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Improvement in mixture risk assessment: A multi-national application of the Pesticide Risk Metric

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ABSTRACT

Significant advancements have been made in the development of methods to assess mixture risk, with the hazard unit and concentration addition methods being commonly used for tier one screening risk assessments. However, a reduction in risk estimate uncertainty is required for higher tier assessments and targeted management action, particularly in the context of policy frameworks such as the European Green Deal, which aims to reduce the use and risk of pesticides that may pollute air, water and soil. This study proposes the Pesticide Risk Metric (PRM), an approach grounded in existing ecotoxicological methods for this purpose. By combining the species sensitivity distribution approach with the independent action model of joint toxicity, the PRM reduces uncertainty and provides more reliable risk estimates for individual toxicants and mixtures. The PRM method was applied to aqueous pesticide monitoring data from eight countries to demonstrate the utility of the method. Of the 706 sites, 61 (8.6 %) did not meet the annual average environmental quality standard (AA-EQS) for individual pesticide active ingredients, with Italy showing the highest number of exceedances. When assessed as a mixture, 359 sites (51 %) failed to meet the AA-EQS, with Italy and the Netherlands showing the highest annual average mixture toxicity. The primary contributors to mixture toxicity were insecticides (61 %), followed by other herbicides (32 %) and photosystem II inhibiting herbicides (6.1 %), with significant variation at the country and site levels. Fourteen pesticides contributed 99 % of the annual average mixture toxicity at the country level. The adaptability and reliable risk estimates of the PRM make it a valuable tool for second-tier risk assessment, further enhancing targeted policy and management decisions.

Introduction

Production and consumption of pesticides and other chemicals are rapidly increasing, particularly in emerging economies (United Nations Environment Programme 2019). Globally, there is concern about the increasing detections of pesticides in waterbodies, as research has shown these chemicals can constitute a major threat to aquatic ecosystem health and function (Beketov et al., 2013; Kellar et al., 2014; Liess et al., 2021; Munz et al., 2017; Stehle and Schulz, 2015). Organisms and ecosystems are more often exposed to mixtures of pesticides than to individual pesticides, with the composition of these mixtures generally influenced by upstream land use practices (Negri et al., 2022; Warne et al., 2020). Research indicates that the combined toxic effects of multiple contaminants tend to exhibit additive behavior (Backhaus et al., 2010; Cedergreen, 2014; Hernández et al., 2017). Policy frameworks such as the European Green Deal (European Commission 2021) and the Water Framework Directive (WFD) (European Commission 2024) recognise this issue and call for reductions in the use and risk of pesticides as well as other toxicants that may impact aquatic ecosystems, both as individual contaminants and as mixtures. The Green Deal outlines a zero pollution ambition by 2050, which aims to reduce water, soil and air pollution to levels no longer considered harmful to the health of natural ecosystems (European Commission 2021). Some European countries share the management of transboundary river basins

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including, for example, the Rhine, Meuse, and Danube (European Commision 2024). Shared water bodies present additional governance challenges and require a coordinated management approach that spans political and regulatory boundaries (Varady et al., 2023). Unfortunately, risk assessment methods often differ markedly between countries due to varying regulatory frameworks, data availability, and technological capability (El Afandi and Irfan, 2024; Evans et al., 2015). In addition, policy controls and on-ground practices typically focus on individual chemicals rather than mixtures, for ease of monitoring, evaluation and management (Cedergreen, 2014). Achieving the 2050 target therefore requires a metric that aligns with the language and goals of the target (ecosystem protection), allows for accurate risk assessment of both mixtures and individual contaminants, and supports the harmonised communication of risk and the coordinated efforts across multiple jurisdictions (Huang and Li, 2025; Tang et al., 2025).

There are several methods available to assess the environmental risk of pesticides, both as individual pesticide active ingredients (PAIs) and as mixtures (e.g. (Navarro et al., 2024; Posthuma et al., 2019; Tang et al., 2021)). The most common is a two-step approach that combines the hazard unit (HU) and multiple-substance potentially affected fraction concentration addition (CA) methods (Bliss, 1939; Posthuma et al., 2001; Traas et al., 2002). The ease of calculation and interpretation makes this approach ideal for tier one screening risk assessments which usually involve preliminary evaluations of large data sets or modelled spatial outputs to identify broad patterns and potential risks (Cedergreen et al., 2008). An array of studies have used this approach to highlight the prevalence and complexity of cumulative mixture risk around the world, providing valuable information for high-level policy and management (e.g. (Carafa et al., 2011; Martínez-Megías et al., 2024; Parkerton et al., 2018; Pistocchi et al., 2023; Posthuma et al., 2019; Rodea-Palomares et al., 2023)). However, the HU approach has been shown to introduce systematic statistical bias and false patterns if the compounds that are being compared (e.g., pesticides) have large differences in their underlying toxicity patterns (Kelley et al., 2017). Similarly, application of the CA model may lead to over- or under-estimation of risk for individual compounds, as it assumes that all chemicals act through the same or similar mode of action and can therefore be suitably described by one 'global average' species sensitivity distribution (SSD) (Rodea-Palomares et al., 2023). The magnitude of estimation error depends on the standard deviation of the underlying toxicity data and slope of the SSD of each contaminant (Gregorio et al., 2013). When the two methods are combined, bias in HU calculations and estimation error in the CA method can compound, leading to cumulative error in risk estimates (Gregorio et al., 2013, see also SI 1). These errors may affect estimates of the total toxicity of mixtures as well as the ranking (importance) of each contaminant within the mixture. When chemical-specific management of risk or allocation of funding is needed, it is better to reduce these areas of uncertainty.

