

Hazard/Risk Assessment

Mix-Tool: An Edge-of-Field Approach to Predict Pesticide Mixtures of Concern in Surface Water From Agricultural Crops

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Abstract: Current regulation on the authorization of plant protection products (PPPs) in the European Union is limited to the evaluation of ecological risks for the single active substances they contain. However, plant protection treatments in agriculture often consist of PPPs already containing more than one active substance; moreover, each cropped field receives multiple applications per year, leading to complex pesticide mixtures in the environment. Different transport processes lead to a multitude of heterogeneous and potentially toxic substances that, for example, may reach water bodies and act simultaneously on natural freshwater ecosystems. In this context, the development of methodologies and tools to manage risks of pesticides mixtures is imperative to improve the current ecological risk assessment procedures and to avoid further deterioration of ecological quality of natural resources. The present study suggests new procedures for identifying pesticide mixtures of potential concern released from agricultural crops in surface water. The approach follows the European Union regulatory context for the authorization of PPPs in the market (edge-of field risk assessment) and requires the use of Forum for the Co-ordination of pesticide fate models and their Use (FOCUS) models (Step 3 and 4) for calculating the concentrations in surface water of mixture components on a daily basis. Moreover, it uses concentration addition models to calculate the toxic potency of the pesticide mixtures released by a treated crop. To implement this procedure, we developed a simple Microsoft-Excel-based tool. We also considered two case studies (maize and apple tree), representative of Italian agricultural scenarios for annual and perennial crops. Moreover, we compared results with 3 years of monitoring data of surface water bodies of the Lombardia region (northern Italy) where the two crops are largely present. *Environ Toxicol Chem* 2022;41:2028–2038. © 2022 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

The European Union agricultural landscape is characterized by a widespread presence of crops and livestock rearing (EUROSTAT, 2020). The management of agricultural activities usually involves the use of different agrochemicals, such as plant protection products (PPPs), fertilizers, and veterinary pharmaceuticals, which show the potential to be transferred into various environmental compartments, resulting in potential risks for ecosystems at different spatial scales. For instance,

PPPs can pose risk to aquatic organisms on a local scale when reaching small water bodies near treated crops (Hartz et al., 2017; Schulz, 2004; Schwarzenbach et al., 2006). Moreover, their transport through air and water can lead to contamination at river basin level (Bonzini et al., 2006; Ccanccapa et al., 2016; Deknock et al., 2019; Morselli et al., 2018; Rabiet et al., 2010) or even on a global scale in remote areas (Daly & Wania, 2005; Rizzi et al., 2019).

Over the decades, the increasing frequency of PPP detection in the environment (Chow et al., 2020; Finizio et al., 2011; Le Cor et al., 2021; Malaj et al., 2014; Schreiner et al., 2016) has raised concern in citizens, scientists, and authorities. For this reason, the use of PPPs is subjected to stringent regulations, at least in most developed countries. Indeed, prior to authorization they undergo standardized human health and environmental risk assessment (HHRA and ERA, respectively) procedures. In ERA procedures, the European Union has adopted a so-called tiered approach for the

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placing of PPPs on the market (European Commission [EC], 2009). For aquatic organisms, the risk is calculated in small edge-of-field water bodies with limited potential for dilution and this is considered as a realistic worst-case scenario (European Food and Safety Agency [EFSA], 2013). The lower tiers are based on the calculation of the exposure/toxicity ratio, which is obtained by dividing a point estimate of exposure (predicted environmental concentration [PEC]) by a point estimate of effects (regulatory acceptable concentration; EC, 2009; EFSA 2013). The latter is assessed using environmental fate models, such as those developed by the Forum for the Coordination of pesticide fate models and their Use (FOCUS) group (FOCUS, 2001). For the evaluation of exposure in surface waters, the FOCUS group proposed different tiers with increasing complexity and realism. In particular, the third tier (Step 3) forecasts the application of predictive models in 10 environmental scenarios, developed by the FOCUS group, designed to represent at least the 90th percentile worst case for surface water exposure resulting from agricultural pesticide use within the European Union (FOCUS, 2001).

According to the European Union regulation for PPPs (EC, 2009), ERA procedures have to be always be conducted on single active substances; furthermore, ERA may be conducted on commercial formulations, which can include different active substances (plus solvents or surfactants), but also in this case the risk is assessed for each single component.

On the other hand, the use of several PPPs during the growth season of a crop can lead to the release in the edge-of-field water bodies of mixtures that are highly variable in the number of constituents and in their relative concentrations (Belden et al., 2007; de Zwart, 2005; Finizio et al., 2005). This high variability will depend on both the choices of farmers in the selection of PPPs and the environmental conditions, which can favor the insurgence of parasites or influence the fate of PPPs (runoff, degradation, and volatilization). The problem is exacerbated at river basin scale where different crops are contemporaneously present. The co-occurrence of pesticides in surface waters has been highlighted in several studies (Curchod et al., 2020; Finizio et al., 2022; Gustavsson et al., 2017; Junghans et al., 2005; Moschet et al., 2014; Relyea, 2008; Schreiner et al., 2016) and this has raised concern also in light of the achievement of a satisfactory ecological status for European water bodies as required by the European Union Water Framework Directive (EC, 2000; EFSA, 2019; Organisation for Economic Co-operation and Development [OECD], 2018; Posthuma et al., 2018; Scientific Committee on Health and Environmental Risks (SCHER), Scientific Committee on Emerging and Newly Identified Health Risks, & Scientific Committee on Consumer Safety, 2012; Vighi et al., 2003). Indeed, the toxicity of mixtures for aquatic organisms can exceed that produced separately by single compounds (SCHER et al., 2012) and this can lead to an underestimation of their effects in water bodies.

The expected toxicity of mixtures can be calculated based on the known toxicities of the components and using two different models, concentration addition and independent action. The first model is based on the assumption that all substances in the

examined mixture show a similar mechanism of action or affect the same target in organisms (Altenburger et al., 2000), whereas the second considers that the mixture components act dissimilarly (Backhaus et al., 2000). In general, the concentration addition model is assumed as a reasonable conservative worst case based on consideration that the mixture toxicity calculated with this model is generally higher than that obtained using the independent action model. For this reason, the concentration addition model is more widely utilized, particularly when no information about the modes of action of mixture constituents is available (SCHER et al., 2012). However, there is still some debate about the predictive capability of this model as some studies have highlighted a deviation up to 300% of the predicted effects compared with those measured (Cedergreen et al., 2008). In this, the availability of tools or methodological approaches useful to identify mixtures of concern is of the utmost importance to elucidate the predictive performances of the models and to develop new risk assessment procedures for mixtures. Earlier studies tried to identify pesticide mixtures using available monitoring data focusing on both small- and large-scale areas (Bundschuh et al., 2014; Finizio et al., 2022; Gustavsson et al., 2017; Petersen et al., 2012; Schreiner et al., 2016). Other suggested approaches are based on the integration, in GIS environments, of relational and spatial databases (environmental characteristics, crops distribution, pesticides use, and their properties) with models to estimate the levels of exposure (PECs) in surface water (Verro et al. 2009a, 2009b) or groundwater (Di Guardo & Finizio, 2016). To the best of our knowledge, very few studies have focused on the assessment of the composition and toxicity of a mixture likely to occur from a single crop (edge-of-field approach). In 2005, Finizio et al. (2005) proposed a methodology to identify pesticide mixtures of potential concern associated with single crops. The approach was based on the integration of the SoilFug model (Di Guardo et al., 1994) for PEC calculation, ecotoxicological data, and the concentration addition model. However, SoilFug was applied in a very generic environmental scenario based on several default assumptions. In the present study, we move a step forward in this methodology and suggest using FOCUS models (Steps 3 and 4) for calculating PECs. There are several advantages in the new tool proposed: (i) we move towards a regulatory context (the FOCUS model is used for the authorization of active substances and PPPs in the market); (ii) the environmental scenarios are not generic but well defined by the FOCUS group; and (iii) the application of the FOCUS model allows an evaluation edge-of-field of the considered crop. This permits the calculation of the daily emission of PPP mixtures during the seasonal cycle and according to the chosen treatment options; consequently, it is possible to identify more appropriately the pesticide mixtures of potential concern.

