

Concentration addition-based approach for aquatic risk assessment of realistic pesticide mixtures in Portuguese river basins

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Abstract A two-tiered outline for the predictive environmental risk assessment of chemical mixtures with effect assessments based on concentration addition (CA) approaches as first tier and consideration of independent action (IA) as the second tier was applied based on realistic pesticide mixtures measured in surface waters from 2002 to 2008 within three important Portuguese river basins ('Mondego', 'Sado' and 'Tejo'). The CA-based risk quotients, based on acute data and an assessment factor of 100, exceeded 1 in more than 39 % of the 281 samples, indicating a potential risk for the aquatic environment, namely to algae. Seven herbicide compounds and three insecticides were the most toxic compounds in the pesticide mixtures and provided at least 50 % of the mixture's toxicity in almost 100 % of the samples with risk quotients based on the sum of toxic units (RQ_{STU}) above 1. In eight samples, the maximum cumulative ratio (MCR) and the Junghan's ratio values indicated that a chemical-by-chemical approach underestimated the toxicity of the pesticide mixtures, and CA predicted higher mixture toxicity than that of IA. From a risk management perspective, the results pointed out that, by deriving appropriate programmes of measures to a limited number of pesticides with the highest contribution to the total mixture toxicity, relevant benefits also on mixture impact could be produced.

Keywords Pesticides · Mixtures · Risk assessment · Concentration addition · Surface waters · Portugal

Introduction

Different agricultural practices can cause the presence of diverse types of pesticide mixtures in the environment varying in terms of their complexity, particularly with regard to the possibility to predict the composition of the mixture, i.e. the identity and concentrations of components (Altenburger et al. 2014). Consequently, with little quantitative data on mixture toxicity for relevant assessment endpoints and numerous mixture possibilities, regulators have to make decisions based on single substance data in combination with mixture extrapolation techniques (Posthuma et al. 2008). The reference models concentration addition (CA) and independent action (IA) allow the calculation of expected combined effects purely based on concentration-effect information for the components of a mixture of concern and their concentrations in the mixture. As CA model predicts the observed toxicity of mixtures with reasonable accuracy (Kortenkamp et al. 2009), a two-tiered outline was suggested by Backhaus and Faust (2012) for predictive environmental risk assessment of chemical mixtures with effect assessments based on CA approaches as the first tier and consideration of IA as the second tier. This makes optimum use of available exposure and toxicity data from the individual chemicals to calculate risk quotients by summing up $EC/PNEC$ (=environmental concentration/predicted no effect concentration) ratios ($RQ_{EC/PNEC}$) or as sums of toxic units (RQ_{STU}) in a stepwise application, since the former provides a more conservative approach. Additional IA studies should be made if the RQ_{STU} is above threshold and only if error estimations indicate the possibility for substantial differences between CA- and IA-based assessments.

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In this context, and in an attempt to provide a general screening level risk assessment of pesticide mixtures measured in the surface waters of three important Portuguese river basins ('Mondego', 'Sado' and 'Tejo') from 2002 to 2008, the outlined approach was used adopting the procedure under the Water Framework Directive (WFD) for individual pesticides, in order (i) to determine the potential threat that pesticide mixtures pose to the aquatic biota (warranting further studies and/or risk management measures), (ii) to select the most sensitive taxonomic group and (iii) to identify the most important compounds in the pesticide mixtures in terms of TUs.

Materials and methods

Study area

In terms of water resources, 'Tejo', 'Sado' and 'Mondego' belong to the largest hydrological basins of continental Portugal occupying 25,666, 12,149 and 6659 km², respectively (APA 2014). Several studies related to surface and ground water contamination have been performed in these basins, since they are located in some of the main Portuguese agricultural areas and, therefore, are potentially at risk. In the 'Médio Tejo' and 'Lezíria do Tejo' regions, located in the 'Tejo' river basin, there are some important irrigated crops like maize, tomato for industry, rice, sugar beet, open-air horticultural crops and potato, as well as wheat and vine (RGA 2001a). Some of these crops are also found in the 'Baixo Mondego' area, particularly maize, rice and potato which occupy an important part of the agricultural area of this region (RGA 2001b). Concerning the 'Sado' river basin, the agricultural area is mainly occupied by paddy rice (RGA 2001c).

