A new and integrated approach to evaluate the environmental and ecotoxicological impact of herbicide mixtures: A case study in maize

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Abstract

An index-based approach for a comprehensive evaluation of the potential risk for active substances and their mixtures to impact the environment was developed. Some of the indices considered already exist (PRISW-1, Priority Index), while others were created *ex novo* from indicators available on open-source platforms (PESTi, ECOi, AGROi). These indices maybe used for an *evaluation before use* of pesticides by farmers and advisers. The present approach was initially validated for herbicides in maize crops, but it can readily be applied to other PPPs and crops. PESTi index underline the physical and chemical characteristics as a whole, not considering the impact of other factors such as application rate or period of application. Hence, this index may underestimate the risk associated to a certain chemical. AGROi has a precautionary approach. The risk associated to a specific mixture derives from a combination of intrinsic characteristics of the chemicals, agronomic impacts, regulation restrictions and potential hazard to water compartment. The ECOi index is focused on the ecotoxicological impact against non-target organisms. The helpfulness of this index stands in its ability to easily discriminate the ecotoxicological impact of chemicals using indicators commonly available in literature and without making complex calculations. PRISW-1 Index discriminate active substances according to their risk against three representative non-target organisms. However, due to the intrinsic characteristics of each pesticide, a high PRISW-1 value could not always mean an easy movement of the chemical via runoff waters. The information deriving from Priority index may certainly help public authorities to select chemicals to be detected in water monitoring campaigns. The application of these indices may represent a valid decision tool for public stakeholders in defining agricultural measures to reduce the externalities of pest control.

Key words: Indices, pesticides, risk assessment

1. INTRODUCTION

A key issue in crop plant protection is maintaining effective methods to combat biotic stress caused by living organisms. Historically, pesticide research centered on the efficacy of a single active substance or of mixtures, on the identification of resistant populations, and on the discovery of new mechanisms of action. Despite the benefit shown on yields and food quality (Cooper and Dobson, 2007; Knutson, 1999; Rathore and Nollet, 2012), pesticides can also affects agricultural sustainability (Wilson and Tisdell, 2001). Moreover, once pesticides have entered the environment, significant negative impacts can occur to living communities of various environmental compartments (Storck et al., 2017; Thiour-Mauprivez et al., 2019).

Plant protection products (PPPs), pesticides can threaten the environment (Pimentel et al., 1992) as surface and/or ground water pollution, soil contamination, or as risks for human health and non-target organisms. Pesticides found in surface and ground waters have the potential to reduce water quality and even limit the availability of water (Batista et al., 2002; De Gerónimo et al., 2014; de Souza et al., 2020; Dougherty et al., 2010; Sjerps et al., 2019; Thurman et al., 1992) . Groundwater protection is critically important in many parts of the world where deep water is used for human consumption (Gennari and Trevisan, 2011; Close et al., 2021). Worldwide, directives and regulations have been issued to protect water from entire categories of chemical substances, including PPPs (*2009/128/CE*, 2009; *2000/60/UE*, 2000). In some areas, PPPs are the most common substances found in water, of which herbicides and fungicides are the most common (Paris et al., 2020).

The future of conventional agriculture requires policymakers and farmers/operators to move to a sustainable approach to pesticide management (Storck et al., 2017). This perspective has already changed the focus of pesticide research to alternative pest control methods, organic farming, integrated pest management, and tools for selecting the most suitable chemicals for crop protection (*2009/128/CE*, 2009). European legislation has also been created to safeguard environmental, human, and non-targeted organism health from harmful pesticide use. Directive 128/2009 (*2009/128/CE*, 2009) forces European countries to adopt measures guaranteeing effective and safe use of pesticides and made necessary the development of specific strategies to ensure the availability of effective and sustainable PPPs in the near term.

The threat of additional local and national bans on specific pesticides demands farmers to adopt a more sustainable and farsighted approach. At the European level, actions have been taken to train various stakeholders on the best management practices (BMPs) to mitigate negative impacts on cropping. By example, two Life projects (TOPPS and TOPPS Prowadis) have identified the BMPs for reducing PPP losses from agricultural areas, however, these strategies fall short of the specification needed to tailor prevention tools to the specific characteristics of a PPP, especially for large-scale diffusion. Pesticide risk indices, developed according to their chemical and physical properties, chemical nature, timing and application method, and toxicological and ecotoxicological impacts, should be included in the risk assessment procedure and had the potential to be a powerful prevention tools to optimize PPP use (Finizio and Villa, 2002). In the last years a common effort was addressed to develop new approaches to define the impact on environment and human health of pesticides. The main objective followed by researchers is providing user-friendly tools (indicators, indices) to qualify the risk associated to chemicals used in crop protection. The information derived from the application of this tools can be used by different stakeholders for planning screening or monitoring programs in natural and agricultural areas, as well as the set-up of management programs in cropland close to protected zones. A prioritization method approach was developed by Tsaboula et al., (2016), to get information regarding the environmental impact of pesticides useful in the selection of pesticides to be monitored in waters. Japanese researchers developed a risk prediction method for the assessment of pesticides in waters (Narita et al., 2014). Sampling and monitoring campaigns may certainly be useful in the assessment of pesticide impact on the environment, but they require time and there are not costly-free (Narita et al., 2014). The optimization of pesticide use in crop protection may derive from the use of environmental indices. Different indices have been developed to evaluate and optimize the use of pesticides in current agricultural practice (Reus et al., 2002).

To this end, we developed an index-based approach for a comprehensive evaluation of the potential risk for active substances and their mixtures to impact the environment. The approach was initially developed for herbicides in maize crops, but it can readily be applied to other PPPs and crops. Some of the indices considered already exist (PRISW-1, Priority Index), while others were created *ex novo* from indicators available on open-source platforms (PESTi, ECOi, AGROi). These indices maybe used for an *evaluation before use* of pesticides by farmers and advisers.

Once the best-describing indices were defined, they were used in a case study for comparison across the most common chemical strategies for maize weeds control in Northern Italy. The approach allowed to discriminate among the most common chemical strategies adopted for maize weed control based on their full environmental and ecotoxicological profiles, rather than their efficacy against weeds alone. Moreover, by attributing an environmental weight to the crop protection strategies adopted at the farm level, local authorities can select a suitable PPP that takes into account environmental risk of a specific chemical strategy in a specific agricultural area.

MATERIALS AND METHODS

2.1 Risk indices for selecting and comparing herbicides

To define the environmental impact of an herbicide or herbicide mixture, a series of indices were considered. These indices were obtained by scoring different indicators referring to the chemical-physical, ecotoxicological, and toxicological properties of each active substance. Indices permit comparison among different active substances expressing similar herbicidal efficacy. To differentiate among the potential for ecological and environmental hazards in the active ingredients, we individuated the following indices: “Pesticide index” (PESTi), “Ecotoxicological index” (ECOi), “Agronomic index” (AGROi), “Short term pesticide risk Index for surface water system” (PRISW-1) and “Priority index for surface and ground waters” (PI).

Risk index differentiation was performed using two approaches: the scoring approach and the ratio approach (Padovani and Capri, 2006). The first one was used for the determination of Pesticide Index (PESTi), Ecological Index (ECOi), Agronomic Index (AGROi), and Priority Index (PI). The ratio approach was used to determine the PRISW-1, calculating the Toxicological Exposure Ratio (TER) by dividing the Predicted Environmental Concentration (PEC) of each substance by the toxicological endpoints for selected target species. In general, the ecotoxicological and physical-chemical indicators of the substance were considered additive, while some other indicators such as the application rate must be considered as multiplicative. In the present paper all indicators were considered additive. In order to define the water contamination profile of a certain maize herbicide mixture the main chemical and physical properties and the ecotoxicological and toxicological properties of each single active substance, were considered and used as indicators (Table 1). For each indicator, specific thresholds have been considered as previously suggested by the Footprint database (Lewis et al., 2016). A specific score has been attributed according to the referring threshold. By attributing to each active substance, a specific score according to its characteristics, it was possible to calculate a general score, which represents the value of the index. In case of mixture of herbicides, the index was calculated by adding the individual score of each active substance of the mixture. For indicators such as “priority substance” and “candidate for substitution”, where specific numeric thresholds were not available, the score was attributed considering the inclusion of the substance to a category. For example, if herbicide X is candidate for substitution, the classification was Yes and the score was 5 (Yes = 5), otherwise the classification was No and the score was 0 (No = 0).