The tool developed is a simple Microsoft-Excel-based tool (Mix-Tool), downloadable from the Supporting Information, which allows the results obtained from all the applications of the FOCUS model (one run for each potential PPPs registered on a particular crop) to be combined temporally. To test the new tool, we applied the procedure to two case studies (maize and apple tree), which are representative of the Italian agricultural context. Moreover, we compared the results with

available monitoring data for surface water bodies in the Lombardia region (northern Italy) where the two crops are largely present. In the present study, we report the obtained results, describe the approach and highlight its potential for the identification of pesticide mixtures of concern.

MATERIALS AND METHODS

General considerations on the methodological approach

A flowchart of the proposed method is shown in Figure 1. The approach starts with the identification of active substances, which are authorized on a specific crop, and their relative application options (good agricultural practices). Data on the physical-chemical and eco-toxicological properties of the active substances are then collected. Physical-chemical properties and the application options are used to calculate PECs in surface water using FOCUS models (Steps 3 and 4). Finally, PECs (calculated on daily basis) and the ecotoxicological data are used as input of the Mix-Tool to identify pesticide mixtures of potential concern.

More details of the proposed approach, applied to the two case studies, are reported in the following sections.

Physical-chemical and ecotoxicological properties of selected pesticides and treatment options.

The procedure starts with the identification of the active substances authorized for use on selected crops. As case studies, we have considered maize and apple tree crops, which are largely representative of the Italian agricultural scenario (588 597 Ha for maize, 114 050 Ha for apple trees; Istituto Nazionale di Statistica [ISTAT], 2022) and in particular in its most intensive agricultural areas, located in the Lombardia region (northern Italy). By consulting the Italian database on pesticides (Sistema Informativo Agricolo Nazionale, 2021) we selected 10 of the most used active substances registered for these crops in Italy in 2018. The final list does not include inorganic active

TABLE 1: Ten of the most widely used pesticides applied on maize and apple trees in Lombardia (Italy)

Active substance	Abbreviation	Classification	Crop	
			Maize	Apple trees
Bentazone	Bent	Fungicide	X	–
Captan	Cap	Fungicide	–	X
Chlorpyrifos	Chlor	Insecticide	X	X
Chlorpyrifos-CH ₃	Chlor-met	Insecticide	X	–
Dicamba	Dica	Herbicide	X	–
Dithianon	Dith	Fungicide	–	X
Flufenacet	Flu	Herbicide	X	–
Fosetyl-aluminium	Fos-Al	Fungicide	–	X
Glyphosate	Gly	Herbicide	X	X
Mancozeb	Manc	Fungicide	–	X
MCPA	MCPA	Herbicide	X	X
Mesotrione	Meso	Herbicide	X	–
Metiram	Meti	Fungicide	–	X
Pendimethalin	Pend	Herbicide	X	X
s-metolachlor	s-Meto	Herbicide	X	–
Tebuconazole	Tebu	Fungicide	–	X
Terbutylazine	Terb	Herbicide	X	–

substances, because FOCUS models are not fully validated for these substances. Moreover, we also excluded those active substances that expired before 2018 to get a realistic overview of recently used substances.

Table 1 lists selected active substances for the two considered crops. To define the application scenarios (rate of application, number of treatments, and application dates), the Pesticidoc database was used (International Centre for Pesticide Safety [ICPS], 2021) to extract data from the labels of at least 10 PPPs containing the selected active substances. Then, we selected the application leading to the worst-case loading in surface water. It should be noted that the results were obtained by considering a single application date for each treatment, and therefore these results must not be taken as a representation of the reality, but just as a potential situation which shows the level of detail that can be achieved by the Mix-Tool. The selected application data are reported in Supporting Information Table S1.

PEC calculation in surface water. The second step of the proposed approach is the calculation of PECs in surface water by using FOCUS models according to the application scenarios previously identified.

Physical-chemical properties of the active substances, as well as their sorption and degradation data are the input needed by FOCUS models to simulate the behavior of the chemicals in the environment. In our study, we retrieved these data from EFSA Conclusion documents or Review reports. Supporting Information Table S2 reports the selected data together with the original reference. We calculated PECs following the European Union guidelines developed by the FOCUS workgroup (FOCUS, 2001) and deployed in the context of the European Union and national registration of pesticides.

For PEC calculation in surface water, FOCUS guidelines suggest a four-step approach with increasing realism

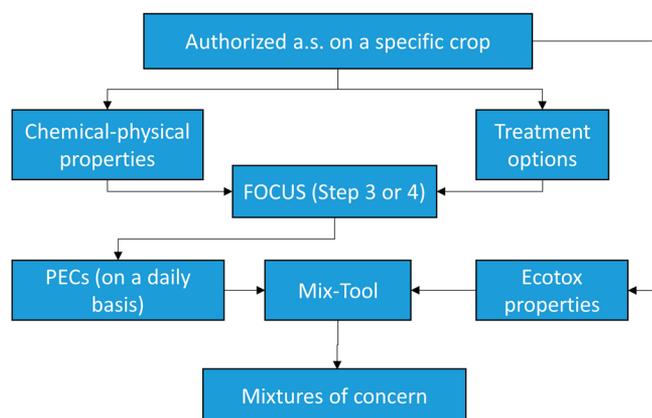


FIGURE 1: Flow diagram of the procedure to identify pesticide mixtures of concern released in surface water from a given crop. a.s. = active substance; FOCUS=Forum for the Co-ordination of pesticide fate models and their Use; PECs=predicted environmental concentrations.

(FOCUS, 2001). For our case studies, we calculated PECs using the three-step approach and, when necessary, the software SWAN 5.0, for the refinement of PEC by considering risk mitigation measures (FOCUS Step 4). For the application of the predictive models according to Step 3, the FOCUS Workgroup identified 10 realistic worst-case environmental scenarios, six representing drainage scenarios (D1–D6) and four representing run-off scenarios (R1–R4). The scenarios were developed taking into account agronomic and climatic conditions relevant to the different crops, and a selection of typical water bodies. In our study, we considered only those scenarios that are relevant for the Italian context (D4, D6, R3, and R4 for maize and D4, R3, and R4 for apple trees).