Pesticide compounds selected for the study

Twenty one herbicides, five insecticides and three metabolites were selected in this study due to their indication in the list of priority substances in the field of water policy (EC 2013), their amount sold in Portugal (DGAV 2014) since 2002, their approval for use in the main crops of the studied agricultural areas (see above section), their detection in previous studies performed in Portugal (Batista 2003; Batista et al. 2001, 2002; Cerejeira et al. 2000, 2003; Pereira 2003; Silva et al. 2006, 2011, 2012a, b) and/or their inclusion in the priority list defined in the European project 'Optimization and evaluation of multiresidue methods for priority pesticides in drinking and related waters' (Jaskulké et al. 1999).

Risk analysis

Exposure data

The exposure data used in this study correspond to 281 surface water samples collected at 43 sampling sites chosen to give a general environmental status of the 'Mondego', 'Sado' and 'Tejo' river basins during the main period of agricultural practices from 2002 to 2008, both in terms of pesticide application and irrigation. The pesticides alachlor, atrazine, chlorfenvinphos, chlorpyrifos, cyanazine, dichlobenil, endosulfan, ethofumesate, lindane, metolachlor, metribuzin, molinate, oxadiazon, pendimethalin, pirimicarb, prometryn, propanil, propazine, simazine, terbuthylazine, terbutryn and trifluralin, and the metabolites 3,4-dichloroaniline (3,4-DCA), desethylatrazine (DEA) and desisopropylatrazine (DIA) were extracted by solid-phase microextraction (SPME) followed by qualitative and quantitative analysis by gas chromatography with mass spectrometric detection (GC-MS) (Silva et al. 2012a, b), while the pesticides cycloxydim, MCPA, profoxydim and triclopyr by solid-phase extraction (SPE) followed by liquid chromatography with mass spectrometric detection (LC-MS) (Silva et al. 2006).

Table 1 presents the mean-median concentration and detection frequency values for the pesticides compounds analysed in surface waters of the 'Mondego', 'Sado' and 'Tejo' river basins. Molinate, 3,4-DCA, metolachlor and atrazine were the most frequently detected pesticides in surface waters of the 'Mondego' river basin with 62, 58, 56 and 53 % detections, respectively. In surface waters of the 'Sado' river basin, molinate, 3,4-DCA and oxadiazon were the most frequently detected pesticide compounds with 77, 61 and 52 % detections, respectively. Atrazine and alachlor were the most frequently detected pesticides in surface waters of the 'Tejo' river basin with 44 and 33 % detections, respectively. These pesticides also match the highest mean and median concentrations.

Characterisation of effects

At least one short-term datum from each of the three taxonomic groups of the basic dossier data that has to be provided within the context of Regulation (EC) No 1107/2009 (EC 2009) was used. Median effective concentration (EC₅₀) values for algae and crustaceans and median lethal concentration (LC₅₀) values for fish of the pesticide compounds selected in the present study were obtained from McBean (2012). In those cases where data was not available, the FOOTPRINT pesticide database (FOOTPRINT 2014) was consulted (Table 2). Limited toxicity data were available for the herbicide metabolites DEA and DIA. This last one was not detected in surface

Table 1 Mean-median concentration and detection frequency values for the pesticide compounds analysed in 281 surface water samples collected at 43 sampling sites in the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins from 2002 to 2008

