Ecotoxicology (2018) 27:945–955
https://doi.org/10.1007/s10646-018-1939-z

Ecotoxicological assessment of the herbicide Winner Top and its active substances—are the other formulants truly inert?

Libânia Queirós[1] · Tânia Vidal[1] · António J. A. Nogueira[1] · Fernando J. M. Gonçalves[1] · Joana Luísa Pereira[1]

Accepted: 11 April 2018 / Published online: 3 May 2018

Abstract

Formulants used in Plant Protection Products (PPPs) to promote their efficiency are normally undisclosed in the PPP documentation, unless they bear a human health or environmental hazardous potential *per se*. PPP regulation also demands the assessment of putative interactions among formulants within each product recipe and consequent effects, but these results are often unavailable. Such a case is that of the herbicide Winner Top (Selectis®, Portugal), which we selected as a model commercial formulation in the present study specifically aiming at (i) characterising its aquatic toxicity towards sensitive eco-receptors (*Raphidocelis subcapitata*, *Chlorella vulgaris*, *Lemna minor* and *Lemna gibba*), as well as that of its active substances (a.s.) nicosulfuron and terbuthylazine; (ii) comparing the ecotoxicity among the commercial formulation, the corresponding mixture of its a.s. and this a.s.'s mixture increasingly enriched with the formulants. Single chemical testing revealed that terbuthylazine was the strongest microalgae growth inhibitor and nicosulfuron was the strongest macrophyte growth inhibitor. On the other hand, the commercial formulation was consistently less toxic than the corresponding mixture of the a.s., suggesting that Winner Top formulants (72.9% of the commercial formulation) interact with the a.s., promoting less than additive effects in the selected non-target species. Importantly, this environmentally protective effect of the formulation can be apparent. Because macrophytes share most physiological features with the weeds targeted by the studied herbicide, it is likely that increased application doses are required to reach desired efficacy levels with the consequent detrimental increase of PPP residues load in edge-of-field freshwater ecosystems.

Keywords Winner Top · Formulant mixture · Nicosulfuron · Terbuthylazine · Non-target aquatic species

Introduction

Plant Protection Products (PPPs) are extensively used in agriculture to keep agricultural production rates (Carlile 2006; Fuentes et al. 2013). Following application, they can reach edge-of-field surface waters through different transport pathways, and indeed, PPP residues have been found frequently in this environmental compartment (DeLorenzo et al. 2001; Abrantes et al. 2009; Abrantes et al. 2010; Papadakis et al. 2015; Silva et al. 2015a; Silva et al. 2015b). Therefore, PPPs represent a putative environmental hazard and their toxic potential over non-target organisms has been addressed frequently in the literature (e.g. Cedergreen and Streibig 2005; Pereira et al. 2009; Vidal et al. 2011; Silva et al. 2015a; Silva et al. 2015b). The overall inability to mitigate the environmental side-effects of PPPs has been attributed to their high toxicity, frequently unspecific as to the target, and non-biodegradability; but also to the lack of a systematic, scientifically-based formulation development process that considers environmental safety as a design variable that does not necessarily represents efficiency losses (Castro et al. 2014). Investment has been made by the agrochemicals' industry regarding both faces of the problem, but the continuous flow of environmental risk reports indicates that the progresses are not yet significant.

PPPs are essentially formulations composed by one or more active substances (a.s.) and a set of other chemicals, that can play a role as safeners, synergists or co-formulants. The general designation of 'formulants' is used (and will

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10646-018-1939-z) contains supplementary material, which is available to authorized users.

✉ Libânia Queirós
libania.queiros@ua.pt

[1] Department of Biology & CESAM (Centre for Environmental and Marine Studies), University of Aveiro, Aveiro 3810-193, Portugal

apply hereinafter) to describe these ingredients, which are typically added to the a.s. as part of the commercial formulation recipe to improve PPP efficacy, stability and delivery of the active substances to their target (Surgan et al. 2010; Castro et al. 2014). An up-to-date controversy regards the role of formulants in the environmental toxicity of PPPs. The European regulation is amongst the most demanding in this context and it actually makes it mandatory to always disclose a.s. involved in any PPP product, and to disclose formulants proven to represent an environmental hazard *per se*; also, testing with the formulation intended to be marketed is required to allow the authorisation process (EC 2009), thus covering the logical interaction between formulation components – a formulant is added for a reason to the PPP recipe, typically because it can interact with other components constituting the PPP mixture to improve its overall efficacy against the target weed, which may share physiological targets with non-target eco-receptors. However, this later requirement seems to be poorly implemented so far (the short period following enforcement may partly justify it), despite the availability of regulatory-driven specific guidelines for the environmental risk assessment of PPPs in edge-of-field waterbodies and soil (EFSA PPR 2013; Ockleford et al. 2017). Very few, if any authorisation dossiers can be found containing ecotoxicological information on the whole PPP as a whole and the product's marketing documentation rarely discloses any information on the whole product ecotoxicity.

The commercial herbicide Winner Top (Selectis Premium; Selectis 2012) configures such a case and was selected in the present study to address the above problematic. Winner Top is an oil dispersion using terbuthylazine and nicosulfuron as a.s. The interaction between these two active components of the formulation deserves attention for a view on the primary environmental hazardous potential of the PPP, adding to effects characterisation regarding each a.s. individually as currently available (EFSA 2008, 2011; Selectis 2012). Considering the percentage of the a.s. present in the formulation (terbuthylazine 25.4% w/w and nicosulfuron 1.7% w/w), undisclosed formulants constitute 72.9% w/w of the product (Selectis 2012). Herbicides are amongst the most used PPPs following fungicides (EEA 2017), and Winner Top has been widely used on maize cultures as a post-emergence, systemic and residual herbicide to combat several weeds (e.g. *Portulaca oleracea*, *Amaranthus* spp., *Poa annua*). Nicosulfuron is a poorly liposoluble sulfonylurea and terbuthylazine is a highly liposoluble 1,3,5-triazine, and both are highly leachable (Selectis 2012; Lewis et al. 2016). The modes of action of both a.s. are well known and characterized. Nicosulfuron prevents the growth of the plant by blocking the plant amino acid synthesis through the inhibition of acetohydroxyacid synthase (Lewis et al. 2016), an enzyme whose site of action exhibits affinity towards different classes of herbicides such as imidazolinones and sulfonylureas (Singh et al. 1988; McCourt et al. 2006; Duggleby et al. 2008). This enzyme only exists in microorganisms and plants, thus its inhibitors are deemed selective, very potent and apparently nontoxic to animals (Duggleby and Pang 2000; McCourt et al. 2006; Duggleby et al. 2008). Terbuthylazine inhibits the photosynthesis by acting as a photosystem II blocker (Lewis et al. 2016). Photosystem II blockers belong to chemical groups such as amides and triazines and are generally assumed selective to photosynthetic organisms (Pfister and Arntzen 1979).