The following software, recommended by the European Union guidance, was used (European Soil Data Center, 2021):

- FOCUS SWASH (Ver 5.3): is a user-friendly shell that is used to input all the data needed for Step 3 calculations, such as physical-chemical properties, degradation rates, and application dates. In our study we have estimated the application dates from the BBCH index (Meier, 2001) and using the AppDate tool (Klein, 2012). Depending on the BBCH index at the time of spreading, the application method varied between direct incorporation to soil or foliar application.
- FOCUS_MACRO (Ver 5.5.4): this model is used to simulate pesticide drainage loadings towards surface water.
- FOCUS_PRZM_SW (Ver 4.3.1): this model is used to simulate pesticide run-off loadings towards surface water.
- FOCUS_TOXSWA (Ver 5.5.3): this model is used to manage the output data from MACRO and PRZM, and to assess the fate of the active substances in surface water, considering the degradation of chemicals in the water bodies alongside the field as well as the repartition in the sediment layer.
- SWAN (Ver 5.0): is a tool to incorporate the effects of mitigation measures (spray drift and run-off) on PEC calculations.

The default setting of FOCUS models predicts only the maximum concentration in surface water (PEC_{sw}) and subsequent concentrations in a few predetermined time intervals after the maximum PEC_{sw} is reached. However, as our approach requires daily concentrations of pesticides during the year we selected the detailed output option in TOXWA, which provides the complete set of PEC data on an hourly basis in the surface water body.

Mix-Tool: Pesticide mixtures released from crops (hedge of field assessment). The third step of the proposed approach foresees the identification of pesticide mixtures in surface water during a year, linked to a given crop (hedge of field assessment) and following the pesticide treatment calendar. We have created a simple Microsoft-Excel-based tool (Mix-Tool) that organizes the data generated by FOCUS models in the previous step. A first sheet has been pre-formatted to receive the output data of the FOCUS models (simply copy the output text file from TOXWA and pasting it

into the sheet). Subsequently, the tool automatically selects the maximum PEC value reached by each active substance during the 24 h (as previously reported, in FOCUS models, PECs are expressed on an hourly basis). Consequently, for each day of the year, it is possible to obtain the composition of the mixture in terms of concentration by simply listing the results of the daily concentrations of each active substance in subsequent columns.

Moreover, the Mix-Tool characterizes the mixtures in terms of toxicity, using the concentration addition model (Altenburger et al., 2000), which is based on the idea that all components in the mixture behave as if they are simple dilutions of one another. In particular, this model considers that one chemical could be replaced by an equivalent concentration in terms of the effect (e.g., a median effect concentration [EC50]) of another chemical, without modifying the total effect on that target. According to the concentration addition model, the overall toxicity of a mixture (TU_{mix}) can be calculated as follows:

$$TU_{mix} = \frac{C_{mix}}{EC_{mix}} \sum_{i=1}^n \frac{C_i}{EC_{x_i}} = \sum_{i=1}^n TU_i \quad (1)$$

where c_{mix} is the total concentration of the mixture components, EC_{mix} is the mixture concentration that produces an $x\%$ effect, c_i and EC_{x_i} are concentration and the $x\%$ effect of a substance i , respectively, and TU_i is the fraction of the toxic unit of the substance i .

TU_{mix} is obtained by summing the toxicity contribution of each component ($c_i/EC_{x_i} = TU_i$).

Algae, Daphnia, and fish were identified as nontarget organisms representative of the trophic levels of aquatic ecosystems, and the mixture toxicity for these organisms was calculated using EC50 or median lethal concentration values (Supporting Information Table S3).

We also considered the possibility of uncertainty arising from the use of a single ecotoxicity datum for each of the three nontarget organisms. To overcome this uncertainty, we applied assessment factors to the toxicity of the mixtures, calculated according to Equation (1; 0.1 for algae and 0.01 for Daphnia and fish). This procedure is in line with the risk assessment strategy for pesticides (EFSA, 2013).

Monitoring data. Surface water monitoring data was acquired from the regional environmental authority (ARPA Lombardia, 2022) for 2015–2017. The input dataset for each sample includes the monitoring station identifier, its geographical coordinates, the sample date and name, measured concentrations, and the limit of quantification of each sampled substance. Data are available in Supporting Information as a separate Microsoft Excel file.

RESULT AND DISCUSSION

As reported in the *Materials and methods* section, we selected maize and apple trees as case studies to test the Mix-tool and to highlight the potential results.

Annual trends of mixtures toxicity released from maize and apple trees, and comparison among scenarios

Figures 2 and 3 show the toxicity/assessment factor annual trends (in logarithmic scale) of mixtures released from a hectare of maize or apple trees during the year (edge of field assessment). Values of log toxicity/assessment factor > 0 indicate that the risk threshold has been exceeded.

If compared with the runoff scenarios (R3 and R4), the drainage scenarios (D4 and D6) show a lower frequency and intensity of risk for the aquatic organisms for both crops. In particular, for maize the risk threshold is slightly exceeded only during April and May/July for algae and fish, respectively, whereas for *Daphnia* a potential risk is present during May and sometimes in winter (probably due to winter rainfall peaks, which can transport some remaining residues in surface water; Figure 2A–C). A similar picture can be observed for the mixtures released from apple trees (Figure 3A–C). Indeed, for the

three aquatic organisms, the threshold of risk is exceeded sporadically during April–July (for *Daphnia* also during November–December). On the contrary, in the runoff scenarios the exceedances are very frequent throughout the year for both crops (Figures 2 and 3D–F).

The toxicity of the potential mixtures released from the two considered crops varies considerably over the year, for both the different aquatic organisms and the considered scenarios. For each crop, the highest toxicity values for algal organisms were obtained by the R4 scenario, particularly during the second half of April (toxicity/assessment factor range 16–42 and 9.3–92 for maize and apple trees, respectively) and during May–June (toxicity/assessment factor range 5–8 and 6–12 for maize and apple trees, respectively; Figures 2D and 3D). Moreover, *Daphnia* seems to be the organism most at risk in all the environmental scenarios. In fact, the risk threshold is frequently surpassed during the year (for maize toxicity/assessment factor > 1 values were found 118 and 129 times in R3

