset for ratios calculated with the endpoints growth rate (E_rC_{50}) and the biomass-related endpoints- area under the curve (E_bC_{50}) or yield (E_yC_{50}) endpoints- ratios E_rC_{50}/E_bC_{50} and E_rC_{50}/E_yC_{50}- for various test species (algae and aquatic macrophytes), status of active substances (approved, not approved) and their class (herbicides or fungicides)

Ratio	Test category		*n*	Median	± 95% CI	Statistical test	*p*-value
Algae							
E_rC_{50}/E_bC_{50}	Test species	All species	177	2.33	2.08–2.53	–	–
		Anabaena flos-aquae[a]	22	2.43	1.71–3.18	Kruskal–Wallis test	0.721
		Desmodesmus subspicatus[b]	20	2.30	1.69–2.70		
		Raphidocelis subcapitata[b]	98	2.37	2.03–2.64		
		Navicula pelliculosa[c]	27	2.17	1.77–2.67		
		Skeletonema costatum[c]	10	1.92	1.22–4.10		
	Status*	Approved	128	2.16	1.98–2.45	Wilcoxon rank sum test	0.066
		Not approved	46	2.55	2.10–3.54		
	Class**	Herbicides	112	2.27	2.08–2.55	Wilcoxon rank sum test	0.946
		Fungicides	63	2.37	1. 91–2.64		
E_rC_{50}/E_yC_{50}	Test species	All species	52	2.96	2.39–3.88	–	–
		Anabaena flos-aquae[a]	6	2.69	1.89–7.62	Kruskal–Wallis test	0.643
		Desmodesmus subspicatus[b]	3	2.59	–		
		Raphidocelis subcapitata[b]	37	3.65	2.39–4.66		
		Navicula pelliculosa[c]	2	2.60	–		
		Skeletonema costatum[c]	4	2.03	–		
	Status*	Approved	31	2.61	1.89–4.19	Wilcoxon rank sum test	0.069
		Not approved	19	3.38	2.67–6.25		
	Class	Herbicides	32	3.72	2.45–4.80	Wilcoxon rank sum test	0.151
		Fungicides	20	2.59	1.75–3.38		
Macrophytes							
E_rC_{50}/E_bC_{50}	Test species	*Lemna* spp.	40	1.60	1.18–1.92	–	–
	Status*	Approved	26	1.56	1.00–1.80	Wilcoxon rank sum test	0.216
		Not approved	13	2.16	0.64–3.58		
	Class	Herbicides	35	1.64	1.20–2.00	Wilcoxon rank sum test	0.513
		Fungicides	5	1.16	–		
E_rC_{50}/E_yC_{50}	Test species	All species	28	2.08	1.79–2.45	–	–
	Status	Approved	20	2.00	1.79–2.34	Wilcoxon rank sum test	0.304
		Not approved	8	2.69	1.29–7.07		
	Class**	Herbicides	23	1.91	1.74–2.34	Wilcoxon	0.082
		Fungicides	4	2.69	–	Rank sum test	

Information is given: "*n*" as number of entries, medians with ± 95% confidence intervals (CI), statistical tests used for comparing different groups and outcomes as *p*-values

*Status "Pending" was excluded from further statistical analysis (for details, see Methods)

**Substances labeled as both herbicide and fungicide (HU, FU) are excluded from further statistical analysis (for details, see Methods)

[a] Cyanobacteria, [b]Green algae, [c]Diatom

The statistics applied to compare the different groups were the non-parametric Kruskal–Wallis test and Wilcoxon rank sum test for unpaired data and the Wilcoxon signed-rank test for paired data with the null hypothesis significance testing (NHST), since the data did not fulfill the requirements for parametric testing (i.e., the assumption of homoscedasticity analyzed with Levene's test and normality checked with Shapiro–Wilk test and quantile–quantile plots). The significance level is set at a *p*-value of 0.05 throughout the entire study.

Mathematical relationship between yield/AUC inhibition and 50% growth rate inhibition

The relationship between yield- and AUC-inhibition with the 50% growth rate inhibition (E_rC_{50}) was investigated, considering the generic formulas for yield and AUC from the current and the previous growth inhibition test for algae, respectively [18]. The mean values for yield and AUC for the control replicates were calculated assuming exponential growth rates (μ). For the treatment group, yield and AUC were calculated assuming a 50% inhibition of these exponential growth rates ($0.5 * \mu$). The resulting formulas presented in the Additional file 1 were used to examine the impact of varying exponential growth rates and test lengths on the inhibition of yield and AUC compared to a fixed E_rC_{50}.

Lower and higher tier endpoints for comparing Tier-1 and Tier-3 RACs

Data collection

The data are from the available EFSA LoEP for each a.s.. In addition to the endpoints from standard toxicity tests used for the tier 1 RA for primary producers (i.e., algae or aquatic macrophytes), the endpoints from reliable micro-/mesocosm studies used in tier 3 RA are considered. The tier 3 endpoints were identified for 18 a.s. from studies often performed with representative mono-formulations of PPPs. Thus, only this subset for the 18 a.s. from the tier 1 dataset established above (under section Endpoints from standard toxicity tests (tier 1 data)) was used in this analysis. These include 15 herbicides and 3 fungicides. For the latter group, it should be noted that primary producers may not be the most sensitive aquatic organism group and thus may not always trigger the highest aquatic risk. This is the case for dodine, for which the tier 1 risk assessment (RA) is driven by invertebrates; however, the tier 3 values are similar for both invertebrates and primary producers. In the case of spiroxamine and fenpropidin, the tier 1 risk assessment is driven by the primary producers and the tier 3 values derived are also for primary producers.

Criteria for selection of data

In most cases, the lowest E_rC_{50} values and the lowest $E_{b/y}C_{50}$ values originate from the same standard toxicity tests and are used for the tier 1 RA during the EU a.s. evaluation. However, in a few cases, there were some deviations either in the reporting or the calculation of endpoints, e.g., in absence of appropriate E_xC_x value in the EFSA LoEP according to our criteria (see Additional file 1: Tables S2 and S3).

Tier-1 RACs based on E_rC_{50} and the $E_{b/y}C_{50}$ values for an a.s. were calculated using the data from the reliable standard toxicity test delivering the lowest endpoint used for the risk assessment in the respective EFSA LoEP and applying a default Assessment Factor (AF) of 10. Before implementation of the aquatic guidance document [6] in January 2015, the default AF of 10 was applied to the lowest endpoint (i.e., lowest E_xC_{50} value).