The WFD operates via the protective benchmark no-effect concept, using Predicted No-Effect Concentrations (PNEC) and environmental quality standards (EQS), to ensure that ecosystems remain sufficiently protected from expected or observed pesticide exposures (European Commission 2024; Lepper, 2005; Posthuma et al., 2019). This approach seeks to safeguard 95 % of species from the harmful effects of pesticide exposure (Lepper, 2005). It is therefore essential to confidently predict the risk at this protection level for both individual contaminants and mixtures. The cost of getting this wrong could be the unnecessary loss of access to vital farming chemicals, misdirection of funding or other resources, or a failure to effectively reduce risk despite management efforts (Meys et al., 2024). Advancement to a second tier of risk assessment necessitates a reduction in uncertainty to inform chemical-by-chemical regulatory actions and targeted on-ground support.

This study proposes an assessment approach called the Pesticide Risk Metric (PRM) that is grounded in existing ecotoxicological methods. The PRM repurposes the SSD method of (Posthuma et al., 2001), typically used for the derivation of water quality standards, for use in probabilistic risk assessment. Species sensitivity distributions are a form of cumulative distribution function, with the shape of each distribution being described by a unique mathematical formula (Iwasaki and Yanagihara, 2025). Traditionally, these formulas are used to estimate concentrations that are protective of a certain portion of the ecosystem (e.g., (Warne et al., 2018)). The PRM inverts these formulas to convert each pesticide concentration datum in a water sample to an estimate of ecosystem impact. The results are expressed as the percentage of species potentially affected in the ecosystem (PAF). In this way, a risk estimate is derived for each pesticide concentration datum without the need to transform the x-axis (concentration in μg/L) to a common range (HU). The PRM provides a predictive ability lacking in the HU method, as differences in risk estimates relate directly to the toxic impact of chemical exposure rather than a magnitude change in concentration (Warne et al., 2023, SI 1). The resulting risk estimates are then combined using the independent action model of joint toxicity which keeps the SSDs separate (Bliss, 1939; Traas et al., 2002). The PRM method therefore provides reliable and transparent risk estimates surrounding the PC95 for individual toxicants as well as the mixture, making it ideal for second-tier risk assessment and the provision of data to inform policy and management decisions. The PRM was applied to pesticide concentration data from eight different countries to demonstrate its compatibility with current risk assessment frameworks such as the WFD. Although the PRM is currently restricted to pesticides, the method can be applied to any mixture of toxicants provided that an SSD is available. To facilitate broader use, the study also provides access to automated code that can be adapted to this purpose by other researchers (Neelamraju, 2025).

Methods

A workflow diagram is presented in Fig. 1 to provide a clear overview of the risk assessment process used for this study. For clarity, a list of acronyms and their definitions can be found in supplementary section SI 2.

Study area

This study examines pesticide mixture risk across eight countries: Belgium, Canada, Croatia, France, Germany, Italy, the Netherlands, and Sweden. Site selection was based on data availability, ensuring robust spatial comparisons of risk estimates.

Selection of pesticide active ingredients

For brevity, all compounds in the PRM are referred to as pesticide active ingredients (PAIs) even though some are metabolites. Pesticide active ingredients were selected for inclusion in the metric based on the following criteria:

1. They were included in the previous version of the PRM (see (Warne et al., 2023; Water Quality and Investigations 2023)), allowing for consistency with historical reporting;
2. They were identified as pesticides of concern either through a hazard assessment of available monitoring data or literature review;
3. They were identified by the authors as pesticides not currently associated with high risk, but expected to pose ecological risk in the future due to anticipated increases in usage, detection frequency, or emerging challenges in management and regulation; or
4. They were identified by the authors as potential replacement chemicals; i.e. lower toxicity PAIs that may result in a potential reduction in risk over time. These PAIs were included to allow for the quantification of improvement in risk before and after practice change.

For point 2 above, PAIs were identified through hazard analysis of

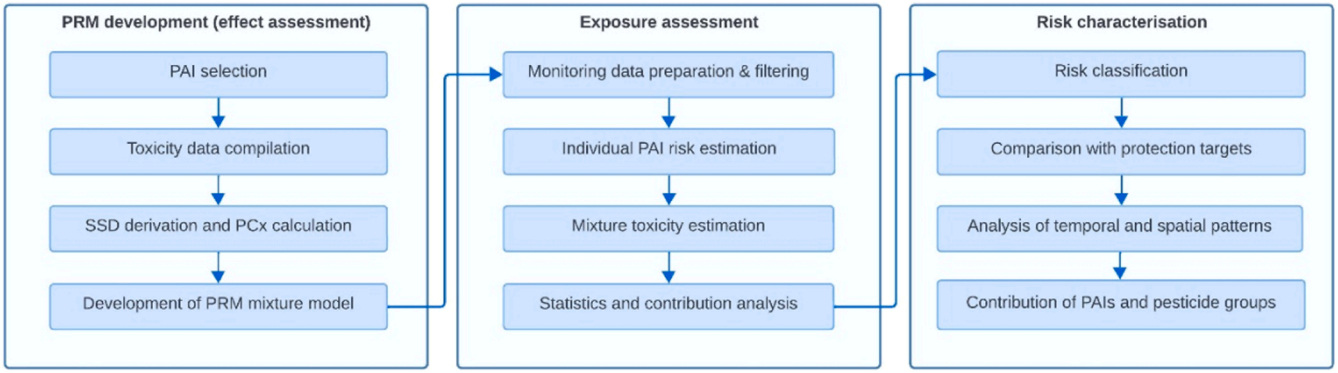

Fig. 1. Workflow diagram illustrating the sequential steps of the pesticide mixture risk assessment conducted in this study. The framework integrates three key components: quantification of toxicological effect using the Pesticide Risk Metric, exposure assessment using empirical monitoring data, and risk characterisation for assessment against regulatory thresholds.

long-term pesticide monitoring data from the Australian Tahbil Water Quality Data Portal (Water Quality & Investigations, 2024), the Canadian National Water Monitoring Program for Pesticides (Government of Canada, 2025), and the Swedish University of Agricultural Sciences pesticide data portal (Swedish University of Agricultural Sciences, 2025). Data were also extracted from the supplementary information of (Navarro et al., 2024), which included pesticide concentration data for 116 analytes from 11 different countries.