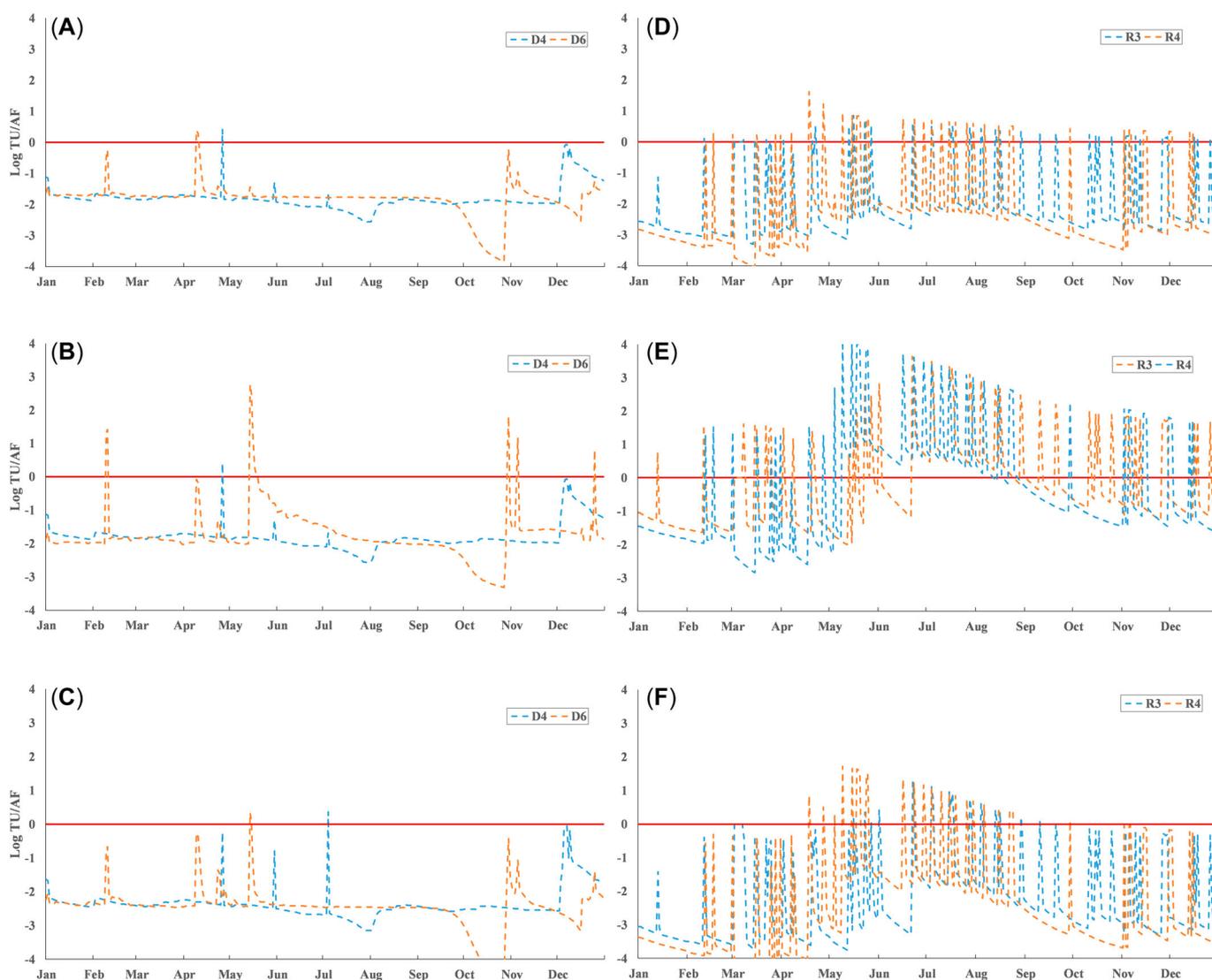


FIGURE 2: Annual trend of the potential mixture toxicities released from one hectare of crop for algae (A and D), *Daphnia* (B and E), and fish (C and F) in relation to different FOCUS models scenarios (D4–D6: drainage scenarios; R3–R4: run-off scenarios). AF = assessment factor; FOCUS = Forum for the Co-ordination of pesticide fate models and their Use; TU = toxicity factor.

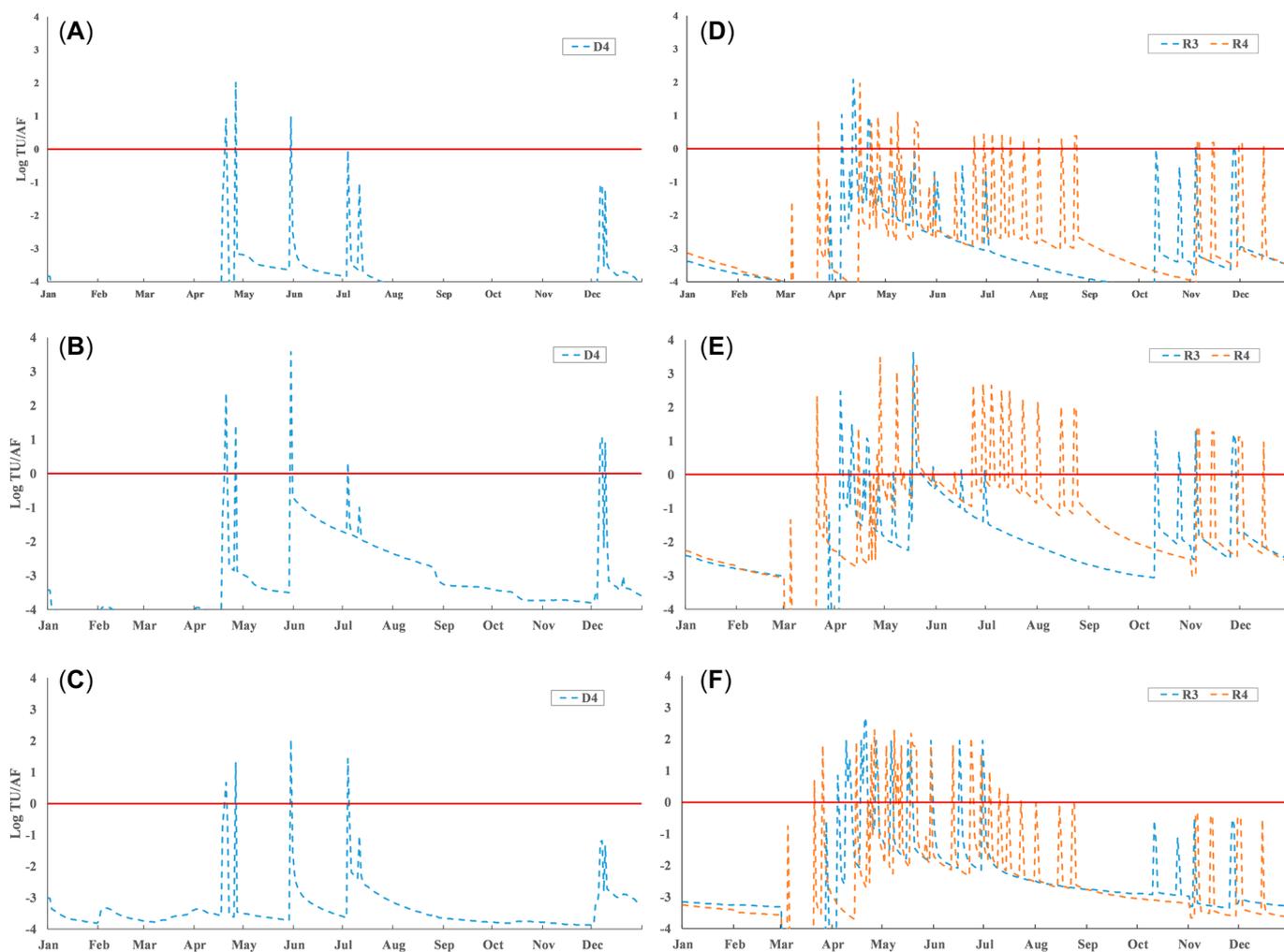


FIGURE 3: Annual trend of the potential mixture toxicities released from one hectare of apple trees for algae (A and D), Daphnia (B and E), and fish (C and F) in relation to different FOCUS models scenarios (D4: drainage scenario; R3–R4: run-off scenarios). AF = assessment factor; FOCUS = Forum for the Co-ordination of pesticide fate models and their Use; TU = toxicity factor.

and R4; for apple tree toxicity/assessment factor > 1 values were found 27 and 46 times in R3 and R4), with peaks during May–July that exceed the risk threshold by three or four orders of magnitude (especially for maize). Finally, for fish, the R4 scenario is the scenario most at risk (toxicity/assessment factor > 1 values were found 29 and 27 times during the year for maize and apple trees, respectively), and especially during April–May.