Tier-3 RACs used for the analyses were calculated using the data from micro-/mesocosm studies applying the recommended AF of 2 or 3 to the effect threshold option (ETO) based on the effect class 1 (no effect)/ or class 2 (slight effects), and using the NOEC/LOEC (No/Lowest Observed Effect Concentration) to derive an ETO-RAC in most cases. In the cases when the NOAEC (No Observed Adverse Effect Concentration) are the only data available, the Tier-3 RACs were calculated by applying the recommended AF of 3 or 4 to the ecological recovery option (ERO) based on effect class 3 (3 A or B; i.e., pronounced effects followed by recovery within 8 weeks after first or last application), using the NOAEC to derive an ERO-RAC (see section 9.3 in [6]). Also, when the calculation based on a NOAEC is leading to a lower Tier-3 RAC value- i.e., a lower ERO-RAC than the ETO-RAC- the Tier-3 RAC used in the analysis is the ERO-RAC. The lowest Tier-3 RAC is selected independently of the group of primary producers that triggered the tier 1 RA, i.e., algae or macrophytes; it is usually based on endpoints (NOEC or NOAEC) expressed in nominal concentrations.

Statistical analysis

For the comparison of Tier-1 RACs of active substances that are based on E_xC_{50} values for aquatic primary producers with the corresponding Tier-3 RACs, Tier-1 RAC values were plotted against Tier-3 RAC values using the R packages *ggplot2* and *gridExtra*. A diagonal black line with a ratio of 1:1 was added illustrating the comparison between RACs of various tiers.

For the cumulative distribution analysis of the protection level of Tier-1 RACs, the Tier-1 RAC/Tier-3 RAC ratios were fitted to various distribution models via maximum likelihood estimation by using the R package *ssdtools* [19]. Model averaging was performed by weighing models based on Akaike's Information Criterion (AIC), and the resulting weighted averaged distribution curve with 95%-confidence intervals (CI) was plotted via the R package *ggplot2*. R version 4.1.1 for Windows was used for all analyses and creation of all figures.

Results

Differences between lowest endpoints and growth rate endpoints

Overall differences

For algae, the largest dataset available is for the comparisons of E_rC_{50} and E_bC_{50} endpoints. The total number of substance data pairs for the calculation of E_rC_{50}/E_bC_{50} ratios was 177, including 112 herbicides and 63 fungicides, with most of them being currently approved in the EU ($n = 128$).

The total number of substance data pairs for the calculation of E_rC_{50}/E_yC_{50} ratios was 52, including 32 herbicides and 20 fungicides, with 31 of them being currently approved in the EU.

The dataset as shown in Table 1 was dominated by results from tests with *R. subcapitata* ($n = 98$). This is followed by *N. pelliculosa* ($n = 27$), *A. flos-aquae* ($n = 22$), *D. subspicatus* ($n = 20$) and *S. costatum* ($n = 10$).

The comparison based on these large datasets shows that the E_rC_{50} values are generally higher than the $E_{b/y}C_{50}$ values (see Fig. 1). This is indicated by the ratios of E_rC_{50}/E_bC_{50} (i.e., 10th percentile = 1.22; median = 2.33; mean = 3.44; 90th percentile = 5.63) and by the ratios E_rC_{50}/E_yC_{50} (i.e., 10th percentile = 1.60; median = 2.96; mean = 3.66; 90th percentile = 6.54; see Fig. 1). The NHST using the Wilcoxon signed-rank test shows statistically significant differences between E_rC_{50} values and E_bC_{50} ($p < 0.001$) and E_yC_{50} values ($p < 0.001$), respectively.

For macrophytes (i.e., *L. gibba* and *L. minor*), the largest dataset available is also for the comparison of E_rC_{50} and E_bC_{50} endpoints. The total number of available substance data pairs for the calculation of E_rC_{50}/E_bC_{50} ratios was 40, including 35 herbicides and 5 fungicides, with most of them being currently approved in the EU ($n = 26$).

The total number of available substance data pairs for the calculation of E_rC_{50}/E_yC_{50} ratios was 28, including 23 herbicides and 5 fungicides, with 20 of them being currently approved in the EU.

The comparison shows that E_rC_{50} values are generally higher than the $E_{b/y}C_{50}$ values, as indicated by the ratios of E_rC_{50}/E_bC_{50} (i.e., mean = 1.79; median = 1.6; 90th percentile = 3.23) and by the ratios E_rC_{50}/E_yC_{50} (i.e., mean = 2.42; median = 2.08; 90th percentile = 3.98; Fig. 2). The NHST using the Wilcoxon signed-rank test shows statistically significant differences between E_rC_{50} values and E_bC_{50} ($p = 0.001$) and E_yC_{50} values ($p < 0.001$), respectively.

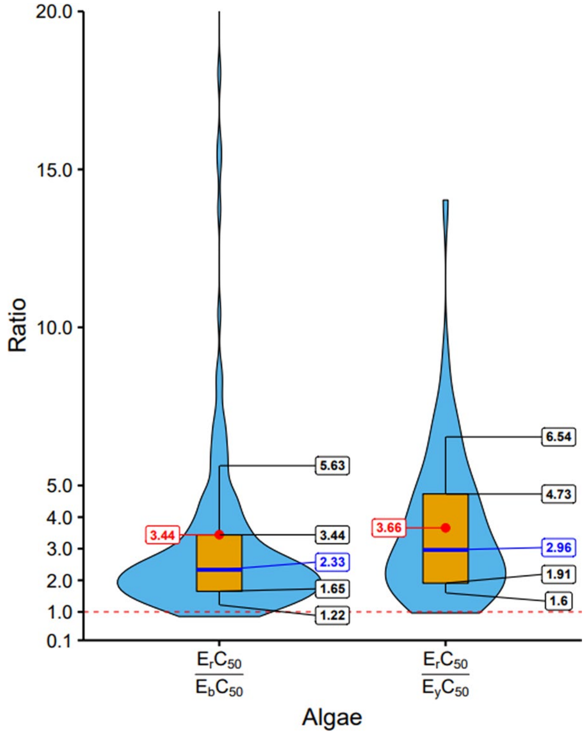

Fig. 1 Ratios between available endpoints for algae: growth rate inhibition (E_rC_{50}) and corresponding endpoints: area under the curve-AUC/"biomass" (E_bC_{50}) or yield (E_yC_{50}). Violin plots (in blue) and boxplots (orange boxes) display the distribution of E_rC_{50} values divided by their corresponding E_bC_{50} values (left; $N = 177$) or E_yC_{50} values (right; $N = 52$). The endpoints are from different algae species from the list of endpoints of the EFSA conclusions of various herbicides and fungicides (for more details, see Additional file 1: Table S1). The boxplots illustrate the 25th, 50th (dark blue line) and 75th percentiles; whiskers illustrate the 10th and 90th percentiles; means are shown as red dots. For statistical analyses, see Table 1

