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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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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*
6 *use* of pesticides by farmers and advisers. The present approach was initially validated for herbicides
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
15 available in literature and without making complex calculations. PRISW-1 Index discriminate active
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
18 easy movement of the chemical via runoff waters. The information deriving from Priority index may
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
21 defining agricultural measures to reduce the externalities of pest control.

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
28 mechanisms of action. Despite the benefit shown on yields and food quality (Cooper and Dobson,
29 2007; Knutson, 1999; Rathore and Nollet, 2012), pesticides can also affects agricultural sustainability
30 (Wilson and Tisdell, 2001). Moreover, once pesticides have entered the environment, significant
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
34 surface and/or ground water pollution, soil contamination, or as risks for human health and non-
35 target organisms. Pesticides found in surface and ground waters have the potential to reduce water
36 quality and even limit the availability of water (Batista et al., 2002; De Gerónimo et al., 2014; de
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
40 have been issued to protect water from entire categories of chemical substances, including PPPs
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).

43 The future of conventional agriculture requires policymakers and farmers/operators to move to a
44 sustainable approach to pesticide management (Storck et al., 2017). This perspective has already
45 changed the focus of pesticide research to alternative pest control methods, organic farming,
46 integrated pest management, and tools for selecting the most suitable chemicals for crop
47 protection (2009/128/CE, 2009). European legislation has also been created to safeguard

48 environmental, human, and non-targeted organism health from harmful pesticide use. Directive
49 128/2009 (2009/128/CE, 2009) forces European countries to adopt measures guaranteeing
50 effective and safe use of pesticides and made necessary the development of specific strategies to
51 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
53 more sustainable and farsighted approach. At the European level, actions have been taken to train
54 various stakeholders on the best management practices (BMPs) to mitigate negative impacts on
55 cropping. By example, two Life projects (TOPPS and TOPPS Prowadis) have identified the BMPs for
56 reducing PPP losses from agricultural areas, however, these strategies fall short of the specification
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
61 prevention tools to optimize PPP use (Finizio and Villa, 2002). In the last years a common effort was
62 addressed to develop new approaches to define the impact on environment and human health of
63 pesticides. The main objective followed by researchers is providing user-friendly tools (indicators,
64 indices) to qualify the risk associated to chemicals used in crop protection. The information derived
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
67 programs in cropland close to protected zones. A prioritization method approach was developed by
68 Tسابoula et al., (2016), to get information regarding the environmental impact of pesticides useful
69 in the selection of pesticides to be monitored in waters. Japanese researchers developed a risk
70 prediction method for the assessment of pesticides in waters (Narita et al., 2014). Sampling and
71 monitoring campaigns may certainly be useful in the assessment of pesticide impact on the

72 environment, but they require time and there are not costly-free (Narita et al., 2014). The
73 optimization of pesticide use in crop protection may derive from the use of environmental indices.
74 Different indices have been developed to evaluate and optimize the use of pesticides in current
75 agricultural practice (Reus et al., 2002).

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

82 Once the best-describing indices were defined, they were used in a case study for comparison across
83 the most common chemical strategies for maize weeds control in Northern Italy. The approach
84 allowed to discriminate among the most common chemical strategies adopted for maize weed
85 control based on their full environmental and ecotoxicological profiles, rather than their efficacy
86 against weeds alone. Moreover, by attributing an environmental weight to the crop protection
87 strategies adopted at the farm level, local authorities can select a suitable PPP that takes into
88 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**

92 To define the environmental impact of an herbicide or herbicide mixture, a series of indices were
93 considered. These indices were obtained by scoring different indicators referring to the chemical-
94 physical, ecotoxicological, and toxicological properties of each active substance. Indices permit
95 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).

100 Risk index differentiation was performed using two approaches: the scoring approach and the ratio
101 approach (Padovani and Capri, 2006). The first one was used for the determination of Pesticide
102 Index (PESTi), Ecological Index (ECOi), Agronomic Index (AGROi), and Priority Index (PI). The ratio
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
105 endpoints for selected target species. In general, the ecotoxicological and physical-chemical
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
109 mixture the main chemical and physical properties and the ecotoxicological and toxicological
110 properties of each single active substance, were considered and used as indicators (Table 1). For
111 each indicator, specific thresholds have been considered as previously suggested by the Footprint
112 database (Lewis et al., 2016). A specific score has been attributed according to the referring
113 threshold. By attributing to each active substance, a specific score according to its characteristics, it
114 was possible to calculate a general score, which represents the value of the index. In case of mixture
115 of herbicides, the index was calculated by adding the individual score of each active substance of
116 the mixture. For indicators such as "priority substance" and "candidate for substitution", where
117 specific numeric thresholds were not available, the score was attributed considering the inclusion
118 of the substance to a category. For example, if herbicide X is candidate for substitution, the

119 classification was Yes and the score was 5 (Yes = 5), otherwise the classification was No and the
120 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)
123 peer review reports, The Pesticide Manual (BCPC, 2012), AGRITOX (Maniere et al., 2011) and
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)**

130 The pesticide index was obtained by attributing a specific score to a selected number of pesticide
131 indicators. These pesticide indicators refer to the physical and chemical properties of the active
132 substance. The pesticide indicators used were: water solubility, soil mobility, soil persistence, water
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
136 et al., 2016) and from The Pesticide Manual (BCPC, 2012) (see Appendix A). The index was calculated
137 for each active substance and for the different herbicide mixtures. For each active substance the
138 index derives from the sum of the individual scores (min. value= 0, max. =40; Table 1) attributed to
139 each indicator weighted for the maximum score obtainable, as follow (Eq. 1):

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

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):

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

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.

