Development of a Method for Estimating the Toxicity of Pesticide Mixtures and a Pesticide Risk Baseline for the Reef 2050 Water Quality Improvement Plan

Reef 2050 Water Quality Improvement Plan

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Glossary

Term	Definition
Basin	An area of land that contains one or more catchments. Examples of basins in the Great Barrier Reef Catchment Area include the Mulgrave-Russell basin that includes the Mulgrave River catchment and the Russell River catchment; the Johnstone basin that includes the North Johnstone River catchment and South Johnstone River catchment; the Haughton basin that includes the Haughton River catchment and Barratta Creek catchment.
Bi-modal distribution	A distribution of data that has two distinctly different peaks.
Catchment	An area of land where all surface water drains to a single point of discharge to marine waters. Examples of catchments in the Great Barrier Reef Catchment Area include the Mulgrave River catchment and the Russell River catchment.
Default guideline values (DGVs)	The numerical values recommended to provide protection to environmental values of Australia's water resources. For chemical toxicants such as pesticides, DGVs are the aqueous concentrations recommended to provide an appropriate degree of protection for aquatic ecosystems (see protective concentrations).
Direct effects	Direct effects are those effects on plants and animals that are directly caused by exposure to a toxicant. Toxicity tests where a test species is exposed to a toxicant measure the direct effects of that toxicant. Direct effects are opposite to indirect effects.
GBR	Great Barrier Reef
Great Barrier Reef Catchment Area (GBRCA)	All the land where the surface water is discharged to the marine waters of the Great Barrier Reef World Heritage Area. There is a spatial hierarchy used in this report. The GBRCA contains 6 Natural Resource Management (NRM) regions. The 6 NRM regions contain 35 basins. Each basin may contain one or more catchments.
Great Barrier Reef Catchment Loads Monitoring Program (GBRCLMP)	A program conducted by the Queensland Department of Environment and Science. It monitors total suspended solids, nine forms of nutrients and a suite of pesticides in selected creeks and rivers that discharge to the Great Barrier Reef lagoon. Further information can be obtained from: https://arcg.is/1TG9e1
Indirect effects	Indirect effects are those effects on plants and animals that are mediated by one or more other species (Preston, 2002). For example, a toxicant may directly affect species A but have no direct effect on species B. But, species B may be indirectly affected if, for example, species B is highly dependent on species A as a food source.

Term	Definition
Insecticides	The term used for insecticides that are included in the Pesticide Risk Metric. The Insecticides are: chlorpyrifos, fipronil and imidacloprid.
Insecticide toxicity	An estimate of the toxicity exerted by Insecticides present in water samples (see definition of Insecticides). The Insecticide toxicity is estimated by the Pesticide Risk Metric.
Multiple imputation	This is a well established statistical method for dealing with missing data while also allowing for the uncertainty of the missing values. It fits a statistical distribution to the observed data and randomly samples values from this distribution according to the number of missing values. This is done a large number of times (typically 1000 or more) to account for the variability of these unknown values. Each imputed dataset is then combined with the observed data to estimate values for parameter of interest. These multiple datasets are then aggregated to estimate an overall average and confidence intervals for the parameter of interest.
Multi-substance potentially affected fraction (msPAF)	This is the fraction (actually the proportion) of species that are estimated will experience adverse effects (toxicity) for a certain mixture of chemicals. In this report the term msPAF is not used, rather the term pesticide mixture toxicity is used.
Natural Resource Management Region (NRM region)	Areas of land and water that have been designated by state and territory governments and are recognised by the Australian Government. Within each NRM region is an organisation responsible for managing the natural resources of the region. There are six NRM regions that contain waterways that discharge to the Great Barrier Reef; these are Cape York, Wet Tropics, Burdekin, Mackay Whitsundays, Fitzroy and the Burnett Mary.
Other Herbicides	A term used to denote all herbicides other than PSII herbicides that are included in the Pesticide Risk Metric. The Other Herbicides are: haloxyfop, imazapic, metsulfuron-methyl, pendimethalin, metolachlor, 2,4-D, MCPA, fluroxypyr, triclopyr, isoxaflutole.
Other Herbicides toxicity	An estimate of the toxicity exerted by Other Herbicides that are present in a water samples (see definition of Other Herbicides). The Other Herbicide toxicity is estimated by the Pesticide Risk Metric.
Pesticide Risk Baseline	An estimate of the risk posed by Total Pesticides (all 22 selected pesticides) to aquatic ecosystems expressed as the percentage of protected species for the period 2015/2016 to 2017/2018. The Pesticide Risk Baseline values are compared to the Pesticide Target to determine if the target has been met or further action is required to meet the target. The Pesticide Risk Baseline is estimated for 35 basins, 6 Natural Resource Management regions and the entire Great Barrier Reef

Term	Definition
	Catchment Area.
Pesticide Risk Metric	The group of methods used to estimate the risk posed by 22 pesticides to aquatic ecosystems. The components of the metric are: combining species sensitivity distributions (SSDs) using the method developed by Traas et al. (2002), the independent action model of joint action and multiple imputation. The risk is expressed as the average daily per cent of species affected during the wet season. The risk is estimated for Photosystem II inhibiting Herbicides, Other Herbicides, Insecticides and Total Pesticides (all 22 pesticides).
Pesticide Target	The acceptable level of risk that can be posed by pesticides at the mouth of waterways that discharge to the Great Barrier Reef lagoon by 2022. The target is to protect at least 99% of aquatic species from the harmful effects of pesticides at the mouth of waterways that discharge to the GBR lagoon. By requiring the target be met at river mouths ensures that the entire Great Barrier Reef will receive at least this level of protection.
Protective concentrations	The aqueous concentration of a chemical that, if not exceeded, should protect a specified percentage of species in an aquatic ecosystem. In Australia, there are four protective concentrations that comprise the Default Guideline Values. These aim to protect 99% of species (PC99), 95% of species (PC95), 90% of species (PC90) and 80% of species (PC80).
PSII Herbicides	These are herbicides that inhibit the photosystem II component of the photosynthetic process. Specifically, they bind to the plastoquinone B (QB) protein binding site on the D1 protein in PSII which prevents the synthesis of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) and therefore prevents the conversion of CO_2 to carbohydrates. PSII Herbicides included in this Pesticide Risk Metric are: ametryn, atrazine, diuron, hexazinone, metribuzin, prometryn, simazine, tebuthiuron and terbuthylazine.
PSII Herbicide toxicity	An estimate of the toxicity exerted by PSII Herbicides that are present in water samples (see definition of PSII Herbicides). The PSII Herbicide toxicity is estimated by the Pesticide Risk Metric.
Reef Water Quality Report Card	A report card, usually released annually, that reports on progress made to meeting the catchment and water quality targets set out in the Reef 2050 Water Quality Improvement Plan.
Reef 2050 Water Quality Improvement Plan (WQIP)	A plan jointly developed by the Australian and Queensland governments that sets out to improve the quality of water entering and in the Great Barrier Reef lagoon. It has an overall goal and catchment and water quality targets that are to be achieved and help drive improvement.