Differences according to species, status of the substances and their mode of action

The data were analyzed to investigate whether factors such as the specific test species, the approval status of the active substances (a.s.) and the class of active substances (fungicides and herbicides) have an impact on the calculated ratios between endpoints: E_rC_{50} (growth rate) and the corresponding E_bC_{50} (Area Under the Curve, AUC) or E_yC_{50} (yield) endpoints (i.e., E_rC_{50}/E_bC_{50} and E_rC_{50}/E_yC_{50}). Table 1 indicates that no statistically significant differences were identified between the various species of algae belonging to different groups (cyanobacteria, green algae, diatoms; Kruskal–Wallis test, *p*-values > 0.05) nor between the two species of *Lemna* spp. (Wilcoxon rank sum test, *p*-values > 0.05).

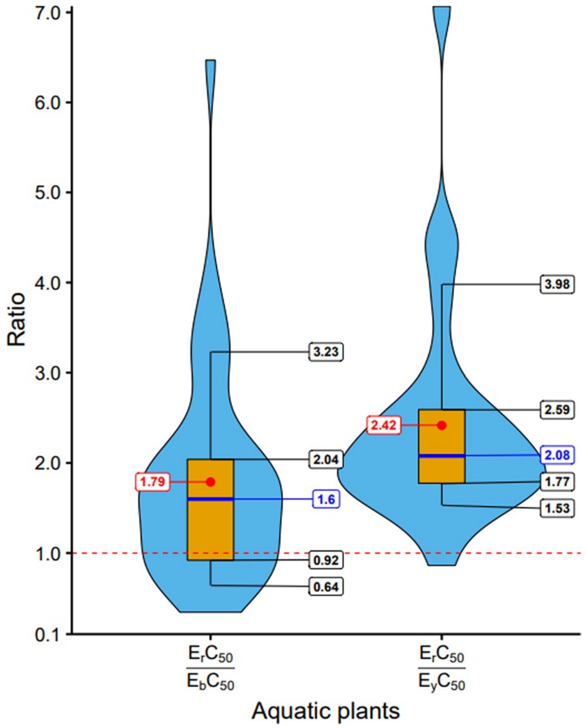

Fig. 2 Ratios between available endpoints for macrophytes (aquatic plants): growth rate inhibition (E_rC_{50}) and corresponding endpoints: area under the curve/"biomass" (E_bC_{50}) or yield (E_yC_{50}). Violin plots (in blue) and boxplots (orange boxes) display the distribution of E_rC_{50} values divided by their corresponding E_bC_{50} values (left; $N=40$) or E_yC_{50} values (right; $N=28$). The endpoints are from the species *Lemna gibba* and *Lemna minor* from the list of endpoints of the EFSA conclusions of various herbicides and fungicides (for more details, see Additional file 1: Table S1). The boxplots illustrate the 25th, 50th (dark blue line) and 75th percentiles; whiskers illustrate the 10th and 90th percentiles; means are shown as red dot. For statistical analyses, see Table 1

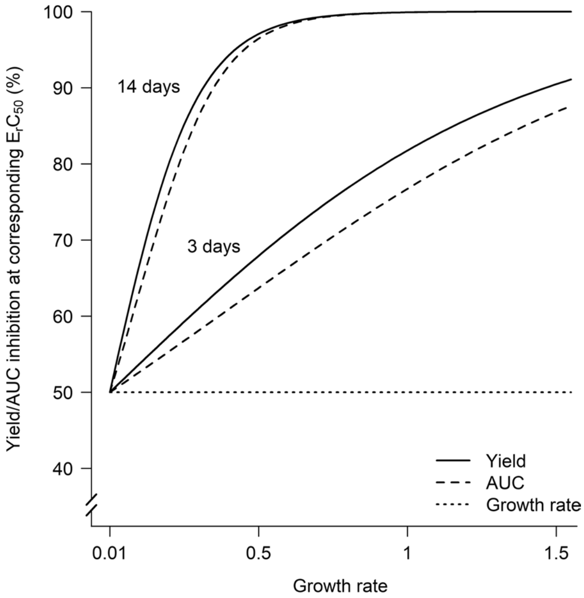

Fig. 3 Relationship between 50% inhibition of growth rate (dotted line) and the inhibition of biomass measured as yield (continuous line) and AUC (area under the curve, dashed line) for exponentially growing species, shown on the y-axis. The relationship depends only on the uninhibited exponential growth rate (control growth rate) shown on the x-axis and the test duration shown exemplary as 3 and 14 days. For comparison, growth rates of 0.1, 0.4, 0.7 and 1 correspond to doubling times of 7, 1.7, 1 and 0.7 days, respectively. For further information on the formulas used, see Additional file 1

Similarly, there is no statistically significant differences identified between the two classes of active substances, nor between the data for approved or not approved active substances.

Mathematical relationship between yield/AUC inhibition and 50% growth rate inhibition

The mathematical relationship between biomass inhibition (AUC" and yield) and 50% inhibition growth rate (E_rC_{50}) is shown in Fig. 3. It illustrates that a 50% inhibition of growth rate (ErC50) results in more than 50% inhibition on biomass. Consequently, the Ey/bC50 are observed at lower test concentrations compared to the ErC50. The extent of biomass inhibition at 50% growth rate inhibition is influenced by the growth rate of the species under assessment and the duration of the test. Higher growth rates and longer test durations lead to greater inhibition of biomass at 50% growth rate inhibition. Biomass inhibition for an exponentially growing species usually exceeds clearly that of the growth rate.

For algae, *Lemna* ssp., and *Myriophyllum spicatum*, the test durations and minimum required growth rates according to the OECD test guidelines (e.g., [16]) are as follows: algae, test duration of 3 days with a minimum growth rate of 0.92 day^{-1}; *Lemna sp.*, test duration of 7 days with a minimum growth rate 0.275 day^{-1}; *M. spicatum*, test duration of 14 days with a minimum growth rate 0.05 day^{-1}.