The values of the different indicators used were retrieved from open source databases such as Pesticide Properties DataBase (Lewis et al., 2016), European food and safety authority (EFSA, 2021) peer review reports, The Pesticide Manual (BCPC, 2012), AGRITOX (Maniere et al., 2011) and TOXNET (PubChem, 2021) . As these indices were applied to the most common chemical strategies adopted to control maize weeds in Northern Italy, data on presence of herbicides in surface and ground waters were obtained from ISPRA annual reports (Paris et al., 2020). The information regarding the number of weed species involved in resistance to specified herbicides were obtained from the International resistant weed database (Heap, 2019).

2.1.1 Pesticide index (PESTi)

The pesticide index was obtained by attributing a specific score to a selected number of pesticide indicators. These pesticide indicators refer to the physical and chemical properties of the active substance. The pesticide indicators used were: water solubility, soil mobility, soil persistence, water persistence, percolation index and bioaccumulation (Table 1). For each indicator, three classes of assignment were individuated. A specific score was attributed to each class of assignment as shown in Table 1. Physical and chemical data were obtained from the Pesticide Properties Database (Lewis et al., 2016) and from The Pesticide Manual (BCPC, 2012) (see Appendix A). The index was calculated for each active substance and for the different herbicide mixtures. For each active substance the index derives from the sum of the individual scores (min. value= 0, max. =40; Table 1) attributed to each indicator weighted for the maximum score obtainable, as follow (Eq. 1):

$$PESTi_{a.s.} = \frac{\sum individual\ scores\ for\ each\ indicator}{40} \qquad [1]$$

For the mixtures, the calculation of the index was weighted to consider the number of active substances of the mixture, as follow (Eq. 2):

$$PESTi_{mix} = \frac{Total\ score\ of\ the\ mixture}{40 * N} \qquad [2]$$

where 40 is the maximum score of the index calculated for a single active substance and N is the number of active substances of the mixture.

The indicators individuated for the determination of the index, saved vapor pressure, were selected considering the content of the report of the Tuscany agency for the environmental protection (ARPAT, 2015) regarding a general indicator of impact of pesticides on the environment based on pesticides properties and other parameters. The classes for the attribution of the relative score are those individuated in the ARPAT document.

Table 1: Indicators selected to calculate PESTi for a certain active substance.

INDICATORS	PROPERTY	SCORE		
		0	**2.5**	**5**
Water solubility in water at 20°C (g/l)	Water affinity	≤ 50	50-500	> 500
***Koc* (ml/g)**	Soil mobility	> 500	75-500	≤ 75
DT50 soil (days)	Soil persistence	≤ 30	30-100	> 100
Aqueous photolysis (days at pH 7)	Water persistence	< 1	1-30	> 30
Aqueous hydrolysis (days at pH 7)	Water persistence	<1	1-30	>30
GUS index	Percolation index	< 1,8	1,8-2,8	> 2,8
***Kow* log P**	Partition coefficient	< 2,7	2,7-3	> 3
Vapour pressure (mPa)	Volatility	<5	5-10	>10
MAXIMUM SCORE				**40**

2.1.2 Ecotoxicological index (ECOi)

The ecotoxicological index (ECOi) was calculated considering the ecotoxicological endpoints of each active substance [Lethal concentration (LC_{50}), lethal dose (LD_{50}), environment concentration (EC_{50})], for mammals, birds, fish, aquatic invertebrates, earthworms, and honeybees (Table 2) retrieved from PPDB and PubChem database (Lewis et al., 2016; PubChem, 2021). As for PESTi, three classes of assignment were individuated for each indicator. A specific score was attributed to each class of assignment as shown in Table 2. Most of the ecotoxicological data for the selected organisms used for the calculation of the index were obtained from the “The pesticide properties database” (Lewis et al., 2016) and The pesticide Manual (LC_{50} fish, LD_{50} birds, EC_{50} algae, LD_{50} bees) (see Appendix A). The mammalian toxicity was evaluated considering the oral acute LD_{50} values for rats expressed in mg/kg. For birds it was considered the oral acute LD_{50} value (mg/kg) for mallard ducks, as for this species the data were available for all the active substances. For fish and earthworms, the endpoint was represented by EC_{50} values. The EC_{50} of fish refer to a period of 96 h for the rainbow trout, while in case of earthworms the period was of 14 days. For algae, the EC_{50} (72h) values for *Pseudokirchneriella subcapitata* was generally used; exceptions were the EC_{50} (72h) values for *Raphidocelis subcapitata* for the herbicides tembotrione, isoxaflutole, prosulfuron, rimsulfuron, flufenacet and sulcotrione, the EC_{50} (72h) values for *Anabena flos aque* in the case of nicosulfuron and foramsulfuron, and the EC_{50} (72h) values for *Navicula pelliculosa* in the case of aclonifen and clomazone. Effective concentrations for invertebrates refer to EC_{50} (48h) data of *Daphnia magna*. The toxicity against honeybees was evaluated considering the oral acute LD_{50} values. The index was calculated for each active substance and for the different herbicide mixtures.

For each active substance the index derives from the sum of the individual scores (min. value= 0, max. =40; Table 2) attributed to each indicator weighted for the maximum score obtainable, as follow (Eq. 3):

$$ECOi_{a.s.} = \frac{\sum individual\ scores\ for\ each\ indicator}{40} \qquad [3]$$

Similarly to PESTi, for the mixtures the calculation of the ECOi was weighted to consider the number of active substances of the mixture, as follows (Eq. 4):

$$ECOi_{mix} = \frac{Total\ score\ of\ the\ mixture}{40 * N} \qquad [4]$$

where 40 is the maximum score of the index calculated for a single active substance and N is the number of active substances of the mixture.

With the exception of BCF factor, the indicators used for the calculation of ECOi index were selected considering the content of the report of the Tuscany agency for the environmental protection (ARPAT, 2015). The classes of ranking for the attribution of the relative score are those individuated in the ARPAT document.

Table 2: Indicators selected to calculate the ecotoxicological index of a certain active substance

INDICATORS	**PROPERTY**	**SCORE**		
		0	**2.5**	**5**
LD 50 acute mammals (rats) – oral mg/kg body weight/day	Toxicity for rats	>2000	100-2000	<100
LD50 acute birds (mg/kg)	Toxicity for birds	>2000	100-2000	<100
LD50 acute honeybees (48 h, µg/kg)	Toxicity for bees	>100	1-100	<1
LC50 acute fish (96 h, mg/l)	Toxicity for fish	>100	0.1-100	<0.1
EC50 algae	Toxicity for algae	>100	0.1-100	<0.1
EC50 daphnia	Toxicity for daphnia	>100	0.1-100	<0.1
LC50 earthworms (mg/kg)	Toxicity for earthworms	>1000	10-1000	<10
BCF - Bioconcetration factor (l/kg)	General risk	<100	5000-100	>5000
MAXIMUM SCORE				**40**

Notes: LD_{50}: dose required to kill half percent of the tested population, LC_{50}: concentration required to kill half percent of the tested population; EC_{50}: concentration required to have a certain effect on the tested population.

2.1.3 Agronomic index (AGROi)

The agronomic index was calculated taking into account indicators relative to three fields of reference: resistance proneness (number of known resistant weed populations, the mode of action of the active substance), regulation constrictions (substance candidate for substitution, priority substance), environmental impact (application rate, relevant metabolites, MacKay fugacity level 1, runoff risk) (Appendix B). Data and formulae used for the calculation of the index were retrieved from different sources (Directive 2013/39/EU, 2013; Heap, 2019; Lewis et al., 2016; Mackay and Paterson, 1981; Regulation 408/2015/EU, 2015) (see Appendix A).