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

151 **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

152

153 **2.1.2 Ecotoxicological index (ECOi)**

154 The ecotoxicological index (ECOi) was calculated considering the ecotoxicological endpoints of each
 155 active substance [Lethal concentration (LC₅₀), lethal dose (LD₅₀), environment concentration (EC₅₀)],
 156 for mammals, birds, fish, aquatic invertebrates, earthworms, and honeybees (Table 2) retrieved
 157 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
161 et al., 2016) and The pesticide Manual (LC₅₀ fish, LD₅₀ birds, EC₅₀ algae, LD₅₀ bees) (see Appendix A).
162 The mammalian toxicity was evaluated considering the oral acute LD₅₀ values for rats expressed in
163 mg/kg. For birds it was considered the oral acute LD₅₀ value (mg/kg) for mallard ducks, as for this
164 species the data were available for all the active substances. For fish and earthworms, the endpoint
165 was represented by EC₅₀ values. The EC₅₀ 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
167 *Pseudokirchneriella subcapitata* was generally used; exceptions were the EC₅₀ (72h) values for
168 *Raphidocelis subcapitata* for the herbicides tembotrione, isoxaflutole, prosulfuron, rimsulfuron,
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
171 clomazone. Effective concentrations for invertebrates refer to EC₅₀ (48h) data of *Daphnia magna*.
172 The toxicity against honeybees was evaluated considering the oral acute LD₅₀ values. The index was
173 calculated for each active substance and for the different herbicide mixtures.

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

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

178

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

181
$$ECOi_{mix} = \frac{\text{Total score of the mixture}}{40 * N} \quad [4]$$

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

184 With the exception of BCF factor, the indicators used for the calculation of ECOi index were selected
 185 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
 187 in the ARPAT document.

188 **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 - Bioconcentration factor (l/kg)	General risk	<100	5000-100	>5000
MAXIMUM SCORE				40

189 Notes: LD₅₀: dose required to kill half percent of the tested population, LC₅₀: concentration required to kill
 190 half percent of the tested population; EC₅₀: concentration required to have a certain effect on the tested
 191 population.

192 **2.1.3 Agronomic index (AGROi)**

193 The agronomic index was calculated taking into account indicators relative to three fields of
 194 reference: resistance proneness (number of known resistant weed populations, the mode of action
 195 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

198 from different sources (Directive 2013/39/EU, 2013; Heap, 2019; Lewis et al., 2016; Mackay and
199 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
201 reasons of this troublesome phenomenon are different: the repeated use of herbicide with the same
202 mechanism of action, the use of herbicides at low dosages, the spread of genetically modified crops
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
205 point of view, the appearance of resistant weeds may induce farmers to make on way changes in
206 the chemical control strategies currently adopted such as increasing the application rate looking for
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
211 of action has been very scarce, it is important adopting a sustainable and rational use of the existing
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
214 companies involved in new herbicide discovery, the advent of GM crops in many parts of the world,
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
217 substance has been evaluated in terms of risk of development of resistant weed populations. For
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
220 of resistance proneness was the mode of action of the active substance according to the *Herbicide*
221 *resistance Action Committee website* (HRAC) classification of herbicides: in this case, a higher score

222 was attributed to herbicides belonging to HRAC groups for which a higher number of cases of
223 resistance have been reported worldwide (groups B, C1, A, G, O). All the information regarding weed
224 resistance were taken from the HRAC.

225 The environmental impact of a certain pesticide has been evaluated considering the application
226 rate, the presence of relevant metabolites, its fugacity according the MacKay model Level 1 and the
227 application timing. The application rate gives information regarding the amount of the active
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).

233 Regulation restrictions were taken into account by considering the indicators “candidate for
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
236 408/2015/EU, 2015) and the list of priority substances according to the Directive 2013/39/EU
237 (Directive 2013/39/EU, 2013). As the application timing is concerned, this indicator has been
238 included for the calculation of the AGROi index to consider the different impact that the period of
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.
241 Moreover, pre-emergence herbicides are intrinsically more persistent, as they should be active (i.e.
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
244 applied in early-post-emergence were considered to have a behavior not dissimilar to those applied
245 in pre-emergence. At this application stage, most of the soil is still bare and crops and weeds (if

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

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

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

263

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

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

267 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.

269 **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

270

271 **2.1.5 Short term pesticide risk Index for surface water system (PRISW-1 index) (Finizio et al., 2001)**

272 The short term pesticide risk for surface waters system (PRISW-1 index) was calculated for selected
 273 non-target organisms according to the classification intervals and the scores indicated in Table 4.

274 The TER value considered for the attribution of the score was obtained dividing EC₅₀ or LC₅₀ by the
 275 corresponding PEC for surface water. The PEC value for surface water (PEC_{sw}) was taken from the
 276 official peer-review report of each active substance released by the European Food and Safety
 277 Authority (EFSA).