Term	Definition
Species sensitivity distribution (SSD)	A cumulative frequency plot of the sensitivity of species to a chemical. Each species is represented by a single value. Using the SSD the percent of species that should be protected (or affected) for any chemical concentration can be determined. Alternatively the SSD can be used to determine the chemical concentration that should not be exceeded in order to protect a selected percentage of species.
Sum of Toxic Units (ΣTU)	A measure of the hazard that mixtures of chemicals pose. It is the combined hazard for every chemical in the mixture (refer to Toxic Units).
Total Pesticides	The 22 pesticides that are included in the Pesticide Risk Metric. These are: 2,4-D, ametryn, atrazine, chlorpyrifos, diuron, fipronil, fluroxypyr, haloxyfop, hexazinone, imazapic, imidacloprid, isoxaflutole, MCPA, metribuzin, metolachlor, metsulfuron-methyl, pendimethalin, prometryn, simazine, tebuthiuron, terbuthylazine and triclopyr.
Total Pesticides Toxicity	An estimate of the toxicity of Total Pesticides that are present in water samples (see definition of Total Pesticides). The Total Pesticide toxicity is estimated by the Pesticide Risk Metric.
Toxic pressure	The toxic effect exerted by individual chemicals expressed as the percentage of affected species. This is equivalent to the phrase 'pesticide toxicity' that is used in this report.
Toxic mixture pressure	The toxic effect exerted by mixtures of chemicals expressed as the percentage of affected species. This is equivalent to the phrase 'pesticide mixture toxicity' that is used in this report.
Toxic unit(s) (TUs)	A toxic unit (TU) is a measure of the hazard that a chemical poses. It is calculated as the measured or predicted environmental concentration (MEC or PEC, respectively) divided by a measure of toxicity of the chemical (e.g. EC50 or a Default Guideline Value, DGV). A TU of greater than one means that the chemical is present at a hazardous concentration, that a toxic effect could have occurred or that the DGV has been exceeded, while values less than one means that the toxic effect could not have occurred or the DGV has not been exceeded.
Uni-modal distribution	A distribution of data that has a single peak.

Executive Summary

The Reef 2050 Water Quality Improvement Plan 2017–2022 (Reef 2050 WQIP) released in 2018 adopted a new water quality target for pesticides. The target is to protect at least 99% of aquatic species at the end of catchments from the harmful effects of all pesticides that discharge to the Great Barrier Reef (GBR). A new baseline (starting point) that stated the current condition of waterways that discharge to the GBR using the same units as the new pesticide target, was required in order to measure progress towards the target. In addition, published and unpublished work has shown relationships between the effects and concentrations of pesticides and land use in catchments. Therefore, pesticide monitoring data were used to develop the baseline. Twenty-two pesticides regularly detected in waterways discharging to the GBR were included in the baseline. The pesticide monitoring data were subsequently used to estimate the toxicity of mixtures of photosystem II inhibiting (PSII) Herbicides, Other Herbicides, Insecticides and then all 22 pesticides combined (Total Pesticides). The method for estimating the toxicity of pesticide mixtures uses three established and published methods — the combining of species sensitivity distributions (SSDs) developed by Traas et al. (2002), the independent action (IA) model of joint action, and multiple imputation. The combining of SSDs and IA methods are used to estimate the combined toxicity of pesticide mixtures in each water sample, which is expressed as the percentage of species affected (also referred to as the multi-substance potentially affected fraction - msPAF). Multiple imputation is used to provide estimates for missing values in order to calculate the average per cent of species affected during the wet season for PSII Herbicides[1], Other Herbicides[2], Insecticides[3] and Total Pesticides[4]. The resulting pesticide mixture toxicity data were divided into two datasets. The larger dataset, containing 80% of the data (the training dataset), was used to develop pesticide mixture toxicity — land use relationships. The smaller dataset, containing 20% of the data (the validation dataset), was used to determine the accuracy of the predictions from the best pesticide mixture toxicity — land use relationships. The pesticide mixture toxicity values in the training dataset were regressed, using forward and backward step-wise regression, against spatial, hydrological and land use variables to develop relationships able to predict pesticide mixture toxicity for the monitored catchments and then scaled up to predict the pesticide mixture toxicity to 35 basins, six natural resource management (NRM) regions and the entire GBR Catchment Area[5] (GBRCA) that are reported on in the Reef Water Quality report cards.

A rigorous process was followed to identify the best pesticide mixture toxicity — land use relationship for each group of pesticides: PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides. Adjusted coefficients of determination (R^2)[6] values ranged between 0.68 and 0.79 for the final pesticide mixture

[1] PSII herbicides were ametryn, atrazine, diuron, hexazinone, metribuzin, prometryn, simazine, tebuthiuron and terbuthylazine.

[2] Other Herbicides were 2,4-D, fluroxypyr, haloxyfop, imazapic, isoxaflutole, MCPA, metolachlor, metsulfuron-methyl, pendimethalin and triclopyr.

[3] Insecticides were chlorpyrifos, fipronil and imidacloprid.

[4] Total Pesticides were all the 22 pesticides (i.e. PSII Herbicides, Other Herbicides and Insecticides)

[5] The GBRCA is all the land that is drained by waterways that discharge to the Great Barrier Reef lagoon.