The development of resistant weed populations is a current issue in many parts of the world. The reasons of this troublesome phenomenon are different: the repeated use of herbicide with the same mechanism of action, the use of herbicides at low dosages, the spread of genetically modified crops resistant to herbicides, and the lack of herbicides with new mechanisms of action (Holt and Lebaron, 1990; Manalil, 2014; Manalil et al., 2011; Powles, 2014; Vidotto et al., 2021). From an environmental point of view, the appearance of resistant weeds may induce farmers to make on way changes in the chemical control strategies currently adopted such as increasing the application rate looking for a highest efficacy or using mixtures of other herbicides. The last update given by the HRAC (Heap, 2020), reported 263 weeds which have evolved resistance to at least one mechanism of action. Of the 31 mechanisms of action known, weeds have developed resistance to 21 of them and to 164 different herbicides (Heap, 2020). As in the last years the introduction of herbicides with new modes of action has been very scarce, it is important adopting a sustainable and rational use of the existing herbicides to limit the appearance of new resistant weed populations. Many factors have determined this current lack of new herbicides on the market, such as the significant attrition of companies involved in new herbicide discovery, the advent of GM crops in many parts of the world, the raising of industrial costs for research and development, the more and more severe regulation restrictions established worldwide by regulatory authorities (Duke, 2012). The profile of each active substance has been evaluated in terms of risk of development of resistant weed populations. For each active substance a resistance index has been determined considering the number of weeds which have developed resistance to it across the world. Another trait considered in the evaluation of resistance proneness was the mode of action of the active substance according to the *Herbicide resistance Action Committee website* (HRAC) classification of herbicides: in this case, a higher score was attributed to herbicides belonging to HRAC groups for which a higher number of cases of resistance have been reported worldwide (groups B, C1, A, G, O). All the information regarding weed resistance were taken from the HRAC.

The environmental impact of a certain pesticide has been evaluated considering the application rate, the presence of relevant metabolites, its fugacity according the MacKay model Level 1 and the application timing. The application rate gives information regarding the amount of the active substance that is introduced into the environment, while the presence of relevant metabolites allows to qualify the environmental indirect risk linked to the degradation products of the parental compound. Data for the application rates were directly obtained from the field case study. The number of relevant metabolites originated from the parental compound applied were obtained from the PPDB database (Lewis et al., 2016).

Regulation restrictions were taken into account by considering the indicators “candidate for substitution” and “priority substance”, obtained by checking the list of substances candidate for substitution according to the European Commission Implementing Regulation 408/2015 (Regulation 408/2015/EU, 2015) and the list of priority substances according to the Directive 2013/39/EU (Directive 2013/39/EU, 2013). As the application timing is concerned, this indicator has been included for the calculation of the AGROi index to consider the different impact that the period of application may have in terms of water pollution. Pre-emergence applications are more prone to runoff and leaching phenomena being applied on bare soils without any presence of weeds or crops. Moreover, pre-emergence herbicides are intrinsically more persistent, as they should be active (i.e. able to prevent germination) for as long as the critical period of weed control is over, which can require, in the case of maize, several weeks from emergence (Knezevic and Datta, 2015). Herbicides applied in early-post-emergence were considered to have a behavior not dissimilar to those applied in pre-emergence. At this application stage, most of the soil is still bare and crops and weeds (if present) are in their very early growth stages. Another important indicator considered in the calculation of this index is the environmental distribution according to the MacKay model. Fugacity is the escaping tendency of a chemical to move from an environmental compartment to another to which it has more affinity (Calliera et al., 2001; Mackay and Paterson, 1981). The fugacity represent the partial pressure of a certain chemical in a specific environmental compartment (Calliera et al., 2001). In order to establish at first the behavior of a certain substance in the environment, the MacKay model Level I was used (Mackay and Peterson, 1981). This multicompartmental model considers a “world unit” constituted of different compartments that occupy an established volume. The world unit that was considered in this study has a 1 km^2 base with a 6 km-high atmosphere with the characteristics indicated in Appendix A. The various environmental compartments and environmental phases used were individuated according the indication of (Calliera et al., 2001; Mackay and Paterson, 1981; Perin, 2004) The index was calculated for each active substance and for the different herbicide mixtures.

For each active substance the index derives from the sum of the individual scores (min. value= 0, max. =40; Table 3) attributed to each indicator weighted for the maximum score obtainable, as follow (Eq. 5):

$$AGROi_{a.s.} = \frac{\sum individual\ scores\ for\ each\ indicator}{40} \quad [5]$$

As for the previous indices, for herbicide mixtures the calculation of the index was weighted to consider the number of active substances of the mixture, as follow (Eq. 6):

$$AGROi_{mix} = \frac{Total\ score\ of\ the\ mixture}{40 * N} \quad [6]$$

where 40 is the maximum score of the index calculated for a single active substance and N is the number of active substances of the mixture.

Table 3: Indicators selected to calculate the environmental index of a certain active substance

INDICATORS	PROPERTY	SCORE		
		0	**2.5**	**5**
Application rate (g/ha)	Environmental load	<100	100-500	>500
Resistance index	N° of resistant weed species	< 5	5-10	> 10
Mode of action (HRAC group)	Resistance proneness	All the other groups	C2, N, K1, E, D	B, C1, A, G, O
Relevant metabolites	Additional risk	0	1-2	> 2
Mackay distribution (% of partition in water)	Fugacity	0-60	61-90	>90
Candidate to substitution	Regulation constriction	No (0)		Yes (5)
Priority substance	Regulation constriction	No (0)		Yes (5)
Application period	Water pollution risk	Post-emergence (0)		Pre-emergence Early post-emergence (5)
MAXIMUM SCORE				**40**

2.1.5 Short term pesticide risk Index for surface water system (PRISW-1 index) (Finizio et al., 2001)

The short term pesticide risk for surface waters system (PRISW-1 index) was calculated for selected non-target organisms according to the classification intervals and the scores indicated in Table 4. The TER value considered for the attribution of the score was obtained dividing EC_{50} or LC_{50} by the corresponding PEC for surface water. The PEC value for surface water (PEC_{sw}) was taken from the official peer-review report of each active substance released by the European Food and Safety Authority (EFSA).

Table 4. Risk classification ranges and corresponding scores for selected non-target organisms (NTO) (fish, daphnia, algae) in surface waters used for PRISW-1 index (modified from Finizio et al., 2001).

Toxicity Exposure ratio (TER) value*	Score
>10000	0
10000-1000	1
1000-100	2
100-10	4

10-2	6
<2	8

*** TER=** $\frac{\mathbf{LC50}}{\mathbf{PEC}}$ **fish ;** $\frac{\mathbf{EC50}}{\mathbf{PEC}}$ **daphnia/algae**

According to Finizio et al., (2001), a specific weight was attributed to each organism: 5.5 for fish, 4 for daphnia, 3 for algae. The general score of each herbicide was calculated as follows (Eq.7):

$$\text{PRISW-1} = score\ of\ fish\ \times\ 5.5 + score\ of\ daphnia\ \times\ 4 + score\ of\ algae\ \times 3$$

[7]

For herbicide mixtures, the PRISW-1 index was calculated considering a general toxicity exposure ratio (TER $_{\text{mix}}$) instead of TER of each active substance alone. The determination of TER $_{\text{mix}}$ was done by adding the toxic unit of each constituent of the mixture for each of the three categories of organism considered, as follow (Travisi et al., 2004) (Eq. 8):

$$TER_{mix} = \frac{1}{\sum \frac{1}{Toxic\ unit}} \qquad [8]$$

Where $Toxic\ unit = \frac{1}{TER\ \ _{single\ substance\ (1>n)}}$; n is the number of components of the mixture

For each active substance and for each category of organism considered (fish, algae and daphnia) it was determined the reciprocal of TER ($\frac{1}{TER}$). TER $_{\text{mix}}$ of fish, algae and daphnia of each herbicide mixture was calculated by adding the reciprocal of TER previously determined for each active substance (Eq. 9):

E.g. TER *mix for fish* = $\frac{1}{\sum(\frac{1}{TER_{S-metolachlor}} + \frac{1}{TER_{dicamba}} + \frac{1}{TER_{nicosulfuron}})}$ [9]

The values of TER *mix* for fish, algae and daphnia were used for calculating the PRISW-1 index of each herbicide mixture using the same equation described above for calculating PRISW-1 index for each active substance alone and the same risk classification ranges and scores indicated in Table 4 (Eq. 10).

$$\text{PRISW-1}_{mix} = score\ of\ fish\ \times\ 5.5 + score\ of\ daphnia\ \times\ 4 + score\ of\ algae\ \times 3$$

[10]

The risk level was classified according to classes of risk as proposed by papers of (Finizio et al., 2001; Travisi et al., 2006) (Table 5).

Table 5: Classes of risk for PRISW-1 index for single active substances and for herbicide mixtures (from Travisi et al., 2004, modified).

PRISW-1 value	Risk classification
≤ 5	Negligible
> 5 - ≤ 15	Low
> 15 - ≤ 40	Medium
> 40 - ≤ 80	High
> 80	Very high

A negligible risk means no impact on aquatic ecosystems, while very high risk leads to severe effects on aquatic living communities with effect on growth and productivity as well as damping off of fishes and invertebrates.