From the above results, it seems that potential mixtures derived from applications of pesticides currently registered on maize and apple trees could represent an environmental issue, particularly in Italian agricultural areas, which can be considered comparable to R3 and R4 scenarios of the FOCUS surface water guidance.

Mixtures of concern

Table 2 and Supporting Information Table S4 list components of those mixtures showing toxicity values above 1

(mixtures of concern) for each aquatic organism considered, for each FOCUS scenario, and in different periods of the year. The relative contribution (as a percentage) of each component to the overall mixture toxicity is also reported. Data are grouped in different intervals of time by considering the homogeneity in the composition of the mixtures and the contribution of components to the mixture toxicity.

Analyzing these data, we can assume that the number of components and their relative contribution to the mixture toxicity varies during the year for both crops. However, toxicity can often be ascribed to one compound (or very few) in both cases, which is in line with previous studies reporting that a few compounds usually drive the toxicity of mixtures (Gustavsson et al., 2017; Rydh Stenström et al., 2021; Verro et al., 2009b). Finally, this finding allows for a better interpretation of the results reported in Figures 2 and 3 and particularly for R3 and R4 scenarios, where the frequency of overcoming the risk threshold is very high. Indeed, in most cases, the risk is due to the release of one active substance residue.

TABLE 2: Pesticide mixtures of concern (toxicity > 1) released by maize during 1 year for different FOCUS scenarios and aquatic organisms

Scenario	Period	Gly	Chlor	Dica	Bent	Meso	Terb	Chlor-met	s-Meto	Pend	MCPA
<i>Algae</i>											
D4	April 26	—	—	—	—	—	14.2	—	18.6	67.3	—
D6	April 9–10	—	—	—	—	—	14–15	—	18–19	66–67	—
R3	February 11–April 21	—	—	—	—	—	—	—	—	100	—
	April 22	—	—	—	—	—	14	—	18	68	—
	May 13–16	—	—	—	—	—	23–46	—	15–24	29–62	—
	May 23–27	—	—	—	—	—	5–9	1–1.4	5–8	79–89	—
R4	June–December	—	≤2.4	—	—	—	—	—	≤0.5	97–99	—
	February–March	—	—	—	—	—	—	—	—	100	—
	April 7	—	—	—	—	—	14	—	18.4	68	—
	April 18–27	—	—	—	—	—	38–61	—	17–22	17–45	—
	May 9–December	—	2.9–3.3	—	—	—	0.3–12	0.6–1.5	0.3–7.1	76–96	—
<i>Daphnia</i>											
D4	May 30	—	—	—	—	—	—	100	—	—	—
	July–December	—	87–100	—	—	—	≤6.5	—	≤6.5	—	—
D6	May 14–18	—	76–88	—	—	—	≤0.6	12–23	≤0.2	—	—
	October 29–May 13	—	99.8	—	—	—	—	—	—	0.2	—
R3	January 13–April 21	—	99–99.7	—	—	—	—	—	—	0.3–1	—
	April 22	—	6.8	—	—	—	24.4	—	20.4	48.4	—
	May 13–16	—	71–85	—	—	—	6–18	—	2.5–6	5–7	—
	May 18–30	—	—	—	—	—	—	97–99.9	—	—	—
R4	June 1–December 26	—	100	—	—	—	—	—	—	—	—
	February 12–April 17	—	97.7–98.7	—	—	—	—	—	—	1.3–2.3	—
	April 18	—	39.4	—	—	—	44.7	—	10.2	5.7	—
	April 19	—	36.8	—	—	—	—	—	10.7	5.4	—
	April 27	—	62.1	—	—	—	21.1	—	6.0	10.3	—
	May 4–31	—	83–98.5	—	—	—	—	1.5–17	—	—	—
	June 1–December 15	—	98.4–100	—	—	—	—	≤1.6	—	—	—
<i>Fish</i>											
D4	July 4	—	99.9	—	—	—	—	—	—	—	—
D6	May 14–15	—	95.0	—	—	—	—	4.8	—	—	—
R3	May 16–27	—	2.2–2.9	—	—	—	0.8–6	≤47	6.5–32	36–58	≤2
	June 1–July 29	—	90–100	—	—	—	—	—	—	≤10	—
	August 4–September 10	—	69–87	—	—	—	—	—	—	13–31	—
R4	April 18–27	—	0.8–1.4	—	≤1.7	—	11–19	—	39–58	22–45	≤1.4
	May 4–25	—	93–96	—	—	—	—	0.7–5	—	2.5–3.6	—
	June 16–September 29	—	59–95	—	—	—	—	—	—	5–41	—

The contribution of each component to the overall toxicity of the mixture is expressed as a percentage (in bold if the contribution is >70%; “—” when the contribution is ≤1%).

Please see Table 1 for abbreviations.

In the following sections, we thoroughly analyse each case study separately.

Maize crop. From Figure 2 and Table 2 the following considerations arise:

Algae. In the drainage scenarios (D4–D6) the mixtures of concern are exclusively released during April (Figure 1A and Table 2) and are composed of three herbicides (Terb, s-Meto, and Pend), each one giving in both scenarios a similar contribution to the toxicity. In the run-off scenarios (R3 and R4) the release of mixtures is more widespread during the year (Figure 1D and Table 2). However, it can be observed that all the mixtures of concern are almost exclusively composed of the herbicide Pend (the percentage of contribution to the toxicity of mixtures ranges from 76% to 100%), with the exception of April to the end of May, where the composition of the mixtures is similar to those observed for the D4–D6 scenarios (Terb, s-Meto, and Pend). These herbicides are applied on maize in pre- or postemergence (April/May). However, Terb and s-Meto are

relatively mobile in soil, with a half-life in soil ($DT_{50\text{soil}}$) of approximately 25 days. On the contrary, Pend shows a higher affinity for soil ($K_{oc} > 15\,000\text{ ml/g}$) and $DT_{50\text{soil}}$ above 100 days. These differences could explain the presence in the mixture of Terb and s-Meto exclusively during the period of treatment and the occurrence of Pend all year.

Daphnia and fish. In all FOCUS scenarios, one or two organophosphorus insecticides (Chlor or Chlor-met) form mixtures of concern. The only exceptions are evident in April, where the mixtures seem to be characterized also by the presence of some herbicides (e.g., in the R3 scenario for Daphnia, during the rain event of 22 April the mixture was composed of Chlor, Terb, s-Meto, and Pend).

Apple trees. From Figure 3 and Table 2 the following considerations arise:

Algae. With a few exceptions, in all the considered scenarios the toxicity of the mixtures is driven by one compound, particularly by the herbicide Pend and the fungicides Manc and

Meti. In R3 and R4 scenarios, the toxic potency of the mixtures can be dependent on a combination of different compounds (R3: April 12 and 20–21; R4: May 18–20).

Daphnia and fish. In all the considered FOCUS scenarios, the toxicity of the mixtures towards these organisms is dependent on the presence of one active substance (the fungicides Manc, Cap, and Dith in April, May, and July, respectively, the herbicide pendimethalin in late April, and the insecticide Chlor in May and December). Finally, in both scenarios during April (and sometimes in May) the toxicity of the mixtures is dependent on the combination of two or more compounds (Chlor, Pend, Cap, and Meti).

