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Original citation:
Warne, M, Neelamraju, C, Strauss, J, Turner, RDR, Smith, RA & Mann, RM 2023, 'Estimating the aquatic risk from exposure to up to twenty-two pesticide active ingredients in waterways discharging to the Great Barrier Reef', Science of the Total Environment, vol. 892, 164632
https://dx.doi.org/10.1016/j.scitotenv.2023.164632

DOI 10.1016/j.scitotenv.2023.164632
ISSN 0048-9697
ESSN 1879-1026

Publisher: Elsevier

Science of the Total Environment 892 (2023) 164632

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Estimating the aquatic risk from exposure to up to twenty-two pesticide active ingredients in waterways discharging to the Great Barrier Reef

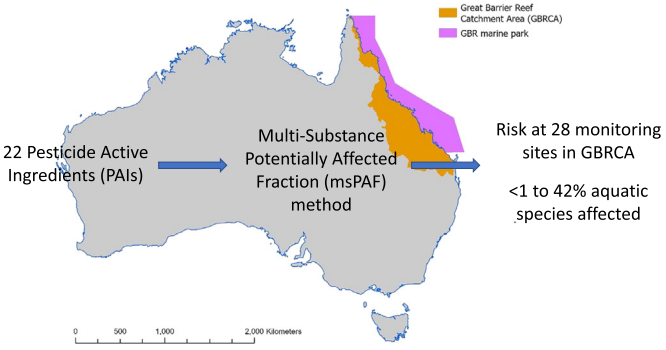
M.St.J. Warne [a,b,c,*], C. Neelamraju [a,b], J. Strauss [b], R.D.R. Turner [a,b,d], R.A. Smith [b,1], R.M. Mann [b]

[a] Reef Catchments Science Partnership, School of Earth and Environmental Sciences, University of Queensland, Brisbane, Queensland, Australia
[b] Department of Environment and Science, Brisbane, Queensland, Australia
[c] Centre for Agroecology, Water and Resilience, Coventry University, West Midlands, United Kingdom
[d] Institute for Future Environments, Queensland University of Technology, Brisbane, Queensland, Australia

HIGHLIGHTS

- Waterways discharging to the Great Barrier Reef (GBR) contain pesticide mixtures.
- The risk from pesticide mixtures has not been fully quantified.
- Risk posed by 22 pesticide active ingredients was assessed using the msPAF method.
- <1 % to 42 % of aquatic species were estimated to be affected.
- Pesticide active ingredients can pose a significant risk to freshwater ecosystems.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Daniel Wunderlin

Keywords:
Pesticide active ingredients
Mixtures
Toxicity
Independent action
Risk
Rivers
Great Barrier Reef

ABSTRACT

Pesticides decrease the quality of water reaching the Great Barrier Reef (GBR), Australia. Up to 86 pesticide active ingredients (PAIs) were monitored between July 2015 to end of June 2018 at 28 sites in waterways that discharge to the GBR. Twenty-two frequently detected PAIs were selected to calculate their combined risk when they co-occur in water samples. Species sensitivity distributions (SSDs) for the 22 PAIs to fresh and marine species were developed. The SSDs, the multi-substance potentially affected fraction (msPAF) method, Independent Action model of joint toxicity and a Multiple Imputation method were combined to convert measured PAI concentration data to estimates of the Total Pesticide Risk for the 22 PAIs (TPR_{22}) expressed as the average percentage of species affected during the wet season (i.e., 182 days). The TPR_{22} and percent contribution of active ingredients of Photosystem II inhibiting herbicides, Other Herbicides, and Insecticides to the TPR_{22} were estimated. The TPR_{22} ranged from <1 % to 42 % of aquatic species being affected. Approximately 85 % of the TPR_{22} estimates were >1 % — meaning they did not meet the Reef 2050 Water Quality Improvement Plan's pesticide target for waters entering the GBR. There were marked spatial differences in TPR_{22} estimates — regions dominated by grazing had lower estimates while those with sugar cane tended to have higher estimates. On average, active ingredients of PSII herbicides contributed 39 % of the TPR_{22}, the active ingredients of Other Herbicides contributed ~36 % and of Insecticides contributed ~24 %. Nine PAIs (diuron, imidacloprid, metolachlor, atrazine, MCPA, imazapic, metsulfuron, triclopyr and ametryn) were responsible for >97 % of TPR_{22} across all the monitored waterways.

* Corresponding author at: Reef Catchments Science Partnership, School of Earth and Environmental Sciences, The University of Queensland, St Lucia QLD 4072, Australia.
E-mail address: michael.warne@uq.edu.au (M.S.J. Warne).
[1] Current address: Australian Institute of Marine Sciences, Townsville, Queensland, Australia.

http://dx.doi.org/10.1016/j.scitotenv.2023.164632
Received 3 March 2023; Received in revised form 20 May 2023; Accepted 31 May 2023
Available online 7 June 2023

1. Introduction

Pesticide active ingredients (PAIs) are used extensively in agricultural and urban settings and many of them can end up in the waterways that drain the land. Pesticide AIs have been routinely detected globally in surface and ground waters (e.g., Smith et al., 2012; Schreiner et al., 2016; Zheng et al., 2016; Schmidt et al., 2019; Kandie et al., 2020) and marine waters (Thai et al., 2020; references cited in Warne and Reichelt-Bruschett, 2023). The studies that have explicitly analysed PAI mixtures in surface waters have generally found mixtures in the majority of samples. These include 100 % of 238 samples from USA rivers (Belden et al., 2007), >99.9 % of midwestern USA streams (Nowel et al., 2018), 99 % of samples from northern German rivers (Schafer et al., 2011), 97 % of samples from wadeable streams in four regions of the USA (Bradley et al., 2021), 90 % from U.S. agricultural and urban surface waters (Gilliom et al., 2006), >80 % of agricultural rivers in Georgia, USA (Glinski et al., 2018) and 66 % in the Ebro River, Spain (Ccanccapa et al., 2016). In addition, 88 % of samples from the USA National Water Quality Network contained five or more pesticides per sample (Covert et al., 2020).

Most ecological risk assessments conducted on PAIs have determined the risk posed by individual AIs, rather than the risk posed by PAI mixtures. Such an approach is likely to underestimate the risk if the risk posed by the mixtures is additive or synergistic and likely to overestimate the risk if the toxicity of the mixtures is antagonistic.

The Great Barrier Reef, the world's largest reef ecosystem, receives water from 35 basins that drain approximately 440,000 km^2 of predominantly agricultural land. The rivers and creeks that drain to the GBR lagoon (henceforth called GBR waterways) also contain PAI mixtures. Both Warne et al. (2020) and Spilsbury et al. (2020) reported >80 % of samples from monitored GBR waterways contained PAI mixtures. Similarly, Vandergragt et al. (2020) found that all of the 22 monitored wetlands in the GBR catchment area contained between 12 and 30 PAIs at each sampling period. To protect the GBR from the harmful effects of PAIs and other aquatic pollutants (i.e., suspended solids (eroded soil), and nutrients (nitrogen and phosphorus)) the Australian and Queensland governments have developed the Reef 2050 Water Quality Improvement Plan (Reef 2050 WQIP) (Australian Government and Queensland Government, 2018). The purpose of the Reef 2050 WQIP is "to identify management and monitoring requirements for all land-based pollution to improve the quality of water flowing from catchments adjacent to the Reef (Australian Government and Queensland Government, 2018)". To do this, a series of land and catchment management and water quality targets were established and the aim was for these to be met by 2025. The 2025 water quality target for pesticides (the pesticide target) was originally based on reducing the annual loads of five photosystem II inhibiting herbicides (Australian Government and Queensland Government, 2013) but this was modified to a risk-based target in 2018 (Brodie et al., 2017; Australian Government and Queensland Government, 2018) in recognition of the fact that PAIs have different toxicities and usually occurred as mixtures in waters entering the GBR. The risk-based pesticide target is to protect at least 99 % of aquatic species from the adverse effects of PAI mixtures at the mouth of waterways (Australian Government and Queensland Government, 2018).