2.1.7 Priority Index for surface and ground waters (PI)

The priority index for surface and ground waters (ISPRA, 2011) is based on different indicators: sales data for the Italian market derived from the Sistema informativo agricolo nazionale database database (SIAN, 2021), the type of application (on the soil, on the crop, on both of them), the environmental distribution according the MacKay model level 1, the soil persistence of the active substance (Table 6). The priority index is calculated using the following equation (Eq. 11):

$$PI = S_s + (S_{ed}\ \times\ S_{use}\ \times\ S_{sp}) \qquad [11]$$

Where S_s is sales score, S_{ed} the environmental distribution score, S_{use} the score relative to type of use and S_{sp} the score that consider the soil persistence.

Table 6. Indicators used to calculate the priority index (ISPRA, 2011).

Score total sales (S_s)		Score environmental distribution (S_{ed})		Score type of use (S_{use})		Score soil persistence (S_{sp})	
1st-10th percentile	5	≥ 99	5	Soil	1	$DT_{50} \leq 10$	0.5
11th-20th percentile	4	> 80-99	4			$> 10\ DT_{50} \leq 30$	0.8
21st-30th percentile	3	> 60-80	3	Crop	0.9	$> 30\ DT_{50} < 90$	1
31st-50th percentile	2	> 30-60	2			$DT_{50} \geq 90$	1.2
51st-100th percentile	1	0-30	1	Soil and crop	0.8	DT_{50} not found	1

Notes: DT_{50} in days

The highest value of the index, corresponding to a substance reaching the highest score for each indicator, is 11, the least 1.4. Sales data refer to the last available dataset on the national yearly sales of pesticide published by the Italian agricultural information system (SIAN, 2021). Although these data refer to 2012, considering that all the active substances used in the field experiment are on the market by many years, great changes in their reciprocal ranking are unlike. Pesticides are listed in a decreasing order according to the sales data. The score relative to the environmental distribution is determined considering the Mackay model Level I (see previous paragraphs).

2.2. Field case study

The indices were applied in a case study that was part of a long-term field study focused on the individuation of the best chemical strategies for maize weed control. The case study was carried out at different conventional farms located in Lombardy region, northern Italy during the period 2014-2019. In particular from 2014 to 2017 field trials were hosted by *Azienda Cerri* at Turano Lodigiano (45°14'23.9"N 9°36'57.4"E), Lodi province, while in the last two years trials were carried out at *Azienda La Madonnina* at Liscate (45°28'37.3"N 9°24'07.7"E), Milano province. During the investigation, the most common chemical weed control strategies adopted in maize were compared. Herbicides were applied according to different control strategies: pre-emergence (PRE strategy), early post-emergence (E-POST) and late post-emergence (L-POST) (POST strategy), pre-emergence followed by post-emergence (PRE+POST strategy). The herbicide mixtures were selected as the most widely used by maize farmers in Northern Italy. All the characteristics of the mixtures used are presented in the supplementary materials files (Appendix C). The acronyms used across the manuscript to indicate a specific active substance are instead reported in Table 7. In all the years, a randomized block design with three blocks and three replications per block was adopted. In each block three untreated plots (control) were individuated. In 2016 and 2017 the number of mixtures compared was reduced to include only the most significant mixtures applied in the fields. Each plot was 50 m^2 large. During the last seasons, 2018-2019, new mixtures chosen by farmers were included in the investigation. All plots were about 1.5 m spaced apart from all sides in order to avoid spray drift or off-site movement of herbicides applied by runoff after spraying. The effects on weed infestation were assessed on key weeds (*Abuthilon theophrasti* Medicus, *Amaranthus retroflexus* L., *Chenopodium album* L., *Echinochloa crus-galli* (L.) Pal. Beauv., *Panicum dichotomiflorum (L.) Michx.*, *Poa annua*, *Portulaca oleracea* L., *Setaria viridis* (L.) Pal. Beauv. and *Solanum nigrum* L.), by measuring plant density (plants/m^2), ground cover (%) and overall efficacy (%) based on plant density data compared to control . Data were statistically analyzed for each year by conducting ANOVA analysis followed by a REGWF test ($\alpha=0.05$) to determine the differences among the efficacy data observed between herbicide mixtures. The values presented are the mean of nine data. SPSS, version 28.00, was used to perform the statistical analysis (SPSS, IBM Corporation, 2008). Weed density and weed cover were assessed on three spots in each plot selected by randomly launching a metal quadrat frame of known area (0.625 m^2). Weed density was assessed by counting the number of weed species present within the measurements area while weed cover was evaluated by estimating the percentage of the area included in the metal frame covered by the weeds. Herbicide application was carried out using a backpack sprayer (Honda Power Sprayer, 25 L. tank, GX 25T air-cooled-single cylinder, 4Stroke OHV, 0.72kw / 7000rpm) which mounted 5 nozzles (Tee Jet 11002) bar able to distribute a volume of 300 l/ha. Pre-emergence applications were performed the day after sowing, while the early post-emergence and the late-post-emergence treatments were carried out with maize ad BBCH stage 12 (2th leaf) and 15 (5th leaf), respectively.

Table 7: Active substances included in the herbicide mixtures used in the field survey with the corresponding acronyms used across the document, mode of action of the active substances according to the HRAC Classification (HRAC, 2021).

ACTIVE SUBSTANCE	ACRONYM	SUBSTANCE GROUP	HRAC – LEGACY HRAC
Terbuthylazine	***TBA***	*TRIAZINES*	1, 2 (C1)
S-Metolachlor	***SMET***	*CHLOROACETAMIDES*	15 (K3)
Petoxamide	***PETO***		

Flufenacet	***FLU***	*OXYACETAMIDES*	
Mesotrione	***MES***	*TRIKETONES*	27 (F2)
Sulcotrione	***SUL***		
Tembotrione	***TEM***		
Prosulfuron	***PRO***	*SULFONYLUREAS*	2 (B)
Nicosulfuron	***NICO***		
Rimsulfuron	***RIM***		
Foramsulfuron	***FORAM***		
Isoxaflutole	***ISO***	*ISOXAZOLES*	27 (F2)
Clomazone	***CLO***	*ISOXAZOLIDINONES*	13 (F4)
Fluroxypir	***FLURO***	*PYRIDYLOXY-CARBOXYLATES*	4 (O)
Pendimetalin	***PENDI***	*DINITROANILINES*	3 (K1)
Aclonifen	***ACLO***	*DIPHENYLETHERES*	32 (S)
Dicamba	***DIC***	*BENZOATES*	4 (O)
Bromoxynil Octanoate	***BROMO***	*NITRILES*	6 (C3)
Thiencarbazone-Methyl	***THIEN***	*TRIAZOLINONES*	2 (B)
Pyridate	***PYRI***	*PHENYLPYRIDAZINES*	6 (C3)

RESULTS

2. Efficacy of weed control strategies adopted for use in the field case study

Differences among three strategies laid the ground for selection of indices used to develop an overall health/hazard pesticide index. We considered three strategies (PRE, PRE+POST, and POST); the highlights are discussed here. We found that all herbicides and herbicide mixtures provided a high degree (above 95%; Appendix D) of weed control when the PRE-emergence strategy was used. Rainfall events that occurred shortly after application boosted the effectiveness of the strategy during the first four seasons. The second strategy, Pre + Post treatment, is more costly to use than any strategy that is applied just once (either pre- or post-emergence), and it is used most often for instances of incomplete pre-emergence application. The results for this strategy, across all seasons, resulted in the addition of little efficacy relative to total efficacy, as the weed control level achieved after the pre-emergence sprayed was already high (Supplementary Material files, Appendix D). Finally, the third strategy (POST strategy) is an alternative to pre-emergence applications in cases of weed infestation not properly controlled by pre-emergence herbicides. The POST strategy consists usually is two herbide applications, one applied in early post-emergence and a second of different chemicals applied in late post-emergence. Generally, this strategy provides adequate weed control throughout the seasons, however very competitive (e.g. *Sorghum halepense*) or newly emerging weeds (e.g. *Cyperus esculentus*) can still breakthrough some chemical mixtures. Reduced efficacy of some early post-emergence mixtures applied is due to the limited action of some active ingredients under unfavorable soil conditions. For instance, during 2014, the mixture of S-metolachlor, terbuthylazine, and mesotrione showed low efficacy in some grasses (*Panicum dichotomiflorum* and *Setaria viridis)*. During that season, soil dryness at the time of early application limited the activity of S-metolachlor (See supplementary materials, Appendix D).