Comparison with monitoring data

According to Ippolito and Fait (2019), one key element in selecting predictive models for the environmental fate of pesticides is the identification of the most appropriate spatial-temporal scale (edge-of-field, catchment, regional, or continental). Indeed, the increase in the spatial-temporal scale increases the complexity of the variables and dynamic processes to be considered (i.e., environmental loads, water body hydrology, degradation, sediment deposition, resuspension, and volatilization). In the context of the current European Union procedures for pesticide authorization (EFSA, 2013), the characterization of exposure for the aquatic organisms relies on the edge-of-field approach and using predictive models applied in artificial environmental scenarios representative of the reasonable worst-case conditions in European Union territory (Linders et al., 2003). Generally, the analysis is performed for each single active ingredient present in the commercial formulations. In our study we suggest an edge-of-field approach to identify the most probable and toxicological relevant mixtures released by crops during the year. This approach has the advantage of reducing the number of input data (e.g., no site-specific data are needed); on the other hand, the spatial relevance of the predictions is unknown. Moreover, when considering mixtures of chemicals, the edge of the field may not be the worst case in terms of aggregate risk, and a catchment-scale (watershed) assessment

should also be considered (Holmes et al., 2018). It can be argued that the results obtained using the edge-of-field approach cannot be extrapolated to a larger spatial-temporal scale (e.g., catchment) and a comparison between our results and those deriving from monitoring campaigns may not be appropriate. On the other hand, the composition of pesticide mixtures in a catchment is highly dependent on the pesticides released from the crops at the edge of the field. In small catchments characterized by homogeneity in the agricultural land use (one or very few crops), the composition of the mixture will be characterized by those pesticides, which are largely used on the prevalent crops present in the considered area.

Based on this consideration, and to validate (at least qualitatively) the proposed approach, we compared our results with data deriving from the monitoring programs (2015–2017 period) performed by Italian regional authorities (ARPA Lombardia, 2022; Istituto Superiore per la Protezione e la Ricerca Ambientale [ISPRA], 2020), particularly in the Lombardia region where both crops are widespread. Unfortunately, Man, Dith, Meti, and Cap are not (or scarcely) included in the list of monitoring programs in Italy (ISPRA, 2020) and this hampered the possibility of comparison with apple trees. Consequently, we have focused our attention on a small river basin of the Lombardia region (lower course of the Adda River, northern Italy) characterized by the intensive presence of maize (~73% of the total agricultural area, according to ISTAT, 2022).

According to the ICPS document (ICPS, 2007) the considered area is better described by the FOCUS scenarios D4 and D6. For this reason, we have compared monitoring data with our results obtained for these scenarios. From Table 2 and Figure 2A–C the mixtures of concern released from maize during a year in the D4 and D6 scenarios are present in April (algae and fish), in May (*Daphnia* and fish), and during October–December (*Daphnia*). Moreover, as previously described, the overall toxicity of the mixtures is strictly dependent on the contribution of one (Chlor or Chlor-met for *Daphnia* and fish) or a few components (Terb s-Meto, Pend for algal organisms). Even if slightly flawed, our results seem to be in agreement with the monitoring data. In Table 3, the mixtures of concern

TABLE 3: Mixtures of concern (toxicity > 1) identified in the lower portion of the Adda River (Lombardia; monitoring program 2015–2017)

Station name	Sampling data	TU	Mixture composition (% of contribution to the overall mixture toxicity)
Algae			
Crema—Molinara (Roggia)	25/06/2015	7.3	Terb (74), s-Meto (25)
Castelnuovo Bocca d'Adda—Adda (Collettore)	14/04/2016	3.4	Terb (67), Flu (28), s-Meto (4.4)
Castelnuovo Bocca d'Adda—Adda (Collettore)	14/05/2015	2.5	Terb (45), s-Meto (36), Flu (13.6)
Castelnuovo Bocca d'Adda—Adda (Collettore)	16/06/2015	1.7	s-Meto (43), Terb (36), Flu (17)
Crema—Cresmiero (Roggia)	25/06/2015	1.6	s-Meto (51), Terb (48);
Castelnuovo Bocca d'Adda—Gandiolo (Colatore)	14/05/2015	1.5	Flu (67), Terb (14%), s-Meto (9)
Daphnia			
Castelnuovo Bocca d'Adda—Adda (Collettore)	14/05/2015	20	Chlor (100)
Castelnuovo Bocca d'Adda—Gandiolo (Colatore)	14/05/2015	20	Chlor (100)
Montodine—Serio (Fiume)	05/07/2017	3	Chlor-met (100)
Fish			
Castelnuovo Bocca d'Adda—Adda (Collettore)	14/05/2015	2	Chlor (88), s-Meto (7), Terb (3),
Castelnuovo Bocca d'Adda—Gandiolo (Colatore)	14/05/2015	2	Chlor (96), s-Meto (1)

TU = toxicity; please see Table 1 for abbreviations.

(toxicity > 1) present in the lower course of the Adda river in 2015–2017 are reported. It is confirmed that the higher risk for the aquatic organisms occurs between May and July. Particularly for algal organisms, the risk is mainly due to the presence of Terb, s-Meto, and flufenacet, whereas for daphnia and fish the risk is associated exclusively with the presence of Chlor or Chlor-met residues. Flufenacet and Pend are the only substances with different values between our results and the monitoring data. A possible explanation for this discrepancy is that the selection of Pend was made based on the quantity sold in Italy of this active substance. However, this compound is registered for many crops (contrarily to flufenacet); moreover, in Lombardia Pend is not included among the most widely utilized pesticides.

CONCLUSIONS

In the present study, we proposed an edge-of-field risk assessment approach to predict pesticide mixtures of concern released from agricultural crops into surface water. The approach follows the standard procedures utilized for the commercial authorization of these substances in the European Union for single active substances, but is expanded for forecasting relevant pesticide mixtures in surface water. The composition of the mixtures released by crops during the year is achieved by taking into account the pest protection programs (i.e., the registered active substances on a crop) and using FOCUS models for PEC calculation of every active substance in surface water. In the proposed Mix-tool, PECs obtained from FOCUS model applications are expressed on daily basis. This allows the calculation of the mixture toxicity released from a given crop during a year through the application of the concentration addition model. To simplify the procedure, we have developed a simple Microsoft-Excel-based tool (Mix-Tool), which is downloadable from the Supporting Information. The application of the procedure to maize and apple tree crops (representative of the Italian agricultural context) allowed the identification of mixtures of concern released from these crops on an edge-of-field scale. The good agreement between predicted and observed mixtures indicated the validity of the Mix-Tool method applied to an area characterized by the intensive presence of maize (lower portion of Adda River). We believe this class of tools could be a useful companion for environmental agencies in Europe to consider the impact of pesticide mixtures in surface water.

Supporting Information—The Supporting Information is available on the Wiley Online Library at <https://doi.org/10.1002/etc.5363>.