[6] The adjusted R^2 value differs from the R^2 value by taking into account the number of variables used in the relationship. The adjusted R^2 value will only increase if additional variables that are added to a relationship actually increase the ability of the relationship to explain the data. In contrast, the R^2 will automatically increase when additional variables are added, irrespective of whether the additional variable increases the ability of the relationship

toxicity – land use relationships, meaning that the explanatory variables explained between 68% and 79% of the observed variation in pesticide mixture toxicity data for the monitored catchments in the training set. The explanatory variables used in these relationships were a combination of spatial, climate and land use variables, although the land use variables were the most common variables in the relationships. The per cent of land in a catchment that was used for horticulture and sugar cane were explanatory variables in the relationships for all four groups of pesticides. Per cent conservation was included in three of the relationships, while per cent urban and per cent dryland cropping were in two relationships.

The best pesticide mixture toxicity – land use relationships were validated by using them to predict the mixture toxicity values of catchments in the validation set and comparing them to the measured values. This showed that the relationships could accurately predict mixture toxicity (i.e., the mean absolute errors between the predicted and actual pesticide mixtures were 1.72, 0.68, 1.00 and 2.78 for PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides, respectively). The relationships were then used to predict the toxicity of PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides for 35 basins, the six NRM regions in the GBRCA and for the GBRCA as a whole. The predicted mixture toxicity values were assessed for reasonableness using several lines of evidence. In most cases the predicted toxicity mixture values were reasonable as they differed only slightly from expected values. The predicted pesticide mixture toxicity values were divided into five risk classes, based on ecological condition classifications used in the Australian and New Zealand Guidelines for Fresh and Marine Water (ANZG, 2018) (i.e., very low[7], low, moderate, high and very high). Maps showing the classification of predicted risk for Total Pesticides, PSII Herbicides, Other Herbicides and Insecticides for the 35 basins were generated (Figure I to Figure IV) and maps for Total Pesticides for the NRM regions and the GBRCA were generated (Figure V and Figure VI).

Key findings from this project are:

- The toxicological risk posed by Total Pesticides was very low for all basins in the Cape York region, very low or low for all basins in the Fitzroy region and mainly very low and low in basins of the Burdekin region (although one basin had a high risk) and most basins in the Burnett Mary region had a low risk (although one basin had a very low risk and another had a moderate risk). Most of the basins in the Wet Tropics faced a moderate risk (but two had a very low risk) while two basins in the Mackay Whitsunday region faced a very high risk, one faced a high risk and another faced a moderate risk.
- The toxicological risk posed by PSII Herbicides for basins was generally very low or low. Two small coastal basins faced moderate risk (one each in the Wet Tropics and Burdekin regions), and two faced a high risk (both in the Mackay Whitsunday region).
- The toxicological risk posed by Other Herbicides was very low or low for all basins with the exception of the Plane basin (Mackay Whitsunday region) that faced a moderate risk.
- The toxicological risk posed by Insecticides was very low for all basins except for all basins in the Mackay Whitsunday regions and one basin in the Wet Tropics.

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to explain the data. The adjusted R^2 is the metric that should be used when assessing multiple regression relationships.

[7] very low (≥ 99% of species protected), low (95% to < 99% of species protected), moderate (90% to < 95% of species protected), high (80% to < 90% of species protected) and very high (< 80% of species protected.

- At a regional level, Cape York faces a very low risk from Total Pesticides, while the Wet Tropics, Burdekin, Fitzroy and Burnett Mary NRM regions face a low risk. The Mackay Whitsunday NRM region faces a high risk.
- At the GBRCA level the risk faced from Total Pesticides is low.
- At the basin level the contribution of the three groups of pesticides was highly variable with no one group being dominant.
- The contribution of the three groups of pesticides was also highly variable at the regional level – with the contribution of PSII Herbicides ranging from 35 to 88%, Other Herbicides ranged from 0 to 65% and Insecticides ranged from 0 to 17%.
- At the GBRCA level the median contribution of PSII Herbicides to the Total Pesticide mixture toxicity was approximately 47% compared to 32% for Other Herbicides and 17% for Insecticides[8]. Thus, PSII Herbicides were the dominant pesticide group in terms of toxicity in the waterways discharging to the Great Barrier Reef lagoon.

Care needs to be exerted in interpreting the basin, region or GBRCA mixture toxicity values as they are aggregate or summary values and may not reflect the risk faced by particular waterways at finer spatial scales. For example, a region with a low risk could contain one or more basins and/or catchments that face a markedly higher or lower risk. This occurs in the Burdekin NRM region, which faces a low risk from Total Pesticides, but the Haughton basin faces a high risk from Total Pesticides. Similar situations occur in the Wet Tropics, Mackay Whitsundays and Burnett Mary regions.

The pesticide mixture toxicity values are not absolute values, rather, they are estimates of the risk posed. Thus, an estimate of 95% species protection should not be interpreted literally to mean that exactly 95% of species will be protected. Rather, the estimates were developed to determine if the pesticide target has been met or whether further land management change is required to reduce pesticide run-off and meet the target. Importantly these estimates of pesticide mixture toxicity can be used for relative assessments: (1) spatially to prioritise catchments, basins, or regions for on-ground improvements; and (2) temporally, to assess changes in the pesticide mixture toxicity at locations over time and improvements towards the target.

[8] The sum of the values for PSII Herbicides, Other Herbicides and Insecticides do not equal 100% as they are the median contribution for each pesticide group in the region of the GBRCA.