Pesticide compound	Mean-median ($\mu\text{g L}^{-1}$)			Detection frequency (%)		
	‘Mondego’ river basin	‘Sado’ river basin	‘Tejo’ river basin	‘Mondego’ river basin	‘Sado’ river basin	‘Tejo’ river basin
Herbicide						
Alachlor	0.002-<DL	–	<DL-1.15	5	–	33
Atrazine	0.16-<0.05	–	<DL-0.57	53	–	44
Cyanazine	–	–	<DL-<DL	–	–	0
Cycloxydim	–	<DL-<DL	–	–	0	–
Dichlobenil	–	–	<DL-<DL	–	–	0
Ethofumesate	–	–	<DL-0.04	–	–	20
MCPA	–	0.02-<DL	–	–	10	–
Metolachlor	0.06-<0.05	–	<DL-0.07	56	–	20
Metribuzin	–	–	<DL-0.007	–	–	2
Molinate	1.03-0.06	5.4-0.14	–	62	77	–
Oxadiazon	0.006-<DL	0.15-<0.05	–	12	52	–
Pendimethalin	–	–	<DL-0.004	–	–	2
Profoxydim	–	0.0002-<DL	–	–	2	–
Prometryn	–	–	<DL-<DL	–	–	0
Propanil	0.007-<DL	0.01-<DL	<DL-0.16	18	14	7
Simazine	0.08-<DL	–	<DL-0.08	47	–	23
Terbuthylazine	–	–	<DL-0.09	–	–	24
Terbutryn	–	–	<DL-0.006	–	–	10
Triclopyr	–	0.07-<DL	–	–	17	–
Trifluralin	–	–	<DL-<DL	–	–	0
Metabolite						
3,4-DCA	0.33-<0.05	0.39-<0.05	<DL-0.35	58	61	7
DEA	–	–	<DL-0.005	–	–	3
DIA	–	–	<DL-<DL	–	–	0
Insecticide						
Chlorfenvinphos	0.03-<DL	0.05-<DL	<DL-0.007	9	18	5
Chlorpyrifos	–	–	<DL-0.02	–	–	16
Endosulfan	0.0008-<DL	0.01-<DL	<DL-0.02	3	7	7
Lindane	–	–	<DL-<DL	–	–	0
Pirimicarb	–	–	<DL-<DL	–	–	0
At least one of the pesticide compounds				93	49	82

– no data, <DL below the detection limit

water samples, while DEA was only detected in four samples in the ‘Tejo’ river basin (see Table 1). In order to derive the first tier (standard test species approach) PNEC values for pesticides, Lepper (2005) proposed to use an assessment factor (AF) of 100 to the lowest acute toxicity value. This methodology was adopted in the technical guidance for deriving maximum acceptable concentration-quality standards (MAC-QS) to protect against possible effects from short-term concentration peaks (EC 2011).

CA-based risk quotients for pesticide mixtures

The first tier of the approach suggested by Backhaus and Faust (2012) used exclusively CA as a basis for the preliminary risk assessment of the pesticide mixture(s) of concern. This tier itself contains two consecutive steps, based on $RQ_{MEC/PNEC}$ and RQ_{STU} . $RQ_{MEC/PNEC}$ (Eq. 1) denotes a final risk quotient for the pesticide mixture that was calculated by simply summing up measured environmental concentration (MEC)/PNEC ratios of all individual

Table 2 Median lethal (effective) concentration (L(E)C₅₀; in mg L⁻¹) for algae, crustaceans and fish, and predicted no effect concentration (PNEC) values (in µg L⁻¹), for the pesticide compounds selected in the present study

Pesticide compound	Algae EC ₅₀ (72 h) (mg L ⁻¹)	Crustaceans (<i>Daphnia</i> sp.) EC ₅₀ (48 h) (mg L ⁻¹)	Fish LC ₅₀ (96 h) (mg L ⁻¹)	PNEC (µg L ⁻¹)
Herbicide				
Alachlor	0.012	13	2.1	0.12
Atrazine	0.043	29	4.3	0.43
Cyanazine	0.2	42	16	2
Cycloxydim	44.9	71	100	449
Dichlobenil	111	6.2	5	50
Ethofumesate	3.9	13.52	10.92	39
MCPA	79.8	190	50	500
Metolachlor	0.1	25	3.9	1
Metribuzin	0.021	49.6	74.6	0.21
Molinate	0.5	14.9	16	5
Profoxydim	33	18.1	13	130
Prometryn	0.002	12.66	5.5	0.02
Propanil	0.11	4.8	5.4	1.1
Propazine	0.18	17.7 ^a	17.5	1.8
Oxadiazon	0.004	2.4	1.2	0.04
Pendimethalin	0.018	0.4	0.707	0.18
Simazine	0.042	100	49	0.42
Terbuthylazine	0.016	69.3	2.2	0.16
Terbutryn	0.0017	2.66	1.1	0.017
Triclopyr	75.8	133	117	758
Trifluralin	0.0122	0.245	0.088	0.122
Metabolite				
3,4-DCA	1.65	0.12	1.94	1.2
DEA	0.1	–	–	1
DIA	–	–	–	–
Insecticide				
Chlorfenvinphos	1.6	0.0003	0.04	0.003
Chlorpyrifos	0.48	0.0017	0.002	0.017
Endosulfan	0.56	0.075	0.002	0.02
Lindane	2.5	1.6	0.022	0.22
Pirimicarb	140	0.017	55	0.17