The Hazard Quotient (HQ) method was implemented to estimate the hazard of each analyte by comparing environmental concentrations observed in the data to relevant guidelines where available, using the following formula (Urban, 1986):

$$HQ = \frac{environmental\ concentration}{PC_x} \quad (1)$$

Where HQ is the hazard quotient, and PCx is the concentration of a toxicant that is likely to protect a certain percent (e.g., 95 %) of species in the aquatic ecosystem. A HQ value greater than 1 indicates an exceedance of the relevant ecosystem protection guideline. A list of 83 guidelines were collated for the analysis from various sources which allowed for the generation of HQ values and plots for each data set (SI 3). In the absence of available monitoring data or guidelines, a reasonable risk was identified through a review of published hazard assessments in the literature (both as individual PAIs and as mixture assessments) from Australia and overseas (Liess et al., 2021; Mitchell et al., 2023; Neale et al., 2024; Negri et al., 2022; Spilsbury et al., 2020; Warne et al., 2020). The list of PAIs was further expanded through consultation with agricultural industry groups and subject matter experts in Australia. This was particularly helpful for identification of pesticides in criteria 3 and 4 above, which required on-ground knowledge of current practices across a broad range of crop types, as well as likely future practice change in chemical usage. A total of 106 PAIs and metabolites were identified through this process, of which 53 were not included as they were either deemed low priority (low hazard and no indication that the PAI would be part of future practice change) or had insufficient toxicity data with which to build an SSD. See SI 4 for the list of 53 PAIs included in the metric, their modes of action, and their physicochemical properties. The PRM R code was designed to allow for the addition of new PAIs (or other toxicants) should the need arise (SI 5, and (Neelamraju, 2025)).

Calculation of species sensitivity distributions and protective concentrations

Toxicity data for each of the 53 PAIs were collated through an extensive search of the USEPA ECOTOX database (USEPA 2021), Office of the Pesticide Program database (USEPA 2021), Australasian Ecotoxicology Database (Warne et al., 1998) and the ANZECC and ARMCANZ toxicant database (Sunderam et al., 2000). This was supplemented with data from the literature where necessary. Some endpoint data were also obtained as part of the complementary project data collation processes (Mitchell et al., 2024; Mitchell et al., 2024; Neale et al., 2024) or revision of the Australian and New Zealand Water Quality Guidelines for Fresh and Marine Water Quality (henceforth termed the ANZ Guidelines) (ANZG, 2025; King et al., 2018; King et al., 2017). All toxicity values represent effects on aquatic organisms exposed to dissolved (aqueous phase) concentrations of each PAI, which is consistent with the exposure pathways relevant to surface water risk assessment and aquatic ecosystem protection (Warne et al., 2018). Only chronic toxicological endpoints of ecological relevance were used, for example lethality, immobilisation, growth, development, reproduction, and population growth. Chronic toxicity herein refers to an effect that occurs following exposure to a chemical for a period of time that represents a substantial portion of an organism's life span (greater than 10 %) (see Table 1 of (Warne et al., 2018)). The quality of the data was assessed and endpoints preferentially selected using the nationally endorsed ANZ Guideline method, which awards a quality score to each toxicity value based on a series of questions regarding the experimental design of the original study (see Appendix 1 and Table 3 of (Warne et al., 2018)). The SSDs and protective concentrations were derived in accordance with the derivation method of the ANZ Guidelines (Batley et al., 2014; Warne et al., 2018) using the ssdtools R package (ssdtools 2018). Five distributions were fitted to the log transformed toxicity data for each pesticide: Gamma, Weibull, log Gumbel (also known as inverse Weibull), log logistic, and log normal. The best fitting distributions were chosen based on both a statistical and a visual assessment. The concentrations that should theoretically protect 99, 95, 90 and 80 % of aquatic species (i.e., PC99, PC95, PC90 and PC80, respectively) were then calculated and the unique shape and scale parameters of each SSD curve were exported to a separate data table. All SSDs were assessed for reliability as per the method of (Warne et al., 2018) which takes into account the number and types of toxicity data (chronic or converted acute) used in each SSD, as well as the resulting fit of the distribution to the data set. A detailed technical account of the calculation of SSDs, reliability gradings, and PC values for each of the 53 PRM pesticides is included in SI 6.

Estimation of risk for individual pesticides in a sample

The estimation of risk from water samples is achieved by converting each PAI concentration datum to PAF the using its respective SSD cumulative distribution function and shape parameters. The mathematical formulas for each distribution type are provided in SI 7, along with a worked example. The results are expressed as the fraction of the ecosystem that is potentially affected (i.e., between 0 and 1) so are multiplied by 100 to achieve a percentage. The PRM was coded in R

Table 1
Mixture toxicity was assessed across all sites and years. The eight countries listed below had sufficient sample sizes and pesticide analyses to be included in the comparisons for this study.