278 **Table 4. Risk classification ranges and corresponding scores for selected non-target organisms**
 279 **(NTO) (fish, daphnia, algae) in surface waters used for PRISW-1 index (modified from Finizio et al.,**
 280 **2001).**

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

10-2	6
<2	8

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

281

282

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

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

286

[7]

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

$$\text{TER}_{\text{mix}} = \frac{1}{\sum_{\text{Toxic unit}} \frac{1}{\text{TER}_{\text{single substance}}}} \quad [8]$$

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

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

$$\text{E.g. } \text{TER}_{\text{mix for fish}} = \frac{1}{\sum \left(\frac{1}{\text{TER}_{\text{S-metolachlor}}} + \frac{1}{\text{TER}_{\text{dicamba}}} + \frac{1}{\text{TER}_{\text{nicosulfuron}}} \right)} \quad [9]$$

298 The values of TER_{mix} for fish, algae and daphnia were used for calculating the PRISW-1 index of
 299 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).

$$302 \quad \text{PRISW-1}_{mix} = \text{score of fish} \times 5.5 + \text{score of daphnia} \times 4 + \text{score of algae} \times 3$$
$$303 \quad [10]$$

304 The risk level was classified according to classes of risk as proposed by papers of (Finizio et al., 2001;
305 Traversi 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 Traversi et al., 2004, modified).**

PRISW-1 value	Risk classification
≤ 5	Negligible
$> 5 - \leq 15$	Low
$> 15 - \leq 40$	Medium
$> 40 - \leq 80$	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)

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

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

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

321 **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})	
1 st -10 th percentile	5	≥ 99	5	Soil	1	$DT_{50} \leq 10$	0.5
11 th -20 th percentile	4	> 80-99	4			$> 10 DT_{50} \leq 30$	0.8
21 st -30 th percentile	3	> 60-80	3	Crop	0.9	$> 30 DT_{50} < 90$	1
31 st -50 th percentile	2	> 30-60	2			$DT_{50} \geq 90$	1.2
51 st -100 th percentile	1	0-30	1	Soil and crop	0.8	DT_{50} not found	1

322 Notes: DT_{50} in days

323

324 The highest value of the index, corresponding to a substance reaching the highest score for each
 325 indicator, is 11, the least 1.4. Sales data refer to the last available dataset on the national yearly
 326 sales of pesticide published by the Italian agricultural information system (SIAN, 2021). Although
 327 these data refer to 2012, considering that all the active substances used in the field experiment are
 328 on the market by many years, great changes in their reciprocal ranking are unlikely. Pesticides are
 329 listed in a decreasing order according to the sales data. The score relative to the environmental
 330 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
 333 individuation of the best chemical strategies for maize weed control. The case study was carried out
 334 at different conventional farms located in Lombardy region, northern Italy during the period 2014-
 335 2019. In particular from 2014 to 2017 field trials were hosted by *Azienda Cerri* at Turano Lodigiano
 336 (45°14'23.9"N 9°36'57.4"E), Lodi province, while in the last two years trials were carried out at
 337 *Azienda La Madonnina* at Liscate (45°28'37.3"N 9°24'07.7"E), Milano province. During the
 338 investigation, the most common chemical weed control strategies adopted in maize were
 339 compared. Herbicides were applied according to different control strategies: pre-emergence (PRE
 340 strategy), early post-emergence (E-POST) and late post-emergence (L-POST) (POST strategy), pre-
 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,
345 a randomized block design with three blocks and three replications per block was adopted. In each
346 block three untreated plots (control) were individuated. In 2016 and 2017 the number of mixtures
347 compared was reduced to include only the most significant mixtures applied in the fields. Each plot
348 was 50 m² large. During the last seasons, 2018-2019, new mixtures chosen by farmers were included
349 in the investigation. All plots were about 1.5 m spaced apart from all sides in order to avoid spray
350 drift or off-site movement of herbicides applied by runoff after spraying. The effects on weed
351 infestation were assessed on key weeds (*Abutilon theophrasti* Medicus, *Amaranthus retroflexus* L.,
352 *Chenopodium album* L., *Echinochloa crus-galli* (L.) Pal. Beauv., *Panicum dichotomiflorum* (L.) Michx.,
353 *Poa annua*, *Portulaca oleracea* L., *Setaria viridis* (L.) Pal. Beauv. and *Solanum nigrum* L.), by
354 measuring plant density (plants/m²), ground cover (%) and overall efficacy (%) based on plant
355 density data compared to control . Data were statistically analyzed for each year by conducting
356 ANOVA analysis followed by a REGWF test ($\alpha=0.05$) 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
362 by estimating the percentage of the area included in the metal frame covered by the weeds.
363 Herbicide application was carried out using a backpack sprayer (Honda Power Sprayer, 25 L. tank,
364 GX 25T air-cooled-single cylinder, 4Stroke OHV, 0.72kw / 7000rpm) which mounted 5 nozzles (Tee
365 Jet 11002) bar able to distribute a volume of 300 l/ha. Pre-emergence applications were performed
366 the day after sowing, while the early post-emergence and the late-post-emergence treatments were
367 carried out with maize ad BBCH stage 12 (2th leaf) and 15 (5th leaf), respectively.

