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

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(Article begins on next page)

1 Abstract

2 An index-based approach for a comprehensive evaluation of the potential risk for active substances 3 and their mixtures to impact the environment was developed. Some of the indices considered 4 already exist (PRISW-1, Priority Index), while others were created *ex novo* from indicators available 5 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 6 7 in maize crops, but it can readily be applied to other PPPs and crops. PESTi index underline the 8 physical and chemical characteristics as a whole, not considering the impact of other factors such 9 as application rate or period of application. Hence, this index may underestimate the risk associated 10 to a certain chemical. AGROi has a precautionary approach. The risk associated to a specific mixture 11 derives from a combination of intrinsic characteristics of the chemicals, agronomic impacts, 12 regulation restrictions and potential hazard to water compartment. The ECOi index is focused on 13 the ecotoxicological impact against non-target organisms. The helpfulness of this index stands in its 14 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 15 16 substances according to their risk against three representative non-target organisms. However, due 17 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 18 19 certainly help public authorities to select chemicals to be detected in water monitoring campaigns. 20 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. 21

22 Key words: Indices, pesticides, risk assessment

23

24 **1. INTRODUCTION**

25 A key issue in crop plant protection is maintaining effective methods to combat biotic stress caused 26 by living organisms. Historically, pesticide research centered on the efficacy of a single active 27 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, 28 2007; Knutson, 1999; Rathore and Nollet, 2012), pesticides can also affects agricultural sustainability 29 (Wilson and Tisdell, 2001). Moreover, once pesticides have entered the environment, significant 30 31 negative impacts can occur to living communities of various environmental compartments (Storck 32 et al., 2017; Thiour-Mauprivez et al., 2019).

33 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-34 35 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 36 37 Souza et al., 2020; Dougherty et al., 2010; Sjerps et al., 2019; Thurman et al., 1992). Groundwater 38 protection is critically important in many parts of the world where deep water is used for human 39 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 40 41 (2009/128/CE, 2009; 2000/60/UE, 2000). In some areas, PPPs are the most common substances 42 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.

52 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 53 various stakeholders on the best management practices (BMPs) to mitigate negative impacts on 54 cropping. By example, two Life projects (TOPPS and TOPPS Prowadis) have identified the BMPs for 55 reducing PPP losses from agricultural areas, however, these strategies fall short of the specification 56 57 needed to tailor prevention tools to the specific characteristics of a PPP, especially for large-scale 58 diffusion. Pesticide risk indices, developed according to their chemical and physical properties, 59 chemical nature, timing and application method, and toxicological and ecotoxicological impacts, 60 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 61 addressed to develop new approaches to define the impact on environment and human health of 62 pesticides. The main objective followed by researchers is providing user-friendly tools (indicators, 63 indices) to qualify the risk associated to chemicals used in crop protection. The information derived 64 65 from the application of this tools can be used by different stakeholders for planning screening or 66 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 67 68 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 69 70 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 71

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.

89

90 MATERIALS AND METHODS

91 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 chemicalphysical, ecotoxicological, and toxicological properties of each active substance. Indices permit comparison among different active substances expressing similar herbicidal efficacy. To 96 differentiate among the potential for ecological and environmental hazards in the active
97 ingredients, we individuated the following indices: "Pesticide index" (PESTi), "Ecotoxicological
98 index" (ECOi), "Agronomic index" (AGROi), "Short term pesticide risk Index for surface water
99 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 100 approach (Padovani and Capri, 2006). The first one was used for the determination of Pesticide 101 Index (PESTi), Ecological Index (ECOi), Agronomic Index (AGROi), and Priority Index (PI). The ratio 102 103 approach was used to determine the PRISW-1, calculating the Toxicological Exposure Ratio (TER) by 104 dividing the Predicted Environmental Concentration (PEC) of each substance by the toxicological endpoints for selected target species. In general, the ecotoxicological and physical-chemical 105 106 indicators of the substance were considered additive, while some other indicators such as the 107 application rate must be considered as multiplicative. In the present paper all indicators were 108 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 109 properties of each single active substance, were considered and used as indicators (Table 1). For 110 each indicator, specific thresholds have been considered as previously suggested by the Footprint 111 112 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 113 114 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 115 the mixture. For indicators such as "priority substance" and "candidate for substitution", where 116 specific numeric thresholds were not available, the score was attributed considering the inclusion 117 of the substance to a category. For example, if herbicide X is candidate for substitution, the 118

classification was Yes and the score was 5 (Yes = 5), otherwise the classification was No and the
score was 0 (No = 0).

121 The values of the different indicators used were retrieved from open source databases such as 122 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 123 124 TOXNET (PubChem, 2021). As these indices were applied to the most common chemical strategies 125 adopted to control maize weeds in Northern Italy, data on presence of herbicides in surface and 126 ground waters were obtained from ISPRA annual reports (Paris et al., 2020). The information 127 regarding the number of weed species involved in resistance to specified herbicides were obtained 128 from the International resistant weed database (Heap, 2019).