Country	N years	N sites	N samples	Max PAIs sampled	Percent mixtures (%)
Belgium	7	36	840	18	95.6
Canada	5	11	170	18	92.5
Croatia	1	1	12	15	85.7
France	9	121	12776	23	90.4
Germany	12	121	6037	16	70.7
Italy	10	294	16398	23	96.6
The Netherlands	8	119	5230	23	96.9
Sweden	10	3	213	19	97.2

(Neelamraju, 2025; RStudio 2020) and includes built-in functions for each distribution type to allow for automated calculation of PAF for every pesticide concentration datum in large, multi-site, multi-year, multi-sample datasets. If an analyte observation is absent in the data (i.e. the PAI was not sampled or analysed for) the code returns 'NA', which is then ignored in subsequent calculations. The PRM code returned stable and accurate PAF estimates when validated using concentrations that aligned to the protective concentrations of each pesticide (SI 8).

Estimation of mixture toxicity

The PRM converts the PAF values for individual pesticides to an estimate of combined mixture toxicity in each water sample using the ms-PAF independent action model of joint toxicity (Bliss, 1939; Posthuma et al., 2001; Traas et al., 2002). The independent action model allows for individual pesticide SSDs to be retained in the calculation, thereby avoiding estimation error associated with the CA 'global average' SSD. See SI 9 for additional discussion and justification. The independent action model of joint action is described by the following formula (Bliss, 1939):

$$\textit{Pesticide mixture toxicity} = 1 - \prod_i (1 - PAF_i) \quad (2)$$

Where $\prod$ represents the product of a sequence of numbers, and PAF_i is the potentially affected fraction of each pesticide in a water sample calculated using the cumulative distribution function approach. As with individual toxicant PAF, mixture toxicity is first expressed as the proportion of species affected then multiplied by 100 to achieve a percentage. This equation was also used to estimate the combined toxicity of the four main pesticide groups: Photosystem-II (PSII) inhibiting herbicides, Other Herbicides (all non-PSII herbicides), Insecticides, and Fungicides. In this way, the PRM provides an estimate of the combined mixture toxicity of all 53 PAIs, as well as the four groups of PAIs, for every water sample in the data set.

Preparation of pesticide water monitoring data

Pesticide monitoring data were acquired from the GemStat Data Portal for all surface water types (rivers, lakes, and reservoirs) and all available countries, regions, and catchments. The initial dataset consisted of monitoring data from a total of 1652 sites across 27 countries, over a 13-year time span from 2011 – 2023 (SI 10). Mixture toxicity was calculated for all observations where at least two PAIs were sampled (80,954 water samples across 23 countries and 6901 site/year combinations), and results are provided as supplementary information (SI 11). To allow for spatial comparison of risk estimates, the data were filtered to site/year combinations with greater than four samples and at least 10 PAIs in a sample, as per (Rodea-Palomares et al., 2023). The final dataset consisted of 41,676 water samples collected from 706 monitoring sites across eight countries between 2010 and 2021, comprising 3343 unique site/year combinations (Table 1, and SI 12).

Of the 53 PAIs in the Pesticide Risk Metric, 37 were present in the filtered GemStat data. These 37 were comprised of 14 Insecticides, nine PSII Herbicides, 11 Other Herbicides, and three Fungicides (Table 2). All concentration data were converted to μg/L. Results less than the analytical limit of reporting (e.g. < 0.02 μg/L) were replaced with 0.0001 μg/L, which is approximately one order of magnitude lower than the lowest PC99 value in the PRM (0.00066 μg/L for bifenthrin). This value was chosen as it would not overly influence the calculation of mixture toxicity once converted to PAF, thereby avoiding the introduction of a false toxicity signature for results that were less than the limit of reporting.

Classification of risk

The PRM estimates the percentage of species in the ecosystem likely to be affected by exposure to individual PAIs, and by mixtures of up to 53 PAIs in a water sample. The protection of 95 % of the ecosystem aligns to a PAF of 5 % (100 % protected – 95 % protected = 5 % affected). A traffic light system of classifying mixture toxicity risk was previously developed for use by the Australian and Queensland governments for reporting against the Reef 2050 Water Quality Improvement Plan (Warne et al., 2023; Water Quality and Investigations 2023) and is applied herein. This system is based on the ecological condition classes used in the ANZ guidelines (ANZG, 2025) and the corresponding percentage of species to be protected i.e. 99 %, 95 %, 90 % and 80 % of species (Table 3). The Low risk category (95 % of species protected) aligns with that used to derive the 5th percentile cutoff value that may be used in the determination of annual average environmental quality standard (termed the AA-EQS) under the European WFD (Lepper, 2005). The WFD considers the biological effects of chemical pollutants acting individually and in mixtures. Therefore, for a water body to be classed as 'Good' status under the WFD, an annual average risk category of Low or Very Low (light green or dark green, respectively) would need to be achieved for individual PAIs and the mixture as a whole. For ease of interpretation, the term AA-EQS (PAI) is used to refer to the protection of 95 % of the aquatic ecosystem from exposure to individual PAIs, whereas AA-EQS (mixture) will refer to the protection of 95 % of the aquatic ecosystem from the effects of all pesticides combined. It is important to note that risk is a continuous vector, and that discrete classes are used for interpretation and comparison only. Refer to SI 13 for notes on interpretation and communication of risk.

Calculation of summary statistics

The European WFD stipulates that to assess long-term ecosystem protection, the average impact of chemical exposure over a year should be compared to the relevant AA-EQS. The mixture toxicity (PAF) values for each of the 3343 site/year combinations were therefore averaged for comparison with the AA-EQS targets under the WFD. In addition, the detection frequency of each analyte was calculated at the site/year level, as was the percentage of mixtures (defined as ≥ 2 PAIs detected in a sample). The relative contribution of each pesticide group (PSII Herbicides, Other Herbicides, Insecticides, and Fungicides) to the toxicity of the entire mixture (all 37 pesticides) was determined using the following equation:

Table 2
The 53 pesticides included in the Pesticide Risk Metric. Pesticides in italics are metabolites, and those in bold occurred in the filtered GEMStat data used for this study. See SI 4 for a summary of physicochemical properties.