Comparison of protection levels achieved when using RACs derived from different tier 1 endpoints

The regulatory acceptable concentrations (RACs) derived in the first tier risk assessment (Tier-1 RACs) based on standard laboratory studies can be expressed as different endpoints (inhibition of growth rate E_rC_{50} or lowest endpoint (AUC or yield) $E_{b/y}C_{50}$). These Tier-1 RACs were compared to the RAC values of the higher tier RA (Tier-3 RACs, based on data from micro-mesocosm studies, and considered as surrogate reference tier). This was done in order to assess the protection levels achieved when using these different endpoints. The comparison was performed for 18 substances (15 herbicides, 3 fungicides). The values used for Tier-1 RACs were either from algae or macrophyte tests, with the lowest of the two being selected. The values used for Tier-3 RACs were mostly based on the effect threshold option (ETO-RACs), i.e., on the NOEC or LOEC values; in the cases when only the NOAEC was delivered, they were then based on the ecological recovery option ERO-RACs (for more information, see Additional file 1: Table S2). Note that for isoproturon, both a NOEC for aquatic plants and a NOAEC for algae with a same value (13.8 μg/L) but different AF (2 and 4, respectively) were reported; the resulting RAC for algae was selected.

The comparisons of Tier-1 RACs based on various options for the selected endpoint in tier 1 (i.e., E_rC_{50},$E_{b/y}C_{50}$ and E_rC_{10} with the default AF of 10) and Tier-3 RACs are illustrated in Fig. 4. The endpoints used to derive the Tier-1 RACs refer either to macrophyte or to algae since the lowest endpoint available drives the risk assessment for aquatic primary producers at tier 1. The datapoints above the line representing the 1:1 ratio of both RAC values indicate that the tier 1 effect assessment is less conservative than the corresponding tier 3 assessment (i.e., Tier-1 RACs are higher than Tier-3 RACs) and thus the general principle of the tiered approach is not fulfilled (see [6]). The tier 3 risk assessment should inform on the effects of a pesticide in the field in a more realistic way than the tier 1 assessment. Thus, such tier 1 cases are considered as not sufficiently protective.

This is the case for a large number of a.s. when Tier-1 RACs values are derived as in the current approach (i.e., E_rC_{50} values and the default AF of 10); indeed 9 out of 20 a.s. are either above the line of the 1:1 ratio or borderline.

When the Tier-1 RACs are derived as previously, i.e., based on the lowest E_xC_{50}—that are the $E_{b/y}C_{50}$ values (Fig. 4B), this situation occurred only for 2 out of 20 substances. This indicates a shift towards a lower protection level between the current approach (always use the E_rC_{50}) compared to the previously agreed approach and protection level (use the lowest available endpoint for aquatic primary producers).

One approach to reach the previously agreed level of protection and consolidate the tiered approach could be to consider using the E_rC_{10} endpoint associated with the default AF of 10 instead of the E_rC_{50} endpoints (data in Additional file 1: Tables S2 and S3; Fig. 4C). However, this approach was not pursued since it has some drawbacks. Another approach (increase of the final AF applied to E_rC_{50} endpoints, by multiplying the default AF of 10 by an extra factor) is described in the section below.

Assessing protection levels achieved according to the different approaches in tier 1

The comparison of Tier-3 RACs and Tier-1 RACs for each active substance (a.s.) results in a specific ratio, which varies among different a.s.. The distribution of these ratios can be represented as a cumulative distribution function since it follows a unimodal distribution. This function provides the ratio at which a certain percentage of a.s. delivers a cumulative probability for a minimum level of protection considered as suitable according to the SPGs (Fig. 5). The ratio corresponds to the factor by which the default assessment factor currently set at 10 in the tier 1 risk assessment would need to be multiplied and show the percentage of active substances that reach a level of protection that is considered as the minimum suitable level.

When the Tier-1 RACs were derived from the lowest endpoint (i.e., $E_{b/y}C_{50}$ value of each a.s.) and the default AF of 10, the cumulative probability of a minimum suitable protection level for aquatic primary producers for the selected dataset was of 87.5% (Fig. 5A). By contrast, when based on E_rC_{50} endpoints, it is 69.2% (Fig. 5B). In order to achieve again a protection level of approx. 88% while using E_rC_{50} endpoints, a shift of a factor of 2.37 would be needed, e.g., by using an extra factor of approx. 2.4 combined with the default AF of 10 to reach an overall AF of approx. 24.

It is also interesting to note that the protection level achieved previously (i.e., when based on lowest endpoint, i.e., $E_{b/y}C_{50}$ values and an AF of 10) was not optimum since it was ensured in approx. 88% of cases "only". In order to fulfill the protection level intended by the SPGs in, e.g., 95% or 99% of cases by using Tier-1 RACs derived from the $E_{b/y}C_{50}$ values, an overall AF of approx. 21 and 85, respectively, would have been needed. With the current approach, to reach a protection level in, e.g., 95% or 99% of cases by using Tier-1 RACs derived from the E_rC_{50}, an overall AF of approx. 48 and 190, respectively, would be needed. It should be noted that these overall factors are variable, especially when referring to 99% of cases.

(See figure on next page.)

Fig. 4 Comparison of regulatory acceptable concentrations (RACs) at different tiers, represented as first tier RACs (Tier-1 RACs) versus higher tier RACs (Tier-3 RACs). **A** Tier-1 RACs based on the growth rate inhibition endpoint (E_rC_{50}) for each active substance (a.s.) and the default AF of 10 (current approach); **B** Tier-1 RACs based on the lowest endpoint for each a.s. and the default AF of 10 (previous approach). Please note that the lowest endpoints correspond always to the area under the curve or yield ($E_{b/y}C_{50}$) since the values were below the E_rC_{50} values; **C** Tier-1 RACs based on the growth rate inhibition endpoint (E_rC_{10}) for each active substance (a.s.) and the default AF of 10