No information on the likelihood of an interactive behaviour between the a.s. was found in the literature while searching for the present study. However, since the formulants constitute a significant part (72.9%) of the product, a modification of the toxicity of the a.s (which can also interact when dosed jointly) driven by these formulants was hypothesised. In this context, the aim of the present study was to characterize the aquatic toxicity of Winner Top towards sensitive organisms. Implicitly, we established the goal of assessing whether unknown formulants can significantly contribute to the overall toxicity of the product, i.e. whether formulants are as inert as they should be from an environmentally precautionary point of view. The toxicity of each of the two a.s., that of the commercial formulation Winner Top, and that of a customized mixture of the a.s. respecting their ratio in the commercial formulation were compared for the purposes. Furthermore, a.s. mixtures enriched with the unknown formulants were tested to gain a direct insight on the toxicity of the latter.

Standard non-target organisms from the aquatic compartment were carefully selected to address these aims. In this way, two microalgae species (*Raphidocelis subcapitata* and *Chlorella vulgaris*) and two macrophyte species (*Lemna minor* and *Lemna gibba*), both primary producer representatives, were used as the most direct non-target aquatic eco-receptors for a product with herbicidal properties. In fact, the risk assessment of both nicosulfuron (EFSA 2008) and terbuthylazine (EFSA 2011) support this option. We expected from the beginning that macrophytes would show higher sensitivity than microalgae since the selected PPP is of systemic action. Although both microalgae species and both macrophyte species are interchangeably accepted as standard test species, differences in sensitivity have been frequently noticed. For example, Vidal et al. (2011) found a difference by more than 7-fold between *R. subcapitata* and *C. vulgaris* in sensitivity to the herbicide phenmedipham; and *Lemna minor* has been found more tolerant than *Lemna gibba* to some metals (Dvorák et al. 2012) and some organic solvents (Cowgill et al. 1991).

Material and methods

Chemicals and test solutions

The herbicide Winner Top was supplied by Selectis®, Portugal, as an oil dispersion with 16.75 g/L nicosulfuron and 250 g/L terbuthylazine as active substances (a.s.). Technical terbuthylazine and nicosulfuron were acquired from Sigma-Aldrich (Pestanal®, Steinheim; 99.4 and 99.6% purity, respectively). Stock solutions were prepared immediately before each assay by dissolving the a.s. or diluting the pesticide formulation in each test medium. No solvents or carriers were used to assist the dissolution of the active ingredients. Both the a.s. are stable as dissolved in water, with aqueous hydrolysis being negligible considering both the exposure lengths and the test incubation conditions (Lewis et al. 2016), hence the use of nominal concentrations while referring exposure treatments in the present study.

Test organisms

Cultures of the green microalgae *R. subcapitata* and *C. vulgaris* were cyclically maintained in the laboratory in sterilized Woods Hole MBL medium (Stein 1973), at 20 ± 2 °C with a 16 h^L:8 h^D photoperiod, with renewal scheduled once a week. Cultures of the macrophytes *L. minor* and *L. gibba* were maintained in Steinberg medium (OECD 2006), at 20 °C with a photoperiod of 16 h^L:8 h^D, with renewal scheduled once a week.

Toxicity tests

Growth inhibition tests with the microalgae followed the recommendations of the OECD guideline 201(OECD 2011), adapted to the use of 24-well microplates (Geis et al. 2000). The exposures of *R. subcapitata* and *C. vulgaris* to each toxicant challenge (see below) were run in triplicate, in 24-well microplates containing 1 mL of test volume per well and using an initial cell density of 10^4 cells/mL. Tests were incubated at 23 ± 1°C under a continuous light cycle for 96 h, and the algal suspension in each well was thoroughly mixed by repetitive pipetting twice a day to prevent cell clumping and promote gas exchange. At the end of the exposure period, optical density of suspensions in each well was read (440 nm; Shimadzu UV-1800) and converted to cell density records on the basis of a previously established calibration curve. The microalgae biomass yield following each test treatment was calculated as the difference between corresponding cell densities at the end and the beginning of the test.

Growth inhibition tests with *L. minor* and *L. gibba* followed the recommendations of the OECD guideline 221 (OECD 2006). Tests were carried out in disposable 6-well microplates as adapted by Kaza et al. (2007) and Kolasińska et al. (2010), at 23 ± 1°C, under continuous light, and using 3 replicates per chemical treatment with 6 replicates assigned to the control treatment (blank Steinberg medium). Each replicate well was inoculated with 3 healthy colonies of 3 fronds at the beginning of the test. Three extra replicates were collected from the culture for determination of the average dry weight until constant weight, at the beginning of the test. After 7 days of exposure period, all colonies were collected from each replicate, fronds were counted, then rinsed with distilled water and dry at 60°C for final dry weight records. *Lemna* biomass yield for each individual treatment was calculated on the basis of either frond number or dry weight, as the difference between records at the end and at the beginning of the test.

Five toxicity testing trials were run independently for the microalgae and the macrophytes. The organisms were exposed to geometric concentration ranges of (i) nicosulfuron (3000–58028 µg/L for *R. subcapitata*, 600–6619 µg/L for *C. vulgaris*, 1–69 µg/L for *L. minor* and *L. gibba*); (ii) terbuthylazine (15–269 µg/L for *R. subcapitata* and *C. vulgaris*, 20–550 µg/L for *L. minor* and 8–550 µg/L for *L. gibba*); (iii) Winner Top, thus a combination of nicosulfuron and terbuthylazine added formulants (Table S1); (iv) a customized mixture of the two a.s. respecting the ratio used in the commercial formulation (i.e. mimicking Winner Top with no formulants), for a direct comparison between the effects of combined a.s. and the commercially formulated a.s. combination (Table S1). As a.s. concentrations increase in trial (iii), the proportion of formulants in test solutions also increase, which may bias conclusions regarding formulants toxicity while comparing with data yield from trial (iv). Therefore, and in order to provide a more direct view on the putative toxicity of formulants, a fifth trial was run. In trial (v) *R. subcapitata* and *L. minor* were exposed to combinations between the commercial formulation and an equivalent mixture of the a.s., representing the EC_{25}, the EC_{50} and the EC_{75} as estimated following trial (iv) (see *Data analysis* for details on the calculations and table S1 for the corresponding concentration levels). At each effective concentration level kept constant, the proportion of formulants in the test solutions was increased, establishing a formulants' treatment range (0, 25, 50, 75 and 100% commercial solution). In practice, we prepared equivalent (i.e. same a.s. concentrations at a given fixed effect level) stock solutions of Winner Top and combined a.s. and mixed them to establish test solutions; by increasing the relative proportion of Winner Top stock in test solutions, unknown formulants were increasingly added (see Table S1 for further details). This allowed testing the effect of increasing concentrations of formulants while keeping the concentration of a.s. intrinsically constant.