PSII Herbicide	Other Herbicide	Insecticide	Fungicide
Ametryn	**2,4-D**	**Acetamiprid**	*4-Hydroxychlorothalonil*
Amicarbazone	**Acifluorfen**	**Bifenthrin**	Carbendazim
Atrazine	**Flumetsulam**	**Chlorantraniliprole**	**Epoxiconazole**
Bromacil	Flumioxazin	**Chlorpyrifos**	**Flutriafol**
Diuron	Fluroxypyr	**Clothianidin**	Mancozeb
Fluometuron	**Glyphosate**	Diazinon	**Propiconazole**
Hexazinone	Halosulfuron-methyl	**Dimethoate**	
Metribuzin	Haloxyfop (acid)	**Dinotefuran**	
Prometryn	**Imazamox**	**Fipronil**	
Simazine	Imazapic	**Flupyradifurone**	
Tebuthiuron	Isoxaflutole	**Imidacloprid**	
Terbuthylazine	**MCPA**	**Methomyl**	
	Metolachlor	**Methoxyfenozide**	
	Metsulfuron-methyl	Spinetoram	
	Paraquat	Tetraniliprole	
	Pendimethalin	**Thiacloprid**	
	Picloram	**Thiamethoxam**	
	Triclopyr		

Table 3
The ecological condition classes and the corresponding percent of species to be protected that are used in the Australian and New Zealand water quality guidelines (ANZG, 2025) and the risk classes allocated to these for this study (adapted from (Warne et al., 2020)).

Pesticide Risk Metric value		Risk category	Colour	Ecological condition class
% species affected	% species protected			
≤ 1	≥ 99	Very low	Dark green	High ecological value (minimally disturbed)
>1 to 5	95 to < 99	Low	Light green	Slightly to moderately disturbed
>5 to 10	90 to < 95	Moderate	Yellow	Highly disturbed
>10 to 20	80 to < 90	High	Orange	
>20	< 80	Very High	Red	

$$Percent\ contribution = \left(\frac{average\ PAF_x}{average\ PAF_{Total}}\right) \times 100 \quad (3)$$

Where the subscript 'x' denotes any of the pesticide groups, and the subscript 'Total' denotes the total mixture toxicity (all 37 pesticides). The same equation was used to determine the contribution of individual PAIs to the total mixture toxicity. When calculating group or PAI contributions, sites with a Low or Very Low annual risk category were removed from the calculations. This was primarily due to the sites already meeting the AA-EQS (mixture) target of 95 % ecosystem protection, but the calculation of proportions can also be unstable at this level. The contributions were calculated at different spatial scales: all data (all countries combined), at the country level (all sites within a country), and at the individual site level.

Discussion and results

Species sensitivity distributions and estimates of risk

Of the 53 PAIs included in the PRM, the lowest PC95 was for bifenthrin (0.0013 μg/L) and the highest was for flutriafol (386 μg/L). The SSDs ranged from Low to Very High reliability according to the classification criteria in Warne M St J, Batley, et al. (Warne et al., 2018). The majority (47 of 53) had at least a Moderate reliability. The six SSDs with a Low reliability included one fungicide metabolite (4-hydroxychlorothalonil), three insecticides (bifenthrin, flupyradifurone, and thiamethoxam) and two herbicides (flumetsulam and imazamox). The Low reliability grading for these PAIs indicates that the robustness of the SSDs may be improved with the addition of more toxicity data. The toxicity data for 23 of the PAIs was deemed to be bimodal so the SSDs were derived using data from only the most sensitive group, as per the recommendations of (Warne et al., 2018) and (Oginah et al., 2023). In the case of herbicides this was phototrophs (algae and plants) and for insecticides this was arthropods (crustaceans and insects). Refer to SI 13 for notes on communication and interpretation of mixture toxicity for bimodal SSDs. Toxicity data used to derive the SSDs can be found in (Neelamraju, 2025).

Toxicity of individual pesticide active ingredients

Of the 706 sites included in the study, a total of 61 sites (8.6 %) did not meet the AA-EQS (PAI) at some point in the 11 years of monitoring data analysed, with the majority of those occurring in Italy (Table 4). Only seven of the 37 monitored PAIs exceeded an annual average PAF of 5 %. The analyte with the greatest number of exceedances was the PSII herbicide terbuthylazine (30 sites, all of which occurred in Italy), followed by metolachlor (20 sites in Italy, two in France). The neonicotinoid insecticide imidacloprid exceeded the AA-EQS (PAI) at 15 sites in the Netherlands, one site in Germany, and one site in Belgium. Six of these exceedances occurred after imidacloprid was banned for outdoor use in Europe in 2018 (European Commission, 2025), although most were in 2019. Site DEU01525 in the Rhine-Meuse in Germany exceeded the AA-EQS (PAI) for imidacloprid in 2020 (7.2 % affected fraction) and again in 2021 (5.7 % affected fraction). The maximum annual average PAF for six of the PAIs occurred in Italy (terbuthylazine 21 % affected fraction, metolachlor 13 %, methomyl 16 %, chlorpyrifos 12.7 %, pendimethalin 11 %). The maximum annual average PAF of the seventh PAI, imidacloprid, occurred in the Netherlands in 2016 (17 % affected fraction).