– no data

^a Unknown species

pesticide compounds. The PNEC was calculated individually for each pesticide compound selected for the study. This was derived by the standard test species (deterministic) approach according to the technical guidance for deriving environmental quality standards (EC 2011): an AF of 100 was applied to the lowest L(E)C₅₀ of the acute toxicity data (Table 2). RQ_{STU} (Eq. 2) in contrast denotes a procedure where sums of toxic units (STU) were first calculated for each trophic level separately. Afterwards, that organism group that was most sensitive to the pesticide mixture (i.e. for which the highest STU was calculated) was selected, and by

applying the corresponding AF (100), a risk quotient for the pesticide mixture RQ_{STU} was then calculated.

$$RQ_{PEC/PNEC} = \sum_{i=1}^n \frac{PEC_i}{PNEC_i}$$

$$= \sum_{i=1}^n \frac{PEC_i}{\min(EC_{50} \text{ algae}, EC_{50} \text{ Daphnid}, EC_{50} \text{ fish})_i} \times \frac{1}{AF_i} \quad (1)$$

$$RQ_{STU} = \max(STU_{\text{algae}}, STU_{\text{Daphnid}}, STU_{\text{fish}}) \times AF$$

$$= \max\left(\sum_{i=1}^n \frac{PEC_i}{EC_{50i, \text{algae}}}, \sum_{i=1}^n \frac{PEC_i}{EC_{50i, \text{Daphnid}}}, \sum_{i=1}^n \frac{PEC_i}{EC_{50i, \text{fish}}}\right) \times AF \quad (2)$$

Table 3 Frequency of exceedance of $RQ_{MEC/PNEC}$ for the pesticides compounds detected in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins

River basin	No. of samples	Freq. $RQ_{MEC/PNEC}$ ^a (%)
Mondego	103	31.1
Sado	56	46.4
Tejo	122	42.6

^a Frequency of exceedance of $RQ_{MEC/PNEC} = \sum n/N$, where n is the number of samples with $RQ_{MEC/PNEC}$ ratios above 1, and N is the total number of samples with analytical measurements for the pesticide compounds selected for the study

The former risk quotient provides the more conservative approach but might violate the assumption of a common biological end point that is inherent to the concept of CA. Hence, if $RQ_{MEC/PNEC}$ is above 1, i.e. indicated potential for reason for concern, RQ_{STU} can be calculated in a next step.

Only if there are still indications for a potential risk (i.e. when $RQ_{STU} > 1$) tier II commences. Tier II takes IA into consideration, either as a single model or in the form of so-called mixed models which have been suggested in the literature as combinations of both CA and IA. This tier will, due to the data demands of IA, often require additional experimental studies.

The critical decision on whether to proceed to a tier II hazard assessment was driven by the possible ratio of the CA- and IA-expected mixture toxicities. A tier II assessment only makes sense when the number of pesticide compounds present in the scenario and their specific toxic unit distribution indicate that the sole application of CA might lead to a substantial risk overestimation. An appropriate approach to predict the maximum possible ratio between the mixture EC_{50} predicted by both concepts has been put forward by Junghans et al. (2006) for mixtures of pesticides and is equal to or smaller than the sum of all toxic units divided by the highest

individual toxic unit of the components that make up the mixture (Eq. 3).

$$\frac{EC_{50IA}}{EC_{50CA}} \leq \frac{\sum_{i=1}^n \frac{ci}{EC_{50i}}}{\max_{i \in (1, \dots, n)} \left(\frac{ci}{EC_{50i}} \right)} \quad (3)$$

The maximal factor by which CA may predict a lower EC_{50} value and thus a higher toxicity than IA equals the number of mixture components, irrespective of the other determining factors (the concentration ratio of mixture components, the slope of the individual concentration response curves and the effect level under consideration). However, if any mixture component has a TU that differs from the TUs of the remaining components, the maximal factor will always be smaller than n (Junghans et al. 2006).