Data analysis

Effective concentrations (EC_{10}, EC_{20} and EC_{50} as standard ecotoxicological benchmarks useful for environmental risk assessment purposes (EFSA PPR 2013), and EC_{25} and EC_{75} as testing benchmarks) corresponding to data retrieved in bioassays with microalgae and macrophytes were estimated by non-linear regression, using the least-squares method to fit the data to the logistic equation. Also because of their relevance in the environmental risk assessment of chemicals, No Observable Effect Concentrations (NOECs) were determined by applying a one-way ANOVA followed by the post-hoc Dunnet test ($p < 0.05$) to each test outcome from trials (i)-(iv). The post-hoc Tuckey was rather applied following ANOVA to the outcome of trial (v) to assign significant differences between treatment combinations. In order to facilitate the comparison between data yield from test trials (iv) and (v), concentration ranges in these cases were transformed into dimensionless, hence fully comparable Toxic Unit (TU) ranges. The sum of the quotients $Ci/EC_{50}i$ (TUsum; Toxic strength of the mixture) was applied for the purpose, considering i^{th} components of the mixture (in this case, nicosulfuron and terbuthylazine), and assuming that C is the concentration of i within the mixture and EC_{50} is the median effect concentration found in single-chemical exposures to i (see e.g. Jonker et al. 2005 for more details on TU approach).

Results

Nicosulfuron and terbuthylazine were able to significantly depress the growth of both microalgae and both macrophytes, regardless dosed singly (Fig. 1; Table S2) or as mixtures (Fig. 2; Table S2). Interestingly, the macrophytes were markedly more sensitive than microalgae only to nicosulfuron and differential sensitivity was found between macrophyte species and especially between microalgae species as detailed below.

Terbuthylazine was more toxic than nicosulfuron by more than two orders of magnitude to the green microalgae *R. subcapitata*, as visually evident when comparing concentration response curves in Fig. 1. This trend was confirmed by confronting corresponding NOEC values below 15 and 3000 µg/L, respectively, and estimated EC_x values for terbuthylazine lower by 2–3 orders of magnitude than those for nicosulfuron (Table 1). The a.s. mixture (respecting the a.s. ratio used in the commercial formulation) was more toxic to *R. subcapitata* than Winner Top, with 70–80% yield inhibition expected at 2–3 TU compared to 6–8 TU, respectively (Fig. 2). Consistently, a lower NOEC value and non-overlapping confidence intervals were generally found for the a.s. mixture (Table 1). The responses of the other microalgae species, *C. vulgaris*, followed similar patterns, although the magnitude of the differential tolerance found following compared exposures was lower. For example, while *C. vulgaris* was also more

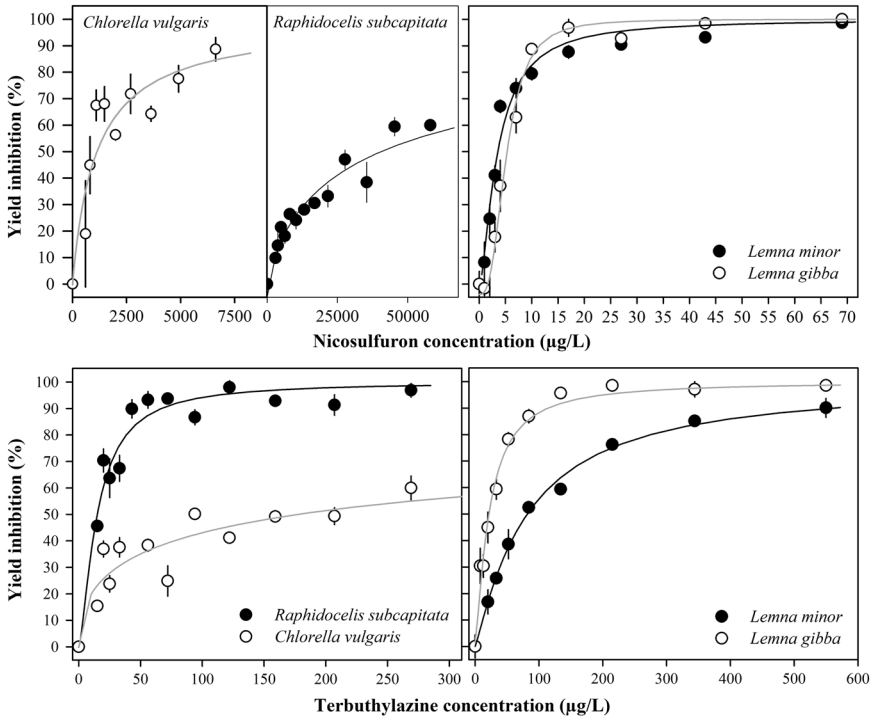

Fig. 1 Biomass yield inhibition (% compared to the blank control) promoted by nicosulfuron and terbuthylazine dosed as technical active substances in microalgae (left-hand panels) and macrophytes (right-hand panels). The points stand for experimental means while the lines represent the best non-linear regression model fitted for the estimation of EC_x values (see Table 1). Error bars stand for the standard error

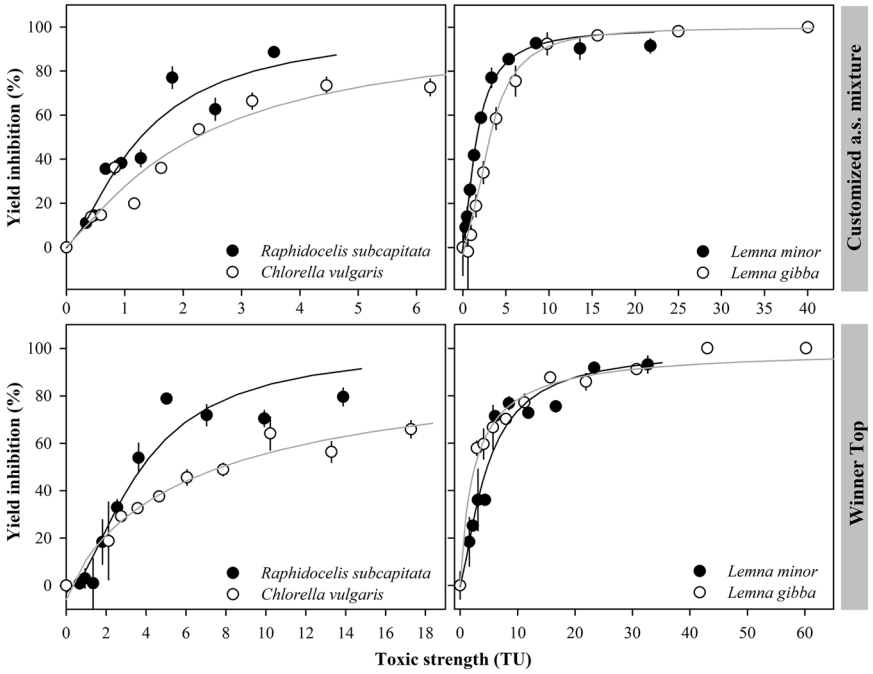

Fig. 2 Microalgae (left-hand panels) and macrophyte (right-hand panels) biomass yield inhibition (% compared to the blank control) promoted by a customised mixture of nicosulfuron and terbuthylazine (upper panels) corresponding to the mixture of the active substances respecting the ratio used in Winner Top, whose effects are depicted in lower panels. The toxic strength reflects the sum of the TU in the mixture. The points stand for experimental means while the lines represent the best non-linear regression model fitted for the estimation of EC_x values (see Table 1). Error bars stand for the standard error