There are two main ways of determining the risk posed by mixtures of PAIs – the hazard or risk quotient or toxic unit (HQ, RQ or TU) approach, and the multi-substance potentially affected fraction (msPAF) method. The former approaches have been used widely (Cruzeiro et al., 2017; Kandie et al., 2020; Spilsbury et al., 2020). The latter approach was developed by Traas et al. (2002) and used for PAI mixtures by Faggiono et al. (2010), Gregorio et al. (2012), Silva et al. (2015), Rämö et al. (2018), Posthuma et al. (2019) and Smith (2018). Of these two methods only the msPAF method can calculate the risk in terms of the proportion or percentage of species affected (or conversely protected) and is therefore consistent with the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018) and with the pesticide target (Australian Government and Queensland Government, 2018). It was therefore adopted in the current study.

The aims of this study were to: develop a msPAF method to estimate the risk posed by mixtures of PAIs with multiple modes of action; to use the msPAF method to estimate the risk posed by mixtures of PAIs that commonly occur in GBR waterways; determine whether the pesticide target for GBR waterways is being met; and finally determine the contribution of PAI groups and individual PAIs to the total risk posed by PAIs.

2. Methods

2.1. Selection of pesticide active ingredients

Pesticide AIs were included if they met all the following criteria:

1. they were registered for use in Australia;
2. they have been detected in GBR waterways; and
3. species sensitivity distributions (SSDs) for fresh and/or marine organisms were available.

The 78 PAIs that have been detected in GBR waterways (i.e., Lewis et al., 2009; Brodie et al., 2012; Kroon et al., 2012; Smith et al., 2012; Turner et al., 2012, 2013; Davis et al., 2012, 2013; Gallen et al., 2013, 2014; O'Brien et al., 2013; Wallace et al., 2014, 2015, 2016; Garzon-Garcia et al., 2015; Smith et al., 2015; O'Brien et al., 2016; Huggins et al., 2017) were compared to the above criteria. A total of 22 PAIs met the criteria and were included in the calculations (Table 1).

2.2. Selection of Waterways

All 31 waterways monitored for PAIs by the Great Barrier Reef Catchment Loads Monitoring Program (GBRCLMP) between 2015/2016 and 2017/2018 were included in the analysis (Table S1, Supplementary Material). Not all waterways or sites were monitored each year. In total, 68 site-year datasets were available. The monitored waterways were in five of the six Natural Resource Management Regions that comprise the Great Barrier Reef Catchment Area (GBRCA) (Fig. 1). No waterways in the Cape York region were monitored due to logistic difficulties and the relatively small proportion of land with pesticides applied. Details of the sites are provided in Table S2, Supplementary Material.

2.3. Collecting water samples and measuring pesticide aqueous concentrations

Grab samples were collected either manually or using refrigerated autosamplers (for details see Huggins et al., 2017; Napel et al., 2019a; Napel

Table 1
The 22 pesticide active ingredients included in the calculation of the risk posed by pesticide mixtures.

2,4-D[a,d]	Ametryn[b]	Atrazine[b]	Chlorpyrifos[c,e]
Diuron[b]	Fipronil[c,f]	Fluroxypyr[a,g]	Haloxyfop[a,h]
Hexazinone[b]	Imazapic[a,i]	Imidacloprid[c,j]	Isoxaflutole[a,k]
MCPA[a,d]	Metribuzin[b]	Metolachlor[a,l]	Metsulfuron-methyl[a,i]
Pendimethalin[a,m]	Prometryn[b]	Simazine[b]	Tebuthiuron[b]
Terbuthylazine[b]	Triclopyr[a,g]		

[a] Other herbicides (i.e. all herbicides included in the calculations that are not PSII herbicides).
[b] Photosystem II inhibiting herbicides.
[c] Insecticides.
[d] Auxin mimic (Phenoxy-carboxylic acid auxins).
[e] Acetylcholine esterase (AChE) inhibitor.
[f] Gamma-aminobutyric acid (GABA) gated chloride channel blocker.
[g] Auxin mimic (Pyridine-carboxylic acid auxins).
[h] Acetyl-coenzyme A carboxylase (ACCase) inhibitor.
[i] Acetolactate synthase (ALS) inhibitor.
[j] Nicotinic receptor agonist.
[k] 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibitor.
[l] Cell division inhibitor.
[m] Microtubule synthesis inhibitor.

Fig. 1. The locations of monitoring sites, waterways and the Natural Resource Management Regions.

et al., 2019b). The PAI analyses were conducted by the Queensland Health Forensic and Scientific Services Organics Laboratory (Coopers Plains, Queensland), which is accredited for these analyses by the Australian National Association of Testing Authorities. The PAIs were analysed for using liquid chromatography-mass spectrometry/mass spectrometry (LCMS/MS). The water samples were analysed using one or more of the following methods:

- Solid Phase Extraction followed by LCMS/MS high concentration analysis (used during periods with elevated river discharge that follow rain when

high PAI concentrations were expected). This was used for most of the 943 samples in 2015/2016.
- Solid Phase Extraction followed by LCMS/MS low concentration analysis (used during the dry season when water samples were expected to contain low PAI concentrations). This method has a 10-fold lower limit of reporting (LOR) than the LCMS/MS high concentration method for all PAIs. This method was used for a minority of the 945 samples in 2016/2017.
- Direct Inject LCMS/MS analysis where a small volume of the water sample is directly injected into the LC column. The LORs for the direct injection method are similar to the LCMS/MS high concentration method (i.e., approximately 10-times larger than for the LCMS/MS low concentration method). This method was used for the majority of 945 samples from 2016/2017 and all 1408 samples from 2017/2018. The LORs for all three methods for the 22 selected PAIs are provided in Table S3, Supplementary Material.

The GBRCLMP quality assurance and quality control procedures (Huggins et al., 2017) were used to determine the accuracy of PAI concentrations. An exception was how concentrations below the LOR were handled where the following rules were used:

- If PAI concentrations were not greater than the LOR at a site for the entire wet season all < LORs for that PAI, site and year combination were changed to 1×10^{-11};
- All < LOR values of a PAI that occurred before the first quantifiable (i.e., >LOR) occurrence of that PAI were changed to a value of 1×10^{-11}; and
- All < LOR values of a PAI after the first quantifiable occurrence of that PAI were substituted by the product of the LOR and the relative Ecotoxicity Threshold Value (relative ETV) (Table S4, Supplementary Material).

The value of 1×10^{-11} is arbitrary and was used instead of zero because a logarithmic function was required in subsequent calculations and so it would not alter, to any meaningful degree, the estimate of Total Pesticide Risk of the 22 selected pesticides (i.e., the TPR_{22}). Ecotoxicity Threshold Values were calculated using the same method as Default Guideline Values (DGV) in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality, (hereafter termed the ANZG guidelines) (ANZG, 2018). Default Guideline Values are the numerical limits for pollutants in Australia and New Zealand (ANZG, 2018). The difference between ETVs and DGVs is that ETVs have not been nationally endorsed. The relative ETVs were calculated by converting the ETV from μg/L to μmol/L and then dividing the ETV for each PAI by the ETV of the least toxic PAI – in this case haloxyfop.

2.4. Collation of toxicity data

To derive ETVs, SSDs were constructed using toxicity data collated by King et al. (2017a, 2017b), ANZECC and ARMCANZ (2000) for chlorpyrifos and the authors for atrazine (Table S5, Supplementary Material). The literature and guidelines, standards and criteria of regulatory authorities (e.g., the US EPA, Environment Canada and the European Union) were searched. In addition, databases (ECOTOX (US EPA, 2015a), Office of the Pesticide Program (US EPA, 2015b), Australasian Ecotoxicology Database (Warne et al., 1998; https://app.australasia.setac.org/) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al., 2000)) were searched.