Recommendations

It is recommended that:

1. **The Pesticide Risk Metric (particularly the pesticide mixture toxicity – land use relationships) and the Pesticide Risk Baseline be periodically updated to be aligned with the updating of the Reef 2050 WQIP, and Paddock to Reef reporting**. The next update of the Reef 2050 WQIP is planned for 2022. The number of pesticide monitoring sites should be expanded from the current base level of monitoring in order to improve the pesticide mixture toxicity – land use relationships developed in the current project.
2. **Basins and regions that currently meet the pesticide target be re-evaluated with each update of the Reef 2050 Reef WQIP to ensure they continue to meet the target, or sooner if significant land use changes have occurred that might increase pesticide run-off**. This could be done by conducting on-going pesticide monitoring at appropriate sites, as well as monitoring changes to land use. But as land use data for any particular region is only updated periodically (e.g. every five years) on-going annual pesticide monitoring is likely to pick up changes more rapidly – but at considerably greater expense. It is recommended that both on-going pesticide monitoring and land use monitoring are continued so that any change in risk can be periodically assessed, particularly for the basins and regions that currently meet the Pesticide Target.
3. **The information on the contribution of different pesticide groups to the total risk posed by pesticides be used to: identify which pesticide groups pose the highest risk within a catchment/basin or region and target on-ground management practice changes to reduce runoff of these higher-risk pesticides**. If an alternative active ingredient is used as a strategy to reduce the higher-risk pesticides in runoff, it is recommended to use the Pesticide Decision Support Tool (Warne and Neale, 2019) to ensure that the replacement is a lower risk to aquatic ecosystems. The Pesticide Decision Support Tool uses the same ecotoxicity information for assessing pesticide risk, as used in this report.
4. **The number and types of pesticides included in the Pesticide Risk Metric be expanded.** This should be done in a structured approach such as an audit of the use of pesticides not currently included in the Pesticide Risk Metric. Analytical methods, SSDs and Default Guideline Values (DGVs) should then be developed for pesticides identified by the audit and if needed, these pesticides should then be included in the Great Barrier Reef Catchment Loads Monitoring Program. Particular attention should be paid to including more insecticides and fungicides in the Pesticide Risk Metric as these groups are currently under-represented.

The findings of this report also have implications beyond the scope of the Reef 2050 WQIP and the Reef Water Quality report cards. Therefore it is also suggested that

1. *The Pesticide Risk Metric be used to predict the toxicity of waterways or reaches/stretches of waterways that discharge to the GBR lagoon and are not currently monitored*. This would permit the ecological risk to be determined along waterways, rather than the current situation where a single risk value is estimated for the entire waterway. This could help guide decisions on the location of future pesticide monitoring sites assist in prioritising catchments or sub-catchments where stakeholder engagement could decrease the risk posed by pesticides. It would provide data that would be extremely useful for the Regional Report Cards.

2. *Laboratory and field-based toxicity tests (effect-based methods[9]) should be added to the chemical-based methods used in this report as another line of evidence on the effects of pesticides on aquatic ecosystems.* Such techniques directly measure the effects of pesticides on aquatic species and ecosystems and provide an independent assessment, as they do not rely on pesticide concentration data. In the first instance a three to five-year project should be established that would test the accuracy of the predicted risk posed by pesticide mixture and seek to identify the effects of pesticides on aquatic species and ecosystems. The on-going or periodic inclusion of laboratory and field-based toxicity tests would provide direct evidence of whether the risk posed by pesticides was changing over time.
3. *Pesticide mixture toxicity – land use relationships be developed for all Queensland waterways.* Since land use and pesticide usage patterns vary spatially, it would be reasonable to assume that the pesticide mixture – land use relationships developed for this project may not be applicable outside of the GBRCA waterways that were used to train the models. Developing such relationships for other regions would permit the estimation of the risk that pesticides pose to other areas such as South East Queensland, the Gulf country and Central Queensland.
4. *The effects of undisturbed stream sections located in the headwaters and elsewhere in catchments on the harmful effects of pesticides in GBRCA waterways be investigated.* If undisturbed stream sections do ameliorate the effects of pesticide pollution, the extent (length or area) could be measured and included as a potential variable in new pesticide mixture toxicity – land use relationships. Their inclusion could improve the predictive capabilities of these relationships.

[9] Effect-based methods use the "response of whole organisms (*in-vivo*) or cellular bioassays (*in-vitro*) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern" (Brack et al., 2019). These are essentially toxicity tests conducted on water samples in the laboratory or in the waterways being studied.