Table 1 Summary of relevant ecotoxicological benchmarks retrieved based on biomass yield following exposure of two green microalgae (*Raphidocelis subcapitata* and *Chlorella vulgaris*) and two macrophytes (*Lemna minor* and *Lemna gibba*) to the active substances terbuthylazine and nicosulfuron, as well as to their commercial formulation Winner Top and to the two active substances mixed respecting the Winner Top formulation ratio

		Nicosulfuron (µg/L)	Terbuthylazine (µg/L)	Winner Top (TU)	Formulation ratio (TU)
R. subcapitata	EC_{10}	1473 (623–2322)	3.33 (1.68–4.97)	1.03 (0.33–1.72)	0.29 (0.17–0.42)
	EC_{20}	4806 (3017–6596)	5.79 (3.65–7.93)	1.64 (0.82–2.47)	0.50 (0.35–0.66)
	EC_{50}	36209 (28242–44177)	14.92 (12.28–17.56)	3.67 (2.61–4.73)	1.27 (1.05–1.48)
	NOEC	<3000	<15	2.55	0.34
C. vulgaris	EC_{10}	115.26[a] (0–262.62)	1.93[a] (0–5.07)	0.54[a] (0–1.11)	0.36 (0.17–0.56)
	EC_{20}	267.20 (24.66–509.74)	10.26[a] (0–20.62)	1.39 (0.42–2.36)	0.71 (0.44–0.98)
	EC_{50}	1122.8 (687.15–1558.4)	177.49 (85.40–269.58)	6.84 (4.72–8.96)	2.23 (1.77–2.70)
	NOEC	600	<15	2.12	0.60
L. minor	EC_{10}	0.80 (0.46–1.14)	11.88 (7.14–16.63)	0.94 (0.46–1.42)	0.36 (0.24–0.47)
	EC_{20}	1.37 (0.94–1.80)	24.22 (17.04–31.40)	1.70 (1.04–2.36)	0.63 (0.48–0.77)
	EC_{50}	3.46 (2.84–4.08)	81.29 (67.62–95.00)	4.70 (3.66–5.74)	1.62 (1.39–1.85)
	NOEC	1	<20	1.58	0.51
L. gibba	EC_{10}	2.36 (1.59–3.13)	4.23 (2.69–5.76)	0.25 (0.00–0.51)	1.09 (0.45–1.73)
	EC_{20}	3.15 (2.38–3.92)	7.78 (5.67–9.89)	0.60 (0.15–1.04)	1.63 (0.91–2.35)
	EC_{50}	5.16 (4.36–5.95)	22.07 (18.61–25.53)	2.57 (1.65–3.50)	3.24 (2.41–4.08)
	NOEC	3	<8	<2.91	2.39

Estimated EC_x values, with 95% confidence intervals within brackets, and NOEC values (see corresponding ANOVA summaries in Table S2) are given

[a]Non-significant

sensitive to the customized mixture of a.s. than to the commercial formulation Winner Top, non-overlapping confidence intervals were only found at the EC_{50} level (Table 1). It is further noticeable that *R. subcapitata* was markedly more tolerant than *C. vulgaris* to nicosulfuron (EC_x and NOEC values differing by one order of magnitude), but the reverse applies when challenging the microalgae with terbuthylazine (Fig. 1; Table 1).

A direct graphical interpretation of Fig. 1 shows terbuthylazine as the least toxic a.s. to macrophytes, which contrasts to the pattern found following microalgae exposure (see above). This trend was confirmed for both *Lemna* species by corresponding NOEC values and distinct EC_x estimates with non-overlapping confidence intervals (Table 1). However, the responses of the two *Lemna* species were not consistent regarding differential sensitivity to each toxicant challenge. *L. gibba* was more tolerant than *L. minor* to nicosulfuron but the reverse applies following exposure to terbuthylazine; and *L. minor* was more tolerant to Winner Top but more sensitive to the a.s. formulation than *L. gibba* (see the benchmarks in Table 1 comparatively). In fact, and among all species tested, *L. gibba* was the most sensitive to Winner Top (Table 1); it was also the single species responding more sensitively to the commercial formulation than to the corresponding customised mixture of the a.s. (Fig. 2), although the difference could not be validated by EC_x estimation, since overlapping confidence intervals were found for all equi-effective benchmarks when comparing between formulations (Table 1).

The combination of the a.s. (both as Winner Top and a.s. mixture) seems to elicit an antagonistic interaction specially at the level of the EC_{50} and higher effect levels, where the TUsum is invariably well above 1 (Fig. 2; Table 1). Also noticeable is the trend for a stronger antagonistic behaviour of the a.s. combination dosed as Winner Top compared to the mixture of a.s. respecting the commercial formulation ratio (see corresponding higher benchmarks in Table 1). This trend is consistent throughout all assessed benchmarks, with likely antagonistic behaviour noticed for the mixture dosed as Winner Top (TUsum above 1) being frequently reversed into a likely synergic behaviour of the corresponding a.s. mixture (TUsum well below 1) at low effect levels (Table 1). These trends triggered the final experimental tier where we intended to understand them further through an inspection on whether formulants can modulate the toxicity of the a.s. combination. So, an increased range of formulants' concentration was tested assessed at discrete equi-effective concentrations of the a.s. combination: EC_{25} corresponding to 0.61 and 0.76 TU for *R. subcapitata* and *L. minor*, respectively; EC_{50} as exposed in Table 1; EC_{75} corresponding to 2.6 and 3.44 TU for *R. subcapitata* and *L. minor*, respectively. It is worth noting that, given the deviation showed by *L. gibba* to the common trend describing a lower toxicity of the commercial formulation compared to the a.s. formulation (Fig. 2; Table 1), we preferred to use *L. minor* along with the microalgae *R. subcapitata* (generally more sensitive than *C. vulgaris*) as the model species to run this final experimental trial.