129 2.1.1 Pesticide index (PESTi)

The pesticide index was obtained by attributing a specific score to a selected number of pesticide 130 131 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 132 133 persistence, percolation index and bioaccumulation (Table 1). For each indicator, three classes of 134 assignment were individuated. A specific score was attributed to each class of assignment as shown 135 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 136 for each active substance and for the different herbicide mixtures. For each active substance the 137 index derives from the sum of the individual scores (min. value= 0, max. =40; Table 1) attributed to 138 139 each indicator weighted for the maximum score obtainable, as follow (Eq. 1):

140
$$PESTi_{a.s.} = \frac{\sum individual \ scores \ for \ each \ indicator}{40}$$
[1]

141 For the mixtures, the calculation of the index was weighted to consider the number of active 142 substances of the mixture, as follow (Eq. 2):

143
$$PESTi_{mix} = \frac{Total \ score \ of \ the \ mixture}{40 \ * \ N}$$
[2]

144 where 40 is the maximum score of the index calculated for a single active substance and N is the

145 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.

151	Table 1: Indicators selected to calculate PESTi	for a certain active substance.
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	DDODEDTV	SCORE		
INDICATORS	PROPERTY	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	\leq 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				

152

153 2.1.2 Ecotoxicological index (ECOi)

The ecotoxicological index (ECOi) was calculated considering the ecotoxicological endpoints of each active substance [Lethal concentration (LC₅₀), lethal dose (LD₅₀), environment concentration (EC₅₀)], 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 158 of assignment were individuated for each indicator. A specific score was attributed to each class of 159 assignment as shown in Table 2. Most of the ecotoxicological data for the selected organisms used 160 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). 161 162 The mammalian toxicity was evaluated considering the oral acute LD₅₀ values for rats expressed in mg/kg. For birds it was considered the oral acute LD₅₀ value (mg/kg) for mallard ducks, as for this 163 species the data were available for all the active substances. For fish and earthworms, the endpoint 164 165 was represented by EC_{50} values. The EC_{50} of fish refer to a period of 96 h for the rainbow trout, while 166 in case of earthworms the period was of 14 days. For algae, the EC₅₀ (72h) values for Pseudokirchneriella subcapitata was generally used; exceptions were the EC₅₀ (72h) values for 167 Raphidocelis subcapitata for the herbicides tembotrione, isoxaflutole, prosulfuron, rimsulfuron, 168 169 flufenacet and sulcotrione, the EC₅₀ (72h) values for Anabena flos aque in the case of nicosulfuron 170 and foramsulfuron, and the EC₅₀ (72h) values for Navicula pelliculosa in the case of aclonifen and clomazone. Effective concentrations for invertebrates refer to EC₅₀ (48h) data of *Daphnia magna*. 171 172 The toxicity against honeybees was evaluated considering the oral acute LD₅₀ values. The index was calculated for each active substance and for the different herbicide mixtures. 173

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):

177
$$ECOi_{a.s.} = \frac{\sum individual \ scores \ for \ each \ indicator}{40}$$
[3]

178

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):

181
$$ECOi_{mix} = \frac{Total \ score \ of \ the \ mixture}{40 \ * \ N}$$
[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
- 186 (ARPAT, 2015). The classes of ranking for the attribution of the relative score are those individuated
- in the ARPAT document.

INDICATODS	DDODEDTV	SCORE		
INDICATORS	PROPERTY	0	2.5	5
LD 50 acute mammals (rats) - oral	Toxicity for rats	>2000	100-2000	<100
mg/kg body weight/day				
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	>1000	10-1000	<10
	earthworms			
BCF - Bioconcetration factor (l/kg)	General risk	<100	5000-100	>5000
MAXIMUM SCORE				40

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

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.

192 2.1.3 Agronomic index (AGROi)

193 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

- 196 substance), environmental impact (application rate, relevant metabolites, MacKay fugacity level 1,
- 197 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).

200 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 201 mechanism of action, the use of herbicides at low dosages, the spread of genetically modified crops 202 203 resistant to herbicides, and the lack of herbicides with new mechanisms of action (Holt and Lebaron, 204 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 205 the chemical control strategies currently adopted such as increasing the application rate looking for 206 207 a highest efficacy or using mixtures of other herbicides. The last update given by the HRAC (Heap, 208 2020), reported 263 weeds which have evolved resistance to at least one mechanism of action. Of 209 the 31 mechanisms of action known, weeds have developed resistance to 21 of them and to 164 210 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 211 212 herbicides to limit the appearance of new resistant weed populations. Many factors have 213 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, 214 215 the raising of industrial costs for research and development, the more and more severe regulation 216 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 217 218 each active substance a resistance index has been determined considering the number of weeds 219 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* 220 221 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.

225 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 226 application timing. The application rate gives information regarding the amount of the active 227 228 substance that is introduced into the environment, while the presence of relevant metabolites 229 allows to qualify the environmental indirect risk linked to the degradation products of the parental 230 compound. Data for the application rates were directly obtained from the field case study. The 231 number of relevant metabolites originated from the parental compound applied were obtained 232 from the PPDB database (Lewis et al., 2016).