Results and discussion

Aquatic risk of pesticide mixtures

The two-tiered approach for predictive environmental risk assessment of chemical mixtures proposed by Backhaus and Faust (2012) was applied to pesticide monitoring data from surface waters in the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins. A database considering the concentration as given of 14 herbicides (alachlor, atrazine, ethofumesate, MCPA, metolachlor, metribuzin, molinate, oxadiazon, pendimethalin, propanil, simazine, terbuthylazine, terbutryn and triclopyr), 3 insecticides (chlorfenvinphos, chlorpyrifos and endosulfan) and 1 metabolite (3,4-DCA) detected at least once provided the input for the study (see Table 1).

The frequency of samples with the summation of MEC/PNEC ratios above 1 was calculated for surface waters of the

Table 4 Frequency of exceedance of RQ_{STU} and frequency of maxSTU for algae, crustaceans and fish for the pesticides compounds detected in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins

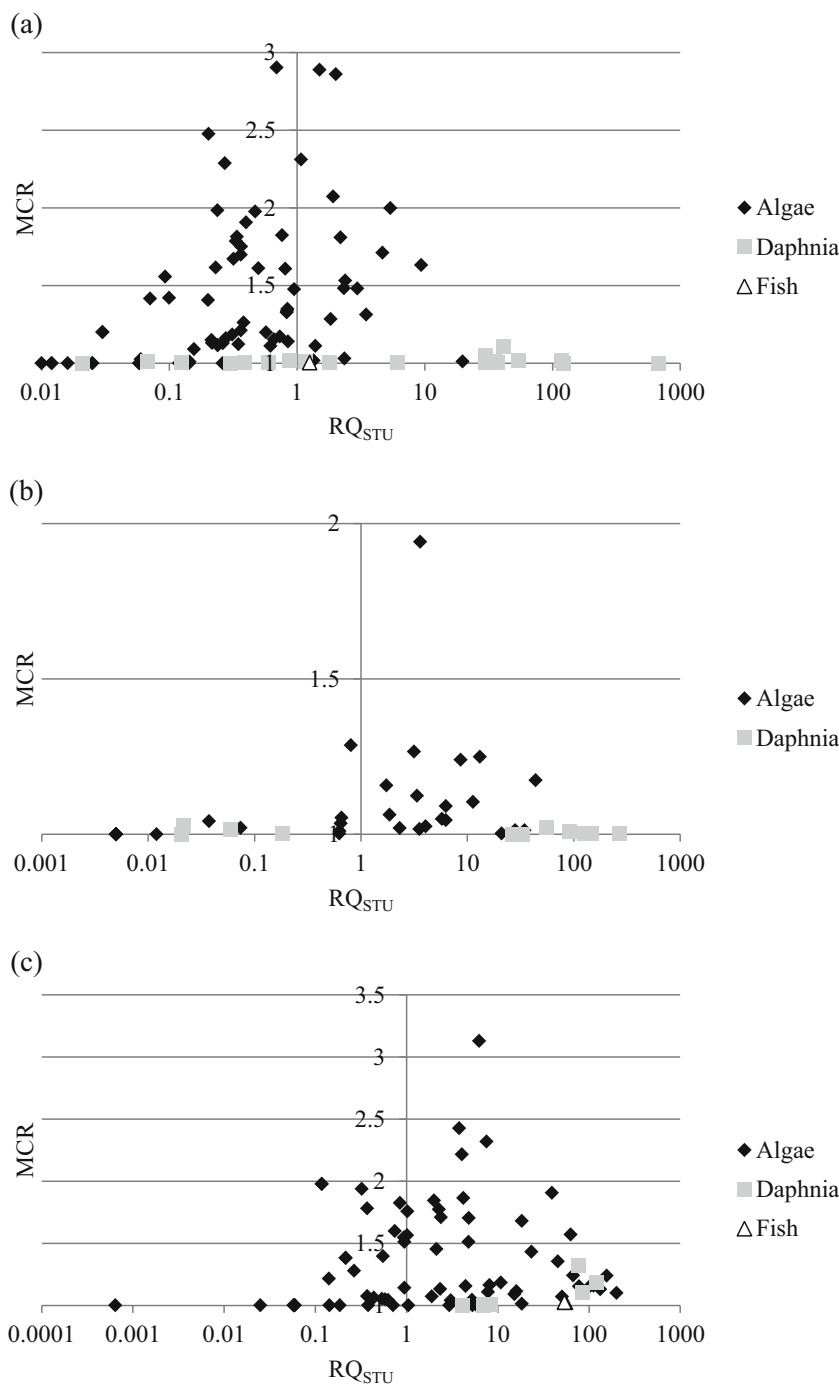
River basin	No. of samples	Freq. RQ_{STU} ^a	Freq. max ^b (%)		
			STUalgae	STUcrustaceans	STUfish
Mondego	32	94	56.7	40	3.3
Sado	26	100	69.2	30.8	–
Tejo	52	100	82.7	13.5	3.8