Figure 3 shows that maximum formulants concentration (100% Winner Top treatments) adding to the combination

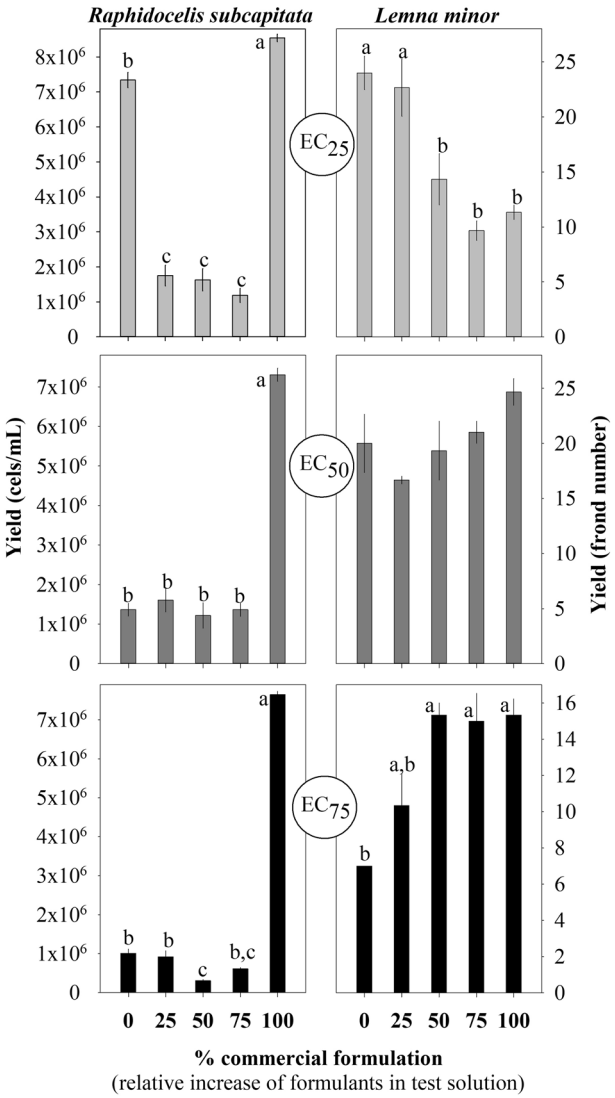

Fig. 3 Mean biomass yield of the microalgae *Raphidocelis subcapitata* (left-hand panels) and the macrophyte *Lemna minor* (right-hand panels) following exposure to nicosulfuron and terbuthylazine at fixed levels (EC_{25}, EC_{50} and EC_{75}, as estimated previously in previous tests with the mixture of the a.s. respecting the ratio used in the commercial PPP, Winner Top) across a range of increased concentration of formulants (0, 25, 50, 75 and 100% commercial PPP solution). Error bars stand for the standard error and low-case letters (a-c) were added as necessary to assign differences between treatments (Tuckey test; $p < 0.05$)

of nicosulfuron and terbuthylazine generally promotes maximum yield, both in microalgae and macrophytes regardless the effect level, again suggesting that formulants decrease the toxicity of a.s. (Fig. 3). The EC_{25} level tested with macrophytes depicted the opposite and the EC_{50} level did not allow statistical confirmation of the trend (Fig. 3; Table S2). Apart from the deviant pattern of the response by *L. minor* tested at the EC_{25} level, increasing formulants load seems to promote the macrophytes growth, thus comparatively depressing the a.s. toxic potential. This monotonic

trend was not confirmed for microalgae, where formulant loads of 25–75% seem not to yield a protective effect towards the a.s. toxicity.

Discussion

As a general outcome, this study showed that Winner Top, the mixture of its a.s. respecting the formulation ratio, and its a.s. nicosulfuron and terbuthylazine *per se*, can significantly affect the growth of all tested species at the tested concentrations. Some published studies report the occurrence of nicosulfuron and terbuthylazine in the aquatic environment, particularly in surface waters, and the integration of these records with our ecotoxicological outcome provides the necessary grounds for a more realistic discussion on the actual hazardous potential of these pesticides.

Terbuthylazine concentrations of 0.02 and 1.65 µg/L were detected in surface water samples collected in different Portuguese reservoirs (Azevedo et al. 2000), while the largest national reservoir in the south of the country (Alqueva) was found to held maximum terbuthylazine surface water concentration of 112 and 532 ng/L (Palma et al. 2009; Palma et al. 2014); concentrations up to 0.24 µg/L (Hildebrandt et al. 2008) and around 4 µg/L (Silva et al. 2015a) were found in the Ebro and Tejo river basins, respectively. These and other studies (Sass and Colangelo 2006; Postigo et al. 2010; Baillie 2016; Tsaboula et al. 2016) suggest that detection of terbuthylazine in surface waters has been widening, which can relate to the ban of atrazine in Europe requiring an adequate replacer for the control of agricultural weeds, and that levels can be at least one order of magnitude higher depending on the time to sampling following application. Maximum terbuthylazine concentrations found in surface waters are very close to the concentrations that cause a significant deleterious effect on the model primary producers tested in the present study. The highest environmental concentration found was around of 4 µg/L (Silva et al. 2015a) while the lowest benchmark noticing negative impacts (LOEC or EC_{20}; ECB 2003) was the EC_{20} value of 5.79 µg/L estimated for *R. subcapitata*. Assuming the increasing trend for terbuthylazine levels in surface waters as mentioned above, and considering that long-term exposure to sequential inflows of the contaminant may translate into more pronounced effects in the aquatic biota, it is reasonable to raise the concern on the real hazardous potential of terbuthylazine at least for primary producers in edge-of-field freshwater ecosystems.

The assessment of nicosulfuron occurrence in surface waters is apparently less frequent. Still, this herbicide was identified in some agricultural streams and rivers at maximum concentrations of 84 ng/L in Baie Saint-François, 266 ng/L across Midwestern United States rivers (Battaglin et al. 2000) and 525 ng/L in Central Canadian surface waters (Struger et al. 2011). Maximum surface water concentrations found so far for nicosulfuron are almost one order of magnitude lower than the lowest LOEC and EC_{20} values determined in our study (2 and 1.37 µg/L, respectively, for *Lemna minor*). Still, the studies available are scarce and not recent, while nicosulfuron is widely authorised and applied, which suggests the need for a wide update on its actual concentrations in aquatic ecosystems nowadays allowing a more comprehensive view on the hazardous environmental potential of this herbicide. In fact, the environmental risk assessment of this pesticide in Europe exposed its very high toxicity to macrophyte species, thereby constraining the pesticide use to the establishment of a mandatory buffer zone between application sites and edge-of-field waterways (EFSA 2008). Such a recommendation denotes a marked concern on the suitability of nicosulfuron to promote adverse environmental effects in surface water ecosystems by affecting primary producers.

The range of microalgae sensitivity to terbuthylazine was wide, with equi-effective concentrations differing by up to one order of magnitude between the most sensitive *R. subcapitata* and the most tolerant *C. vulgaris*. Differential sensitivity to terbuthylazine was also found for macrophytes, with *L. gibba* being significantly more sensitive than *L. minor*. Amongst the species tested, the microalgae *R. subcapitata* was the most sensitive to this herbicide hence the one delivering the most environmentally protective benchmarks. Our results were consistent with the literature, where the high sensitivity of microalgae to triazines has been demonstrated (Fairchild et al. 1997; Ma et al. 2006; Pérez et al. 2011). For example, Pérez et al. (2011) found a terbuthylazine 72 h-EC_{50} of 24 µg/L for *R. subcapitata* growth, and Sbrilli et al. (2005) determined an even lower 72 h-EC_{50} (9 µg/L) than the equivalent in the present study for *R. subcapitata*, using natural samples. Munkegaard et al. (2008) recorded an estimated terbuthylazine 7 d-EC_{50} value of 157 µg/L for *L. minor*, which is almost twice the value obtained in our study. However, the parameter evaluated in this case was the growth rate, whose magnitude is buffered by normalising to the logarithmic time range of the test thus possibly explaining a higher responsiveness of yield inhibition as used here. Consistently with our results, Cedergreen and Streibig (2005) found that *R. subcapitata* was more sensitive to terbuthylazine than the macrophyte species *L. minor*.