3.2 Risk indices

3.2.1 PESTi index

The pesticide index (PESTi) was calculated for each active substance (Appendix E), then used to determine the value of different chemical strategies and herbicide mixtures for comparison in the field survey. The highest PESTi values were found in FLURO and sulfonylureas, while the lowest resulted in PYRI, ISO, and SUL the lowest. The herbicide mixtures were categorized based on their application timing. The PESTi value determined for mixtures used during pre-emergence varied from a minimum of 0.25 (three mixtures, all containing active ingredient ISO) to a maximum of 0.35 (MES+PENDI+SMET). Averaging among the different mixtures adopted in pre-emergence strategy, the PESTi value was 0.29. More than 90% of the mixtures applied in pre-emergence had a PESTi value below 0.4, indicating that majority of the herbicides applied in pre-emergence had a chemical profile not particularly negative in terms of soil-water properties. The PESTi index calculated for mixtures adopted in POST strategy revealed a not dissimilar framework. Less than 80% of the mixtures had a PESTi value below 0.40, whit an average value of the strategy of 0.36. The highest PESTi value was calculated for the mixtures FOR+MES+FLURO (0.46) and NICO+PRO+DIC+FLURO

(0.47). The lowest PESTi value was calculated for the mixture containing SUL+PET (0.25). The highest PESTi values observed for post-emergence mixtures compared to the pre-emergence ones can be related to the presence in the mixtures of active substances of the sulfonylurea family, which are characterized by high water solubility and low *Koc*. The mixtures applied in PRE+POST strategies showed the highest PESTi values. This was not unexpected considering the highest chemical complexity of this kind of strategy. The average value was 0.38, a bit over the average PESTi value observed in POST strategy. However, all herbicides mixtures had a PESTi index ranging from 0.35 to 0.40 (Figure 1).

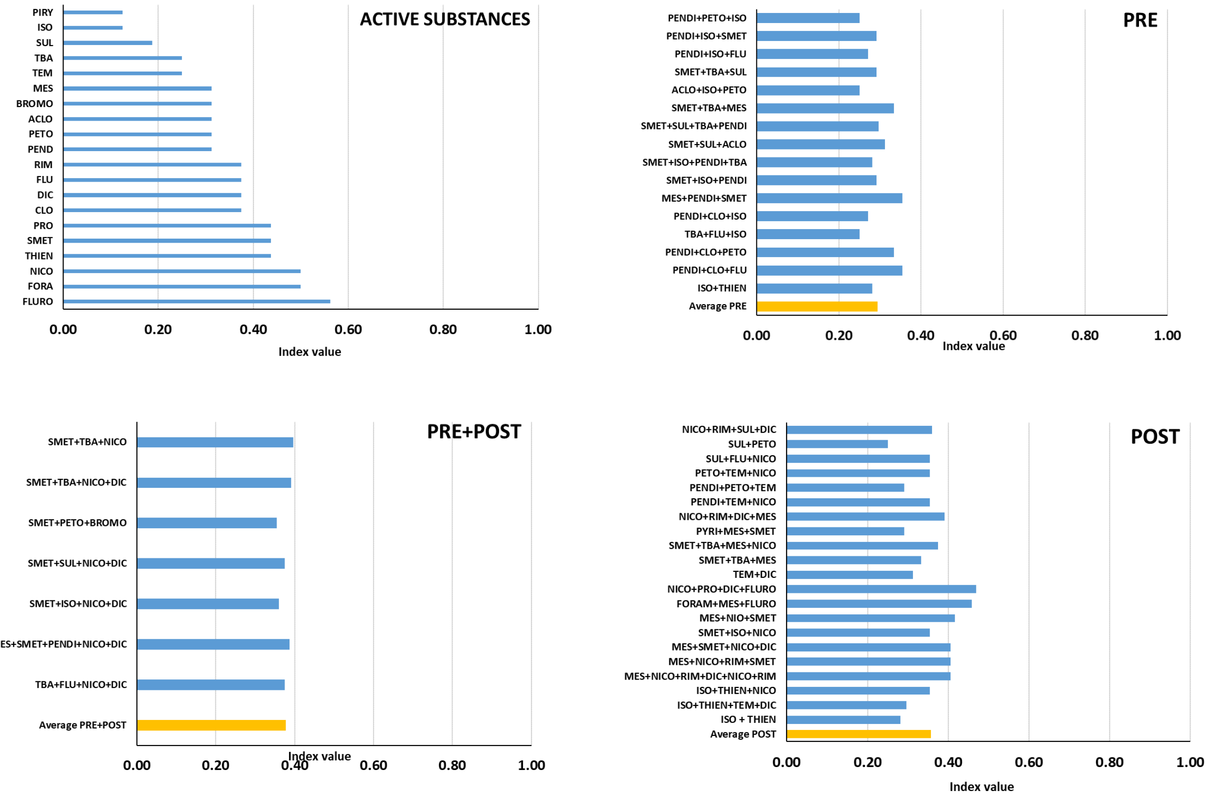


Figure 1: PESTi index calculated for each single active substance and for all the mixtures compared in the field case study divided according the strategy.

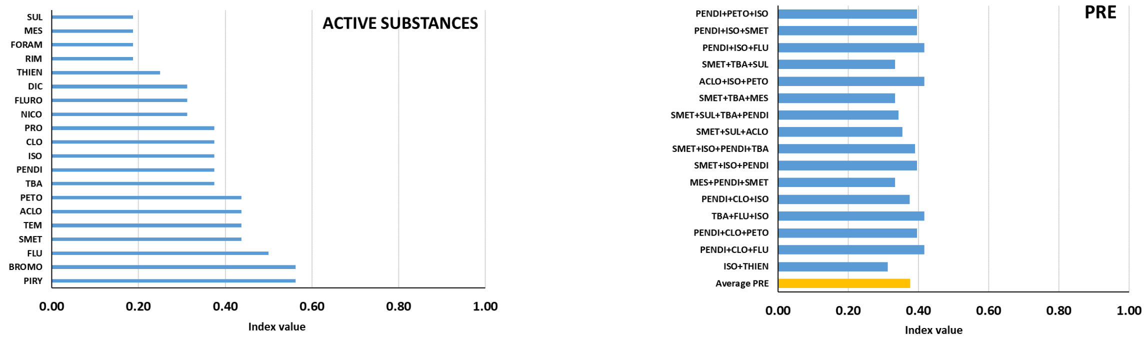
3.2.2 AGROi index

The index was calculated for each active substance (see Appendix F), then for each mixture. Among the active substances under investigations, TEM, BROMO and PYRI got the lowest AGROi value. Pre-emergence chemicals were negatively affected by the score gained by certain indicators, such as the period of application, by the number of relevant metabolites and by the soil persistence. TBA and NICO reached the highest value; the second one, in particular, was affected by a high proneness to develop resistance. The average AGROi value determined in pre-emergence strategy was 0.45. The lowest AGROi value was calculated for the mixture CLO+PENDI+ISO (0.38). All the other mixtures applied in pre-emergence showed an AGROi value equal or above 0.41. The AGROi index for post-emergence mixtures was, on average, lower compared to pre-emergence treatments (0.38). PRE+POST strategy had an AGROi average value of 0.47. This was mainly due the presence in the strategy of active substances which gained an individual high score such as SMET, TBA or a sulfonylurea. The highest AGROi values was reached by the mixture SMET+TBA+NICO (0.53), the lowest by the mixture of SMET+PETO+BROM (0.31) (Figure 2).

Figure 2: AGROi index calculated for each single active substance and for all the mixtures compared in the field case study divided according the strategy.

3.2.3 ECOi index

The index was calculated for each active substance (see Appendix G), then for each mixture. PIRY, BROM and FLU showed the highest ECOi index among all the active substances compared. RIM, FORA and MES had the best profile. The ECOi index calculated for pre-emergence mixtures showed values ranging from 0.31 (ISO+TIEN and NICO+MES+S-MET) to 0.42 (in four different mixtures). The average value was 0.38. The pre-emergence mixtures were characterized by higher ECOi values compared to the post-emergence mixtures. This is because the former generally contain at least one active substance of old introduction on the market such as PENDI, TBA, FLU, SMET. The ECOi for post-emergence mixtures varied from 0.25 to 0.42. The highest ECOi value (0.42) was obtained in the mixture PENDI+PETO+TEM. PRE+POST strategy account for an average ECOi index similar to that observed for pre-emergence mixtures (0.37). The bad performance of this strategy can be attributed to the worst ecological profile of the pre-emergence herbicides (Figure 3).