Regulation restrictions were taken into account by considering the indicators "candidate for 233 234 substitution" and "priority substance", obtained by checking the list of substances candidate for 235 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 236 237 (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 238 239 application may have in terms of water pollution. Pre-emergence applications are more prone to 240 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. 241 242 able to prevent germination) for as long as the critical period of weed control is over, which can 243 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 244 in pre-emergence. At this application stage, most of the soil is still bare and crops and weeds (if 245

present) are in their very early growth stages. Another important indicator considered in the 246 247 calculation of this index is the environmental distribution according to the MacKay model. Fugacity 248 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 249 the partial pressure of a certain chemical in a specific environmental compartment (Calliera et al., 250 2001). In order to establish at first the behavior of a certain substance in the environment, the 251 MacKay model Level I was used (Mackay and Peterson, 1981). This multicompartmental model 252 considers a "world unit" constituted of different compartments that occupy an established volume. 253 The world unit that was considered in this study has a 1 km² base with a 6 km-high atmosphere with 254 the characteristics indicated in Appendix A. The various environmental compartments and 255 environmental phases used were individuated according the indication of (Calliera et al., 2001; 256 257 Mackay and Paterson, 1981; Perin, 2004) The index was calculated for each active substance and 258 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):

262
$$AGROi_{a.s.} = \frac{\sum individual \ scores \ for \ each \ indicator}{40}$$
 [5]

263

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):

266
$$AGROi_{mix} = \frac{Total \, score \, of \, the \, mixture}{40 * N}$$
[6]

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

	DDODEDTV	SCORE			
INDICATORS	PROPERTY	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)	(0) Yes (5)		Yes (5)
Application period	Water pollution risk	Post-emergence (0)		Pre-emergence Early post- emergence (5)	
MAXIMUM SCORE					40

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

270

271 **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 EC₅₀ or LC₅₀ 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



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): 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 _{mix}) instead of TER of each active substance alone. The determination of TER _{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):

291
$$TER_{mix} = \frac{1}{\Sigma \frac{1}{Toxic unit}}$$
 [8]

292 Where *Toxic unit* = $\frac{1}{TER \text{ single substance (1>n)}}$; 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 _{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):

297 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 300 each active substance alone and the same risk classification ranges and scores indicated in Table 4

301 (Eq. 10).

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

303

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

[10]

305 Travisi et al., 2006) (Table 5).

306 **Table 5: Classes of risk for PRISW-1 index for single active substances and for herbicide mixtures** 307 **(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

308

309 A negligible risk means no impact on aquatic ecosystems, while very high risk leads to severe effects

310 on aquatic living communities with effect on growth and productivity as well as damping off of fishes

311 and invertebrates.

312 **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):

318
$$PI = S_s + (S_{ed} \times S_{use} \times S_{sp})$$
[11]

- 319 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.

Score total sales (S _s)		Score environmental distribution (S _{ed})		Score type of use (S _{use})		Score soil persistence (S _{sp})	
1 st -10 th percentile	5	≥ 99	5	Soil	1	DT ₅₀ ≤ 10	0.5
11 th -20 th percentile	4	> 80-99	4	3011	T	> 10 DT ₅₀ ≤ 30	0.8
21 st -30 th percentile	3	> 60-80	3	Cron	0.0	> 30 DT ₅₀ < 90	1
31 st -50 th percentile	2	> 30-60	2	Сгор	0.9	DT ₅₀ ≥ 90	1.2
51 st -100 th percentile	1	0-30	1	Soil and crop	0.8	DT ₅₀ not found	1

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

322 Notes: DT₅₀ in days

323

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).

331 2.2. Field case study

332 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 333 334 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 335 (45°14'23.9"N 9°36'57.4"E), Lodi province, while in the last two years trials were carried out at 336 Azienda La Madonnina at Liscate (45°28'37.3"N 9°24'07.7"E), Milano province. During the 337 investigation, the most common chemical weed control strategies adopted in maize were 338 339 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-340 341 emergence followed by post-emergence (PRE+POST strategy). The herbicide mixtures were selected 342 as the most widely used by maize farmers in Northern Italy. All the characteristics of the mixtures 343 used are presented in the supplementary materials files (Appendix C). The acronyms used across 344 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 345 block three untreated plots (control) were individuated. In 2016 and 2017 the number of mixtures 346 compared was reduced to include only the most significant mixtures applied in the fields. Each plot 347 was 50 m² large. During the last seasons, 2018-2019, new mixtures chosen by farmers were included 348 in the investigation. All plots were about 1.5 m spaced apart from all sides in order to avoid spray 349 drift or off-site movement of herbicides applied by runoff after spraying. The effects on weed 350 351 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., 352 Poa annua, Portulaca oleracea L., Setaria viridis (L.) Pal. Beauv. and Solanum nigrum L.), by 353 measuring plant density (plants/m²), ground cover (%) and overall efficacy (%) based on plant 354 density data compared to control. Data were statistically analyzed for each year by conducting 355 356 ANOVA analysis followed by a REGWF test (α =0.05) to determine the differences among the efficacy 357 data observed between herbicide mixtures. The values presented are the mean of nine data. SPSS, 358 version 28.00, was used to perform the statistical analysis (SPSS, IBM Corporation, 2008). Weed 359 density and weed cover were assessed on three spots in each plot selected by randomly launching 360 a metal quadrat frame of known area (0.625 m²). Weed density was assessed by counting the 361 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. 362 Herbicide application was carried out using a backpack sprayer (Honda Power Sprayer, 25 L. tank, 363 364 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 365 the day after sowing, while the early post-emergence and the late-post-emergence treatments were 366 carried out with maize ad BBCH stage 12 (2th leaf) and 15 (5th leaf), respectively. 367