On the other hand, the microalgae *C. vulgaris* was more sensitive than *R. subcapitata* to nicosulfuron, both showing equi-effective concentrations more than three orders above their terbuthylazine counterparts. Ma (2002) estimated a nicosulfuron 96 h-EC_{50} value almost one order of magnitude below ours for *C. vulgaris*, but a similar one for *R. subcapitata* (Ma et al. 2006). The macrophytes *L. minor* and

L. gibba showed similar sensitivity to this herbicide, with *L. minor* being the most sensitive species among all species tested. The sensitivity of *Lemna* sp. to nicosulfuron has been sparsely recorded in the literature, with 7 days-EC_{50} values for different endpoints within 2–14.5 µg/L (Mohammad et al. 2005; Lewis et al. 2016). Also the noticed difference in sensitivity between microalgae and macrophytes is supported by the literature. Fairchild et al. (1997) compared the sensitivity of *R. subcapitata* and *L. minor* to sixteen herbicides, and the latter was more sensitive to the two sulfonylureas tested by four orders of magnitude. This trend for a higher sensitivity of macrophytes compared to microalgae was confirmed for the sulfonylureias metsulfuron-methyl and triasulfuron (Cedergreen and Streibig 2005; Munkegaard et al. 2008).

As a summary from the above, it is worth remarking that macrophytes were much more sensitive to nicosulfuron while microalgae were much more sensitive to terbuthylazine, revealing opposite trends within producers. This difference in sensitivity to each herbicide, as well as the inversion in sensitivity order between macrophytes and microalgae can relate to several physical, chemical and biological features involved in the test systems. First, the amount of chemical that reached the physiological site of action may have been distinct given that distinct uptake pathways are placed when comparing microalgae (surface-contact absorption only) and rooted macrophytes (systemic and surface-contact absorption). Linked with absorption pathways is the octanol-water partition coefficient (Kow) of the tested chemicals. The *log* Kow of terbuthylazine is much higher than that of nicosulfuron (3.4 and 0.61, respectively; Lewis et al. 2016), meaning that the former has higher liposolubility. Since lipid-soluble substances easily enter cells through the cell membranes (Reddy and Locke 1996), it is reasonable to consider that terbuthylazine can readily be uptake through the surfaces exposed to the waterborne herbicides although its touting as a systemic herbicide. This uptake route is significant both in microalgae and macrophytes, likely contributing to a lower distance in sensitivity between the organisms compared to the poorly lipid-soluble nicosulfuron. Consistently, high sensitivity of microalgae was already reported for other systemic herbicides that may be ruled by the rationale above (see e.g. Bražėnaitė and Sakaliene 2006 for the toxicity of pendimethalin towards *R. subcapitata*, with a high *log* Kow of 5.4). Also, a parallel can be made with the study by Rioboo et al. (2002), where an higher Kow value was argued to drive an accelerated uptake of a triazine by microalgae. The converse evidences regarding herbicides touted similarly as of systemic action, such as terbuthylazine and nicosulfuron highlight that care must be taken in the selection of the most appropriate non-target organism depending on the specific aims of each study, with physico-chemical properties and intake routes of pesticides being key intertwining properties featuring the expected outcomes.

Also noteworthy is the distance between the toxicity response of the two macrophyte species (belonging to the same genus) or the two microalgae species. *R. subcapitata* and *L. gibba* were markedly more sensitive to terbuthylazine than their counterparts. Regarding the exposure to nicosulfuron, *C. vulgaris* and *L. minor* were the most sensitive organisms tested in the present study but the distance in sensitivity ranges was not as marked as noticed for terbuthylazine. In fact, such a species-dependent variation within similar organisms responding to a same herbicide is common. Differential sensitivity of green microalgae species by more than one order of magnitude was found for e.g. propanil and phenmedipham (Ferraz et al. 2004; Vidal et al. 2011). Cedergreen and Streibig (2005) determined a pendimethalin EC_{50} of 0.634 mg/L for *L. minor* while Turgut and Fomin (2002) determined much higher pendimethalin EC_{50} values (10.74–24.13 mg/L) for another rooted macrophyte, *Myriophyllum aquaticum*. The interplay between the herbicides lipophilicity and the organism's surface-to-volume ratio may play a role in explaining these sensitivity variations. The shape and size of *R. subcapitata* cells compared to that by *C. vulgaris* cells is likely to translate into greater surface-to-volume ratios; also, the contact of *L. gibba* with the waterborne toxicant should be greater than that by *L. minor* provided the much larger area of the inner frond surface and much longer and larger roots. Under this rationale, the comparative sensitivity ranges to terbuthylazine found within the present study seem consistent.

The comparative sensitivity to the formulations tested (Winner Top and corresponding formulation of the a.s. terbuthylazine and nicosulfuron) was mostly species-dependent, rather than depended on the type of producer (microalgae or macrophytes). Unexpectedly (the systemic nature of the PPP would suggest that *Lemna* sp. should be by far the most sensitive non-target indicator), *L. minor* was as sensitive to the formulation ratio as *R. subcapitata*. Furthermore, in all cases but for *L. gibba*, the commercial formulation Winner Top seemed to have a protective effect compared with the customized mixture of the a.s. respecting its formulation ratio, which should probably due to interactions between formulants and the a.s. amending bioavailability of the latter. This tendency and the distinct toxicity confirmed as comparing these two formulations indirectly suggest that there is an actual contribution of the so-called inert ingredients to the overall toxicity of the product. There are previous studies confirming the non-inert property of formulants within pesticide formulations, although their capacity to increase the toxicity of the formulation was more frequently reported. Cedergreen and Streibig (2005) reported this stimulating role of the formulants of a glyphosate PPP, but no repression or

enhancement of herbicide toxicity towards aquatic species by formulants was noticed regarding formulations of terbuthylazine, metsulfuron-methyl, pendimethalin and triasulfuron. Pereira et al. (2009) also verified that Spasor® was more toxic than the respective a.s., glyphosate to the microalgae *R. subcapitata*. Pereira et al. (2000) found similar outcome for other pesticides (e.g. propanil, MCPA, molinate) using *Daphnia* and *Thamnocephalus* but, consistently with our results, *R. subcapitata* showed significantly higher sensitivity to propanil and molinate compared to the respective commercial formulations.

This study supports the idea that formulants are not necessarily inert ingredients as they are supposed to be, and confirms the importance of studying the formulations as a whole and the combination of their active substances. Unlike most published studies, the present one innovatively allowed the comparison of commercial formulation with a version of itself bearing increasing formulant concentrations, thus allowing a direct view of the contribution of formulants to the ecotoxicity of the commercial product at different equi-effective levels. The results suggest that formulants contribute to the decrease in the active substances toxicity, but the protective trend was not consistent as their concentrations increased in test solution, which evidences the need for a better understanding of the interactive effects within formulation recipes towards more efficient (concomitant target efficacy and environmental friendliness) PPP. Despite the apparent protective role of formulants used here, the fact that they were proven non-inert raises concern per se about other formulations. As such and following on our results, the immediate recommendation is towards more comprehensive assessment of the toxicity and efficacy of formulations based not only on a.i. and formulants per se, but also considering potential interactions between them within the formulation.