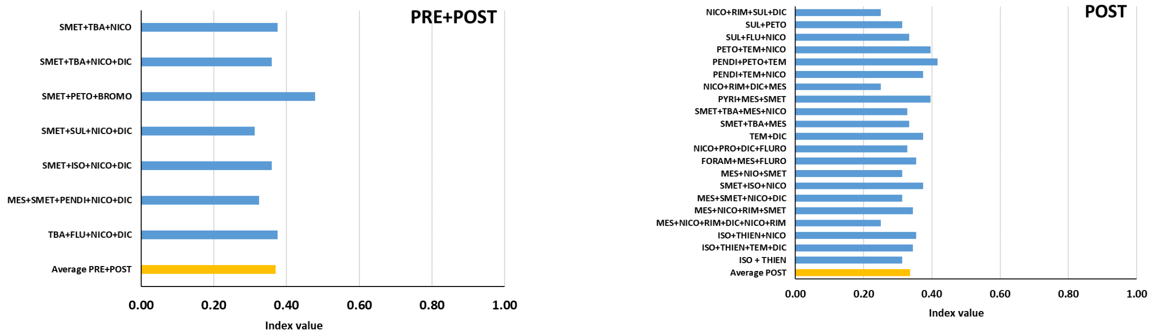


Figure 3: ECOi index calculated for each active substance and for all the mixtures compared in the field case study, divided according the strategy.

3.2.5 PRISW-1 index

The PRISW-1 was calculated for fish, daphnia and algae. In Appendix H are reported the parameters used to calculate the index for each active substance contained in the herbicide mixtures.

Analyzing the PRISW-1 values obtained for all the active substances used in the field survey (Figure 4), it was possible to observe how chemicals with the highest PRISW-1 values were generally “old herbicides”, namely substances on the market since many decades. SMET, TBA, ACLO, BROMO and PENDI were characterized by high risk for surface water system (PRISW-1 > 40). As concern the mixtures, 45% of them reached a high risk (> 40 PRISW-1 >80). The highest risk (PRISW-1 = 73) was reached by several mixtures, regardless of the period of application (Appendix I). All these mixtures include old herbicides such as SMET, PENDI and BROMO. Most of pre-emergence mixtures showed a medium to high risk for surface waters (the only exception was represented by the mixture containing ISO+TIEN). Eleven of the forty-four mixtures presented a medium classification risk (PRISW-1 <40), the majority of them are applied in post-emergence. PRE+POST mixtures had a high risk mainly due to the ecological profile of pre-emergence active substances. These herbicide mixtures contain active substances with low TER values, belonging to chloroacetamide, dinitroaniline and nitrile families. The high complexity of the mixture did not necessary affect the risk classification. The ranking is mostly affected by the specific active substances in the mixture rather than the number of actives substances. Only three mixtures applied in post-emergence had a risk close to the low classification risk category (PRISW-1 = 15,5); they all contain sulfonylureas, triketones and synthetic auxin.

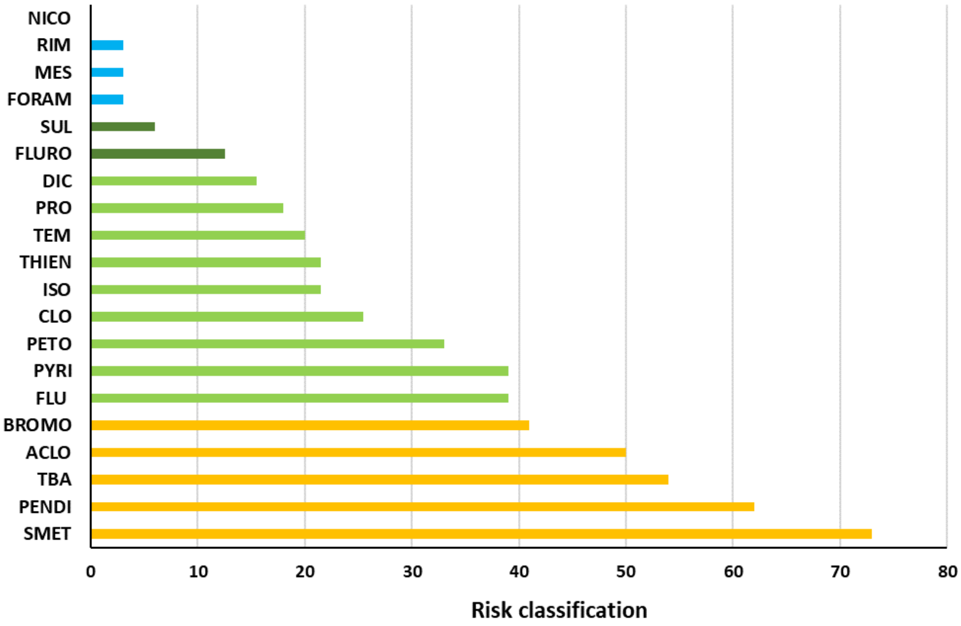


Figure 4: Risk classification of individual herbicide active substances to PRISW-1 index. Explanation of colors: turquoise= PRISW<5 (PRISW-1 value of NICO equal to 0); dark green: >5 PRISW-1 ≤ 15; light green= >15 PRISW-1 ≤40; light orange= >40 PRISW-1 ≤80; red= PRISW-1 >80.

3.2.7 Priority index

The priority index calculated for surface waters confirmed a tendency already highlighted by previous indices. In Figure 5 A are reported the values of the index for each active substance. SMET had the highest value of the index. Most of the chemicals that reach a PI value above 5 are substances with a long history on the market (CLO, FLURO, DIC, PENDI, TBA). Some active substances, such as MES, THIEN and ISO, that showed a good profile in other indices have a PI value above 5, just below the chemicals placed in the top of the list. Their position in the upper part of the list is mainly due to their environmental distribution characteristics (environmental distribution score) and to the fact that are among the most used chemicals (sales score). As concern the comparison between the herbicide mixtures (Figure 5B, 5C and 5D), among pre-emergence herbicides, the highest values were recorded for the most complex mixtures containing certain active substances such as PENDI, SMET, DICA, and TBA. The least problematic mixtures were constituted by ISO+TIEN and ISO+ACLO+PETO. The 55% of post-emergence mixtures had a priority index higher than 15; only two mixtures (TEMB+DIC and SUL+PETO) have a priority index below 10. PRE+POST mixtures reached higher values compared to the other two strategies. The most complex mixture was the worst in term of PI; it was applied in pre+post and it was composed by MES+SMET+PENDI+NICO+DICA.