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	CULOBOACETANAIDES	15 (12)
Petoxamide	ΡΕΤΟ	CHLOROACETAMIDES	12 (K3)

Flufenacet FLL		OXYACETAMIDES	
Mesotrione	MES		
Sulcotrione	SUL	TRIKETONES	27 (F2)
Tembotrione	TEM		
Prosulfuron	PRO		
Nicosulfuron	NICO		2 (ח)
Rimsulfuron	RIM	SULFONTLOREAS	2 (В)
Foramsulfuron	FORAM		
Isoxaflutole	ISO	ISOXAZOLES	27 (F2)
Clomazone	CLO	ISOXAZOLIDINONES	13 (F4)
Fluroxypir		PYRIDYLOXY-	4 (O)
	FLURO	CARBOXYLATES	
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)

371

372 **RESULTS**

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

374 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); 375 376 the highlights are discussed here. We found that all herbicides and herbicide mixtures provided a 377 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 378 379 during the first four seasons. The second strategy, Pre + Post treatment, is more costly to use than 380 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, 381 382 resulted in the addition of little efficacy relative to total efficacy, as the weed control level achieved 383 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 384 weed infestation not properly controlled by pre-emergence herbicides. The POST strategy consists 385

386 usually is two herbide applications, one applied in early post-emergence and a second of different 387 chemicals applied in late post-emergence. Generally, this strategy provides adequate weed control 388 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 389 some early post-emergence mixtures applied is due to the limited action of some active ingredients 390 under unfavorable soil conditions. For instance, during 2014, the mixture of S-metolachlor, 391 terbuthylazine, and mesotrione showed low efficacy in some grasses (*Panicum dichotomiflorum* and 392 393 Setaria viridis). During that season, soil dryness at the time of early application limited the activity 394 of S-metolachlor (See supplementary materials, Appendix D).

395 3.2 Risk indices

396 3.2.1 PESTi index

397 The pesticide index (PESTi) was calculated for each active substance (Appendix E), then used to 398 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 399 resulted in PYRI, ISO, and SUL the lowest. The herbicide mixtures were categorized based on their 400 401 application timing. The PESTi value determined for mixtures used during pre-emergence varied from 402 a minimum of 0.25 (three mixtures, all containing active ingredient ISO) to a maximum of 0.35 403 (MES+PENDI+SMET). Averaging among the different mixtures adopted in pre-emergence strategy, 404 the PESTi value was 0.29. More than 90% of the mixtures applied in pre-emergence had a PESTi 405 value below 0.4, indicating that majority of the herbicides applied in pre-emergence had a chemical 406 profile not particularly negative in terms of soil-water properties. The PESTi index calculated for 407 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 408 409 PESTi value was calculated for the mixtures FOR+MES+FLURO (0.46) and NICO+PRO+DIC+FLURO 410 (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 411 related to the presence in the mixtures of active substances of the sulfonylurea family, which are 412 characterized by high water solubility and low Koc. The mixtures applied in PRE+POST strategies 413 showed the highest PESTi values. This was not unexpected considering the highest chemical 414 complexity of this kind of strategy. The average value was 0.38, a bit over the average PESTi value 415 observed in POST strategy. However, all herbicides mixtures had a PESTi index ranging from 0.35 to 416 0.40 (Figure 1). 417



418 Figure 1: PESTi index calculated for each single active substance and for all the mixtures compared

419 *in the field case study divided according the strategy.*

420

421 3.2.2 AGROi index

422 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-423 424 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 425 and NICO reached the highest value; the second one, in particular, was affected by a high proneness 426 to develop resistance. The average AGROi value determined in pre-emergence strategy was 0.45. 427 The lowest AGROi value was calculated for the mixture CLO+PENDI+ISO (0.38). All the other 428 mixtures applied in pre-emergence showed an AGROi value equal or above 0.41. The AGROi index 429 for post-emergence mixtures was, on average, lower compared to pre-emergence treatments 430 (0.38). PRE+POST strategy had an AGROi average value of 0.47. This was mainly due the presence in 431 the strategy of active substances which gained an individual high score such as SMET, TBA or a 432 433 sulfonylurea. The highest AGROi values was reached by the mixture SMET+TBA+NICO (0.53), the 434 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.

438

439 3.2.3 ECOi index

The index was calculated for each active substance (see Appendix G), then for each mixture. PIRY, 440 BROM and FLU showed the highest ECOi index among all the active substances compared. RIM, 441 FORA and MES had the best profile. The ECOi index calculated for pre-emergence mixtures showed 442 443 values ranging from 0.31 (ISO+TIEN and NICO+MES+S-MET) to 0.42 (in four different mixtures). The 444 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 445 one active substance of old introduction on the market such as PENDI, TBA, FLU, SMET. The ECOi 446 447 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 448 449 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). 450





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

453

454 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 457 458 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 459 PENDI were characterized by high risk for surface water system (PRISW-1 > 40). As concern the 460 mixtures, 45% of them reached a high risk (> 40 PRISW-1 > 80). The highest risk (PRISW-1 = 73) was 461 reached by several mixtures, regardless of the period of application (Appendix I). All these mixtures 462 463 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 464 containing ISO+TIEN). Eleven of the forty-four mixtures presented a medium classification risk 465 (PRISW-1 <40), the majority of them are applied in post-emergence. PRE+POST mixtures had a high 466 risk mainly due to the ecological profile of pre-emergence active substances. These herbicide 467 mixtures contain active substances with low TER values, belonging to chloroacetamide, 468 dinitroaniline and nitrile families. The high complexity of the mixture did not necessary affect the 469 risk classification. The ranking is mostly affected by the specific active substances in the mixture 470

- 471 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,



473 triketones and synthetic auxin.