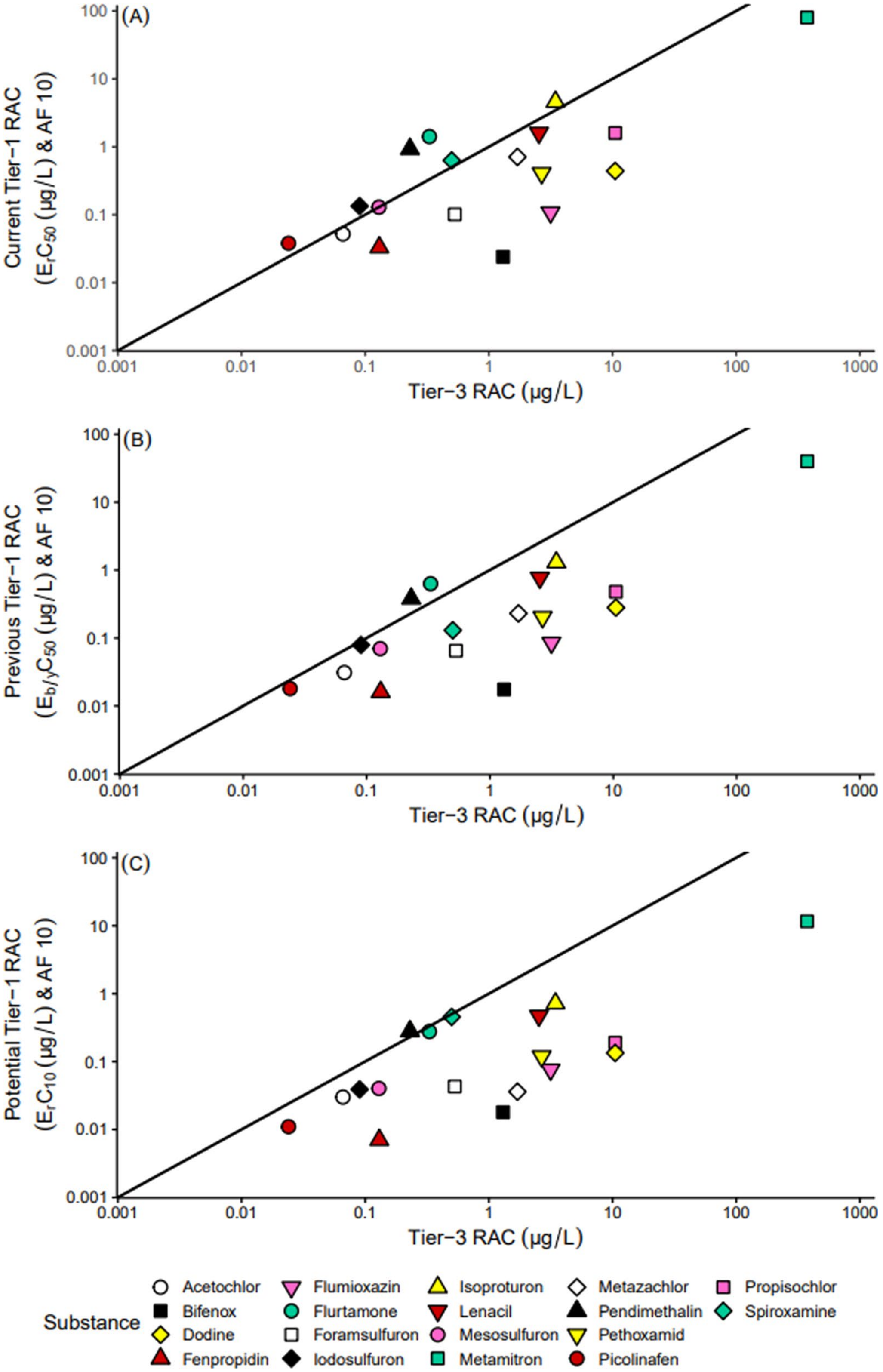

Fig. 4 (See legend on previous page.)