Funding The Portuguese Foundation for Science and Technology (FCT, Portugal) financed Libânia Queirós (SFRH/BDP/129871/2017), Tânia Vidal (SFRH/BDP/94562/2013) and Joana Luísa Pereira (SFRH/BPD/101971/2014) by means of individual grants. Thanks also due to CESAM (UID/AMB/50017) for financial support and FCT through national funds and co-funding FEDER, within the PT2020 Partnership Agreement.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval No research was conducted in the present study involving human or animal participants.

References

Abrantes N, Pereira R, De Figueiredo DR et al. (2009) A whole sample toxicity assessment to evaluate the sub-lethal toxicity of water and sediment elutriates from a lake exposed to diffuse pollution. Environ Toxicol 24:259

Abrantes N, Pereira R, Gonçalves F (2010) Occurrence of pesticides in water, sediments, and fish tissues in a lake surrounded by agricultural lands: Concerning risks to humans and ecological receptors. Water, Air, Soil Pollut 212:77–88

Azevedo D, Lacorte S, Vinhas T et al. (2000) Monitoring of priority pesticides and other organic pollutants in river water from Portugal by gas chromatography-mass spectrometry and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Chromatogr A 879:13–26. https://doi.org/10.1016/S0021-9673(00)00372-1

Baillie BR (2016) Herbicide concentrations in waterways following aerial application in a steepland planted forest in New Zealand. New Zeal J For Sci. https://doi.org/10.1186/s40490-016-0072-0

Battaglin WA, Furlong ET, Burkhard MR, Peter CJ (2000) Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. Sci Total Environ 248:123–133

Bražėnaitė J, Sakaliene O (2006) Availability and toxicity of pendimethalin to aquatic microorganisms. Biologija 3:59–62

Carlile WR (2006) Pesticide selectivity, health and the environment, 1st edn. Cambridge University Press, Cambridge

Castro MJL, Ojeda C, Cirelli FA (2014) Advances in surfactants for agrochemicals. Environ Chem Lett 12:85–95. https://doi.org/10.1007/s10311-013-0432-4

Cedergreen N, Streibig JC (2005) The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. Pest Manag Sci 61:1152–1160. https://doi.org/10.1002/ps.1117

Cowgill U, Milazzo D, Landengberger B (1991) The sensitivity of Lemna gibba G-3 and four clones of Lemna minor to eight common chemicals using a 7-day test. Res J Water Pollut Control Fed 63:991–998

de Almeida Azevedo D, Lacorte S, Vinhas T et al. (2000) Monitoring of priority pesticides and other organic pollutants in river water from Portugal by gas chromatography-mass spectrometry and liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. J Chromatogr A 879:13–26. https://doi.org/10.1016/S0021-9673(00)00372-1

DeLorenzo ME, Scott GI, Ross PE (2001) Toxicity of pesticides to aquatic microorganisms: a review. Environ Toxicol Chem 20:84–98. https://doi.org/10.1897/1551-5028(2001)0200084

Duggleby RG, McCourt JA, Guddat LW (2008) Structure and mechanism of inhibition of plant acetohydroxyacid synthase. Plant Physiol Biochem 46:309–324. https://doi.org/10.1016/j.plaphy.2007.12.004

Duggleby RG, Pang SS (2000) Acetohydroxyacid synthase. Korean Soc Biochem Mol Biol 33:1–36. https://doi.org/10.1107/S0907444901011635

Dvorák V, Caldová J, Trnková L (2012) Differential sensitivity of the Lemnaceae species to chromium and zinc. Proc ECOpole 6:505–510

EC (2009) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32009R1107&from=EN

ECB (2003) Technical guidance document on risk assessment in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances. Part II. EUR 20418 EN/2. https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf

EEA (2017) Environmental indicator report 2017. In support to the monitoring of the 7th Environment Action Programme. https://www.eea.europa.eu/airs/2017

EFSA (2008) Conclusion regarding the peer review of the pesticide risk assessment of the active substance nicosulfuron. Eur Food Saf Auth J 6:91. https://doi.org/10.2903/j.efsa.2008.120r

EFSA (2011) Conclusion on the peer review of the pesticide risk assessment of the active substance terbuthylazine. Eur Food Saf Auth J 9:1–133. https://doi.org/10.2903/j.efsa.2011.1969

EFSA PPR (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA J 11:3290. https://doi.org/10.2903/j.efsa.2013.3290

Fairchild JF, Ruessler DS, Haverland PS, Carlson AR (1997) Comparative sensitivity of Selenastrum capricornutum and Lemna minor to sixteen herbicides. Arch Environ Contam Toxicol 32:353–357. https://doi.org/10.1007/s002449900196

Ferraz DG, Sabater C, Carrasco JM (2004) Effects of propanil, tebufenozide and mefenacet on growth of four freshwater species of phytoplankton: a microplate bioassay. Chemosphere 56:315–320. https://doi.org/10.1016/j.chemosphere.2004.01.038

Fuentes MS, Briceño GE, Saez JM et al. (2013) Enhanced removal of a pesticides mixture by single cultures and consortia of free and immobilized Streptomyces strains. Biomed Res Int 2013:1–9. https://doi.org/10.1155/2013/392573

Geis SW, Leming KL, Korthals ET et al. (2000) Modifications to the algal growth inhibition test for use as a regulatory assay. Environ Toxicol Chem 19:36–41. https://doi.org/10.1002/etc.5620190105

Hildebrandt A, Guillamón M, Lacorte S et al. (2008) Impact of pesticides used in agriculture and vineyards to surface and groundwater quality (North Spain). Water Res 42:3315–3326. https://doi.org/10.1016/j.watres.2008.04.009

Jonker MJ, Svendsen C, Bedaux JJM et al. (2005) Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose-response analysis. Environ Toxicol Chem 24:2701–2713. https://doi.org/10.1897/04-431r.1

Kaza M, Mankiewicz-Boczek J, Izydorczyk K, Sawicki J (2007) Toxicity assessment of water samples from rivers in central Poland using a battery of microbiotests—a pilot study. Pol J Environ Stud 16:81–89

Kolasińska J, Bielińska M, Nałęcz-Jawecki G (2010) Assessment of fluoroquinolones toxicity with application to lemna minor microbiotest. Fresenius Environ Bull 19:1453–1457

Lewis KA, Tzilivakis J, Warner DJ, Green A (2016) An International Database for Pesticide Risk Assessments and Management. Hum Ecol Risk Assess 22:1050–1064. https://doi.org/10.1080/10807039.2015.1133242