Mixture toxicity estimates

The annual average mixture toxicity (expressed as the average PAF for all 37 PAIs combined over the year) was calculated for each of the 3343 unique site-year combinations across the eight countries. When assessed as a mixture, a total of 359 sites out of 706 (51 %) did not meet the WFD AA-EQS (mixture) target at some point across the 11 years of monitoring data (Fig. 2), with the largest number (177 sites) occurring in Italy, followed by the Netherlands (84 sites). By considering the effect of PAIs on their own, 298 of these sites would have been allocated a 'Good' status under the WFD when in fact they are not sufficiently protected from the combined effects of pesticide mixture exposure. Based on this finding and the outcomes of other studies (e.g., (Merga and

Table 4
The number of sites with exceedances of the AA-EQS (PAI) for any compound. Note that multiple PAIs may exceed at the same site in a year, and that multiple exceedances of one PAI at a site (over several years) is counted as a single exceedance. Croatia, Canada, and Sweden are not included in this summary as there were no exceedances of the AA-EQS (PAI) when pesticides were considered individually.

		Number of sites exceeding for each PAI (all years combined)						
Country	**n sites > AA-EQS (PAI)**	**Terbuthylazine**	**Metolachlor**	**Imidacloprid**	**Methomyl**	**Chlorpyrifos**	**Pendimethalin**	**Thiacloprid**
Italy	42	30	20	0	10	2	1	1
The Netherlands	15	0	0	15	0	0	0	0
France	2	0	2	0	0	0	0	0
Germany	1	0	0	1	0	0	0	0
Belgium	1	0	0	1	0	0	0	0
Count	**61**	**30**	**22**	**17**	**10**	**2**	**1**	**1**

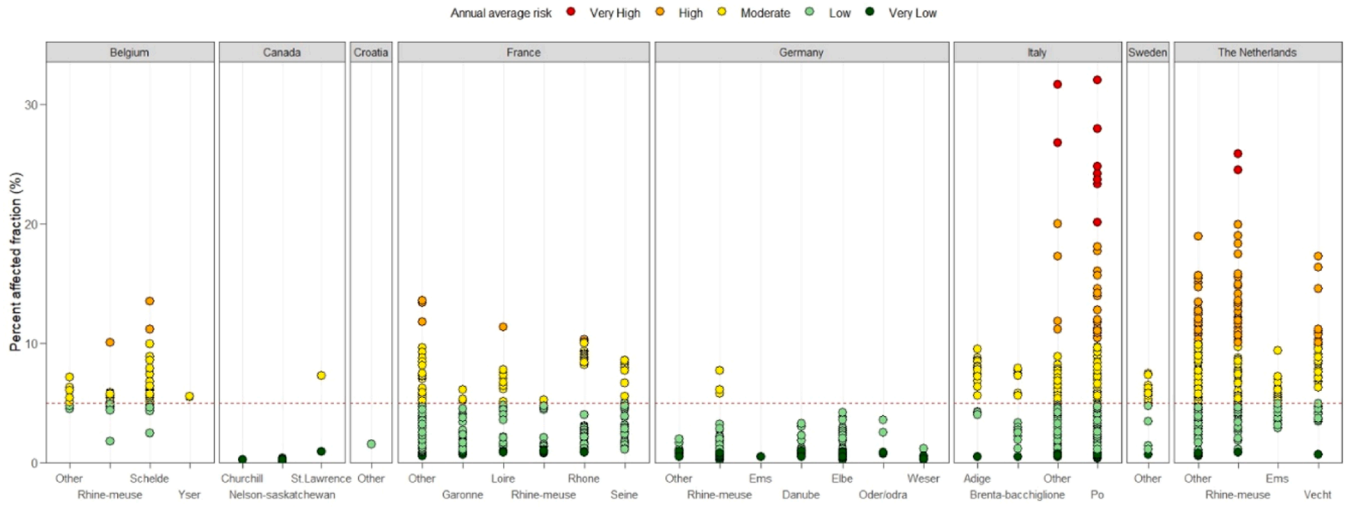

Fig. 2. Annual average mixture toxicity for the 3343 unique site/year combinations across eight countries and their major river basins between 2010 – 2021 (n = 41,676 water samples). The red dashed line refers to the AA-EQS (mixture) target for protection of 95 % of the aquatic species.

Van den Brink, 2021; Mohr et al., 2012; Rico et al., 2018; Thunnissen et al., 2020)), there is a likelihood of significant impact on the structure and function of aquatic communities in the waterways of some countries (e.g., Italy and the Netherlands). This estimate is based on the average annual mixture toxicity and does not account for the impact of higher concentrations during episodic runoff or other delivery events. Some of these waterways traverse multiple countries, such as the Rhine River, which originates in Switzerland and then flows through Austria, Germany, and France before it enters the North Sea via the Netherlands. The Danube and the Meuse also share transnational boundaries. Further research may be needed to understand the cumulative mixture pressure in these systems as the water moves downstream, and where policy and risk mitigation strategies would be best applied. Neelamraju and Turner (2025) contains an interactive dashboard of the risk and contribution data at the site and year level that may help with this assessment. See SI 15 for the maximum mixture toxicity for each country (as annual average PAF for all 37 PAIs) and a breakdown of the numbers of site/-year combinations that fall into each of the risk categories from Table 3.