– no data

^a Frequency of exceedance of $RQ_{STU} = \sum n/N$, where n is the number of samples with RQ_{STU} ratios above 1, and is the total number of samples with $RQ_{MEC/PNEC}$ ratios above 1

^b Frequency of maxSTU = $\sum n/N$, where n is the number of samples with maxSTU for algae, crustaceans and fish, and N is the total number of samples with $RQ_{MEC/PNEC}$ ratios above 1

Fig. 1 Log-linear plot of MCR versus maxRQ_{STU} for algae, crustaceans and fish for the pesticides compounds detected in surface waters of the ‘Mondego’ (a), ‘Sado’ (b) and ‘Tejo’ (c) river basins



‘Mondego’, ‘Sado’ and ‘Tejo’ river basins using measured concentrations per sample. Subsequently, the number of samples where the threshold was exceeded was divided by the total number of samples where the pesticide compounds were monitored. Table 3 shows that the RQ_{MEC/PNEC} ranges from 31.1 % (‘Mondego’ river basin) to 46.4 % (‘Sado’ river basin). For those samples that indicate potential for reason for concern (RQ_{MEC/PNEC}>1), RQ_{STU} was calculated in a next step. The frequency of samples with the summation of STU ratios above 1 was calculated for surface waters of the ‘Mondego’,

‘Sado’ and ‘Tejo’ river basins using measured concentrations per sample. Subsequently, the number of samples where the threshold was exceeded was divided by the total number of samples where the frequency of samples with summation of PEC/PNEC ratios was above 1. Table 4 shows that RQ_{STU} was above 1 in almost all samples where the frequency of samples with the summation of PEC/PNEC ratios was above 1. The ratio between those two mixture risk quotients was smaller than the number of considered trophic levels, as proven by Backaus and Faust (2012), reaching 2.055 in a

surface water sample from the ‘Mondego’ river basin due to differences in the sensitivities of the mixture compounds for the three organism groups.

The taxonomic group with the highest predicted sensitivity to the pesticide mixtures (maximum STU of all analysed trophic levels) measured in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins was algae, followed by crustaceans and fish (Fig. 1, Table 4). This pattern is consistent with the ecotoxicity profiles of the individual pesticide compounds, mainly of herbicides to which algae are more sensitive than aquatic animals, and with higher detection frequency and measured values. Long-term trends of pesticide exposure in Swedish agricultural streams normalised to their potential effects for algae (chronic), invertebrates and fish (both acute) were also assessed using the toxic unit approach. Although in this study, the monitoring data showed a continuous but rather low toxic potential of pesticides for all three trophic levels throughout the year (Bundschuh et al. 2014).