474

475 Figure 4: Risk classification of individual herbicide active substances to PRISW-1 index. Explanation

- 476 of colors: turquoise= PRISW<5 (PRISW-1 value of NICO equal to 0); dark green: >5 PRISW-1 \leq 15;
- 477 light green= >15 PRISW-1 ≤40; light orange= >40 PRISW-1 ≤80; red= PRISW-1 >80.

478 3.2.7 Priority index

The priority index calculated for surface waters confirmed a tendency already highlighted by 479 previous indices. In Figure 5 A are reported the values of the index for each active substance. SMET 480 had the highest value of the index. Most of the chemicals that reach a PI value above 5 are 481 482 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 483 above 5, just below the chemicals placed in the top of the list. Their position in the upper part of the 484 485 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 486 comparison between the herbicide mixtures (Figure 5B, 5C and 5D), among pre-emergence 487 488 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.



495

496 Figure 5: Priority index calculated for the active substances used in the field survey (A) and 497 for herbicide mixtures used in the field survey divided by strategy (other three boxes).

498

499 3.3 Risk classification framework

500 In Appendix L is reported a frame of the risk classification applied to the active substances used in 501 the study as resulted from the calculation of six indices (PESTi, AGROi, ECOi, PRISW-1 and PI). Among 502 the active substances under investigation, SMET, TBA and PENDI showed the worst performances 503 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 504 505 chemical of old introduction on the market is often associated with а an ecotoxicological/environmental bad profile; however, some chemicals are out of this frame, as in 506 the case of PYRI, which it was introduced on the market in 1980 (Lewis et al, 2016), but it showed 507 an overall good classification. Despite the potential risk of water contamination, its rapid 508 degradation in the environment greatly reduces the general risk. The same risk classification 509 methodology was applied to the different herbicide mixtures used in pre, pre+post and post-510 511 emergence (Appendix from L to O). The results showed that post-emergence mixtures have a best 512 environmental and eco-toxicologic profile. The only mixtures with a more critical profile are those 513 containing SMET. Pre+post emergence mixtures characterized by a good profile in term of PESTi and 514 ECOi indices, but they present a more pronounced risk for waters. Pre-emergence mixtures had an 515 overall good profile for PESTi and AGROi indices but they may pose a certain risk for surface and 516 ground waters.

517 **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.

525 The indices proposed in this study had the objective of ranking pesticides and pesticide mixtures 526 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 532 chemical were selected to build or calculate risk indices. Based on the grouping of like 533 environmental affinities, such an index may better describe the behavior of a chemical. For each 534 indicator, ranking ranges were individuated. Chemicals received an individual score for each 535 536 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 537 538 environmental impact of pesticide use was evaluated comparing individual active substances and 539 several mixtures commonly adopted to control weeds in maize cultivation. A total of 20 active 540 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 541 542 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 543 544 meantime highlighting differences among strategies or mixtures where the same chemical could be 545 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 546 547 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 548 sources used (Finizio et al., 2001). In the present paper we refer to solid free-access databases where 549 550 the essential information for the calculation is always available, and the reported data derived from 551 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-552 553 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 554 the results of the application of these indices can be used to prevent or reduce the impact of 555 pesticides use. The development of a unique index enables to qualify the potential risk associated 556 to a pesticide or a pesticide mixture is a complicated effort (Levitan et al., 1995). This kind of index 557 should consider different aspects (risk towards waters, non-target organisms, humans) and several 558 compartments (ground waters, surface waters, sediment, air, soil, biota, etc). However, higher the 559 560 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 561 562 pesticide application should include information regarding application rate, application factors and 563 environmental conditions. Reus et al., (2002) compared 8 different pesticide environmental indicators developed in Europe indicating the main requirements they should have and giving 564 recommendations for their use and harmonization. To be adopted at large scale, even by farmers 565 566 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 567 568 criterion, while PRISW-1 and Priority Index need a more accurate attention in gathering the data. 569 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, 570 571 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.

575 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 576 577 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, 578 triazines are historically found in waters, but according to the PESTi index they have a better profile 579 than sulfunylureas. Sulfonylureas showed a PESTi index higher compared to other chemicals, mainly 580 due to their water solubility (high) and K_{oc} (low) values. PESTi index underlines the physical and 581 chemical characteristics as a whole, not considering the impact of other factors such as application 582 rate or period of application. Hence, this index may underestimate the risk associated to a certain 583 584 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 585 586 substances in natural waters. The reason of this contrasting behavior can be explained by the fact 587 that these two herbicides are applied in PRE-emergence (in Italy 90% of herbicide treatments in 588 maize is carried out in PRE-emergence), they are used on a large cultivated area and, lastly, they have high application rates (SIAN, 2021). 589