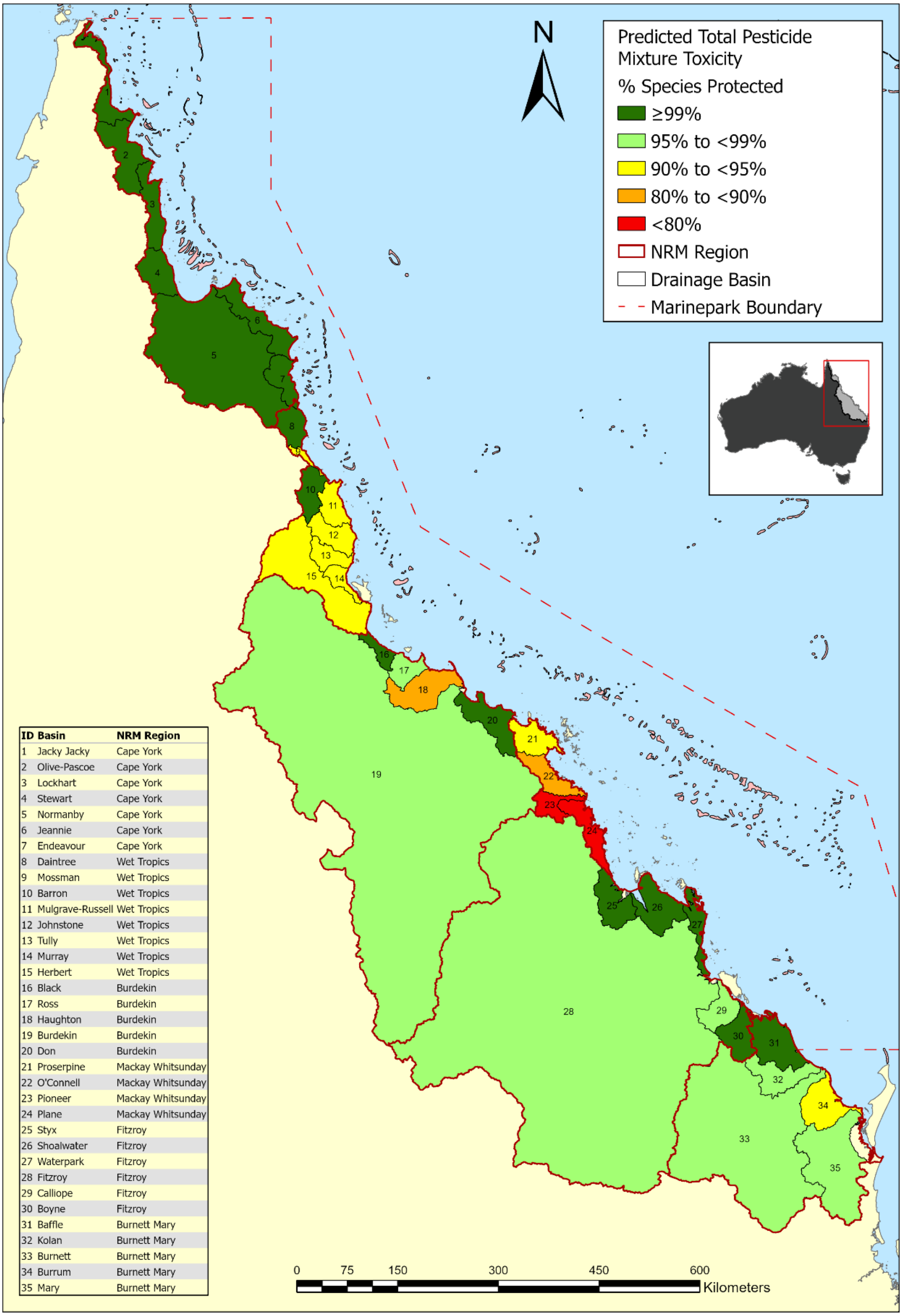

Figure I. Map of predicted mixture toxicity values of all 22 selected pesticides (Total Pesticides) for basins that drain to the Great Barrier Reef lagoon

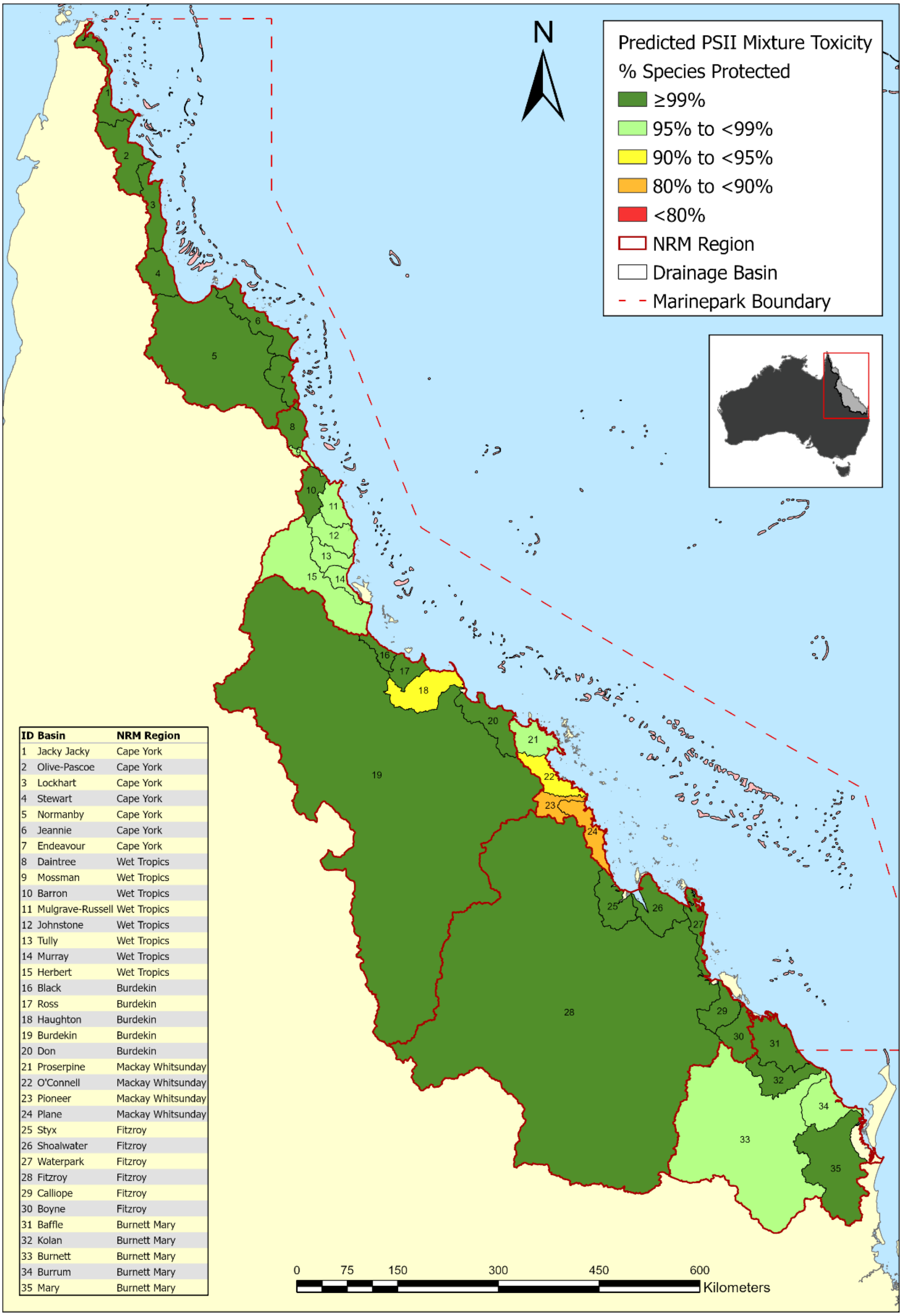

Figure II. Map of the predicted mixture toxicity values of photosystem II inhibiting herbicides (PSII Herbicides) for basins that drain to the Great Barrier Reef lagoon