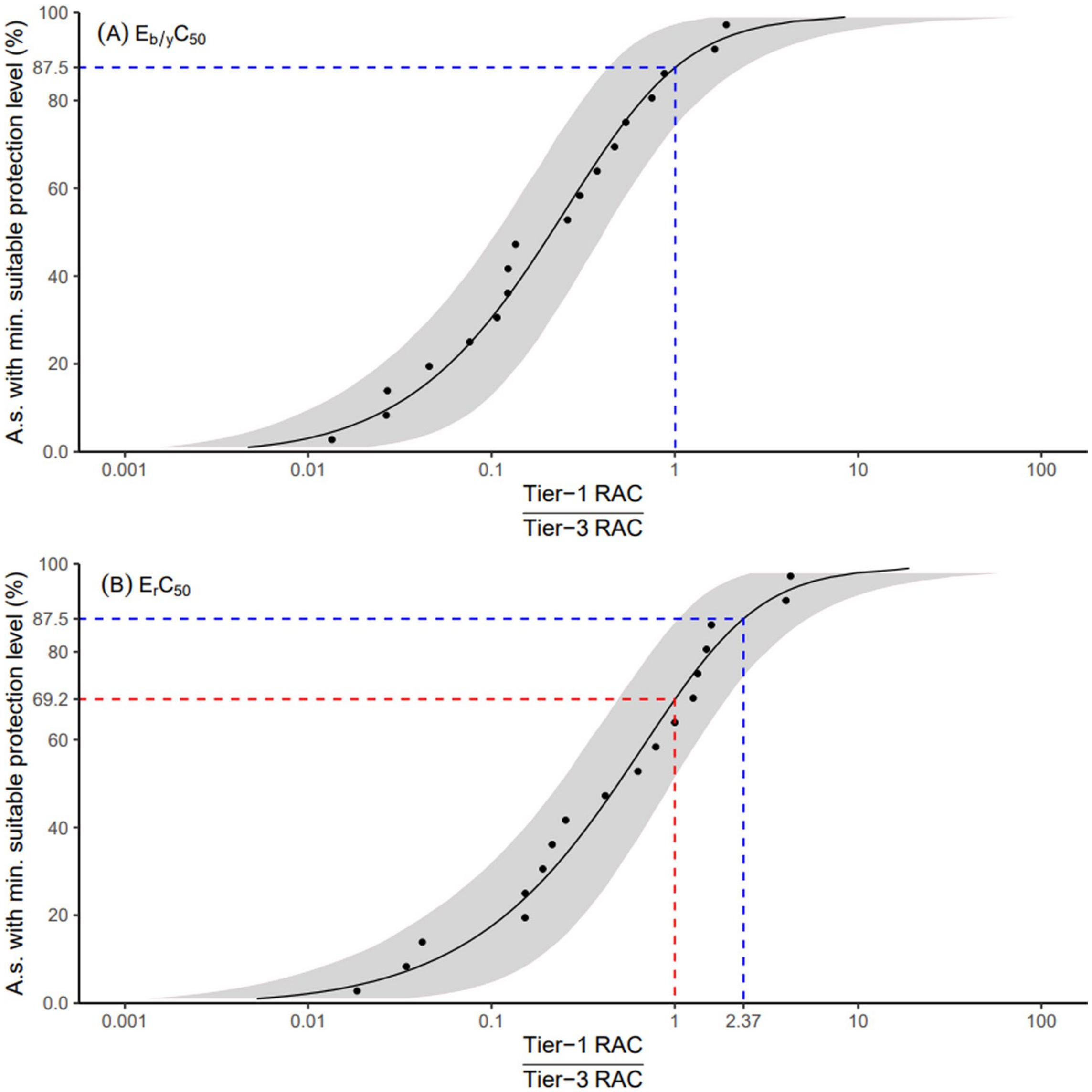

Fig. 5 Protection level indicated by the cumulative distribution of the first tier regulatory acceptable concentrations versus the higher tier regulatory acceptable concentrations (i.e., Tier-1 RAC/Tier-3 RAC ratios) of various active substances. X-axis: Tier-1 RAC/Tier-3 RAC ratios with Tier-1 RAC based on different types of endpoints (**A** lowest endpoint $E_{b/y}C_{50}$; **B** growth rate endpoint E_rC_{50}) and an assessment factor of 10. Y-axis: percentage of active substances with first tier RACs being lower than higher tier RACs, i.e., fulfilling the minimum suitable protection level, as set in the specific protection goals

Discussion

There has been a shift in the procedure to perform the tier 1 aquatic risk assessment of pesticides for primary producers. In the past (i.e., when [4], was in force), the common practice was to select the lowest available endpoint among yield (E_yC_{50}), Area Under the Curve (AUC or "biomass"; E_bC_{50}) and growth rate (E_rC_{50}) endpoints; AUC and yield with E_bC_{50} and E_yC_{50} endpoints were in most cases the lowest. Nowadays—i.e., since the aquatic guidance document [6] was put in force—the endpoint recommended for tier 1 is the growth rate E_rC_{50} endpoint. This change in the selection of the endpoint is justified since the growth rate E_rC_{50} endpoint is a more robust estimate compared to AUC and yield (E_bC_{50} or E_yC_{50}) endpoints. However, using this endpoint while keeping the default AF of 10 has significant implications on the protection level of primary producers. These aspects and implications are presented.

Change of endpoint selected and shift in conservatism of tier 1 assessment

To compare the current protection levels set by the tier 1 risk assessment for primary producers to the former procedure, we used a large and representative EU regulatory dataset and compared the E_rC_{50} values with the $E_{b/y}C_{50}$ values for algae and macrophytes.

Our results indicate a clear change since the tier 1 endpoint currently used (E_rC_{50}) is on average approximately 3.5 and 2.1 times higher than the endpoints previously used (E_bC_{50} or E_yC_{50}) for algae and macrophytes, respectively.

For algae, the 90th percentiles of the ratios between the endpoint for growth rate and those for AUC and yield (i.e., E_rC_{50}/E_bC_{50} and E_rC_{50}/E_yC_{50} ratio) are 5.6 and 6.5, respectively. For macrophytes, these ratios are 3.2 and 4.0, respectively. The shift between the currently used growth rate endpoint and the previously used lowest endpoints is slightly larger when yield values are considered rather than AUC. The shift is also greater for algae compared to macrophytes and they are consistent across different species tested within each group (i.e., algae and the representative macrophyte *Lemna* spp.).

These results indicate that the level of conservatism of the tier 1 risk assessment for aquatic primary producers has decreased since the growth rate inhibition endpoint (E_rC_{50}) is now being used instead of the lowest AUC (or ”biomass”) and yield endpoints ($E_{b/y}C_{50}$). This confirms the results reported by Swarowsky et al. [20], who demonstrated that $E_{b/y}C_{50}$ endpoints are consistently lower than the corresponding E_rC_{50} endpoint (with 90th percentiles of E_rC_{50}/E_yC_{50} ratio values of 7.8 and 3.5 for algae and macrophytes, respectively).

Our findings from the comparisons between growth rate inhibition and biomass-related endpoints based on a large dataset are supported by the derived mathematical relationships, which show that the inhibition observed in biomass surpasses that of the growth rate and that the difference increases as growth rate and test duration increase. It is expected that the shifts between E_rC_{50} and E_yC_{50} are slightly higher compared to that between E_rC_{50} and E_bC_{50}, while the substantial difference between algae and macrophytes can be attributed to significantly lower growth rates of macrophytes, having more influence than longer test durations. To derive the exact mathematical relationship between the growth rate inhibition endpoint (E_rC_{50}) and the lowest endpoint ($E_{b/y}C_{50}$), additional test specific information regarding the dose–response slopes for biomass inhibition is required. Further insights into the mathematical relationship between biomass and growth-related endpoints can be found in [7]. The trend of systematically higher inhibitions in yield and AUC compared to the growth rate explains the difference in the levels of conservatism achieved, using the different EC_{50} values. Consequently, replacing the use of the lowest available endpoint (E_xC_{50}) with the growth rate inhibition endpoint (E_rC_{50}) while maintaining the same assessment factor (AF) of 10 when deriving the regulatory acceptable concentration (RACs) results in a shift to lower conservatism and a reduction in the level of protection set at tier 1. However, such reduction in protection level does not necessarily result in a level which is sufficiently protective, considering the protection goal. In order to assess this aspect, we compared the protection levels achieved currently (based on the growth rate inhibition endpoint) and previously (based on the lowest endpoint) to the level of protection derived from tier 3 studies considered to be the surrogate reference tier, as discussed in the following.

Suitability of the tier 1 protection levels achieved when using different endpoints

In the comparison of protection levels achieved currently (i.e., with Tier-1 RAC based on the E_rC_{50} growth rate inhibition endpoint) and previously (i.e., with Tier-1 RAC based on the lowest E_bC_{50} or E_yC_{50} endpoint—AUC/ “biomass” or yield), we selected the data in a strict way to limit the uncertainties around the analysis. First, we performed our analysis on tier 1 data for active substance (a.s.) by using the current EFSA list of endpoints (LoEPs). By doing so, we ensure that the selected data are those used in EU-wide harmonized tier 1 risk assessment, and thus correspond to those setting the level of protection in the authorization procedures of PPPs in the Member States. In a second step, we limited the dataset to those substances for which relevant tier 3 information is available (i.e., from model ecosystem experiments/ micro-mesocosm studies as published in the same EFSA LoEPs).