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REFERENCES

- Altenburger, R., Backhaus, T., Boedeker, W., Faust, M., Scholze, M., & Grimme, L. H. (2000). Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri*: Mixtures composed of similarly acting chemicals. *Environmental Toxicology and Chemistry*, 19, 2341–2347.
- ARPA Lombardia. (2022). Dati e Indicatori. <https://www.arpalombardia.it/Pages/ricerca-Dati-ed-Indicatori.aspx?sottotema=Acque%20superficiali>
- Backhaus, T., Altenburger, R., Boedeker, W., Faust, M., Scholze, M., & Grimme, L. H. (2000). Predictability of the toxicity of multiple mixtures of dissimilarly acting chemicals to *Vibrio fischeri*. *Environmental Toxicology and Chemistry*, 19, 2348–2356.
- Belden, J. B., Gilliom, R. J., Martin, J. D., & Lydy, M. J. (2007). Relative toxicity and occurrence patterns of pesticide mixture in streams draining agricultural watersheds dominated by corn and soybean production. *Integrated Environmental Assessment and Management*, 3, 90–100.
- Bonzini, S., Verro, R., Otto, S., Lazzaro, L., Finizio, A., Zanin, G., & Vighi, M. (2006). Experimental validation of a geographical information systems-based procedure for predicting pesticide exposure in surface water. *Environmental Science and Technology*, 40(24), 7561–7569.
- Bundschuh, M., Goedkoop, W., & Kreuger, J. (2014). Evaluation of pesticide monitoring strategies in agricultural streams based on the toxic-unit concept—experiences from long-term measurements. *Science of the Total Environment*, 484, 84–91. <https://doi.org/10.1016/j.scitotenv.2014.03.015>
- Ccancappa, A., Masiá, A., Navarro-Ortega, A., Picó, Y., & Barceló, D. (2016). Pesticides in the Ebro River basin: Occurrence and risk assessment. *Environmental Pollution*, 211, 414–424. <https://doi.org/10.1016/j.envpol.2015.12.059>
- Cedergreen, N., Christensen, A. M., Kamper, A., Kudsk, P., Mathiassen, S. K., Streibig, J. C., & Sørensen, H. (2008). A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. *Environmental Toxicology and Chemistry*, 27, 1621–1632. <https://doi.org/10.1897/07-474.1>
- Chow, R., Scheidegger, R., Doppler, T., Dietzel, A., Fenicia, F., & Stamm, C. (2020). A review of long-term pesticide monitoring studies to assess surface water quality trends. *Water Research X*, 9, 100064. <https://doi.org/10.1016/j.wroa.2020.100064>
- Curchod, L., Oltramare, C., Junghans, M., Stamm, C., Dalvie, M. A., Rössli, M., & Fuhrmann, S. (2020). Temporal variation of pesticide mixtures in

- ivers of three agricultural watersheds during a major drought in the Western Cape, South Africa. *Water Research X*, 6(100039), 12. <https://doi.org/10.1016/j.wroa.2019.100039>
- Daly, G. L., & Wania, F. (2005). Organic contaminants in mountains. *Environmental Science and Technology*, 39, 385–398. <https://doi.org/10.1021/es048859u>
- de Zwart, D. (2005). Ecological effects of pesticide use in the Netherlands: Modelled and observed effects in the field ditch. *Integrated Environmental Assessment and Management*, 1, 123–134.
- Deknock, A., De Troyer, N., Houbraken, M., Dominguez-Granda, L., Nolivos, I., Van Echelpoel, W., Eurie Forio, M. A., Spanoghe, P., & Goethals, P. (2019). Distribution of agricultural pesticides in the freshwater environment of the Guayas river basin (Ecuador). *Science of the Total Environment*, 646, 996–1008.
- Di Guardo, A., Calamari, D., Zanin, G., Consalter, A., & Mackay, D. (1994). A fugacity model of pesticide runoff to surface water: Development and validation. *Chemosphere*, 28(3), 511–531. [https://doi.org/10.1016/0045-6535\(94\)90295-X](https://doi.org/10.1016/0045-6535(94)90295-X)
- Di Guardo, A., & Finizio, A. (2016). A moni-modelling approach to manage groundwater risk to pesticide leaching at regional scale. *Science of the Total Environment*, 545, 200–209.
- European Commission (EC). (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Union*, 1–51.
- European Commission. (2009). Regulation (EC) 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. OJ L 309, 24.11.2009.
- European Food and Safety Agency. (2013). Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. *EFSA Journal*, 11(7), 3290.
- European Food and Safety Agency. (2019). Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. *EFSA Journal*, 17(3), 77. <https://doi.org/10.2903/j.efsa.2019.5634>
- European Soil Data Center. (2021). FOCUS DG Sante. <https://esdac.jrc.ec.europa.eu/projects/focus-dg-sante>
- EUROSTAT. (2020). *Agriculture, forestry and fishery statistics, 2020 edition*. <https://ec.europa.eu/eurostat/documents/3217494/12069644/KS-FK-20-001-EN-N.pdf/a7439b01-671b-80ce-85e4-4d803c44340a?t=1608139005821>
- Finizio, A., Azimonti, G., & Villa, S. (2011). Occurrence of pesticides in surface water bodies: A critical analysis of the Italian national pesticide survey programs. *Journal of Environmental Monitoring*, 13, 49–57. <https://doi.org/10.1039/C0EM00192A>
- Finizio, A., Grenni, P., Petrangeli, A. B., Barra Caracciolo, A., Santoro, S., & Di Guardo, A. (2022). Use of large datasets of measured environmental concentrations for the ecological risk assessment of chemical mixtures in Italian streams: A case study. *Science of the Total Environment*, 806(Pt 2), 150614. <https://doi.org/10.1016/j.scitotenv.2021.150614>
- Finizio, A., Villa, S., & Vighi, M. (2005). Predicting pesticide mixtures load in surface waters from a given crop. *Agriculture, Ecosystems & Environment*, 111, 111–118. <https://doi.org/10.1016/j.agee.2005.05.009>
- Forum for the Co-ordination of pesticide fate models and their Use (FOCUS). (2001). *FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC*. Report of the FOCUS working group on surface water scenarios, EC Document Reference SANCO/4802/2001-rev.2, 2001.
- Gustavsson, M., Kreuger, J., Bundschuh, M., & Backhaus, T. (2017). Pesticide mixtures in the Swedish streams: Environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation. *Science of the Total Environment*, 598, 973–983. <https://doi.org/10.1016/j.scitotenv.2017.04.122>
- Hartz, K. E. H., Edwards, T. M., & Lydy, M. J. (2017). Fate and transport of furrow-applied granular tefluthrin and seed-coated clothianidin insecticides: Comparison of field-scale observations and model estimates. *Ecotoxicology*, 26, 876–888.
- Holmes, C. M., Brown, C. D., Hamer, M., Jones, R., Maltby, L., Posthuma, L., Silberhorn, E., Teeter, J. S., Warne, M. S. J., & Weltje, L. (2018). Prospective aquatic risk assessment for chemical mixtures in agricultural landscapes. *Environmental Toxicology and Chemistry*, 37, 674–689. <https://doi.org/10.1002/etc.4049>
- International Centre for Pesticide Safety. (2007). Scenari di simulazione per le acque superficiali. <https://www.icps.it/it/pubblicazioni/scenari-xls>
- International Centre for Pesticide Safety. (2021). Il database Pestidoc. <https://www.icps.it/pestidoc/>
- Ippolito, A., & Fait, G. (2019). Pesticides in surface waters: From edge-of-field to global modelling. *Current Opinion in Environmental Sustainability*, 36, 78–84. <https://doi.org/10.1016/j.cosust.2018.10.023>
- Istituto Nazionale di Statistica. (2022). Censimento Agricoltura 2010. <http://dati-censimentoagricoltura.istat.it/Index.aspx>
- Istituto Superiore per la Protezione e la Ricerca Ambientale. (2020). Rapporto nazionale pesticidi nelle acque – dati 2017-2018. Rapporti 334/2020.
- Junghans, M., Backhaus, T., Faust, M., Scholze, M., & Grimme, L. H. (2005). Application and validation of approaches for the predictive hazard assessment of realistic pesticide mixtures. *Aquatic Toxicology*, 76, 93–110. <https://doi.org/10.1016/j.aquatox.2005.10.001>
- Klein, M. (2012). *Estimation of reasonable application dates dependent on BBCH crop development stages for PELMO (all FOCUS groundwater locations)*. Report Umweltbundesamt. Fraunhofer IME.
- Le Cor, F., Slaby, S., Dufour, V., Iuretic, A., Feidt, C., Dauchy, X., & Banas, D. (2021). Occurrence of pesticides and their transformation products in headwater streams: Contamination status and effect of ponds on contaminant concentrations. *Science of the Total Environment*, 788, 147715. <https://doi.org/10.1016/j.scitotenv.2021.147715>
- Linders, J., Adriaanse, P., Allen, R., Capri, E., Gouy, V., Hollis, J., Jarvis, N., Klein, M., Lolos, P., Maier, W. M., Maund, S., Pais, C., Russell, M., Smeets, L., Teixeira, J. L., Vizantinopoulos, S., & Yon, D. (2003). *FOCUS surface water scenarios in the EU Evaluation process under 91/414/EEC*. Report prepared by the FOCUS Working group on surface water Scenarios. European Commission SANCO/4802/2001–rev 2).
- Malaj, E., von der Ohe, P. C., Grote, M., Kühne, R., Mondy, C. P., Usseglio-Polatera, P., Brack, W., & Schäfer, R. B. (2014). Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 9549–9554. <https://doi.org/10.1073/pnas.1321082111>
- Meier, U. (Ed.). (2001). *Growth stages of mono- and dicotyledonous plants, BBCH—Monograph* (2nd ed., 158pp.). Federal Biological Research Centre for Agriculture and Forestry.
- Morselli, M., Vitale, C. M., Ippolito, A., Villa, S., Giacchini, R., Vighi, M., & Di Guardo, A. (2018). Predicting pesticide fate in small cultivated mountain watersheds using the DynAPlus model: Toward improved assessment of peak exposure. *Science of the Total Environment*, 615, 307–318. <https://doi.org/10.1016/j.scitotenv.2017.09.287>
- Moschet, C., Wittmer, I., Simovic, J., Junghans, M., Piazzoli, A., Singer, H., Stamm, C., Leu, C., & Hollender, J. (2014). How a complete pesticide screening changes the assessment of surface water quality. *Environmental Science and Technology*, 48, 5423–5432. <https://doi.org/10.1021/es500371t>
- Organisation for Economic Co-operation and Development. (2018). *Considerations for assessing the risks of combined exposure to multiple chemicals* (Series on testing and assessment no. 296). Environment, Health and Safety Division, Environment Directorate.
- Petersen, J., Grant, R., Larsen, S. E., & Blicher-Mathiesen, G. (2012). Sampling of herbicides in streams during flood events. *Journal of Environmental Monitoring*, 14, 3284–3294. <https://doi.org/10.1039/C2EM30771E>
- Posthuma, L., Brown, C. D., de Zwart, D., Diamond, J., Dyer, S. D., Holmes, C. M., Marshall, S., & Burton, G. A., Jr. (2018). Prospective mixture risk assessment and management prioritizations for river catchments with diverse land uses. *Environmental Toxicology and Chemistry*, 37, 715–728.
- Rabiet, M., Margoum, C., Gouy, V., Carluet, N., & Coquery, M. (2010). Assessing pesticide concentrations and fluxes in the stream of a small vineyard catchment—Effect of sampling frequency. *Environmental Pollution*, 158, 737–748. <https://doi.org/10.1016/j.envpol.2009.10.014>
- Relyea, R. A. (2008). A cocktail of contaminants: How mixtures of pesticides at low concentrations affect aquatic communities. *Oecologia*, 159, 363–376. <https://doi.org/10.1007/s00442-008-1213-9>
- Rizzi, C., Finizio, A., Maggi, V., & Villa, S. (2019). Spatial-temporal analysis and risk characterisation of pesticides in Alpine glacial streams. *Environmental Pollution*, 248, 659–666.
- Rydh Stenström, J., Kreuger, J., & Goedkoop, W. (2021). Pesticide mixture toxicity to algae in agricultural streams—Field observations and