2.5. Quality assurance and screening of toxicity data

The quality of the collated toxicity data was assessed using the method in the ANZG guidelines (Warne et al., 2018), which was based on Hobbs et al. (2005). Only data with quality scores >50 % were deemed acceptable and used in subsequent calculations. These data were subsequently screened using the methods described in Warne et al. (2018) to ensure they were suitable for calculating ETVs. For example, pesticide formulations and endpoints not considered ecologically relevant were removed (Warne et al., 2018).

2.6. Calculating species sensitivity distributions and protective concentrations

Toxicity data that passed the quality assurance and screening procedures are presented in King et al. (2017a, 2017b), in ANZECC and ARMCANZ (2000) for chlorpyrifos and in Supplementary Material Table S5 for atrazine. These were used to construct SSDs using the method specified by ANZG (2018) for deriving DGVs for toxicants (Batley et al., 2018; Warne et al., 2018). The one exception was that data for both fresh and marine species were combined to derive a single SSD for each PAI. This was done because the Reef pesticide target is intended to protect freshwater, estuarine and marine ecosystems. The calculation of the single value that represents each species and determining whether the toxicity data were uni-modal were conducted using the method of Warne et al. (2018). When the toxicity data were uni-modal, the data for all species were used to calculate the SSD. If the data were multi-modal, then only toxicity data for the most sensitive group of organisms were used (Warne et al., 2018).

The single toxicity value of a PAI for each available species was entered into the Burrlioz software (CSIRO, 2016) and the best fitting SSD (Table S6, Supplementary Material) and the concentrations that should theoretically protect 99, 95, 90 and 80 % of aquatic species (i.e., PC99, PC95, PC90 and PC80 respectively) were calculated. The SSD equations for the 22 PAIs are presented in Tables S7 and S8, Supplementary Material and details of the SSD derivation are presented in Section B, Supplementary Material. The level of confidence (reliability) associated with the SSD and DGVs for each PAI was assessed using the method of Warne et al. (2018) and reported as very high, high, moderate, low or very low reliability (Table S10, Supplementary Material).

2.7. Estimating the risk posed by individual pesticide active ingredients and mixtures

The concentrations of the 22 PAIs in each water sample were entered into the appropriate SSD equations to calculate the percentage of species affected by each PAI in each sample. This SSD-based method was selected in preference to the Toxic Unit approach to be consistent with the ANZG guidelines (ANZG, 2018) and the reef pesticide target (Brodie et al., 2017; Australian Government and Queensland Government, 2018) both of which are expressed in terms of the percent of aquatic species protected.

The PAIs included in the calculations have multiple modes of action (Table 1). There are two established methods for estimating the toxicity of mixtures - the Concentration Addition (CA) model for chemicals with similar modes of action (MoA) and the Independent Action (IA) model for chemicals with different MoA. Initially, it was planned to use the two-step method (Backhaus and Faust, 2012; Altenburger et al., 2013) applying the CA model to chemicals of the same MoA and then the IA model to estimate the joint toxicity of the CA groupings. Chemicals with the same MoA should have parallel SSDs. Analysis for parallelism of SSDs revealed that the majority of PAIs with the same MoA were not parallel, a finding also observed by Smith (2018). Therefore, when the SSDs for PAIs with the same MoA were merged, the resulting SSDs did not accurately predict the toxicity of individual PAIs, confirming earlier work by Smith (2018). For these reasons neither the CA or two-step methods were used to estimate TPR_{22}, rather the IA model of joint action was used.

Advantages of using the IA model are that PAI SSDs remain separate, therefore improving the predictive power of each, the calculations are considerably simpler and it allows the user to estimate the contribution of individual PAIs or groups of PAIs to the TPR_{22}. Because the IA model of joint action consistently yields lower estimates of mixture toxicity than the CA model (Backhaus et al., 2000; Faust et al., 2000, 2003; Dyer et al., 2010; Spilsbury et al., 2020), using the IA model will likely lead to lower estimates of the risk posed by the pesticide mixtures. Having said that, the estimates of mixture toxicity derived using the CA and IA models are often not

statistically different (Dyer et al., 2010). Spilsbury et al. (2020) using pesticide monitoring data between 2011 and 2016 from the GBRCLMP found that the ratio of median and mean CA and IA pesticide mixture toxicity estimates were 1.0 and 1.1, respectively.

The IA model of joint action was used to estimate the risk posed by:

- Total Pesticide Active Ingredients (i.e., the 22 selected PAIs);
- PSII Herbicide AIs;
- Other Herbicide AIs (all herbicide AIs other than PSII AIs); and
- Insecticide AIs

The PAIs allocated to the above groups are shown in Table 1.These calculations were conducted for every water sample collected for 2015/2016 to 2017/2018. It is important to note that the TPR_{22} is the combined risk of the 22 PAIs included in the PRM – it is not the risk posed by all PAIs present in the water samples.

2.8. Selecting an appropriate risk window for Pesticide Risk Reporting

The wet season (typically November to April), was chosen as the most suitable time window to represent pesticide risk in the GBRCA because:

- Rainfall in the GBRCA is highly seasonal with distinct wet and dry seasons with the vast majority of rain falling during the wet season resulting in a greater probability of pesticides being transported to waterways than the dry season. Estimating the risk during the dry season or for the entire year would dramatically underestimate the risk during the wet season;
- For the majority of sites included in the present study, pesticides are applied prior to, or close to the start of the wet season;
- Monitoring by the GBRCLMP indicates that in the majority of monitored catchments in the GBRCA, pesticide concentrations are low over the dry season (e.g., Water Quality and Investigations, 2020)
- Aquatic ecosystem productivity is highest during the wet season because of increased hydrological connectivity, and an influx and rapid redistribution of nutrients (Petit et al., 2017).
- Exposure to pesticides in waterways is episodic and therefore organisms are exposed to a pulse-recovery regime. The 182-day risk window is of sufficient duration to capture the majority of detrimental effects and subsequent recovery that result from exposure to pulses of pesticides, particularly when comparing risk between waterways where different hydrological regimes exist (Smith, 2018).

Generally, when assessing the risk associated with single pesticides, the 95th percentile of the monitoring data is compared with the DGV (ANZG, 2018). If the 95th percentile is greater than the DGV, then the potential risk to the aquatic community warrants further investigation (ANZG, 2018). This highly conservative approach was not adopted. Rather, the average over the wet season was chosen as a suitable statistic as it provides a more stable estimate for comparison between sites and years and to the pesticide target.

The wet season was defined as the six-month period following the first flush in each monitored waterway. A fixed period was set (182 days), based on the hydrological and pesticide monitoring data across all waterways, in order to make temporal and spatial comparisons of exposure between years and waterways. The first flush was identified as the first day after July 1 of each year when river flow and pesticide concentrations simultaneously increased. An additional factor in determining the first flush was to ensure the six months after the first flush covered as much of the period with elevated pesticide concentrations as possible. The dates of the first flush are presented in Table S9, Supplementary Material.

2.9. Calculation of the total pesticide risk over the wet season

If multiple samples were collected within a 24-hour period, the TPR_{22} estimates were estimated for each sample and then averaged to provide a single estimate for each day.

Ideally, the risk posed by pesticides over the wet season would be estimated using concentration data for all 182 days in the wet season. However, PAI concentration data were never available for all 182 days at a site. This limitation was overcome using a multiple imputation method. This method is well accepted for dealing with missing data (e.g., Rubin, 1996; Patrician, 2002; Donders et al., 2006) and is widely used in the fields of statistics, epidemiology and social and political sciences.

A non-parametric Kernel Density function[2] was fitted to the distribution of TPR_{22} estimates for each site/year combination. 1000 imputed datasets were created for each site and year combination calculated and an estimated (imputed) percentage of species affected was generated for each day that did not have a measured risk value. The measured and imputed Pesticide Risk estimates were then combined and the TPR_{22} over the wet season calculated, i.e., the average percentage of species affected over the wet season.

All of the preceding calculations in the methods section, which are collectively termed the Pesticide Risk Metric (PRM), were calculated using the "R" program (Strauss et al., 2019).