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

ACTIVE SUBSTANCE	ACRONYM	SUBSTANCE GROUP	HRAC – LEGACY HRAC
Terbutylazine	<i>TBA</i>	<i>TRIAZINES</i>	1, 2 (C1)
S-Metolachlor	<i>SMET</i>	<i>CHLOROACETAMIDES</i>	15 (K3)
Petoxamide	<i>PETO</i>		

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

371

372 **RESULTS**

373 **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
375 overall health/hazard pesticide index. We considered three strategies (PRE, PRE+POST, and POST);
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.
378 Rainfall events that occurred shortly after application boosted the effectiveness of the strategy
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
381 instances of incomplete pre-emergence application. The results for this strategy, across all seasons,
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).
384 Finally, the third strategy (POST strategy) is an alternative to pre-emergence applications in cases of
385 weed infestation not properly controlled by pre-emergence herbicides. The POST strategy consists

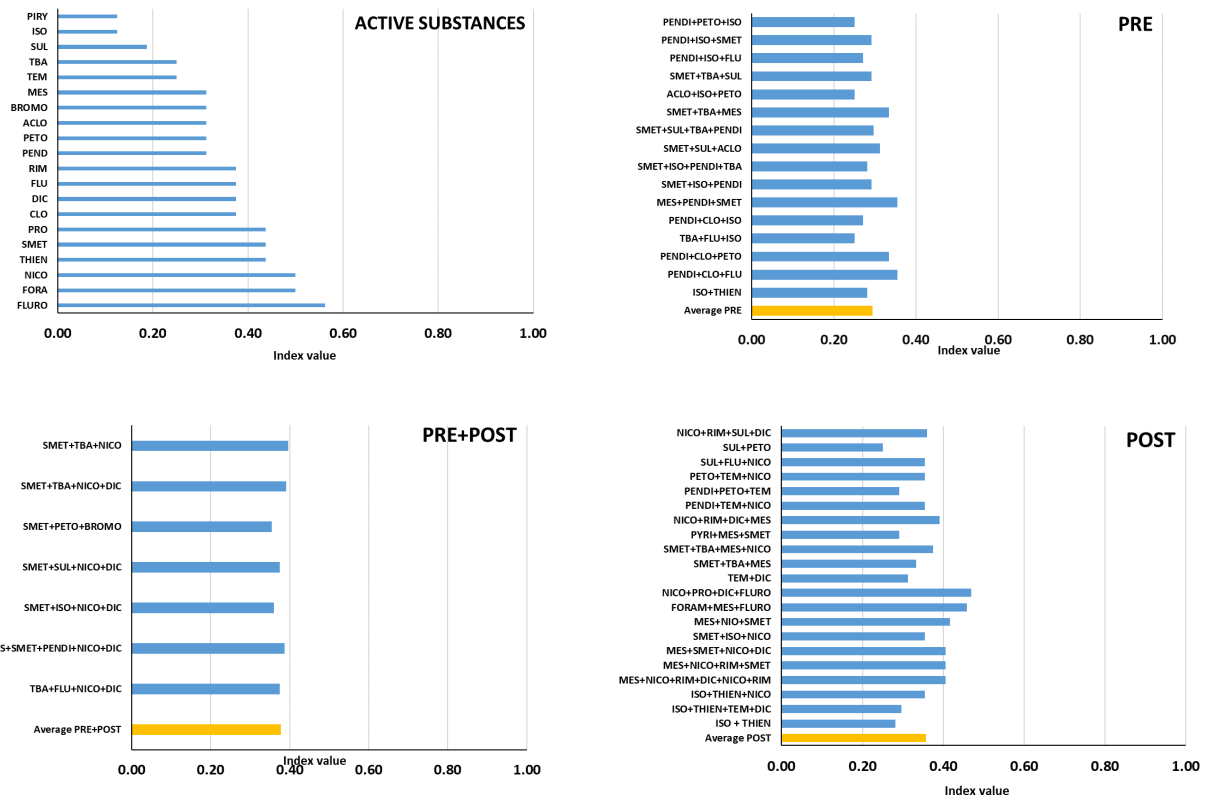
386 usually is two herbicide applications, one applied in early post-emergence and a second of different
387 chemicals applied in late post-emergence. Generally, this strategy provides adequate weed control
388 throughout the seasons, however very competitive (e.g. *Sorghum halepense*) or newly emerging
389 weeds (e.g. *Cyperus esculentus*) can still breakthrough some chemical mixtures. Reduced efficacy of
390 some early post-emergence mixtures applied is due to the limited action of some active ingredients
391 under unfavorable soil conditions. For instance, during 2014, the mixture of S-metolachlor,
392 terbuthylazine, and mesotrione showed low efficacy in some grasses (*Panicum dichotomiflorum* and
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
399 field survey. The highest PESTi values were found in FLURO and sulfonylureas, while the lowest
400 resulted in PYRI, ISO, and SUL the lowest. The herbicide mixtures were categorized based on their
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
408 mixtures had a PESTi value below 0.40, with an average value of the strategy of 0.36. The highest
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
 411 PESTi values observed for post-emergence mixtures compared to the pre-emergence ones can be
 412 related to the presence in the mixtures of active substances of the sulfonylurea family, which are
 413 characterized by high water solubility and low Koc. The mixtures applied in PRE+POST strategies
 414 showed the highest PESTi values. This was not unexpected considering the highest chemical
 415 complexity of this kind of strategy. The average value was 0.38, a bit over the average PESTi value
 416 observed in POST strategy. However, all herbicides mixtures had a PESTi index ranging from 0.35 to
 417 0.40 (Figure 1).