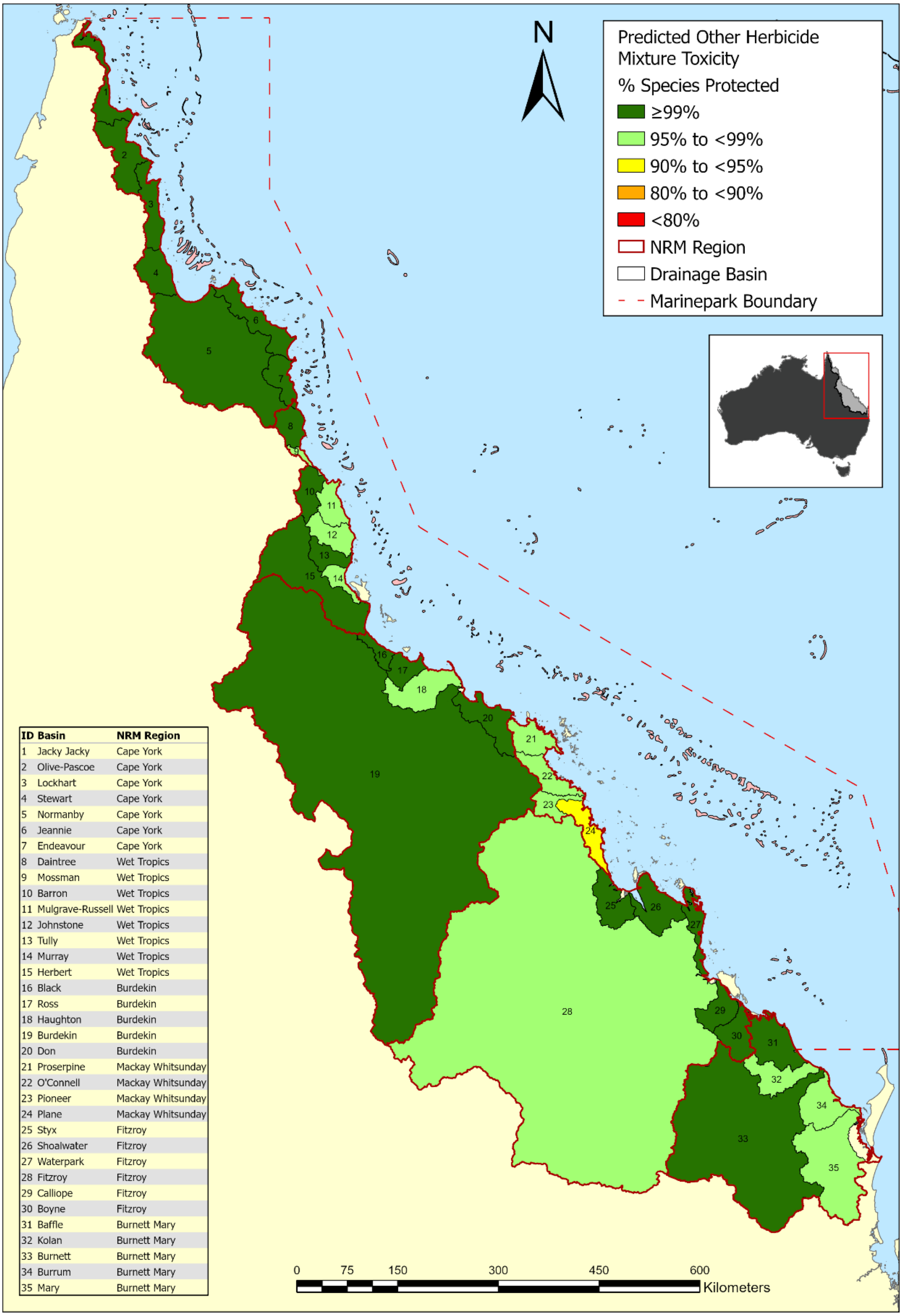

Figure III. Map of predicted mixture toxicity values of Other Herbicides for basins that drain to the Great Barrier Reef lagoon