2.10. Classification of the risk posed by pesticide mixtures

The percent of species that would be affected by pesticide mixtures were converted to the corresponding percent of species protected using the following equation.

$$\text{Percent species protected} = 100 - \text{percent species affected} \quad (1)$$

This was done to permit comparison with the reef pesticide target which is expressed in terms of the percentage of species protected.

The TPR_{22} estimates were classed as posing a very low, low, moderate, high or very high risk that corresponded to protecting >99 %, 95 to <99, 90 to <95, 80 to <90 and <80 of aquatic species, respectively. These risk classes were based on the ecological condition classes used in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC and ARMCANZ, 2000; ANZG, 2018) and the corresponding percentage of species to be protected i.e., 99 %, 95 %, 90 % and 80 %.

2.11. Calculation of the contribution of active ingredient groups to total pesticide risk

The contribution of individual PAIs to the TPR_{22} in each sample was calculated by:

$$\%\text{contribution} = \left(\%\text{affected species}_{y} / \%\text{affected species}_{\text{Total}}\right) \times 100 \quad (2)$$

where 'y' denotes an individual PAI and 'Total' is the TPR_{22}.

The contribution of the active ingredients in PSII Herbicides, Other Herbicides (refer to Table 1) and Insecticides to the TPR_{22} was determined using the same equation except that y denotes PSII Herbicides, Other Herbicides (refer to Table 1) or Insecticides groupings.

The resulting contribution values indicate which group of PAIs or individual PAIs contribute most to the estimated TPR_{22}, and therefore, should be the focus of management actions or policy initiatives to reduce the risk posed by PAIs in discharge to the GBR. It should be noted that these contribution values for individual PAIs and mixtures of PAIs are expressed as a percentage of the risk posed by the 22 PAIs included in the PRM, not all PAIs that might be present in water samples. When the percent affected species values were <1 % the contribution estimates for each PAI group and each PAI became unstable and were not included in the analysis of the contribution of PAI groups or individual PAIs to the TPR_{22}.

[2] In developing the multiple imputation method several distributions (including Log-Normal, Exponential, Weibull, Gamma, Beta and Kernel Density) were tested for their suitability. Both the Beta and Kernel Density distributions were flexible enough to fit the majority of site/year combinations; however, the Kernel Density was a better fit overall as it could deal with datasets with many zero values.

3. Results and discussion

Modality analysis of the toxicity data for each PAI indicated that eight were uni-modal (i.e., different types of organisms all had similar sensitivity) and that 14 were bi- or multi-modal (i.e., there was a marked difference in the sensitivity of different groups of organisms) (Table S10, Supplementary Material). SSDs for the unimodal PAIs were derived using toxicity data for all aquatic species, while for bi- or multi-modal PAIs the SSDs were derived using only toxicity data for the most sensitive group of organisms as recommended by Warne et al. (2018). The ETVs for uni-modal PAIs should theoretically protect the stated percentage of all aquatic species. While the ETVs for bi- or multi-modal pesticides should theoretically protect the stated percentage of the most sensitive group of organisms and protect a higher percentage of all aquatic species (as other organism types are less sensitive). For ease and consistency of interpreting the results, all estimates of the percent of species affected or protected were considered to apply to all aquatic species. Thus, irrespective of whether the pesticide was uni-, bi- or multi-modal the estimated TPR_{22} estimates (i.e., percentage of species affected) were compared to the pesticide target. The reliability of the ETVs for the 22 selected PAIs was low for two PAIs, moderate for eight PAIs, high for six PAIs and very high for six PAIs (Table S10, Supplementary Material).

3.1. Pesticides detected, frequency and concentrations

All 22 PAIs in the PRM were detected at least once in the 3211 samples that were collected and analysed (Table 11, Supplementary material). At individual sites the PAIs occurred in 0 % to 100 % of samples. Across all the samples, five PAIs occurred in <2 % of the samples (in ascending order chlorpyrifos, pendimethalin, prometryn and fipronil). Eleven PAIs occurred in 2 % to 50 % of the samples (in ascending order terbuthylazine, metsulfuron-methyl, simazine, triclopyr, haloxyfop, ametryn, metribuzin, tebuthiuron, isoxaflutole, MCPA, fluroxypyr and metolachlor). Imazapic, 2,4-D, MCPA, hexazinone, atrazine, diuron in ascending order all occurred in between 52 % and 75 % of the samples. The minimum concentrations for most PAIs were their LOR values.

For 11 PAIs the highest concentration was recorded at Barratta Creek (Table 11, Supplementary material), followed by Sandy Creek with six. In terms of median concentrations Sandy Creek had six PAIs with the highest median concentration, while Barratta Creek, the Fitzroy Haughton, and Proserpine rivers each had three PAIs with the highest median concentrations. The range of concentrations of the main contributors to TPR_{22} (see a following section) were: 10^{-4} to 2.4 μg/L for metsulfuron-methyl, imazapic, imidacloprid and triclopyr; 10^{-4} to ~10 μg/L for ametryn, MCPA and metolachlor; 10^{-4} to 19 μg/L for diuron and 10^{-4} to 52 μg/L for atrazine.

PAI concentrations were generally low in the dry season but rapidly increased with the first rainfall event that caused elevated river discharge (e.g., Water Quality and Investigations, 2020) (Figs. S1 to S5 Supplementary Material). The concentrations then typically dissipated during the wet season, returning to the dry season concentrations (Smith et al., 2011; Water Quality and Investigations, 2020). An exception to this typical pattern, was Barratta Creek, where high concentrations of multiple pesticides are often present before the wet season commences (Water Quality and Investigations, 2020). This occurs because the sugarcane in the Barratta Creek catchment is mainly irrigated (Davis et al., 2013; O'Brien et al., 2016). Another exception to the typical exhaustion pattern occurs when pesticides are re-applied during the wet season, leading to rapid increases in pesticide concentrations followed by the typical dissipation. In such cases, a plot of pesticide concentrations consists of a downward curve with one or more spikes like teeth on a saw.

3.2. Total pesticide risk (TPR_{22})

The TPR_{22} for the monitored waterways between 2015/2016 and 2017/2018 (Table 3) ranged from <1 % (very low risk) to 42 % (very high risk) of aquatic species theoretically experiencing adverse chronic sub-lethal effects (Table 2). The mean and median TPR_{22} estimates for the 68 site/year datasets (calculated by replacing the <1 % estimates by 0.5) were 8.0 and 4.5 % of aquatic species being affected respectively, which corresponds to posing a moderate and low risk, respectively. The Reef pesticide target is that at least 99 % of aquatic species are protected at the mouth of GBR waterways (Brodie et al., 2017; Australian Government and Queensland Government, 2018). However, most of the monitoring sites are located upstream of the mouth of waterways for logistical reasons and therefore estimate the risk at that monitoring point rather than the mouth of the waterway. Nonetheless, these provide the best available estimate of the risk posed at the river mouth. Only approximately 16 % of the monitored waterways met the Reef pesticide target (i.e., <1 % species affected, a very low risk), while for another 39 % of the waterways 1 to ≤5 % species would be affected (i.e., a low risk), leaving 44 % of waterways having >5 % to 42 % of species affected (i.e., a moderate to very high risk) in at least one year.