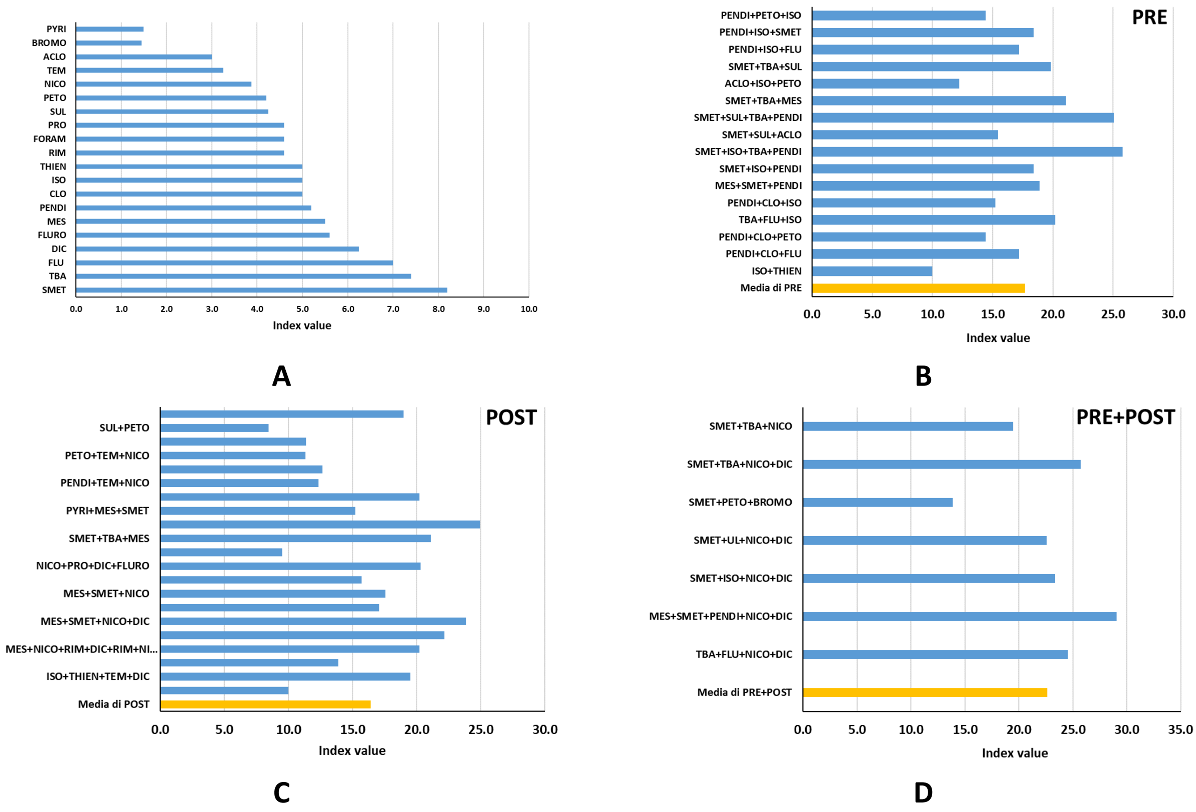


Figure 5: Priority index calculated for the active substances used in the field survey (A) and for herbicide mixtures used in the field survey divided by strategy (other three boxes).

3.3 Risk classification framework

In Appendix L is reported a frame of the risk classification applied to the active substances used in the study as resulted from the calculation of six indices (PESTi, AGROi, ECOi, PRISW-1 and PI). Among the active substances under investigation, SMET, TBA and PENDI showed the worst performances in most of the indices. Sulfonylureas and triketones had a good classification in terms of short/long term risk for surface waters and a better ecotoxicological profile compared to other chemicals. Being a chemical of old introduction on the market is often associated with an ecotoxicological/environmental bad profile; however, some chemicals are out of this frame, as in the case of PYRI, which it was introduced on the market in 1980 (Lewis et al, 2016), but it showed an overall good classification. Despite the potential risk of water contamination, its rapid degradation in the environment greatly reduces the general risk. The same risk classification methodology was applied to the different herbicide mixtures used in pre, pre+post and post-emergence (Appendix from L to O). The results showed that post-emergence mixtures have a best environmental and eco-toxicologic profile. The only mixtures with a more critical profile are those containing SMET. Pre+post emergence mixtures characterized by a good profile in term of PESTi and ECOi indices, but they present a more pronounced risk for waters. Pre-emergence mixtures had an overall good profile for PESTi and AGROi indices but they may pose a certain risk for surface and ground waters.

3. Discussion

Indices, created to classify the risk profile of a chemical, are commonly used in crop protection decision making (Finizio and Villa, 2002; Travisi et al., 2006). Pesticides introduced into the environment undergo processes that may lead to the contamination of different environmental compartments (Damalas, 2018). The ecotoxicological and environmental profile of a pesticide, which derives from its physical and chemical characteristics, can greatly influence the impact on humans and other non-targeted organisms. The challenge lies in protecting crops while reducing negative pesticide effects.

The indices proposed in this study had the objective of ranking pesticides and pesticide mixtures according to their ecotoxicological and environmental impact. The results of this classification may help technicians and policy makers in preparing the best suitable crop protection plans in terms of efficacy and environmental protection. The main advantage in using these indices in evaluating pesticide environmental risk is that they can be easily calculated starting from information regarding pesticide properties, use and fate that are already available. These indices may offer preliminary insights about the *a priori* risk of pesticides use.

Using free-access databases, several indicators that capably described the profile of an individual chemical were selected to build or calculate risk indices. Based on the grouping of like environmental affinities, such an index may better describe the behavior of a chemical. For each indicator, ranking ranges were individuated. Chemicals received an individual score for each indicator of the index. The individual scores were then added each other or combined in an algorithm to calculate the indices. The usefulness of these indices in profiling the ecological and environmental impact of pesticide use was evaluated comparing individual active substances and several mixtures commonly adopted to control weeds in maize cultivation. A total of 20 active substances and 44 mixtures of them were evaluated. They are representative of the most common mixtures used by farmers in Europe in maize cultivation. It is important to note that 80% of the active substances considered were introduced on the market before 2000. The advantage of this two-step approach is pointing out the most critical aspects of each active substance, but in the meantime highlighting differences among strategies or mixtures where the same chemical could be present. PESTi, AGROi and ECOi indices were created ex-novo using available dataset; PRISW-1 and Priority Index were already used by the scientific community. One of the problems in the application of the indices in risk assessment could be the variability of the input data (physical-chemical properties of pesticide, environmental fate) or the absence of some needed data, according to the sources used (Finizio et al., 2001). In the present paper we refer to solid free-access databases where the essential information for the calculation is always available, and the reported data derived from several studies. To the aims of this paper, the proposed indices (newly created or already in use) were not applied for a risk assessment of monitoring data collected in a certain area (evaluation *ex-post*), but for an evaluation *ex-ante* of pesticides or pesticides mixtures. This is a difference compared to other approaches based on risk indices. The main advantage of this approach is that the results of the application of these indices can be used to prevent or reduce the impact of pesticides use. The development of a unique index enables to qualify the potential risk associated to a pesticide or a pesticide mixture is a complicated effort (Levitan et al., 1995). This kind of index should consider different aspects (risk towards waters, non-target organisms, humans) and several compartments (ground waters, surface waters, sediment, air, soil, biota, etc). However, higher the number of factors to be included and considered in the index calculation, more complicated its determination. According to Reus et al., (2002), an ideal indicator focused on the risk associated to pesticide application should include information regarding application rate, application factors and environmental conditions. Reus et al., (2002) compared 8 different pesticide environmental indicators developed in Europe indicating the main requirements they should have and giving recommendations for their use and harmonization. To be adopted at large scale, even by farmers or local advisers, indices must be comprehensible, easy to be calculated with simple algorithms and based on ready and free to access data. PESTi, AGROi and ECOi indices generally respect this criterion, while PRISW-1 and Priority Index need a more accurate attention in gathering the data. The indices proposed in the present approach are focused on different aspects: general profile of pesticides and mixtures (PESTi), impact on non-target organisms in different compartments (ECOi, PRISW-1, Priority Index), agronomic and regulation implications (AGROi).

The main objective of the survey was to compare the efficacy of different weed control strategies. The use of the proposed indices may represent a valid tool for public stakeholders in risk assessment evaluation of pesticides at a territory scale.

The PESTi index consider only the physical and chemical properties of a generic chemical. It gives a framework of the potential risk associated to a specific chemical only on the basis of its chemical nature. However, the physical and chemical features alone does not explain *in toto* the potential risk associated to a chemical or a mixture. This could represent a limit for this index. For instance, triazines are historically found in waters, but according to the PESTi index they have a better profile than sulfunylureas. Sulfonylureas showed a PESTi index higher compared to other chemicals, mainly due to their water solubility (high) and K_{oc} (low) values. PESTi index underlines the physical and chemical characteristics as a whole, not considering the impact of other factors such as application rate or period of application. Hence, this index may underestimate the risk associated to a certain chemical. As an example, despite the PESTi index for terbuthylazine and S-metolachlor resulted better than most sulfonylureas, these two chemicals are among the most frequently found substances in natural waters. The reason of this contrasting behavior can be explained by the fact that these two herbicides are applied in PRE-emergence (in Italy 90% of herbicide treatments in maize is carried out in PRE-emergence), they are used on a large cultivated area and, lastly, they have high application rates (SIAN, 2021).

The innovation of AGROi index stands in the set of input indicators used to calculate it. The index considers the direct effect on the environment of the use of chemicals in terms of evolution of weed resistant populations, hazard to water resources, presence of relevant metabolites, fugacity, environmental load, and existing regulation restrictions. The risk associated to a specific mixture derives from a combination of intrinsic characteristics of the chemicals, agronomic impacts and potential hazard to water compartment. This index has a precautionary approach and may represent an important decision tool for local authorities in defining crop protection strategies within the integrated pest management plans. Some sulfonylureas got the highest AGROi index mainly due to the proneness to develop resistance in weed populations. This class of herbicides, firstly introduced in 1975, showed favorable environmental and toxicological properties. However, their tendency to develop resistance in weed populations have jeopardized their efficacy (Brown and Cotterman, 1994). Even S-metolachlor and terbuthylazine had high AGROi index, because of their high application rates, the period of application and, in a lesser important magnitude, the development of resistant populations. While chloroacetamide resistance in weeds is not frequent despite the longtime widespread use of some chemicals of this family, many weeds have evolved resistance to triazines worldwide (Heap, 1997). According to the AGROi index, in maize weed control, POST strategy is less problematic than PRE or PRE+POST strategies.