In the tiered approach of the aquatic risk assessment, assuming that tier 3 studies are an appropriate surrogate reference tier and the Tier-3 RAC values are appropriately set (i.e., not considerably under- or over-protective), it can then be assumed that Tier-1 RAC values lower than Tier-3 RAC values is indicative of a suitable minimum level of protection in tier 1 risk assessment. Inversely, Tier 1-RAC values higher than Tier-3 RAC values would indicate an under-protective tier 1 risk assessment. When comparing the achieved protection levels, we found that the current tier 1 RA based on E_rC_{50} endpoints and the default AF of 10 is more frequently insufficiently protective (i.e., in ca 39% of cases; Fig. 4A) than when based on $E_{b/y}C_{50}$ endpoints with the AF of 10 used previously (i.e., in approx. 11% of cases; Fig. 4B).

Hartmann et al. [14] conducted a similar evaluation, using a smaller dataset of 16 substances in total; among them, 14 substances were also included in our study (see Additional file 1: Table S4). The authors concluded that the current and previous risk assessments provided "equal protectivity" in over 80% of cases. Our findings appear to contradict their outcomes. Our analysis indicates that 13 out of 18 substances remained in the same "category of protectiveness" (i.e., either above or below the 1:1 line as presented in Fig. 4) while this was shown for 14 out of 16 substances in Hartmann et al. [14]. Overall, a minimum protection level considered as suitable according to the specific protection goals (SPGs) is reached only for 11 out of 18 substances in our dataset (i.e., 61% of cases), compared to 11 out of 15 substances in [19] (i.e., 74% of cases).

The minimum protection level considered as suitable is ensured when Tier-1 RACs are below Tier 3-RACs and this is currently achieved for a significantly lower number of cases than previously (i.e., in 61% versus 89% of cases; Fig. 4A and B). Similar work by Duquesne et al. [16, 17] on slightly different datasets also showed that using the E_rC_{50} instead of the $E_{b/y}C_{50}$ resulted in a lower number of cases having a protective Tier 1 RA (i.e., around 40% vs. 75% of cases). However, in Hartmann et al. [14], the difference was not as large, with 74% of cases currently achieving such protection levels compared to 87% previously. Wjingaarden and Arts [13] also performed a similar evaluation and found that selecting E_rC_{50} as the regulatory endpoint instead of the lowest endpoint, all with the default AF of 10, generally maintained the protection level. They showed that using E_rC_{50} endpoints or the lowest endpoints resulted in protection levels as intended by the SPGs for similar percentages of cases (i.e., 75–80% of cases). However, there were differences in the data selection between their study and ours. They used a variety of sources, i.e., from draft assessment reports published by the EFSA and from the USEPA-ECOTOX database, whereas we restricted our data to the EFSA LoEPs, resulting in only 6 substances common to both datasets, which might explain the discrepancies between the conclusions.

Our approach offers the advantage of characterizing the level of protection achieved specifically when applying the current regulation practice in Europe since the a.s. evaluation on EU-level involves various Member states and delivers expert agreed endpoints and RACs for all tiers of the risk assessment. Differences between our study and the studies of [13, 14] include the fact that they exclusively selected substances with herbicidal mode of action, whereas we also considered fungicides to broaden the dataset; for some of these fungicides, primary producers are not the group most at risk but the higher tier data deliver regulatory acceptable concentrations also applicable for primary producers (i.e., for dodine). These authors also selected the Tier-3 RACs based only on the effect threshold option (ETO-RAC), whereas we selected the ERO-RAC when it was the only tier 3 RAC available (i.e., only NOAECs were delivered in cases of metamitron, metazachlor, acetochlor, iodosulfuron, lenacil, bifenox and dodine)or in the case of isoproturon (see section Results).

Bergtold and Dohmen [15] also performed a comparison of E_bC_{50} and E_rC_{50} endpoints for primary producers to assess their relevance for the aquatic RA of herbicides, referring to higher tier studies using the effect classes approach (e.g., [6, 21]). They concluded that using E_rC_{50} values in combination with the default AF of 10 is sufficient to exclude unacceptable risk to algae and aquatic plants in the environment. This finding again diverges from our scientific evaluation.

Overall, different studies have been conducted to evaluate the protection level achieved for primary producers in the first tier risk assessment by comparing Tier-1 RACs and higher tier RACs derived from microcosm and mesocosm studies. They indicate two different types of outcomes, i.e., a shift towards a lower protection level when using E_rC_{50} (as observed in our study) versus a tendency for the protection level to be maintained (as observed in other studies). They also show that small changes in the data selection can lead to significant variations in outcomes, given the relatively limited datasets (with a maximum of 18 substances in our study). To address this issue and conduct a more robust analysis as well as to assess if the SPGs are reached for each substance (i.e., negligible effects on survival/growth/abundance/biomass or some effects of limited magnitude and duration), we applied the concept of the cumulative distribution function. This analysis shows that the minimum protection level considered as suitable was ensured in 87.5% of the cases previously versus 69.2% currently (Fig. 5A and B) which confirms the extent of the shift in the protection level achieved.

Should the protection level currently achieved be considered sufficient?

The EFSA Opinion on the development of specific protection goals for pesticide risk assessment [9] requires that new risk assessment methods should be calibrated to ensure that they meet the SPGs. While some member states have noted the aquatic guidance document [6], they have also called for calibration to be checked for primary producers following the change of the type of endpoint used in RA. Therefore, it was necessary to evaluate the consequences of the shift in the protection level achieved for primary producers resulting from the current use of E_rC_{50} values with the default AF 10, in order to determine whether the SPGs are being met under the practice proposed in the aquatic guidance document [6], or if it should be adapted.

We question whether the current shift in the level of conservatism for the tier 1 risk assessment of primary producers is acceptable, since the protection level intended by the SPGs considered as the minimum suitable is reached for 69.2% versus 87.5% of active substances when using Tier-1 RACs derived from the E_rC_{50} instead of $E_{b/y}C_{50}$ values. In other words, should such a protection level ensured only in approx.. 69% of the a.s. be considered sufficient, or should it be modified? To achieve the previous level reached for approx. 88% of a.s., an extra factor of approx.. 2.4. to reach an overall AF of approx. 24 instead of the default AF of 10 should be used for the current tier 1 RA. We are aware that these values may be approximative since our dataset is limited to a total of 18 substances; however, they are indicative of the trend. Our recommendation to increase the overall AF—by combining the default AF of 10 that was set by Uniform Principles [5] to an extra factor when associated to E_rC_{50} endpoint—differs from other authors' conclusions on the suitability of the current protection level. Another approach could be to use an alternative E_rC_x, e.g., the growth rate endpoint equivalent to 10% inhibition (i.e., E_rC_{10} instead of E_rC_{50}) and keep the default AF of 10. However, E_xC_{10} endpoints are statistically less robust than E_xC_{50} endpoints and are not according to the Uniform Principles [5] that also set the use of an E_xC_{50}. Thus, this approach was not pursued.