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
 423 the active substances under investigations, TEM, BROMO and PYRI got the lowest AGROi value. Pre-
 424 emergence chemicals were negatively affected by the score gained by certain indicators, such as
 425 the period of application, by the number of relevant metabolites and by the soil persistence. TBA
 426 and NICO reached the highest value; the second one, in particular, was affected by a high proneness
 427 to develop resistance. The average AGROi value determined in pre-emergence strategy was 0.45.
 428 The lowest AGROi value was calculated for the mixture CLO+PENDI+ISO (0.38). All the other
 429 mixtures applied in pre-emergence showed an AGROi value equal or above 0.41. The AGROi index
 430 for post-emergence mixtures was, on average, lower compared to pre-emergence treatments
 431 (0.38). PRE+POST strategy had an AGROi average value of 0.47. This was mainly due the presence in
 432 the strategy of active substances which gained an individual high score such as SMET, TBA or a
 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).

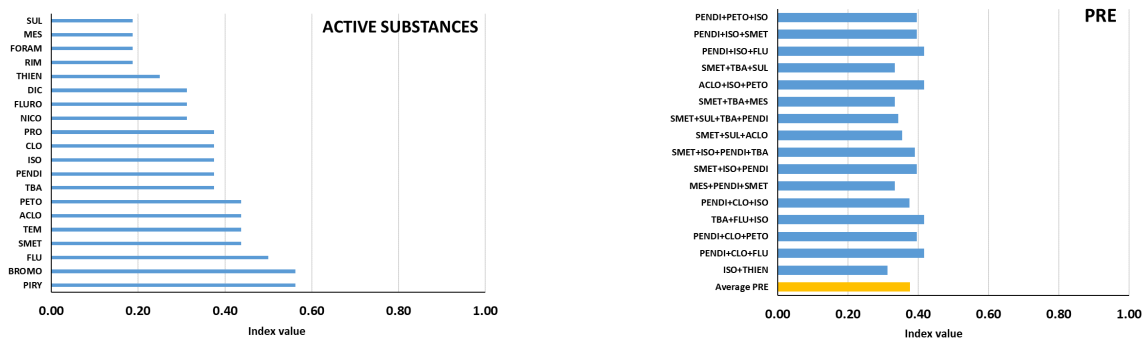


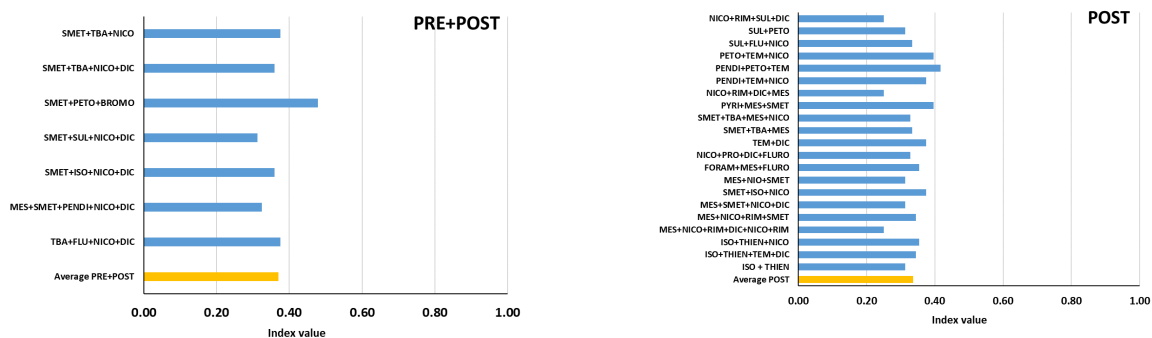
436 **Figure 2: AGROi index calculated for each single active substance and for all the mixtures**
437 **compared in the field case study divided according the strategy.**

438

439 3.2.3 ECOi index

440 The index was calculated for each active substance (see Appendix G), then for each mixture. PIRY,
441 BROM and FLU showed the highest ECOi index among all the active substances compared. RIM,
442 FORA and MES had the best profile. The ECOi index calculated for pre-emergence mixtures showed
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
445 compared to the post-emergence mixtures. This is because the former generally contain at least
446 one active substance of old introduction on the market such as PENDI, TBA, FLU, SMET. The ECOi
447 for post-emergence mixtures varied from 0.25 to 0.42. The highest ECOi value (0.42) was obtained
448 in the mixture PENDI+PETO+TEM. PRE+POST strategy account for an average ECOi index similar to
449 that observed for pre-emergence mixtures (0.37). The bad performance of this strategy can be
450 attributed to the worst ecological profile of the pre-emergence herbicides (Figure 3).