Caution is required when comparing msPAF estimates from this and other studies due to differences in the methods (e.g., acute versus chronic toxicity data, the number of chemicals included, the summary statistic of the msPAF estimates reported and the length of time covered by the msPAF estimates). Nonetheless, Gregorio et al. (2012) and Faggiono et al. (2010) reported maximum msPAF estimates of 3.2 % (in a Swiss lake) and 4.4 % (in south-west France) of species affected, respectively, which are consistent with the very low and low risk sites from the current study. In contrast, Silva et al. (2015) in Portugal, Rämö et al. (2018) in Costa Rica and Posthuma et al. (2019) in rivers throughout Europe all reported markedly higher msPAF estimates. Silva et al. (2015) reported msPAF estimates for primary producers ranging from 0 to 100 % and a median msPAF value of 49 % for three large Portuguese rivers. Rämö et al. (2018) used acute toxicity data and reported mean msPAF estimates of 1–10 % and maximum msPAF estimates of 5–75 % of primary producers. Posthuma et al. (2019) assessed the combined toxicity of up to 1760 chemicals, including many pesticides, in all 22,728 sub-catchments in the European Union. They estimated that most of the sub-catchments, except for large parts of Scandanavia, would have >25 % of aquatic species affected due to chronic chemical exposure and that numerous sub-catchments were estimated to have >50 % of aquatic species affected. In comparison, only in 9 % of monitored GBR catchments were >25 % of aquatic species estimated to be affected by chronic exposure and in no catchments were >50 % of aquatic species estimated to be affected.

msPAF estimates are estimates of the percent of species that should theoretically experience adverse environmental effects. A number of studies have compared msPAF estimates with biological effects in natural waterbodies in order to determine what msPAF estimates mean in the real-world. Posthuma and De Zwart (2006) found that msPAF estimates of between 10 and 50 % for fish corresponded to 10,000-fold change in the observed to expected fish species ratio in rivers in Ohio, USA. Posthuma and De Zwart (2012) estimated msPAF estimates for mixtures of 45 pollutants (no pesticides) using chronic NOEC data for Dutch freshwater that ranged from 0 % to ~90 %, which corresponded with up to 40 % of macroinvertebrates species experiencing a 50 % reduction in abundance and up to 30 % of macroinvertebrate species experiencing a 75 % reduction in abundance. They stated that 'acute toxic pressure (msPAF) was associated almost 1:1 with the observed fraction of taxa exhibiting an abundance reduction of 50% or more' (Posthuma and De Zwart, 2012). msPAF estimates of at least 5 % are considered to correspond to observable ecological changes in the field as at least 5 % of species are expected to experience harmful effects. However, Smetanova et al. (2014) found that changes to the SPEAR index, a measure of aquatic macroinvertebrate composition, commenced at msPAF estimates much lower than 5 %. They found that 5 and 10 % changes in the SPEAR index began at chronic toxicity msPAF estimates of 0.00023 % and 0.0013 %, respectively. Munz et al. (2017) found that acute toxicity msPAF estimates of between 0 and 2.1 % corresponded to decreases in SPEAR index estimates from 50 to 15 in Swiss rivers. These studies indicate that GBR waterways with msPAF estimates between 5 and 42 % of aquatic species are highly likely to have experienced large

Table 2
Total Pesticide Risk (TPR_{22}) estimated for each waterway and year with the percent contribution of Photosystem II inhibiting Herbicide Active Ingredients (PSII), Other Herbicide Active Ingredients (OH) and Insecticide Active Ingredients (I) to the TPR_{22}.

Waterway	Year	Total pesticide risk (% species protected)	% of TPR_{22}		
			PSII	OH	I
Baffle	2017/2018	<1	NC[a]	NC	NC
Barratta	2015/2016	19	65	27	9
	2016/2017	27	80	17	3
	2017/2018	22	82	17	1
Barron	2017/2018	<1	NC	NC	NC
Black	2017/2018	<1	NC	NC	NC
Boyne	2017/2018	<1	NC	NC	NC
Burdekin	2015/2016	<1	NC	NC	NC
	2016/2017	1	6	94	0
	2017/2018	<1	NC	NC	NC
Burnett	2015/2016	2	14	86	0
	2016/2017	2	12	88	0
	2017/2018	3	26	70	4
Burrum	2017/2018	<1	NC	NC	NC
Calliope	2017/2018	<1	NC	NC	NC
Comet	2015/2016	8	26	74	0
	2016/2017	9	36	64	0
	2017/2018	11	49	51	0
East Barratta	2017/2018	9	74	26	0
Elliott	2017/2018	5	34	48	18
Fitzroy	2015/2016	2	14	86	0
	2016/2017	2	3	82	14
	2017/2018	2	15	85	0
Gregory	2017/2018	8	47	39	15
Haughton	2015/2016	7	81	19	0
	2016/2017	6	69	31	0
	2017/2018	3	26	30	45
Herbert	2015/2016	3	26	10	64
	2016/2017	5	35	24	41
	2017/2018	4	22	16	62
Johnstone	2015/2016	3	21	15	64
	2016/2017	3	42	10	49
	2017/2018	5	34	18	48
Kolan	2017/2018	6	70	25	6
Mary	2015/2016	2	10	90	0
	2016/2017	3	12	88	0
	2017/2018	3	27	72	2
Mossman	2017/2018	3	57	36	7
Mulgrave	2015/2016	2	52	42	6
	2016/2017	4	56	37	7
	2017/2018	6	48	39	14
North Johnstone	2015/2016	2	2	3	95
	2016/2017	4	6	8	86
	2017/2018	2	6	1	94
O'Connell (Stafford's Crossing)	2016/2017	12	33	20	46
	2017/2018	8	47	23	29
O'Connell (Caravan Park)	2015/2016	8	23	26	51
	2016/2017	12	31	16	53
	2017/2018	8	45	24	31
Pioneer	2015/2016	18	54	16	29
	2016/2017	17	51	17	32
	2017/2018	25	67	13	19
Proserpine	2016/2017	27	39	20	40
	2017/2018	29	45	17	38
Russell	2015/2016	3	58	17	25
	2016/2017	4	63	21	16
	2017/2018	7	53	29	18
Sandy	2015/2016	40	55	18	27
	2016/2017	39	53	19	28
	2017/2018	42	56	21	23
Styx	2017/2018	<1	NC	NC	NC
Tinana	2015/2016	10	39	55	5
	2016/2017	4	37	60	4
Tully	2015/2016	5	41	10	49
	2016/2017	7	43	13	44
	2017/2018	7	53	8	39
Waterpark	2017/2018	<1	NC	NC	NC

[a] NC = not calculated. These percent contributions were not calculated as the TPR_{22} estimates are very small, therefore small absolute changes in PAI concentrations lead to very large changes in the percent contribution values.

Table 3
The percentage of Total Pesticide Risk (TPR_{22}) classifications, rounded to the nearest integer, that occurred in each Natural Resource Management (NRM) region.

NRM region	No. risk estimates	Percentage of values of each risk class in each region				
		Very low risk	Low risk	Moderate risk	High risk	Very high risk
Burdekin	11	36	27	9	9	18
Burnett Mary	12	8	75	17		
Fitzroy	10	40	30	20	10	
Mackay Whitsunday	14			21	36	43
Wet tropics	19	5	74	21		

biological change and waterways with msPAF estimates of <5 % (the very low and low risk classes used in the current study) may have experienced ecological changes to species that are sensitive to PAIs (e.g., aquatic algae and plants to herbicide AIs, and crustaceans and insects to insecticide AIs).

There have been limited studies that have sought to determine if PAIs in GBR waterways have measurable impacts on aquatic ecosystems. Wood et al. (2019) found a statistically significant negative relationship between the percentage of herbicide sensitive diatom algae species and the combined toxicity of pesticides present in 14 GBR waterways between 2011/2012 and 2012/2013. The combined pesticide toxicity was calculated using a toxic equivalency quotient (TEQ) method. They found that the number of sensitive diatoms expressed as a percentage of the total number of algae present decreased from 30 % to 10 % as the TEQ estimates increased in the 14 waterways. The % of herbicide sensitive diatom species was also inversely related to salinity and nutrient concentrations. The TEQ estimates are not directly comparable to the msPAF estimates from the current study, in addition, Wood et al. (2019) and the current study used pesticide monitoring data from different years. Nonetheless, the 14 waterways used in Wood et al. (2019) were all used in the current study and the average msPAF estimates for these waterways ranged from <1 % to ~40 %.

Kroon et al. (2015) found a strong relationship between extent of *vitellogenin* transcription (an indicator of endocrine disruption) in the liver of Barramundi fish (*Lates calcarifer*) and the percentage of the catchment where the fish were captured that was used to grow sugar cane. They also found significant relationships between the extent of *vitellogenin* expression and the concentrations of several PAIs applied to sugar cane in the waterways where the fish were captured. These endocrine disrupting effects observed by Kroon et al. (2015) in 2011/2012 occurred in seven waterways that were included in the current study. If the 14 sites used in Wood et al. (2019) and the seven waterways used by Kroon et al. (2015) had similar msPAF estimates to the same waterways considered in the current study (i.e., between 2015/2016 and 2017/2018), then biological effects could be expected to occur in these waterways in the current study.