Based on the AGROi data, in hydrogeological areas vulnerable to pesticides, regional authorities may define the most protective strategy or the list of less problematic active substances to be adopted or selected. Similarly, in crop systems with an history of herbicide-resistant weed populations, weed control plans may exclude the mixtures characterized by the active substances with the highest risk of developing resistance and/or forcing farmers to alternate chemicals with different mechanism of action.

The ECOi index is focused on the ecotoxicological impact against non-target organisms. In general, pre-emergence herbicides showed the worst performances mainly because of the presence in the mixtures of chemicals of old introduction on the market. Sulfonylureas can be considered the least critical in terms of toxicity against non-target organisms, while some oldest chemicals such as PIRY and BROMO (and consequently the related mixtures) showed the highest ECOi values. In particular, these two chemicals had low LD_{50}/LC_{50} values for almost all the categories of organisms. In crop lands placed within the border or in proximity to protected or sensible areas the selection of allowed chemicals could be based on this index. The helpfulness of this index stands in its ability to easily discriminate the ecotoxicological impact of chemicals using indicators commonly available in literature and without making complex calculations.

The contamination of water resources by herbicides and metabolites forced public authorities to lay down restriction of use or banning of certain pesticides. However, these measures may represent a strong limit for farmers, particularly when the pesticides portfolio is limited due to the scarcity of authorized products. The potential hazard of chemicals and mixtures of them against water resources was determined using PRISW-1 Index which gave an overview in terms of risk for surface waters and the priority index which help to individuate the substances to pay attention both for surface and ground waters. The usefulness of PRISW-1 index has been already validated (Köck-Schulmeyer et al., 2012). Differently from the common application of PRISW-1 index to pesticide monitoring data (Köck-Schulmeyer et al., 2012; Kouzayha et al., 2013), we use this index for an *a priori* risk assessment of herbicides and herbicides mixtures. PRISW-1 index considers the risk of contamination of surface waters in a short-term horizon. As demonstrated by several studies, the risk of pesticide runoff is related to the time interval elapsed from application to the first runoff event and most of the pesticide losses by runoff occurred in the first two weeks after pesticide spraying (Milan et al., 2013). This is particularly true for herbicides, which are the chemicals with the highest risk of runoff due to their application period and type of use. According to the monitoring campaigns carried out by the Italian regional environmental authorities, one third of the substances found in surface and ground water belong to herbicides and herbicide metabolites (Paris et al., 2020). Considering the PRISW-1 values, PRE-emergence mixtures pose generally the highest hazard potential for surface waters, mostly due to the presence of old active substances with a critical environmental profile (high application rates, widespread use, persistence). Post-emergence mixtures with high risk contain active substances usually applied during pre-emergence but that can be applied even in early post-emergence. PRISW-1 Index discriminate active substances according to their risk against three representative non-target organisms. However, due to the intrinsic characteristics of each pesticide, a high PRISW-1 value could not always mean an easy movement of the chemical via runoff waters. This is the case of chemicals such as PENDI and PIRY. Despite their high risk towards aquatic non-target organisms and their use in pre-emergence on bare soils, they are unfrequently found in surface water because they are strongly bounded to soil matrix or rapidly degraded. This means that PRISW-1 index alone does not clearly represent the real risk linked to a pesticide. Priority index allow to identify pesticides with the highest risk to contaminate water resources considering both environmental and statistical indicators. Some of the parameters used in priority index were included in a risk predictor method, developed by Narita et al., (2021), to individuate priority pesticides in drinking water based on quantity of sales, chemical properties and intensity of toxicity. The application of priority index to a case study demonstrated once again the worst performances of the PRE+POST strategy mostly due to the typology of active substances applied in PRE-emergence. The information deriving from Priority index may certainly help public authorities to select chemicals to be detected in water monitoring campaigns. In areas hydrologically vulnerable to pesticide leaching or sensible to runoff, the chemical ranking deriving from the calculation of priority index may represent a protective strategy towards water resources. The indices presented in this paper may be used individually or together in the evaluation of the environmental profile of a certain chemical or strategy. Each index could be addressed to a principal auditor; for instance, ECOi and PRISW-1 Indices may assume a great importance in cropped areas close to ecosystems to be protected. Priority index could be an interesting way to reduce or avoid water contamination by pesticides used in crop protection by individuating the most critical compounds or strategies. AGRi index put together different critical aspects related to a generic pesticide outlining its environmental profile. For instance, this index could be used in the definition of sustainable crop protection plans within the regional rural development programs prepared by local authorities.

The indices presented in this study were selected to be applied for all categories of pesticides; however, some of the indicators can be adjusted, added or substituted in order to consider some important factors. For instance, the indicator "risk for period of application" in the AGROi index, must be considered in different terms depending on the category of pesticide used. Herbicides are applied on bare soil or in presence of crops/weeds, insecticides/fungicides for orchards use are applied on plants on vegetation or on vegetative rest. Once established the overall efficacy of the strategies adopted, the next step was trying to profile the different mixtures in terms of their environmental impact. The combined use of the proposed indices could help individuating the best strategies and the less critical mixtures to be adopted. Active substances belonging to the same chemical family may had a comparable toxicity on non-target organisms as well as a similar environmental behavior (Finizio et al., 2001). Overall, old chemicals generally got the worst performance in all the indices considered.

The main advantage of performing this kind of risk classification for herbicides mixtures used in maize to control weeds is that the proposed indices are based on data easily obtainable of free data set available online. The application of these indices to a real case study, allowed drawing some additional agronomical conclusions:

a) There is lack of pesticides with new mechanisms of action and with a more environmental and ecotoxicological friendly profile. In maize crop protections, most of the chemicals on the market are of old introduction. Despite these chemicals still have good performances against weeds, they generally had a critical ecotoxicological or environmental profile. There is some exception as, PYRI, an old herbicide that demonstrate having a good environmental profile compared to other old herbicides, but with ecotoxicological issues on non-target organisms.

b) Sulfonylureas generally had a low impact on environment and terrestrial and aquatic communities. They showed a limited impact on the environment and on non-target organisms in spite of some potentially negative chemical properties (high solubility, high GUS index). This is due to their low application field rates and low persistency in water and soil compartments. Similar findings were obtained by (Finizio et al., 2001). The most critical aspect is their tendency to develop weed resistant populations, in particular in case of repeated use.

c) High water solubility alone it is not a sufficient parameter to individuate the risk of water contamination for a certain pesticide (see sulfonylureas).

d) PENDI, SMET and TBA are still very used PRE-emergence herbicides, despite they are frequently found both in surface and ground waters. The presence of these chemicals in a mixture increases the possibility of environmental or ecotoxicological issues. The results of the field study, demonstrated that there are already valid alternative mixtures able to ensure an equal efficacy against weeds than that guaranteed by the old mixtures.

e) Chemicals belonging to the same family generally showed similar environmental behavior and toxicological effect on non-target organisms. Differences can be linked to changes in the field rate used.

f) Pre-emergence herbicides showed the highest risk for aquatic organism both in short and long-time horizon. This is expected considering that these chemicals are applied in greatest quantity compared to the post-emergence herbicides. In this regard, the worst mixtures contained SMET, TBA and SMET. However, there are some chemicals with a moderate or low risk, in spite of their long history on the market (DIC and FLURO).

g) Considering the risk both for surface and ground waters, the priority index pointed out the risk associated to some chemicals of widespread use in maize cultivation. Most of them are pre-emergence herbicides, generally of old introduction on the market, largely applied in maize cultivation.

The assumptions made in this paper are based on the evaluation of indices built using indicators selected on the base of their ability to define the behavior of a chemical. The attribution of weights and scores was based on previous papers or defined during the data analysis. Nevertheless, the application of these indices may represent a valid decision tool for public stakeholders in defining agricultural measures to reduce the externalities of pest control. The present approach was applied to a specific crop protection system (maize and weeds), but it can be used also for other crops and different pests.

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