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

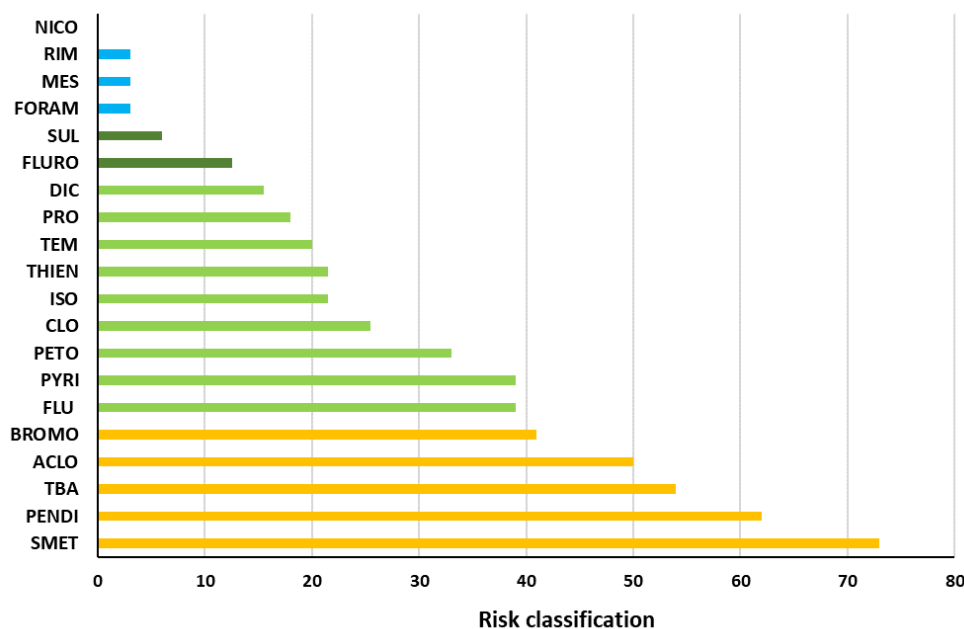
453

454 3.2.5 PRISW-1 index

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

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

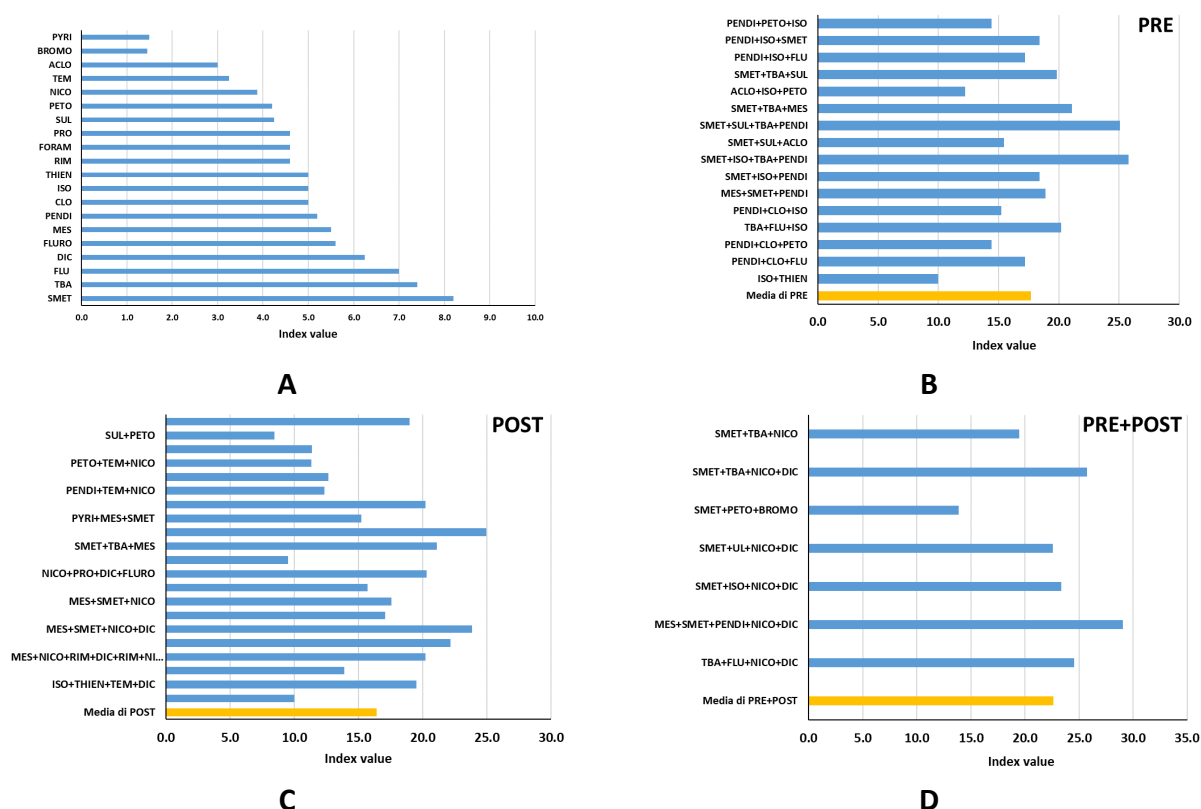
471 rather than the number of actives substances. Only three mixtures applied in post-emergence had
472 a risk close to the low classification risk category (PRISW-1 = 15,5); they all contain sulfonylureas,
473 triketones and synthetic auxin.