Stone et al. (2021) used a multi-species (three) algae toxicity test to determine the toxicity of water from the Tully River and Sandy Creek during the 2016 wet season. Samples were collected between 15 and 19 January (during the wet season) and 18 and 19 April (at the tail end of the wet season) 2016. They had selected the Tully River to act as a control, but they observed statistically significant inhibition of algal growth for one of the three algae species in the samples from both sampling times. In addition, significant decreases in algal growth occurred for two of the three algae species in three of the four samples from Sandy Creek. The wet-season msPAF estimates for those two waterways in 2016 were 5 % and 40 % for the Tully River and Sandy Creek, respectively. The results of Kroon et al. (2015), Wood et al. (2019) and Stone et al. (2021) are consistent with those for non-GBR waterways that show biological effects occurring within the range of msPAF estimates observed in the current study.

There was little temporal variation in the TPR_{22} estimates (Table 3). Of the 19 waterways with TPR_{22} estimates for multiple years, only three had estimates that differed in absolute terms by >4 % — these were for Tinana Creek (4 to 10 % of aquatic species affected), Barratta Creek (19 to 27 % of aquatic species affected) and the Pioneer River (17 to 25 % of aquatic species affected). The three waterways where TPR_{22} varied by >4 % did not

change monitoring site so, these temporal changes might reflect changes in pesticide use or the effects of annual climatic variation. The temporal variation at Barratta and Tinana creeks was not related to a clear change in the percent contribution of PAI groups to the TPR_{22} (Table 3). However, in the Pioneer River the increase in TPR_{22} was associated with an absolute increase of 13 % in the contribution of PSII AIs and a commensurate decrease in the contribution of Insecticide AIs (Table 3).

There was clear spatial variation in the TPR_{22} estimates (Table 3). Based on the percentage of datasets with each risk classification (Table 3) the risk was lowest in the Fitzroy region, slightly higher in the Burnett Mary and Wet Tropics regions, higher in the Burdekin region and markedly higher in the Mackay Whitsunday region. In terms of major land uses the Burnett Mary, Fitzroy and Burdekin are all dominated by grazing (over 70 %), which is not a large user of pesticides apart from tebuthiuron. The higher TPR_{22} associated with the Burdekin compared to the Burnett Mary and Fitzroy is most likely due to the concentration of sugar cane in the Barratta and East Barratta creeks, located in the coastal plain. The percentage of the Wet Tropics and Mackay Whitsundays devoted to sugar cane is considerably higher than in the other regions. The risk in the Mackay Whitsundays is greater as the percentage of sugar cane is double that in the Wet Tropics with half the rainfall.

3.3. Contribution of active ingredient groups to total pesticide risk

Sites with TPR_{22} estimates of <1 % tended to have very small PAI concentrations and small absolute changes in the concentrations of PSII AIs, Other Herbicide AIs and Insecticide AIs made large changes to their percent contribution values. In such cases, the contributions of pesticide groups (PSII AIs, Other Herbicide AIs and Insecticide AIs) to TPR_{22} were deemed to be unstable and therefore were not reported. Of the remaining TPR_{22} estimates (i.e., ≥1 %), the percent contribution of PSII AIs, Other Herbicide AIs and Insecticide AIs were found to be highly variable (Table 3). The contribution of PSII AIs ranged from 2 % to 82 %, Other Herbicide AIs ranged from 1 % to 94 % and Insecticide AIs ranged from 0 to 95 %. Approximately 46 % of the TPR_{22} estimates had PSII AIs as the largest contributor, 30 % had Other Herbicide AIs as the largest contributor, 17 % had Insecticide AIs as the largest contributor and the remainder had either PSII and Insecticide AIs or PSII and Other Herbicide AIs combined as the main contributors (Table 3). Comparisons of the contributions of various PAI groups in the current and other studies was not possible – as the individual pesticides and groupings of pesticides were not the same.

The percent contribution of the PAI groups to the TPR_{22} for each waterway generally showed little variation overtime (Table 3) — although there were some noticeable exceptions including the Haughton and O'Connell (both sites) rivers. Some waterways did not have large temporal changes but exhibited a consistent trend. For example, in the Barratta Creek, Burnett River, and Comet River the contribution of PSII AIs increased with commensurate decreases in Other Herbicide AIs and/or Insecticide AIs.

3.4. Contribution of individual active ingredients to total pesticide risk

Based on their average contribution across all waterways nine PAIs (in descending order of importance: diuron, imidacloprid, metolachlor, atrazine, MCPA, imazapic, metsulfuron-methyl, triclopyr and ametryn) were responsible for >99 % of TPR_{22} (Fig. 2). With the addition of another five PAIs (in descending order of importance: fipronil, isoxaflutole, hexazinone, chlorpyrifos and 2,4-D), on average, at least 99.9 % of the TPR_{22} was accounted for. Ten of the 22 PAIs, on average, each contributed <0.1 % of the TPR_{22}.

A similar analysis but based on the ranking of each PAI's contribution resulted in a similar list of the main contributors to TPR_{22}. Eight of the nine main contributing PAIs were the same, but metsulfuron-methyl was replaced by chlorpyrifos and the order of each PAI's contribution differed between the two analyses.

Spilsbury et al. (2020) also analysed pesticide monitoring data generated by the GBRCLMP at many of the same sites for the period 2011/

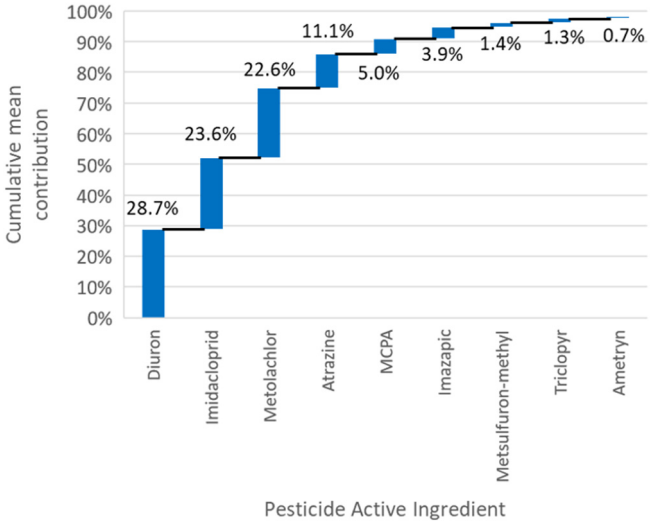


Fig. 2. Mean contribution and cumulative mean contribution of individual pesticide active ingredients to the Total Pesticide Risk (TPR_{22}) across all monitored Great Barrier Reef waterways. Samples where the TPR_{22} were <1 % were not included.

2012 to 2015/2016 – the five years immediately before the data used in the current study. They used the RQ method for determining the risk posed by pesticide mixtures. They also found that the vast majority of the risk was caused by a limited number of PAIs (i.e., 95 % was caused by seven PAIs and 99 % caused by 15 PAIs). They identified the seven main contributors (in descending order) as diuron, imidacloprid, atrazine, metolachlor, hexazinone, imazapic and isoxaflutole. While the current study and Spilsbury et al. (2020) identified many of the same PAIs as the main contributors, with some changes in their contribution, the main difference was the contribution by diuron which was ~27.5 % in the current study but 45.7 % in Spilsbury et al. (2020). This large change in the contribution of diuron could be caused by the use of different methods (RQ in Spilsbury et al. and msPAF in the current study), the introduction of greater restrictions on its use in January 2014 (King et al., 2013) but it could also be due to increased use of other herbicide AIs.