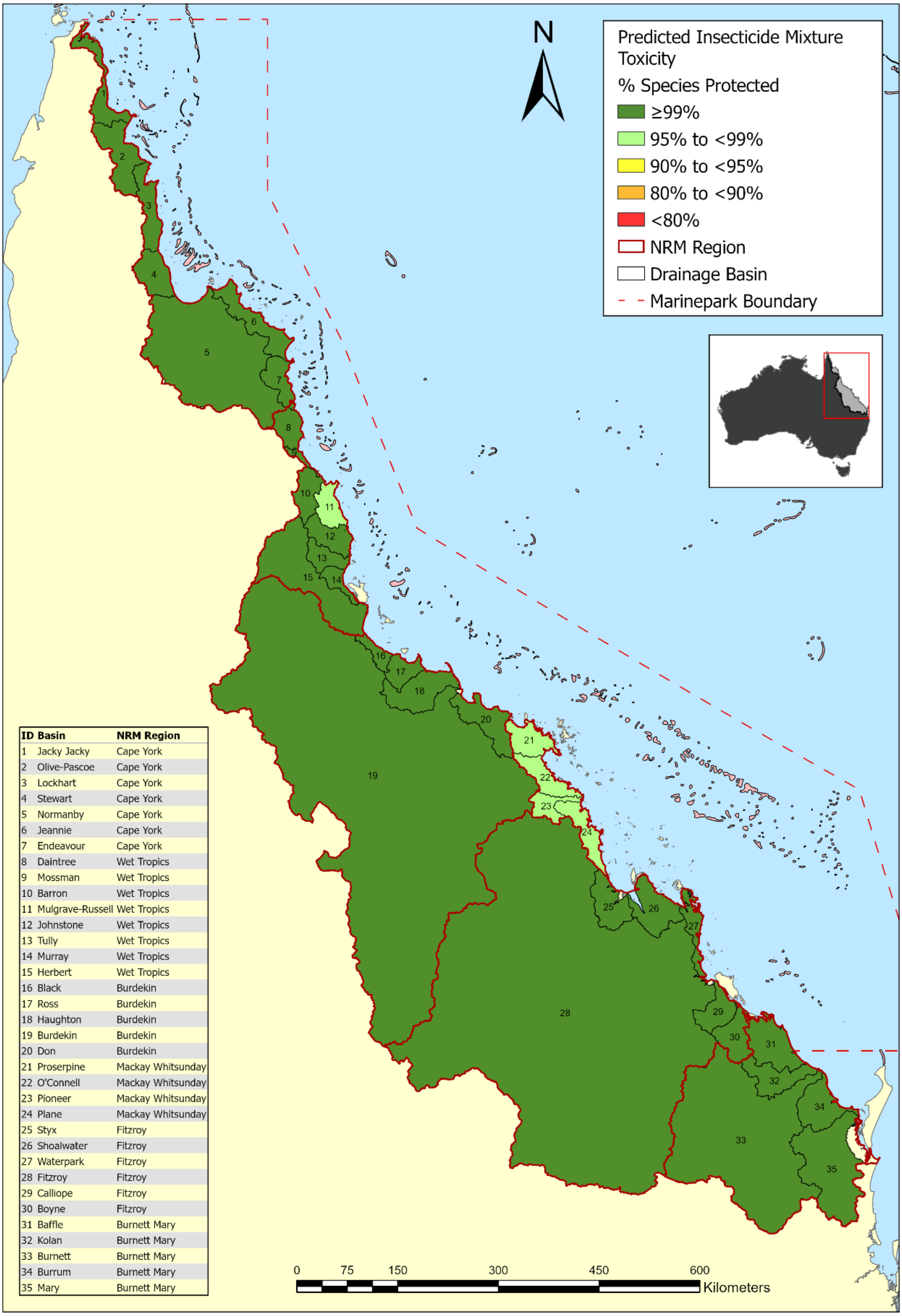

Figure IV. Map of predicted mixture toxicity values of Insecticides for basins that drain to the Great Barrier Reef lagoon

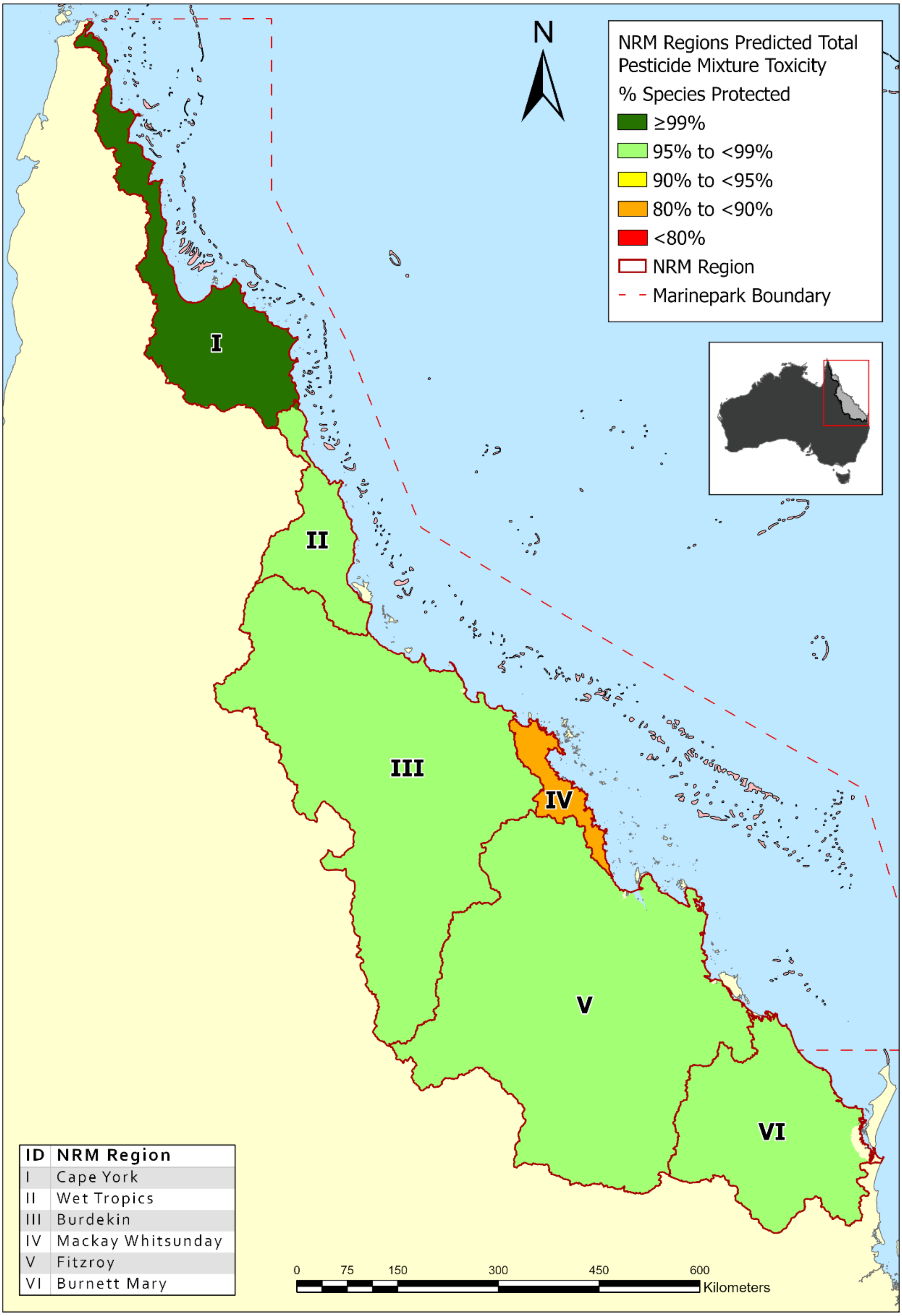

Figure V. Map of predicted mixture toxicity values of Total Pesticides for Natural Resource Management (NRM) regions that contain waterways that drain to the Great Barrier Reef lagoon