The ratio cumulative toxicity/maximum toxicity from one chemical defined as maximum cumulative ratio (MCR) provides a quantitative measure of the magnitude of the toxicity that is underestimated by not performing a cumulative risk assessment (Price and Han 2011). Figure 1 presents the MCR- RQ_{STU} plots for algae, crustaceans and fish for the pesticide compounds detected in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins. In these plots, RQ_{STU} is plotted on a log scale, and MCR is plotted on a linear scale with a

minimum value of 1. As Fig. 1 indicates, in almost all samples with RQ_{STU} above 1, the MCR values were smaller than 2 indicating that one pesticide compound provides at least 50 % of the mixture’s toxicity. For four samples in the ‘Mondego’ river basin (Fig. 1a) and four in the ‘Tejo’ river basin with maxSTU for algae (Fig. 1b), the MCR values averaged 2.528 and ranged from 2.073 to 3.127. These values indicate that the fraction of toxicity that comes from the most toxic pesticide compound averages 40 % and ranges from 32 to 48 %.

Seven herbicide compounds and two organophosphate insecticides were accountable for the highest risk of toxicity for algae and crustaceans, respectively, in samples with RQ_{STU} ratios above 1 in the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins. Despite crustaceans are not the primary ecological receptors of the herbicide metabolite 3,4-DCA, it also contributed with the greatest expected mixture toxicity to the sumTU in surface waters of the ‘Mondego’ and ‘Sado’ river basins. For fish, the insecticide organochloride endosulfan was responsible for the highest mTUs in three samples of the ‘Mondego’ and ‘Tejo’ river basins (Table 5). The mTU values were predominantly associated with herbicide compounds for which detection frequency, measured concentration and/or acute toxicity values for algae were relatively high. It has to be noted that the risk of more polar, thermo-labile and less volatile pesticide compounds was not included in the analytical survey of the present study. This can lead to a considerable underestimation of the overall toxicity of the

Table 5 Pesticide compounds and number of times accountable for mTU for algae, crustaceans and fish in samples with RQ_{STU} ratios above 1 in the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins

Pesticide compound	‘Mondego’ river basin (<i>n</i> =30)			‘Sado’ river basin (<i>n</i> =26)			‘Tejo’ river basin (<i>n</i> =52)		
	No. mTU for algae	No. mTU for crustaceans	No. mTU for fish	No. mTU for algae	No. mTU for crustaceans	No. mTU for fish	No. mTU for algae	No. mTU for crustaceans	No. mTU for fish
Herbicide									
Alachlor	–	–	–	–	–	–	24	–	–
Atrazine	5	–	–	–	–	–	10	–	–
Metolachlor	2	–	–	–	–	–	1	–	–
Molinate	6	–	–	4	–	–	–	–	–
Oxadiazon	2	–	–	14	–	–	–	–	–
Simazine	2	–	–	–	–	–	1	–	–
Terbuthylazine	–	–	–	–	–	–	–	–	–
Terbutryn	–	–	–	–	–	–	7	–	–
Metabolite									
3,4-DCA	–	3	–	–	–	–	–	–	–
Insecticide									
Chlorfenvinphos	–	9	–	–	8	–	–	3	–
Chlorpyrifos	–	–	–	–	–	–	–	4	–
Endosulfan	–	–	1	–	–	–	–	–	2

– no data

surface water samples. In a study carried out in medium-sized Swiss rivers, it was possible to evaluate how a complete pesticide screening changes the assessment of surface water quality (Moschet et al. 2014).

Junghans et al. (2006) observed that the ratio STU/mTU (MCR) could also be used to predict when dose additive and independent action models of a mixture’s toxicity produce similar or divergent estimates of toxicity. Table 6 presents the maximal possible ratio between the EC₅₀ values of the pesticide mixtures predicted by IA and by CA taking into account the number of mixture components and the taxonomic group with maxSTU in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins. For each of these taxon-exposure scenarios, the maximal value by which CA may predict a higher toxicity than IA was lower than the value given by the number of mixture components (between one and seven) and never exceeded

a factor of 3.127. This indicates that the toxicity of all pesticide mixtures is dominated by a very small fraction of the compounds present.