590 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 591 592 resistant populations, hazard to water resources, presence of relevant metabolites, fugacity, 593 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 594 595 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 596 within the integrated pest management plans. Some sulfonylureas got the highest AGROi index 597 598 mainly due to the proneness to develop resistance in weed populations. This class of herbicides, 599 firstly introduced in 1975, showed favorable environmental and toxicological properties. However, their tendency to develop resistance in weed populations have jeopardized their efficacy (Brown 600 601 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 602 development of resistant populations. While chloroacetamide resistance in weeds is not frequent 603 despite the longtime widespread use of some chemicals of this family, many weeds have evolved 604 605 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. 606

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, 613 pre-emergence herbicides showed the worst performances mainly because of the presence in the 614 mixtures of chemicals of old introduction on the market. Sulfonylureas can be considered the least 615 616 critical in terms of toxicity against non-target organisms, while some oldest chemicals such as PIRY 617 and BROMO (and consequently the related mixtures) showed the highest ECOi values. In particular, these two chemicals had low LD₅₀/LC₅₀ values for almost all the categories of organisms. In crop 618 619 lands placed within the border or in proximity to protected or sensible areas the selection of allowed 620 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 621 622 literature and without making complex calculations.

623 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 624 625 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 626 resources was determined using PRISW-1 Index which gave an overview in terms of risk for surface 627 waters and the priority index which help to individuate the substances to pay attention both for 628 surface and ground waters. The usefulness of PRISW-1 index has been already validated (Köck-629 Schulmeyer et al., 2012). Differently from the common application of PRISW-1 index to pesticide 630 monitoring data (Köck-Schulmeyer et al., 2012; Kouzayha et al., 2013), we use this index for an a 631 632 priori risk assessment of herbicides and herbicides mixtures. PRISW-1 index considers the risk of 633 contamination of surface waters in a short-term horizon. As demonstrated by several studies, the 634 risk of pesticide runoff is related to the time interval elapsed from application to the first runoff 635 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 636 the highest risk of runoff due to their application period and type of use. According to the monitoring 637 638 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., 639 640 2020). Considering the PRISW-1 values, PRE-emergence mixtures pose generally the highest hazard 641 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 642 643 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 644 to their risk against three representative non-target organisms. However, due to the intrinsic 645 646 characteristics of each pesticide, a high PRISW-1 value could not always mean an easy movement 647 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 648 649 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 650 pesticide. Priority index allow to identify pesticides with the highest risk to contaminate water 651 resources considering both environmental and statistical indicators. Some of the parameters used 652 in priority index were included in a risk predictor method, developed by Narita et al., (2021), to 653 individuate priority pesticides in drinking water based on quantity of sales, chemical properties and 654 intensity of toxicity. The application of priority index to a case study demonstrated once again the 655 worst performances of the PRE+POST strategy mostly due to the typology of active substances 656 applied in PRE-emergence. The information deriving from Priority index may certainly help public 657 658 authorities to select chemicals to be detected in water monitoring campaigns. In areas 659 hydrologically vulnerable to pesticide leaching or sensible to runoff, the chemical ranking deriving 660 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 661 environmental profile of a certain chemical or strategy. Each index could be addressed to a principal 662 auditor; for instance, ECOi and PRISW-1 Indices may assume a great importance in cropped areas 663 664 close to ecosystems to be protected. Priority index could be an interesting way to reduce or avoid 665 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 666 667 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 668 local authorities. 669

670 The indices presented in this study were selected to be applied for all categories of pesticides; 671 however, some of the indicators can be adjusted, added or substituted in order to consider some 672 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 673 applied on bare soil or in presence of crops/weeds, insecticides/fungicides for orchards use are 674 applied on plants on vegetation or on vegetative rest. Once established the overall efficacy of the 675 strategies adopted, the next step was trying to profile the different mixtures in terms of their 676 677 environmental impact. The combined use of the proposed indices could help individuating the best 678 strategies and the less critical mixtures to be adopted. Active substances belonging to the same 679 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 680 681 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:

686 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 687 market are of old introduction. Despite these chemicals still have good performances against 688 weeds, they generally had a critical ecotoxicological or environmental profile. There is some 689 exception as, PYRI, an old herbicide that demonstrate having a good environmental profile 690 compared to other old herbicides, but with ecotoxicological issues on non-target organisms. 691 b) Sulfonylureas generally had a low impact on environment and terrestrial and aquatic 692 693 communities. They showed a limited impact on the environment and on non-target 694 organisms in spite of some potentially negative chemical properties (high solubility, high GUS 695 index). This is due to their low application field rates and low persistency in water and soil 696 compartments. Similar findings were obtained by (Finizio et al., 2001). The most critical 697 aspect is their tendency to develop weed resistant populations, in particular in case of 698 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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729 References

730 ARPAT, 2015. Fitofarmaci. Proposta di un indicatore di pressione elaborando proprietà ambientali e

731 dati di utilizzo dei prodotti fitosanitari.