Ma J (2002) Differential toxicity of 30 herbicides among populations of two green algae Scenedesmus obliquus and Chlorella Pyrenoidosa. Bull Environ Contam Toxicol 68:275–278. https://doi.org/10.1016/j.ecoenv.2004.12.001

Ma J, Wang S, Ma L et al. (2006) Toxicity of 40 herbicides to the green alga Chlorella vulgaris. Ecotoxicol Environ Saf 63:456–462. https://doi.org/10.1006/eesa.2001.2113

McCourt JA, Pang SS, King-Scott J et al. (2006) Herbicide-binding sites revealed in the structure of plant acetohydroxyacid synthase. Proc Natl Acad Sci USA 103:569–573. https://doi.org/10.1073/pnas.0508701103

Mohammad M, Kishimoto T, Itoh K et al. (2005) Comparative sensitivity of Pseudokirchnetiella subcapitata vs. Lemna sp. to eight sulfonylurea herbicides. Bull Environ Contam Toxicol 75:866–872. https://doi.org/10.1007/s00128-005-0830-9

Munkegaard M, Abbaspoor M, Cedergreen N (2008) Organophosphorous insecticides as herbicide synergists on the green algae Pseudokirchneriella subcapitata and the aquatic plant Lemna minor. Ecotoxicology 17:29–35. https://doi.org/10.1007/s10646-007-0173-x

Ockleford C, Adriaanse P, Berny P et al. (2017) Scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA J. https://doi.org/10.2903/j.efsa.2017.4690

OECD (2006) OECD guidelines for the testing of chemicals. Lemna sp. Growth Inhibition Test, Paris

OECD (2011) OECD guidelines for the testing of Chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test, Paris

Palma P, Köck-Schulmeyer M, Alvarenga P et al. (2014) Risk assessment of pesticides detected in surface water of the Alqueva reservoir (Guadiana basin, southern of Portugal). Sci Total Environ 488–489:208–219. https://doi.org/10.1016/j.scitotenv.2014.04.088

Palma P, Kuster M, Alvarenga P et al. (2009) Risk assessment of representative and priority pesticides, in surface water of the alqueva reservoir (South of Portugal) using On-line solid phase extraction-liquid chromatography-tandem mass spectrometry. Environ Int 35:545–551. https://doi.org/10.1016/j.envint.2008.09.015

Papadakis EN, Tsaboula A, Kotopoulou A et al. (2015) Pesticides in the surface waters of Lake Vistonis Basin, Greece: Occurrence and environmental risk assessment. Sci Total Environ 536:793–802. https://doi.org/10.1016/j.scitotenv.2015.07.099

Pereira JL, Antunes SC, Castro BB et al. (2009) Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: commercial formulation versus active ingredient. Ecotoxicology 18:455–463. https://doi.org/10.1007/s10646-009-0300-y

Pereira T, Cerejeira MJ, Espírito-Santo J (2000) Use of microbiotests to compare the toxicity of water samples fortified with active ingredients and formulated pesticides Environ Toxicol 15:401–405. https://doi.org/10.1002/1522-7278(2000)15:5 <401::AID-TOX7>3.0.CO;2-H

Pérez J, Domingues I, Soares AMVM, Loureiro S (2011) Growth rate of Pseudokirchneriella subcapitata exposed to herbicides found in surface waters in the Alqueva reservoir (Portugal): a bottom-up approach using binary mixtures. Ecotoxicology 20:1167–1175

Pfister K, Arntzen CJ (1979) The mode of action of Photosystem II-specific inhibitors in herbicide-resistant weed biotypes. J Biosci 34:996–1009. https://doi.org/10.1515/znc-1979-1123

Postigo C, López de Alda MJ, Barceló D et al. (2010) Analysis and occurrence of selected medium to highly polar pesticides in groundwater of Catalonia (NE Spain): An approach based on on-line solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry detection. J Hydrol 383:83–92. https://doi.org/10.1016/j.jhydrol.2009.07.036

Reddy KN, Locke MA (1996) Molecular properties as descriptors of octanol-water partition coefficients of herbicides. Water Air Soil Pollut 86:389–406. https://doi.org/10.1007/BF00279169

Rioboo C, González O, Herrero C, Cid A (2002) Physiological response of freshwater microalga (Chlorella vulgaris) to triazine and phenylurea herbicides. Aquat Toxicol 59:225–235. https://doi.org/10.1016/S0166-445X(01)00255-7

Sass JB, Colangelo A (2006) European Union bans atrazine, while the United States negotiates continued use. Int J Occup Environ Health 12:260–267. https://doi.org/10.1179/oeh.2006.12.3.260

Sbrilli G, Bimbi B, Cioni F et al. (2005) Surface and ground waters characterization in Tuscany (Italy) by using algal bioassay and pesticide determinations: Comparative evaluation of the results and hazard assessment of the pesticides impact on primary productivity. Chemosphere 58:571–578. https://doi.org/10.1016/j.chemosphere.2004.07.012

Selectis (2012) Winner Top Safety data sheet [PT]. pp 1–8

Silva E, Daam MA, Cerejeira MJ (2015a) Aquatic risk assessment of priority and other river basin specific pesticides in surface waters of Mediterranean river basins. Chemosphere 135:394–402. https://doi.org/10.1016/j.chemosphere.2015.05.013

Silva E, Daam MA, Cerejeira MJ (2015b) Predicting the aquatic risk of realistic pesticide mixtures to species assemblages in

Portuguese river basins. J Environ Sci (China) 31:12–20. https://doi.org/10.1016/j.jes.2014.11.006

Singh BK, Stidham MA, Shaner DL (1988) Assay of acetohydroxyacid synthase. Anal Biochem 171:173–179. https://doi.org/10.1016/0003-2697(88)90139-X

Stein JR (1973) Handbook of phycological methods: culture methods and growth measurements. Cambridge University Press, Cambridge

Struger J, Grabuski J, Cagampan S et al. (2011) Occurrence and distribution of sulfonylurea and related herbicides in central Canadian surface waters 2006-2008. Bull Environ Contam Toxicol 87:420–425. https://doi.org/10.1007/s00128-011-0361-5

Surgan M, Condon M, Cox C (2010) Pesticide risk indicators: unidentified inert ingredients compromise their integrity and utility. Environ Manag 45:834–841. https://doi.org/10.1007/s00267-009-9382-9

Tsaboula A, Papadakis E-N, Vryzas Z et al. (2016) Environmental and human risk hierarchy of pesticides: A prioritization method, based on monitoring, hazard assessment and environmental fate. Environ Int 91:78–93. https://doi.org/10.1016/j.envint.2016.02.008

Turgut C, Fomin a (2002) Sensitivity of the rooted macrophyte Myriophyllum aquaticum (Vell.) Verdcourt to seventeen pesticides determined on the basis of EC50. Bull Environ Contam Toxicol 69:601–608. https://doi.org/10.1007/s00128-002-0103-9

Vidal T, Abrantes N, Gonçalves AMM, Gonçalves F (2011) Acute and chronic toxicity of Betanal Expert and its active ingredients on nontarget aquatic organisms from different trophic levels. Environ Toxicol 27:537–548