Contribution by pesticide group

Information on the contribution of each pesticide group (Insecticides, Fungicides, PSII Herbicides, and Other Herbicides) to the total risk posed by pesticide mixtures can be used to prioritise on-ground management practice change that might result in reduced runoff of higher-risk pesticides. Group contributions were calculated on sites with a moderate, high, or very high risk category, as these had not yet met the AA-EQS (mixture) target. River basins with all sites meeting the AA-EQS (mixture) target were not included in the calculation of group contributions for this study (e.g., the Churchill and Nelson-Saskatchewan basins in Canada, see Fig. 2). For the remainder of sites across all countries, the primary contributors to mixture toxicity were found to be Insecticides (61 %), followed by Other Herbicides (32 %), then PSII Herbicides (6.1 %) (Fig. 3). However, at the country and site level, there was considerable variation in the relative contributions of each pesticide group to the total mixture toxicity (Fig. 3 and SI 15, respectively). For example, Insecticides contributed, on average, 96 % of the mixture toxicity in the Rhine-Meuse in Germany (all other river basins met the target, so were not included). The lowest contribution of Insecticides was 44 % for Canada. The contribution of Other Herbicides ranged from 3.5 % (Germany) to 56 % (Canada). The contribution of PSII Herbicides ranged from 0 % (Sweden and Germany) to 12 % (Italy). At the country level, Fungicides contributed a maximum of 0.04 % (Italy). Aquatic insects, crustacea, and plants (including algae) can be impacted by the presence of insecticides and herbicides. Taxa in these groups can represent a range of functional ecosystem services that collectively regulate algal growth, process organic matter, cycle nutrients, aerate sediments, and serve as prey to higher trophic levels (Rosenberg and Resh, 1984). More sensitive species would experience higher impacts.

Contribution by pesticide active ingredient

When analysed at the PAI level across all countries, 99 % of the annual average mixture toxicity was contributed by 14 PAIs (Fig. 4). The major contributor (22 %) was the herbicide metolachlor, followed by the neonicotinoid insecticide thiacloprid (21 %), then the organophosphate insecticide chlorpyrifos (11 %). The neonicotinoid insecticides

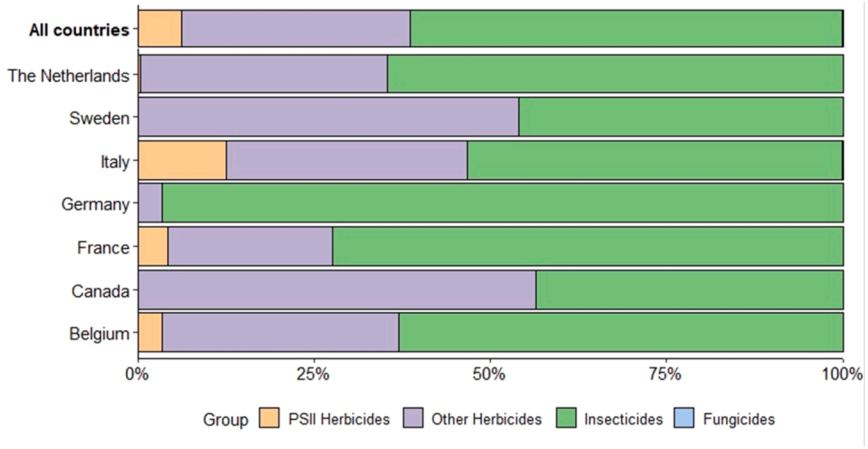

Fig. 3. The relative contribution of pesticide groups was calculated on sites with a Moderate to Very High risk category, as these are the ones that had not yet met the AA-EQS (mixture) target.

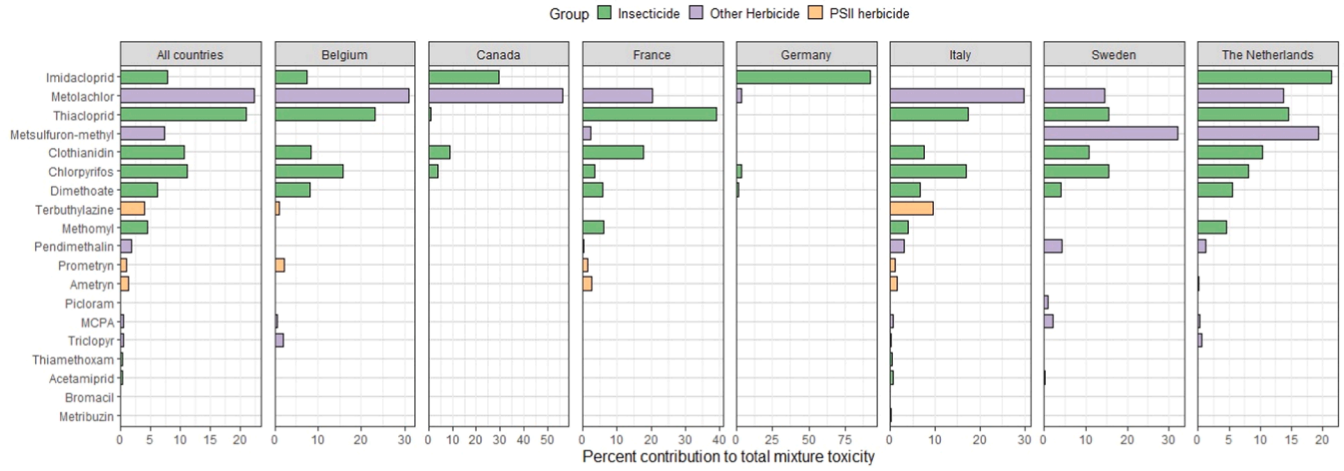

Fig. 4. The percent contribution of individual pesticide active ingredients (PAIs) to the annual average mixture toxicity for each country (Note: sites that met the AA-EQS (mixture) target were removed from this analysis). PAIs contributing < 0.1 % to the total mixture toxicity are not visible on plot.