- Batista, S., Silva, E., Galhardo, S., Viana, P., José Cerejeira, M., 2002. Evaluation of pesticide
 contamination of ground water in two agricultural areas of Portugal. International Journal of
 Environmental Analytical Chemistry 82, 601–609.
- BCPC, 2012. The Pesticide manual. A world compendium. The British Crop Protection Council, Alton,
 UK.
- Brown, H., Cotterman, J., 1994. Recent advances in sulfonylurea herbicides. Herbicides Inhibiting
 Branched-Chain Amino Acid Biosynthesis 47–81.
- Calliera, M., Finizio, A., Otto, S., Vighi, M., 2001. Malerbologia. Valutazione ecotossicologica degli
 erbicidi, Cap. 7., Prima Edizione. ed. Pàtron editore, Bologna.
- Cooper, J., Dobson, H., 2007. The benefits of pesticides to mankind and the environment. Crop
 Protection 26, 1337–1348.
- Damalas, C.A., 2018. Pesticides in agriculture: Environmental and health risks. Current Opinion in
 Environmental Science & Health 4, iv–v. https://doi.org/10.1016/j.coesh.2018.08.001
- De Gerónimo, E., Aparicio, V.C., Bárbaro, S., Portocarrero, R., Jaime, S., Costa, J.L., 2014. Presence
 of pesticides in surface water from four sub-basins in Argentina. Chemosphere 107, 423–
 431. https://doi.org/10.1016/j.chemosphere.2014.01.039
- de Souza, R.M., Seibert, D., Quesada, H.B., de Jesus Bassetti, F., Fagundes-Klen, M.R., Bergamasco,
 R., 2020. Occurrence, impacts and general aspects of pesticides in surface water: A review.
 Process Safety and Environmental Protection 135, 22–37.

- 751 https://doi.org/10.1016/j.psep.2019.12.035
- Directive 128/2009/EC of the European parliament and of the council of 21 October 2009
 establishing a framework for Community action to achieve the sustainable use of pesticides,
 2009.
- 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, 2000.
- Directive 2013/39/EU, 2013. Directive 2013/39/EU of the European Parliament and of the council
 of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority
 substances in the field of water policy.
- Dougherty, J.A., Swarzenski, P.W., Dinicola, R.S., Reinhard, M., 2010. Occurrence of herbicides and
 pharmaceutical and personal care products in surface water and groundwater around
 Liberty Bay, Puget Sound, Washington. Journal of environmental quality 39, 1173–1180.
- Duke, S.O., 2012. Why have no new herbicide modes of action appeared in recent years? Pest
 management science 68, 505–512.
- EFSA, E.F. and S.A., 2021. Peer review of the pesticide risk assessment of the active substances.
 Conclusion on pesticides.
- Finizio, A., Calliera, M., Vighi, M., 2001. Rating systems for pesticide risk classification on different
 ecosystems. Ecotoxicology and Environmental Safety 49, 262–274.
- Finizio, A., Villa, S., 2002. Environmental risk assessment for pesticides: A tool for decision making.
 Environmental Impact Assessment Review 22, 235–248.
- Gennari, M., Trevisan, M., 2011. Gestione e qualità delle acque. Origini, dinamiche, previsioni,
 mutamenti sociali. Alberto Perdisa Editore Bologna.
- Heap, I., 2020. The International Herbicide-Resistant Weed Database [WWW Document]. URL
 http://weedscience.org/Home.aspx (accessed 6.1.20).
- Heap, I., 2019. The International Survey of Herbicide Resistant Weeds.
- Heap, I.M., 1997. The occurrence of herbicide-resistant weeds worldwide. Pesticide science 51,
 235–243.
- Holt, J.S., Lebaron, H.M., 1990. Significance and distribution of herbicide resistance. Weed
 technology 4, 141–149.
- Knezevic, S.Z., Datta, A., 2015. The critical period for weed control: revisiting data analysis. Weed
 Science 63, 188–202.
- Knutson, R.D., 1999. Economic impacts of reduced pesticide use in the United States: measurement
 of costs and benefits. Agricultural and Food Policy Center, Department of Agricultural
 Economics
- Köck-Schulmeyer, M., Ginebreda, A., González, S., Cortina, J.L., de Alda, M.L., Barceló, D., 2012.
 Analysis of the occurrence and risk assessment of polar pesticides in the Llobregat River
 Basin (NE Spain). Chemosphere 86, 8–16.
 https://doi.org/10.1016/j.chemosphere.2011.08.034
- Kouzayha, A., Al Ashi, A., Al Akoum, R., Al Iskandarani, M., Budzinski, H., Jaber, F., 2013. Occurrence
 of pesticide residues in Lebanon's water resources. Bulletin of environmental contamination
 and toxicology 91, 503–509.
- Levitan, L., Merwin, I., Kovach, J., 1995. Assessing the relative environmental impacts of agricultural
 pesticides: the quest for a holistic method. Agriculture, Ecosystems & Environment 55, 153–
 168. https://doi.org/10.1016/0167-8809(95)00622-Y
- Lewis, K.A., Tzilivakis, J., Warner, D.J., Green, A., 2016. An international database for pesticide risk
 assessments and management. Human and Ecological Risk Assessment: An International
 Journal 22, 1050–1064.
- 798 Mackay, D., Paterson, S., 1981. Calculating fugacity. Environmental Science & Technology 15, 1006–

799 1014.