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 ≤ 15;**
477 **light green= >15 PRISW-1 ≤40; light orange= >40 PRISW-1 ≤80; red= PRISW-1 >80.**
478 **3.2.7 Priority index**

479 The priority index calculated for surface waters confirmed a tendency already highlighted by
480 previous indices. In Figure 5 A are reported the values of the index for each active substance. SMET
481 had the highest value of the index. Most of the chemicals that reach a PI value above 5 are
482 substances with a long history on the market (CLO, FLURO, DIC, PENDI, TBA). Some active
483 substances, such as MES, THIEN and ISO, that showed a good profile in other indices have a PI value
484 above 5, just below the chemicals placed in the top of the list. Their position in the upper part of the
485 list is mainly due to their environmental distribution characteristics (environmental distribution
486 score) and to the fact that are among the most used chemicals (sales score). As concern the
487 comparison between the herbicide mixtures (Figure 5B, 5C and 5D), among pre-emergence
488 herbicides, the highest values were recorded for the most complex mixtures containing certain

489 active substances such as PENDI, SMET, DICA, and TBA. The least problematic mixtures were
 490 constituted by ISO+TIEN and ISO+ACLO+PETO. The 55% of post-emergence mixtures had a priority
 491 index higher than 15; only two mixtures (TEMB+DIC and SUL+PETO) have a priority index below 10.
 492 PRE+POST mixtures reached higher values compared to the other two strategies. The most complex
 493 mixture was the worst in term of PI; it was applied in pre+post and it was composed by
 494 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
504 term risk for surface waters and a better ecotoxicological profile compared to other chemicals. Being
505 a chemical of old introduction on the market is often associated with an
506 ecotoxicological/environmental bad profile; however, some chemicals are out of this frame, as in
507 the case of PYRI, which it was introduced on the market in 1980 (Lewis et al, 2016), but it showed
508 an overall good classification. Despite the potential risk of water contamination, its rapid
509 degradation in the environment greatly reduces the general risk. The same risk classification
510 methodology was applied to the different herbicide mixtures used in pre, pre+post and post-
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**

518 Indices, created to classify the risk profile of a chemical, are commonly used in crop protection
519 decision making (Finizio and Villa, 2002; Traversi et al., 2006). Pesticides introduced into the
520 environment undergo processes that may lead to the contamination of different environmental
521 compartments (Damalas, 2018). The ecotoxicological and environmental profile of a pesticide,
522 which derives from its physical and chemical characteristics, can greatly influence the impact on
523 humans and other non-targeted organisms. The challenge lies in protecting crops while reducing
524 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

527 help technicians and policy makers in preparing the best suitable crop protection plans in terms of
528 efficacy and environmental protection. The main advantage in using these indices in evaluating
529 pesticide environmental risk is that they can be easily calculated starting from information regarding
530 pesticide properties, use and fate that are already available. These indices may offer preliminary
531 insights about the *a priori* risk of pesticides use.

532 Using free-access databases, several indicators that capably described the profile of an individual
533 chemical were selected to build or calculate risk indices. Based on the grouping of like
534 environmental affinities, such an index may better describe the behavior of a chemical. For each
535 indicator, ranking ranges were individuated. Chemicals received an individual score for each
536 indicator of the index. The individual scores were then added each other or combined in an
537 algorithm to calculate the indices. The usefulness of these indices in profiling the ecological and
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
541 mixtures used by farmers in Europe in maize cultivation. It is important to note that 80% of the
542 active substances considered were introduced on the market before 2000. The advantage of this
543 two-step approach is pointing out the most critical aspects of each active substance, but in the
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
546 Priority Index were already used by the scientific community. One of the problems in the application
547 of the indices in risk assessment could be the variability of the input data (physical-chemical
548 properties of pesticide, environmental fate) or the absence of some needed data, according to the
549 sources used (Finizio et al., 2001). In the present paper we refer to solid free-access databases where
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)
552 were not applied for a risk assessment of monitoring data collected in a certain area (evaluation *ex-*
553 *post*), but for an evaluation *ex-ante* of pesticides or pesticides mixtures. This is a difference
554 compared to other approaches based on risk indices. The main advantage of this approach is that
555 the results of the application of these indices can be used to prevent or reduce the impact of
556 pesticides use. The development of a unique index enables to qualify the potential risk associated
557 to a pesticide or a pesticide mixture is a complicated effort (Levitan et al., 1995). This kind of index
558 should consider different aspects (risk towards waters, non-target organisms, humans) and several
559 compartments (ground waters, surface waters, sediment, air, soil, biota, etc). However, higher the
560 number of factors to be included and considered in the index calculation, more complicated its
561 determination. According to Reus et al., (2002), an ideal indicator focused on the risk associated to
562 pesticide application should include information regarding application rate, application factors and
563 environmental conditions. Reus et al., (2002) compared 8 different pesticide environmental
564 indicators developed in Europe indicating the main requirements they should have and giving
565 recommendations for their use and harmonization. To be adopted at large scale, even by farmers
566 or local advisers, indices must be comprehensible, easy to be calculated with simple algorithms and
567 based on ready and free to access data. PESTi, AGROi and ECOi indices generally respect this
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
570 pesticides and mixtures (PESTi), impact on non-target organisms in different compartments (ECOi,
571 PRISW-1, Priority Index), agronomic and regulation implications (AGROi).

572 The main objective of the survey was to compare the efficacy of different weed control strategies.
573 The use of the proposed indices may represent a valid tool for public stakeholders in risk assessment
574 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
576 framework of the potential risk associated to a specific chemical only on the basis of its chemical
577 nature. However, the physical and chemical features alone does not explain *in toto* the potential
578 risk associated to a chemical or a mixture. This could represent a limit for this index. For instance,
579 triazines are historically found in waters, but according to the PESTi index they have a better profile
580 than sulfonylureas. Sulfonylureas showed a PESTi index higher compared to other chemicals, mainly
581 due to their water solubility (high) and K_{oc} (low) values. PESTi index underlines the physical and
582 chemical characteristics as a whole, not considering the impact of other factors such as application
583 rate or period of application. Hence, this index may underestimate the risk associated to a certain
584 chemical. As an example, despite the PESTi index for terbuthylazine and S-metolachlor resulted
585 better than most sulfonylureas, these two chemicals are among the most frequently found
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
589 have high application rates (SIAN, 2021).