Posthuma et al. (2019) found that 5 and 15 chemicals, only one of which (chlorpyrifos) was a PAI, accounted for >96.6 % and ~99.8 % of the mixture toxicity in EU waterways, respectively. Rämö et al. (2018) found that just three PAIs (ametryn, diuron and difenoconazole in decreasing order) accounted for ~96 % of risk posed by pesticide mixtures to primary producers and five PAIs (chlorpyrifos, diazinon, ethoprophos, difenoconazole and carbaryl in decreasing order) accounted for >99 % of risk posed by pesticide mixtures to fish and arthropods. Munz et al. (2017) found that five PAIs, which always included clothianidin, diazinon and diclofenac, accounted for all the risk posed by pesticide mixtures in Swiss rivers. Schuler and Rand (2008), Price and Han (2011), Backhaus and Karlsson (2014), Gustavsson et al. (2017) and Makert et al. (2020) also found that between one and eight chemicals contributed to mixture risk in a range of countries in Europe and North America.

The above results (Fig. 2) could be interpreted to indicate that policy and management actions need only focus on these nine PAIs. However, there is considerable variation in the contribution of each PAI to TPR_{22} and to the risk of each water sample collected (Fig. 3). For example, the contribution of atrazine, MCPA and metolachlor in individual water samples all range from essentially zero to 100 %. The contribution of some of the minor contributors to TPR_{22} was also highly variable, e.g., isoxaflutole had an average contribution of 0.16 % but it contributed up to 33 % in one sample. In fact, 17 of the 22 PAIs contributed up to 5 % of TPR_{22} in at least one sample.

The variation in the contribution of each PAI to TPR_{22} observed in Fig. 3 also occurred at the NRM region scale (Fig. 4) and in individual waterways (Fig. 5). For example, the largest contributors to TPR_{22} in the Mackay Whitsunday and Wet Tropics regions are diuron and imidacloprid, whereas in

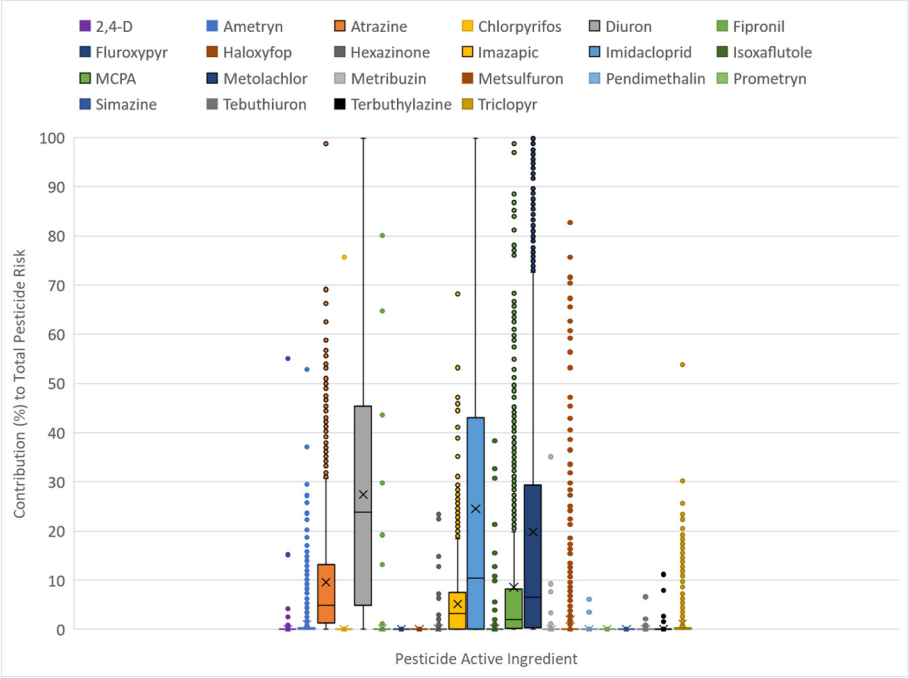


Fig. 3. Variation in the contribution (percent) of individual pesticide active ingredients to the Total Pesticide Risk (TPR_{22}) for all water samples collected except where the TPR_{22} was <1 %. The lower and upper edges of the boxes are the 25th and 75th percentiles. The cross and horizontal line within the boxes are the mean and median estimates. The lower and upper whiskers are the 5th and 95th percentile estimates. Estimates above the upper whisker are greater than the 95th percentile.

the Burnett and Fitzroy regions the main contributor is metolachlor. In individual waterways the main contributor to TPR_{22} was at times atrazine, diuron, imidacloprid, MCPA or metolachlor. This variation most likely reflects the different types and amounts of agriculture in regions and catchments and regional differences in pesticide spraying regimes even for the same crop.

These results point to a dilemma – whether to have a single, simple pesticide risk reduction plan that focusses on the nine or ten most important PAIs across all the monitored waterways or to have multiple plans that focus on the most important PAIs for a particular region or catchment. While focussing management actions on only the nine main PAIs contributing to the TPR_{22} should decrease the overall risk posed by PAIs, it will not address all situations, could misdirect management actions, cause undesired consequences and lead to stakeholders losing faith in the underpinning science. Clearly, pesticide reduction strategies need to be targeted. The finer the spatial resolution of the reduction strategies the more relevant the strategies will be, but the greater the potential confusion amongst stakeholders due to there being multiple strategies.

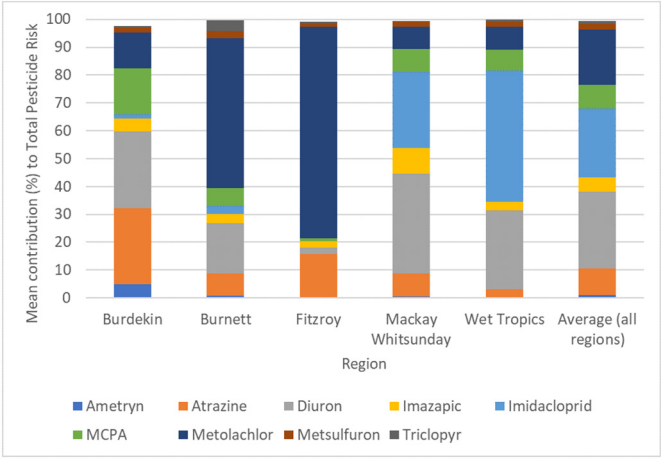


Fig. 4. Mean contribution (percent) of individual pesticide active ingredients to the Total Pesticide Risk (TPR_{22}) in waterways of each Natural Resource Management Region and the average across all regions. Waterways where the TPR_{22} was <1 % were not included.

It should be noted that the current version of the Pesticide Risk Metric has a number of limitations. First, all the above discussion of the contribution of individual PAIs only relates to the 22 PAIs included in the Pesticide Risk Metric. While these are some of the most frequently detected PAIs in waterways discharging to the GBR lagoon they represent only a modest percentage of PAIs that are applied to land (agricultural, commercial, industrial and urban) that is adjacent to the GBR. For example, 47 PAIs are registered for use on sugar cane in Queensland, Australia (Warne et al., 2020; APVMA, 2019) and another 52 are registered for application to four major crops (i.e., soybean, mung bean, rice and corn) grown in rotation with sugar cane (Warne et al., 2020; APVMA, 2022). Given, 99 PAIs can be applied to land used for sugar cane, it is highly likely that considerably more PAIs will be applied on land adjacent to the GBR lagoon where sugar cane is just one, albeit large, agricultural crop. It is quite possible that a number of these additional PAIs could, currently or in the future, contribute significantly to the TPR_{22}.

The second limitation is that the Pesticide Risk Metric predominantly includes PAIs that are registered for application to sugar cane; 17 out of the 22 PAIs included can be applied to sugar cane. The five other PAIs (metsulfuron-methyl, prometryn, simazine, tebuthiuron and triclopyr) included in the calculations can be used for a number of landuses and crops. Metsulfuron-methyl, simazine and triclopyr can be used in forestry, management of native woodlands, commerce and industry. Tebuthiuron is not registered for application to any crops in Australia; rather, its main use is controlling woody plants in grazing. Metsulfuron-methyl, prometryn, simazine and triclopyr can also be used on a variety of horticulture crops, but the use of prometryn is the most restricted. As such, these five pesticides give an incomplete indication of contribution of forestry and native woodlands, horticulture and commerce and industry to the risk measured by the Pesticide Risk Metric. The number of PAIs included in the Pesticide Risk Metric is currently being expanded and this will improve the representivity of the various agricultural sectors.