combined (thiacloprid, imidacloprid, and clothianidin) contributed 40 % of the total mixture toxicity. When assessed for each country separately, the numbers and types of PAIs contributing to annual average mixture toxicity varied (SI 15). For example, 12 PAIs contribute 99 % of the mixture toxicity in Italy: metolachlor (30 %), thiacloprid (17 %), chlorpyrifos (17 %), terbuthylazine (10 %), clothianidin (8 %), dimethoate (7 %), methomyl (4 %), pendimethalin (3 %), ametryn (2 %), prometryn (1 %), MCPA (1 %), and acetamiprid (1 %). For France, the neonicotinoid insecticide thiacloprid is the dominant contributor to mixture toxicity (39 %), followed by metolachlor (20 %), clothianidin (18 %), methomyl (6 %), dimethoate (6 %), chlorpyrifos (4 %), ametryn (3 %), metsulfuron methyl (2 %), and prometryn (2 %). These findings align to those of who also found mixture toxicity was driven by approximately 15 specific chemicals, although some of those were metals, PAHs, and pharmaceuticals, which were not included in this study.

Considerable variation occurred in both annual average mixture toxicity and the relative contribution of each PAI at the site and year level. For example, there is a pattern of deceasing influence of imidacloprid across most sites (Fig. 5). Neelamraju and Turner (2025) contains an interactive dashboard of group and PAI contributions for all sites and years included in the study, allowing for detailed investigation of spatial and temporal patterns. Differences in compound mobility, solubility, and persistence may explain some of these results (see SI 4).

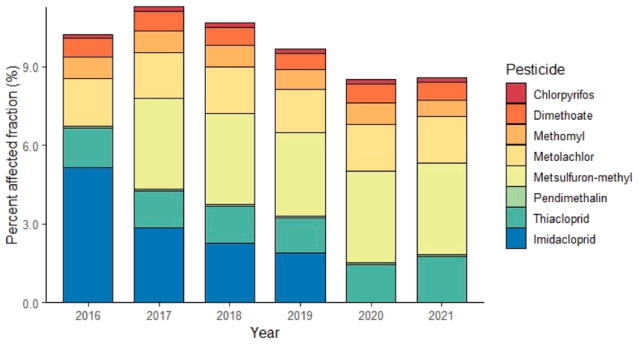

Fig. 5. An example of temporal changes in pesticide active ingredient contribution to total mixture toxicity at site NLD00073 in the Rhine-Meuse, Netherlands. The contribution of imidacloprid appears to decrease from 2016 to 2019, after which it disappears completely. See Neelamraju and Turner (2025) for an interactive dashboard of plots for all sites and years included in the study.

Conclusion

The PRM takes an existing method that was computationally difficult (and therefore difficult to apply to large and complex data sets) and

makes it more accessible through the development of automated R code (see SI 5, and (Neelamraju, 2025)). The SSD approach, combined with the independent action model of joint toxicity, results in more reliable risk estimates for individual pesticides and their mixtures. The PRM was built using the same method and toxicity data used to derive environmental quality standards, providing linkage to ecosystem protection benchmarks such as those of the WFD. The resulting risk estimates are directly related to ecological condition and can be used on measured or modelled water quality monitoring data, making it an ideal tool for second-tier risk assessment. The data can also be interrogated both spatially and temporally to inform a range of assessment outcomes and reporting requirements (see SI 16: Broader Application).

Transboundary river basins present unique challenges for contaminant risk assessment and management, as waterways may pass through countries with varying agricultural practices, monitoring programs, and regulatory frameworks. When applied to pesticide monitoring data from eight countries, more than half of all sites (51 %) failed to meet the AA-EQS of 95 % of the ecosystem protected from the effects of pesticide mixtures. Many of these exceedances occurred in countries that share transboundary river basins, such as the Rhine. These findings illustrate the need for harmonised risk assessment and the joint management of pesticide risk across international boundaries in Europe. The PRM aligns with both the ecosystem protection requirements of the WFD as well as the 2050 zero pollution target of the European Green Deal, making it well suited to this purpose. By providing transparent and comparable estimates of mixture toxicity, the PRM can facilitate dialogue between jurisdictions, support joint monitoring programs, and inform coordinated management in shared watersheds.

Where harmonised regulatory controls are identified as a mechanism of change, PRM results can be used alongside tools like the Pesticide Standard Value proposed by (Huang and Li, 2024), which supports cross-border alignment of pesticide regulations. Conversely, where agricultural practices are identified as the primary driver of risk, the PRM can be paired with the Pesticide Projector (Truii Pesticide Projector 2025; Warne et al., 2023) to inform targeted, on-farm pesticide management and practice change. Project Bluewater is a prime example that employed these two tools (the PRM and the Pesticide Projector) to improve pesticide practices and reduce aquatic ecosystem risk (CANERISE Mackay Whitsunday 2025; NQ Dry Tropics 2025). The project engaged with 75 growers across 169 farms covering 11,396 ha in the Mackay Whitsunday region of Australia and resulted in a 26 % reduction in aquatic ecosystem risk with no net loss of farm productivity. The combined toolkit of the PRM - Pesticide Standard Value – Pesticide Projector provides a comprehensive end-to-end pesticide management framework that integrates risk assessment, regulatory controls, and practical decision support. Together, these tools offer a scalable pathway toward achieving the 2050 zero pollution target and ecosystem protection goals of the Water Framework Directive.

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CRediT authorship contribution statement

Cath A. Neelamraju: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Reinier M. Mann:** Writing – review & editing, Supervision, Methodology. **Zoe T. Bainbridge:** Writing – review & editing, Supervision. **Hannah C. Mitchell:** Writing – review & editing, Data curation. **Ryan D.R. Turner:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envadv.2025.100676.

Data availability

Data will be made available on request.

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