Uncertainties

The suggested approach for a predictive assessment of the mixture toxicity from a given agricultural exposure scenario is intended to give a reasonable, protective and pragmatically useful scheme for further use within risk assessment procedures. Nevertheless, one circumstance can challenge the precautionary character of the approach. Potential synergistic interactions that results in more-than-concentration-additive effects were not covered. There are a number of cases in the literature of synergistic interactions between pesticides. In some cases, the interactions were designed to be synergistic in nature (e.g. where one chemical inhibited the detoxification pathway of another) and thus were predictable. In other cases, a greater degree of synergism was observed only at higher exposure concentrations that may have affected secondary, non-target sites of action. Studies that were designed to determine mixture toxicity at environmentally relevant concentrations did not produce effects beyond those predicted by assuming concentration addition (Rodney et al. 2013).

It is also important to highlight that for the derivation of the PNEC for the pesticide compounds selected in the present study, the deterministic approach was the preferred method. However, the EQSs for some of them were already laid down in the law as a value (EC 2013), but in that case, their derivation followed a weight-of-evidence approach according to the WFD. This implicitly means that the resulting QS, whether it is derived using the AF method, the species sensitivity distribution (SSD) method, or using model ecosystem studies, are all considered reliable.

Table 6 Maximal value by which CA may predict a higher toxicity than IA taking into account the number of mixture components and the taxonomic group with maxSTU in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins

No. of mixture components	Taxon	$\sum_{i=1}^n TU_i / \max\{TU_i\}_{i \in \{1, \dots, n\}}^a$		
		‘Mondego’ river basin	‘Sado’ river basin	‘Tejo’ river basin
1	Algae	–	–	1
2	Algae	1.031	1.942	1.757
3	Algae	1.810	1.174	2.425
4	Algae	2.073	1.250	2.318
5	Algae	2.312	1.013	3.127
6	Algae	2.890	1.104	1.905
7	Algae	2.861	–	–
1	Crustaceans	–	–	–
2	Crustaceans	–	1.000	–
3	Crustaceans	1.004	–	–
4	Crustaceans	1.002	1.001	1.001
5	Crustaceans	1.012	1.022	1.000
6	Crustaceans	1.110	1.001	1.315
7	Crustaceans	1.048	–	1.007
1	Fish	–	–	–
2	Fish	–	–	–
3	Fish	–	–	–
4	Fish	–	–	1.025
5	Fish	1.003	–	1.025
6	Fish	–	–	–
7	Fish	–	–	–

– no data

^a Calculated according to Eq. (3): the ratio between EC_x^{IA} and EC_x^{CA} cannot exceed the given value

Conclusions

The proposed approach provides a general ‘screening level’ risk assessment of pesticide mixtures in surface waters of the ‘Mondego’, ‘Sado’ and ‘Tejo’ river basins and support the view that CA provides a pragmatic precautionous but not an overprotective approach, irrespective of their modes/mechanisms of action. The data generated are of importance for identifying sites of noncompliance and for obtaining a priority list of pesticide mixtures with potential aquatic risk. This list could be very useful for the evaluation of the current control measures that are mostly based on assessments of chemical risks focused on single pure toxicants.

For a given number of pesticide compounds between one and seven and a given response level of 50 %, the maximal factor by which IA may predict a higher EC_{50mix} value than

CA was lower than 2 in almost 100 % of the samples with RQ_{STU} above 1, i.e. it indicates that only one pesticide compound provides at least 50 % of the mixture's toxicity.

With respect to non-target group of organisms, they often were the primary ecological receptors of the most toxic compounds of the pesticide mixtures (e.g., herbicides on algae and insecticides on crustaceans and fish). Despite having a different mode of action (e.g., the herbicide metabolite 3,4-DCA on crustaceans), this also contribute with the highest toxic unit in three pesticide mixtures.

Algae are most at frequent risk because of higher herbicide exposure within this analytical survey to pesticides in surface waters of the 'Mondego', 'Sado' and 'Tejo' river basins and their sensibility to these compounds.

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