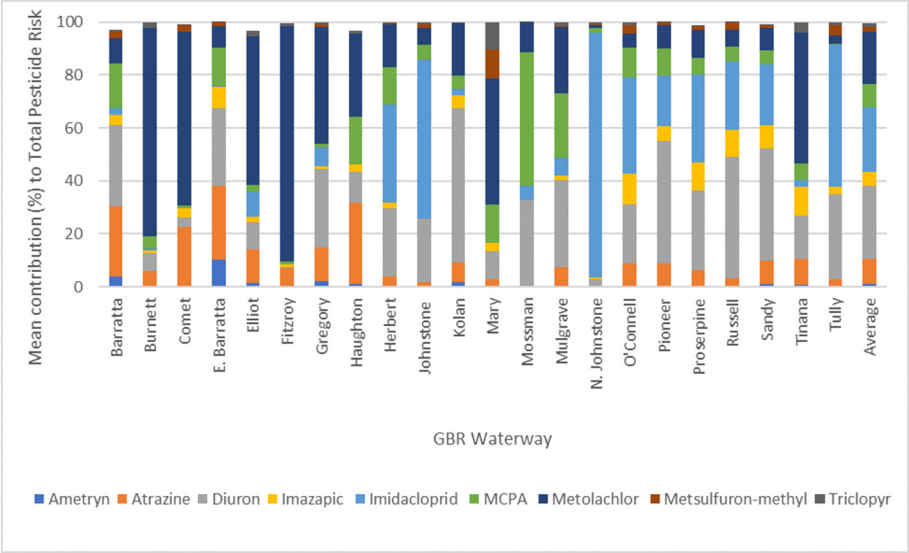


Fig. 5. Mean contribution (percent) of individual pesticide active ingredients to the Total Pesticide Risk (TPR_{22}) in individual waterways and the average across all Great Barrier Reef waterways. Waterways where the $TPR_{22}$was <1 % were not included. Only the nine main contributors to TPR_{22} are included.

Third, only three groups of PAIs are currently included in the PRM – PSII Herbicide AIs, Other Herbicide AIs and Insecticide AIs. No AIs of fungicides, rodenticides, miticides, or nematocides are included. The existing PAI groupings could also be refined and divided into small groups based on mode of action. Including more pesticide groups would provide a more comprehensive analysis of which groups of PAIs are contributing most to the TPR_{22} and that should therefore be the focus of management actions. Fourth, there is a bias in the number of PAIs included in each PAI group. For example, there were only 3 Insecticide AIs, 10 Other Herbicide AIs and 9 PSII herbicide AIs. Until all or most AIs used in pesticides applied to land adjacent to the GBR are included in the Pesticide Risk Metric, the contributions of individual PAIs and groups of PAIs should be considered as indicative/preliminary.

The contribution of individual PAIs to the TPR_{22} provides information that can be used to change management practices, for example, changing the suite of PAIs applied to a farm with the aim of decreasing the TPR_{22} to a neighbouring waterway. This could be done by identifying the risk posed by alternate PAIs and if appropriate (based on cost and efficacy) then these lower risk PAIs could be substituted for the higher risk PAIs. Such a system, called the Pesticide Decision Support Tool (PDST) (Warne et al., 2022) has been developed for the Queensland Sugar Cane industry. The PDST has been implemented as part of a large scale (10,500 ha) land management change project (Project Bluewater funded by the Great Barrier Reef Foundation). By providing financial support for upgrading and improving spray equipment and developing pesticide management plans for each paddock of a farm using the PDST, large reductions in both the total amount of herbicides applied (approximately 30 %) and the risk they pose to aquatic ecosystems (approximately 50 %) have been achieved over two years. The work on Project Bluewater will be the subject of a subsequent paper. Suffice to say that identification of the main contributors of pesticide risk and identifying and applying PAIs that pose a lower risk to aquatic ecosystems can make dramatic reductions in the risk posed by pesticides and improvements to water quality and presumably ecosystem health.

4. Conclusions

A method for estimating the total toxicity of 22 pesticide active ingredients (PAIs) commonly detected in waterways that discharge to the Great Barrier Reef has been developed using the multi-substance potentially affected fraction method. The pesticide reduction target set to improve the health and resilience of the GBR ecosystems (>99 % of aquatic species being protected) was met at only 15 % of the 68 datasets from 28 sites measured over three years. It was estimated that <1 to 42 % of aquatic species in GBR waterways would experience adverse effects due to PAIs, with an average of 8 % and a median of 4.5 % of aquatic species predicted to be affected. However, these are likely to underestimate the risk posed by all PAIs and markedly underestimate the risk posed by all pollutants in GBR waterways as these estimates were based only on the presence of 22 PAIs. International studies that compared msPAF estimates with measured biological effects suggest that the 45 % of monitored GBR waterways with msPAF estimates between 5 and 42 % of aquatic species are highly likely to have experienced large biological changes and the 55 % of monitored GBR waterways with msPAF estimates of <5 % probably experienced ecological changes to species that are sensitive to pesticides (e.g., aquatic algae, crustaceans, insects and plants). This prediction is supported by the limited number of studies conducted in GBR waterways. There were marked spatial differences in TPR_{22} estimates; regions dominated by grazing had lower estimates than those where sugar cane was a significant land use. On average 39 % of the TPR_{22} estimates was contributed by PSII herbicide AIs, ~36 % was contributed by Other Herbicide AIs and ~ 24 % was contributed by insecticide AIs. Nine PAIs (diuron, imidacloprid, metolachlor, atrazine, MCPA, imazapic, metsulfuron-methyl, triclopyr and ametryn) were responsible for >97 % of TPR_{22} across all the monitored waterways.

CRediT authorship contribution statement

MStJW and CN conceived the project and conducted initial data analysis. MStJW and CN did the initial data analysis, while CN did the final statistical analyses. MStJW calculated the species sensitivity distributions and the ecotoxicity threshold values for the pesticide active ingredients. RS developed an earlier msPAF method for five photosystem II inhibiting herbicides. All authors contributed to the development of the method and to the writing and reviewing of drafts and the final version.

Data availability

Data will be made available on request.

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.

Acknowledgements

This project involved contributions and support from numerous people and organisations. These include: Dr. Melanie Shaw (DNRME) for information on Source Catchment models, Rohan Wallace (Queensland Department of Environment and Science, DES) for information on sites and sampling design, Marije Ten Napel (DES) for providing the shapefiles for catchments, basins, and regions, the associated percent land use data and the maps. We also greatly appreciated the computational support from the Remote Sensing Group of DES. The authors acknowledge all the members of the Water Quality and Investigations Unit that conducted the monitoring that generated the pesticide concentration data used in this project: Leigh Anderson, Ben Ferguson, Shaun Fisher, Dane Goddard, Marije Ten Napel, Rohan Wallace, Stephen Wallace, David N Orr, Rae Huggins, Cameron Roberts, Sarah Simpson, Kylee Welk, Olivia King, Jessica-Lee Orreal, Theresa Gayle. Special thanks for Jordan Glen (also of WQI) for providing Fig. 1. We gratefully acknowledge the review, suggestions and support of the Reef Independent Science Panel (ISP) (Prof. Roger Shaw, Dr. Eva Abal, Dr. Andrew Ash, Dr. Graham Bonnett, Dr. Peter Doherty, Professor Bronwyn Harch, Dr. Jenny Stauber, Mr. Hugh Yorkston), the support of Nyssa Henry and Carl Mitchel of the Office of the Great Barrier Reef. We also thank Prof. Bronwyn Harch (University of Queensland and ISP) for statistical advice.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.164632.

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