- Manalil, S., 2014. Evolution of herbicide resistance in Lolium rigidum under low herbicide rates: an
 Australian experience. Crop Science 54, 461–474.
- Manalil, S., Busi, R., Renton, M., Powles, S.B., 2011. Rapid evolution of herbicide resistance by low
 herbicide dosages. Weed Science 59, 210–217.
- Maniere, I., Bouneb, F., Fastier, A., Courty, B., Dumenil, J., Poupard, M., Mercier, T., 2011.
 AGRITOX—Database on pesticide active substances. Toxicology Letters 205, S231–S232.
 https://doi.org/10.1016/j.toxlet.2011.05.792
- Milan, M., Vidotto, F., Piano, S., Negre, M., Ferrero, A., 2013. Buffer strip effect on terbuthylazine,
 desethyl-terbuthylazine and S-metolachlor runoff from maize fields in Northern Italy. null
 34, 71–80. https://doi.org/10.1080/09593330.2012.680919
- Narita, K., Matsui, Y., Iwao, K., Kamata, M., Matsushita, T., Shirasaki, N., 2014. Selecting pesticides
 for inclusion in drinking water quality guidelines on the basis of detection probability and
 ranking. Environment International 63, 114–120.
 https://doi.org/10.1016/j.envint.2013.10.019
- Padovani, L., Capri, E., 2006. Esposizione delle acque superficiali agli agrofarmaci, Quaderni di
 tecniche di protezione ambientale. Protezione e risamento delle acque superficiali. Pitagora
 Editrice, Bologna.
- Paris, P., Pace, E., Maschio, G., Ursino, G., 2020. Rapporto nazionale pesticidi nelle acque. Dati 2017 2018. Edizione 2020.
- Perin, G., 2004. Il sistema ambientale. Ecotossicologia, cap. III Sistema ambiente. Corso di
 ecotossicologia. Università Cà Foscari di Venezia.
- Pimentel, D., Acquay, H., Biltonen, M., Rice, P., Silva, M., Nelson, J., Lipner, V., Giordano, S.,
 Horowitz, A., D'amore, M., 1992. Environmental and economic costs of pesticide use.
 BioScience 42, 750–760.
- 824 Powles, S., 2014. Global herbicide resistance challenge. Wiley.
- PubChem, N.C. for B.Information.N.L. of M., 2021. Open chemistry database.
- Rathore, H.S., Nollet, L.M., 2012. Pesticides: evaluation of environmental pollution. CRC press.
- Regulation 408/2015/EU, 2015. Regulation 2015/408/EU of 11 March 2015 of the European Parliament and of the Council on implementing Article 80(7) of Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and establishing a list of candidates for substitution.
- Reus, J., Leendertse, P., Bockstaller, C., Fomsgaard, I., Gutsche, V., Lewis, K., Nilsson, C., Pussemier,
 L., Trevisan, M., van der Werf, H., Alfarroba, F., Blümel, S., Isart, J., McGrath, D., Seppälä, T.,
- 833 2002. Comparison and evaluation of eight pesticide environmental risk indicators developed
- in Europe and recommendations for future use. Agriculture, Ecosystems & Environment 90,
 177–187. https://doi.org/10.1016/S0167-8809(01)00197-9
- SIAN, (Sistema informativo agricolo nazionale), 2021. Riepiloghi dichiarazioni di vendita dei prodotti
 fitosanitari (D.P.R. n. 290/2001 art. 42 Dati nazionali, anno 2012.
- 838Sjerps, R.M.A., Kooij, P.J.F., van Loon, A., Van Wezel, A.P., 2019. Occurrence of pesticides in Dutch839drinkingwatersources.Chemosphere235,510–518.840https://doi.org/10.1016/j.chemosphere.2019.06.207
- Storck, V., Karpouzas, D.G., Martin-Laurent, F., 2017. Towards a better pesticide policy for the
 European Union. Science of the Total Environment 575, 1027–1033.
- Thiour-Mauprivez, C., Martin-Laurent, F., Calvayrac, C., Barthelmebs, L., 2019. Effects of herbicide
 on non-target microorganisms: Towards a new class of biomarkers? Science of The Total
 Environment 684, 314–325. https://doi.org/10.1016/j.scitotenv.2019.05.230
- 846 Thurman, E.M., Goolsby, D.A., Meyer, M.T., Mills, M.S., Pomes, M.L., Kolpin, D.W., 1992. A

- reconnaissance study of herbicides and their metabolites in surface water of the midwestern
 United States using immunoassay and gas chromatography/mass spectrometry.
 Environmental science & technology 26, 2440–2447.
- Travisi, C.M., Nijkamp, P., Vighi, M., Giacomelli, P., 2006. Managing pesticide risks for non-target
 ecosystems with pesticide risk indicators: a multi-criteria approach. International Journal of
 Environmental Technology and Management 6, 141–162.
- Tsaboula, A., Papadakis, E.-N., Vryzas, Z., Kotopoulou, A., Kintzikoglou, K., Papadopoulou Mourkidou, E., 2016. Environmental and human risk hierarchy of pesticides: A prioritization
 method, based on monitoring, hazard assessment and environmental fate. Environment
 International 91, 78–93. https://doi.org/10.1016/j.envint.2016.02.008
- Vidotto, F., Dalla Valle, N., Fogliatto, S., Milan, M., De Palo, F., Tabacchi, M., Ferrero, A., 2021. Rapid
 increase of herbicide resistance in Echinochloa spp. consequent to repeated applications of
 the same herbicides over time. Archives of Agronomy and Soil Science 67, 620–632.
- Wilson, C., Tisdell, C., 2001. Why farmers continue to use pesticides despite environmental, health
 and sustainability costs. Ecological economics 39, 449–462.
- 862