590 The innovation of AGROi index stands in the set of input indicators used to calculate it. The index
591 considers the direct effect on the environment of the use of chemicals in terms of evolution of weed
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
594 derives from a combination of intrinsic characteristics of the chemicals, agronomic impacts and
595 potential hazard to water compartment. This index has a precautionary approach and may
596 represent an important decision tool for local authorities in defining crop protection strategies
597 within the integrated pest management plans. Some sulfonylureas got the highest AGROi index
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,
600 their tendency to develop resistance in weed populations have jeopardized their efficacy (Brown
601 and Cotterman, 1994). Even S-metolachlor and terbuthylazine had high AGROi index, because of
602 their high application rates, the period of application and, in a lesser important magnitude, the
603 development of resistant populations. While chloroacetamide resistance in weeds is not frequent
604 despite the longtime widespread use of some chemicals of this family, many weeds have evolved
605 resistance to triazines worldwide (Heap, 1997). According to the AGROi index, in maize weed
606 control, POST strategy is less problematic than PRE or PRE+POST strategies.

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

613 The ECOi index is focused on the ecotoxicological impact against non-target organisms. In general,
614 pre-emergence herbicides showed the worst performances mainly because of the presence in the
615 mixtures of chemicals of old introduction on the market. Sulfonylureas can be considered the least
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,
618 these two chemicals had low LD₅₀/LC₅₀ values for almost all the categories of organisms. In crop
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
621 discriminate the ecotoxicological impact of chemicals using indicators commonly available in
622 literature and without making complex calculations.

623 The contamination of water resources by herbicides and metabolites forced public authorities to lay
624 down restriction of use or banning of certain pesticides. However, these measures may represent a
625 strong limit for farmers, particularly when the pesticides portfolio is limited due to the scarcity of
626 authorized products. The potential hazard of chemicals and mixtures of them against water
627 resources was determined using PRISW-1 Index which gave an overview in terms of risk for surface
628 waters and the priority index which help to individuate the substances to pay attention both for
629 surface and ground waters. The usefulness of PRISW-1 index has been already validated (Köck-
630 Schulmeyer et al., 2012). Differently from the common application of PRISW-1 index to pesticide
631 monitoring data (Köck-Schulmeyer et al., 2012; Kouzayha et al., 2013), we use this index for an *a*
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
636 spraying (Milan et al., 2013). This is particularly true for herbicides, which are the chemicals with
637 the highest risk of runoff due to their application period and type of use. According to the monitoring
638 campaigns carried out by the Italian regional environmental authorities, one third of the substances
639 found in surface and ground water belong to herbicides and herbicide metabolites (Paris et al.,
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
642 environmental profile (high application rates, widespread use, persistence). Post-emergence
643 mixtures with high risk contain active substances usually applied during pre-emergence but that can
644 be applied even in early post-emergence. PRISW-1 Index discriminate active substances according
645 to their risk against three representative non-target organisms. However, due to the intrinsic
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
648 high risk towards aquatic non-target organisms and their use in pre-emergence on bare soils, they
649 are unfrequently found in surface water because they are strongly bounded to soil matrix or rapidly
650 degraded. This means that PRISW-1 index alone does not clearly represent the real risk linked to a
651 pesticide. Priority index allow to identify pesticides with the highest risk to contaminate water
652 resources considering both environmental and statistical indicators. Some of the parameters used
653 in priority index were included in a risk predictor method, developed by Narita et al., (2021), to
654 individuate priority pesticides in drinking water based on quantity of sales, chemical properties and
655 intensity of toxicity. The application of priority index to a case study demonstrated once again the
656 worst performances of the PRE+POST strategy mostly due to the typology of active substances
657 applied in PRE-emergence. The information deriving from Priority index may certainly help public
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.
661 The indices presented in this paper may be used individually or together in the evaluation of the
662 environmental profile of a certain chemical or strategy. Each index could be addressed to a principal
663 auditor; for instance, ECOi and PRISW-1 Indices may assume a great importance in cropped areas
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
666 compounds or strategies. AGRi index put together different critical aspects related to a generic
667 pesticide outlining its environmental profile. For instance, this index could be used in the definition
668 of sustainable crop protection plans within the regional rural development programs prepared by
669 local authorities.

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,
673 must be considered in different terms depending on the category of pesticide used. Herbicides are
674 applied on bare soil or in presence of crops/weeds, insecticides/fungicides for orchards use are
675 applied on plants on vegetation or on vegetative rest. Once established the overall efficacy of the
676 strategies adopted, the next step was trying to profile the different mixtures in terms of their
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
680 environmental behavior (Finizio et al., 2001). Overall, old chemicals generally got the worst
681 performance in all the indices considered.

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

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

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

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

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

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

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

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

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727 supervision, F.V.; writing—original draft preparation, M.M.; writing—review and editing, M.M, F.V.,
728 and S.F.

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