- laboratory studies with in situ samples and reconstituted water. *Ecotoxicology and Environmental Safety*, 215, 112153. <https://doi.org/10.1016/j.ecoenv.2021.112153>
- Scientific Committee on Health and Environmental Risks, Scientific Committee on Emerging and Newly Identified Health Risks, & Scientific Committee on Consumer Safety. (2012). *Toxicity and assessment of chemical mixtures*. <https://doi.org/10.2772/21444>
- Schreiner, V. C., Szöcs, E., Bhowmik, A. K., Vijver, M. G., & Schäfer, R. B. (2016). Pesticide mixtures in streams of several European countries and the USA. *Science of the Total Environment*, 573, 680–689. <https://doi.org/10.1016/j.scitotenv.2016.08.163>
- Schulz, R. (2004). Field studies on exposure, effects, and risk mitigation of aquatic nonpoint-source insecticide pollution: A review. *Journal of Environmental Quality*, 33, 419–448. <https://doi.org/10.2134/jeq.2004.4190>
- Schwarzenbach, R. P., Escher, B. I., Fenner, K., Hofstetter, T. B., Johnson, C. A., von Gunten, U., & Wehrli, B. (2006). The challenge of micro-pollutants in aquatic systems. *Science*, 313, 1072–1077. <https://doi.org/10.1126/science.1127291>
- Sistema Informativo Agricolo Nazionale. (2021). Banca+Dati+Fitofarmaci. <https://www.sian.it/portale-sian/attivaservizio.jsp?sid=174%26pid=6%26servizio=Banca%2BDati%2BFitofarmaci%26bottoni=no>
- Verro, R., Finizio, A., Otto, S., & Vighi, M. (2009a). Predicting pesticide environmental risk in intensive agricultural areas. I: Screening level risk assessment of individual chemicals in surface waters. *Environmental Science and Technology*, 43, 522–529. <https://doi.org/10.1021/es801855f>
- Verro, R., Finizio, A., Otto, S., & Vighi, M. (2009b). Predicting pesticide environmental risk in intensive agricultural areas. II: Screening level risk assessment of complex mixtures in surface waters. *Environmental Science and Technology*, 43(2), 530–537. <https://doi.org/10.1021/es801858h>
- Vighi, M., Altenburger, R., Arrhenius, Å., Backhaus, T., Bødeker, W., Blanck, H., Consolaro, F., Faust, M., Finizio, A., Froehner, K., Gramatica, P., Grimme, L., Grönvall, F., Hamer, V., Scholze, M., & Walter, H. (2003). Water quality objectives for mixtures of toxic chemicals: problems and perspectives. *Ecotoxicology and Environmental Safety*, 54, 139–150. [https://doi.org/10.1016/S0147-6513\(02\)00047-7](https://doi.org/10.1016/S0147-6513(02)00047-7)