Furthermore, it is important to question whether the previous minimum level of protection of the tier 1 risk assessment approach based on the lowest endpoint and the default AF 10, which was reached for only ca 88% of a.s., was actually sufficient to protect populations and communities in the field. The approval of an a.s. for use in PPP after the EU evaluation implies that the SPGs are being met, and by consequence, that no unacceptable effects on populations will occur in the field for each group of organisms. Having a failing rate of approximately 12% of the a.s. is an issue to be aware of, especially when it is expected that the tier 1 risk assessment of each single a.s. is suitable to protect the primary producers. It seems essential to ensure a suitable level of protection of tier 1 RA, e.g., by increasing the overall AF value of approx. 48 in the current situation based on the growth rate E_rC_{50} endpoint (corresponding to approx.. 21 in the previous situation based on the lowest endpoint $E_{b/y}C_{50}$) for achieving the aim of an adequate protection level reached in, e.g., 95% of the active substances evaluated and PPPs authorized. Increasing the requirements to, e.g., 95% of the cases is recommended. Indeed, it is crucial to (i) maintain the function of primary producers since they are the lowest trophic level and have thus a fundamental role in the aquatic food web, and eventually (ii) maintain and restore biodiversity of aquatic communities especially threatened in agricultural landscapes [22, 23].

In addition to this comparison and calibration between tiers related to the shift of endpoints selected in the tier 1 RA, it is also worth noting some general aspects and concerns of the aquatic risk assessment, also relevant in the context of the previous assessment based on the lowest endpoints. In the concept of the tiered approach, the lower tier risk assessment should be simpler and more conservative than the more complex and realistic higher tier risk assessment (e.g., [6]). The extent of this high conservatism to be ensured at tier 1 is intrinsically linked to the tier 3 RA. Indeed, there are uncertainties about the suitability of the protection level being actually achieved at tier 1 for borderline cases (i.e., close to 1:1 regression line between Tier-1 RACs and Tier-3 RACs) and this could be solved by clarifying the level of conservatism of the tier 3 risk assessment. This tier 3 aquatic RA is considered as a surrogate reference tier for the field situation, which is the actual reference tier. However, the final step of calibration, which involves the evaluation of the protectiveness of the assessment based on the surrogate reference tier (and thus the adequacy of the tiered approach) compared to the reference tier, needs to be documented. This is a significant concern because for example the representativeness of the assemblages tested in micro-/mesocosm studies for real field conditions may be limited [24].

Also a recent monitoring study of agricultural streams of southern Sweden indicates that pesticide contamination can induce inhibitory effects on algae and that it should thus be seen as one significant stressor among others [25]; the latter is also supported by [26], an investigation on the impact of multiple stressors (inter alia pesticides) on algal communities in lentic small water bodies in a German lowland agricultural area. In addition, a large-scale ecological and chemical monitoring study of surface waters in agricultural areas of Germany identified that (i) about half of the 20 substances with measured environmental concentrations that exceed most the RACs are substances with herbicidal mode of action, and (ii) the current authorization of PPPs underestimates the actual ecological risk to invertebrates [27]; the primary producers were not investigated but they may well be affected, especially long-living macrophytes species that are repeatedly exposed over years. It would be thus important to verify that the prospective risk assessment is appropriately calibrated to the field situation, in order to deliver suitable estimates that ensure sufficient protection for primary producers. It should be also noted that referring to the E_rC_{50} is most suitable for species that have exponential growth such as algae and *Lemna* spp. as the calculation of inhibition of the average specific growth rate is based on logarithmic transformation. For some other species of macrophytes that do not present an exponential growth, the growth rate endpoint E_rC_{50} is less suitable and may lead to an underestimation of the risk due to PPP exposure although it is however more robust than the biomass-related endpoints ($E_{b/y}C_{50}$) when considering varying test conditions.

These aspects indicate a lower degree of conservatism and various types of uncertainties when assessing the minimum level of protection to be actually achieved in the field. Hence, it is important to acknowledge these aspects while conducting risk assessments; this is even emphasized under the current practice of using the E_rC_{50} and thus less conservative endpoints in the ERA.

Conclusion

Our results indicate that the current tier 1 risk assessment for primary producers performed according to the recommendations of EFSA [6] is not sufficiently conservative to protect adequately aquatic communities in the field. Therefore, by contrast to other investigations that performed similar comparisons, we advocate for the (re-) establishment of a more appropriate protection level, so that it becomes at least equal (or better) to that previously reached when based on the lowest biomass-related $E_{b/y}C_{50}$ endpoints. Indeed, disturbances of the primary producer communities should definitely be avoided in order to preserve their structural and functional integrity. The regulatory framework and protection level currently set do not account for higher risk than anticipated by a tiered approach based on single pesticide assessment. But biodiversity loss is a reality of significant concern, especially in the context of agricultural landscapes where aquatic communities are exposed to multiple stressors, including different pesticides applied simultaneously or successively in spraying sequences.

Abbreviations

a.s.	Active substance
E_bC_x	Integral biomass "Area Under the Curve-AUC", for x% growth inhibition
E_rC_x	Inhibition of the average specific growth rate for x% growth inhibition
E_yC_x	Reduction in biomass, calculated from yield for x% growth inhibition
EFSA	European Food Safety Authority
ERA	Environmental risk assessment
ERO	Ecological recovery option
ETO	Effect threshold option
EU	European Union
LoEP	List of endpoints
PPP	Plant protection products
RA	Risk assessment
RAC	Regulatory acceptable concentration
RAR	Registration assessment report
SPG	Specific protection goal

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-023-00767-8.

Additional file 1. Supplementary information (Tables S1 to S4 and Relationship between Yield/AUC inhibition and 50% growth rate inhibition).

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Author contributions

DS: conceptualization, methodology, investigation, data curation, writing—original draft, project administration; BS: conceptualization, formal analysis, writing—review and editing, visualization; KM: conceptualization, formal analysis, investigation, data curation, writing—review and editing, visualization; HL and MS: conceptualization, methodology, data curation, writing—review and editing; WJ and PS: conceptualization, writing—review and editing.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

No, I declare that the authors have no competing interests as defined by Springer, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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