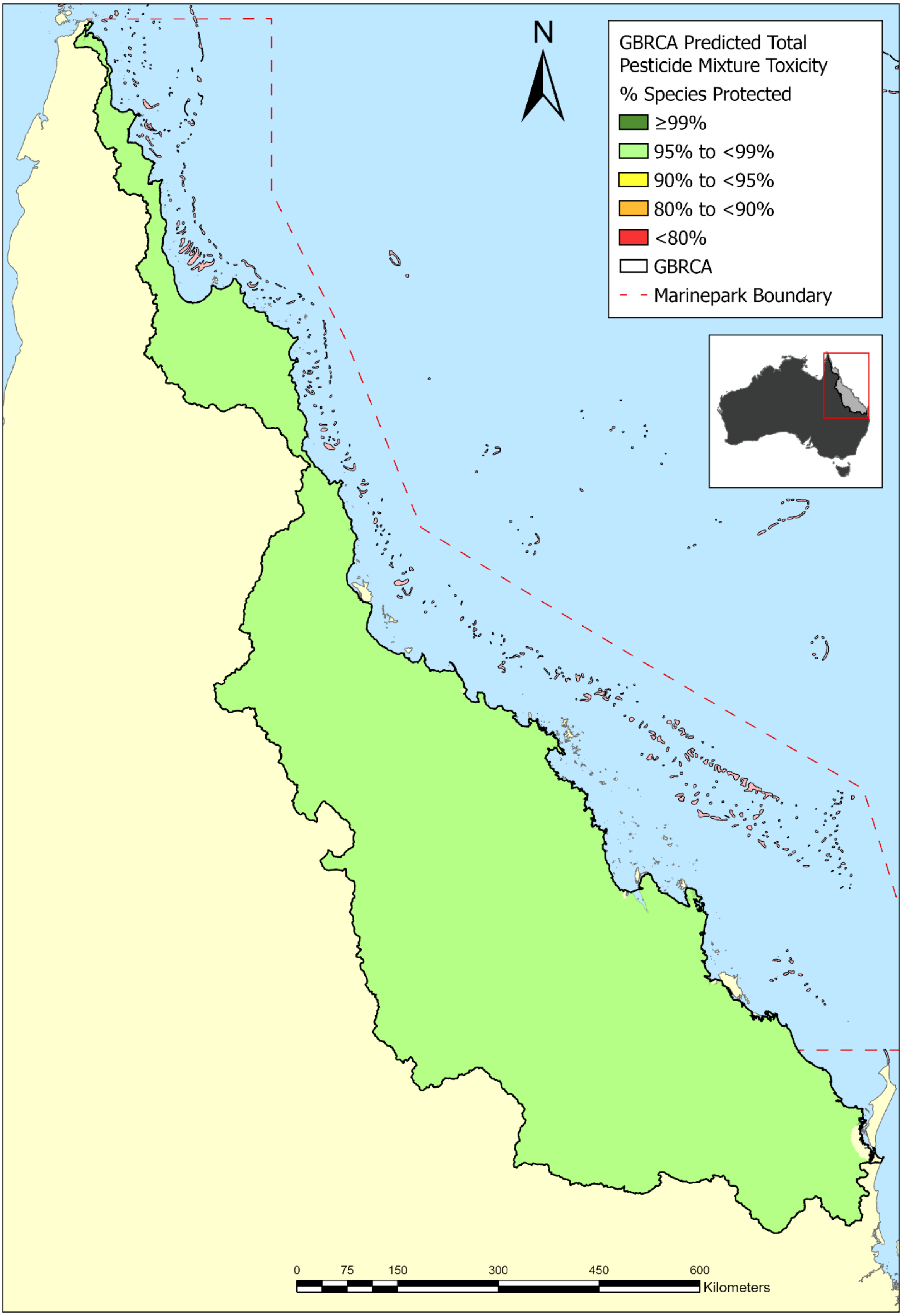

Figure VI. Map of predicted mixture toxicity values of Total Pesticides for the Great Barrier Reef Catchment Area

Introduction

The Great Barrier Reef (GBR) is the world's largest reef ecosystem, a national park, a World Heritage Site (GBRMPA, 2020a) and an international icon. The Reef is home to "600 types of soft and hard corals, more than 100 species of jellyfish, 3000 varieties of molluscs, 500 species of worms, 1625 types of fish, 133 varieties of sharks and rays, more than 30 species of whales and dolphins" (GBRMPA, 2020a) and a variety of marine reptiles (turtles, crocodiles and sea snakes). The Reef is also very important economically – it has been estimated that the GBR generates approximately $5.6 billion annually (QAO, 2015). It is located adjacent to the east Queensland coast. The adjacent land that is drained by rivers and creeks that discharge to the GBR is termed the Great Barrier Reef Catchment Area (GBRCA). The dominant land use in the GBRCA is agriculture (Brodie et al., 2013) and its waterways transport large quantities of suspended solids (eroded soil), nutrients (of both natural and human origin), and pesticides that are predominantly associated with agriculture (Kroon et al., 2013; Negri et al., 2015) but also a range of contaminants associated with urban development (Kroon et al., 2016). The suspended solids, nutrients and pesticides have been identified as key stressors decreasing the health and resilience of the GBR (e.g., Waterhouse et al., 2017). The close proximity of the GBR and agricultural and urban areas has led to a dynamic tension between the desire to produce food and to provide long-term protection of the GBR.

The Australian and Queensland governments have long recognised the deleterious effects of poor quality water entering the GBR. In response, they developed Reef Water Quality Protection Plans (Australian Government and Queensland Government, 2003, 2009, 2013) that aimed to improve the quality of water entering the GBR and to improve the health and resilience of the Reef. Acknowledging that other stressors were also affecting the GBR, the governments developed the Reef 2050 Long-Term Sustainability Plan (Australian Government and Queensland Government, 2015) and the Reef 2050 WQIP (Australian Government and Queensland Government, 2018a). These plans acknowledge the tension and the value of both the agriculture (worth $4.7 billion annually; QAO, 2015) and the reef (worth $5.6 billion annually; QAO, 2015). For these reasons they have focussed on a variety of measures to encourage farmers to improve land management practices which in turn will improve the quality of water entering the GBR. These measures include funding the development and implementation of education programs; the development of Best Management Practices and co-investing with farmers to undertake land management improvements.

The Reef 2050 Water Quality Improvement Plan (Australian Government and Queensland Government, 2018a) is similar in many ways to the European Union's Water Framework Directive (WFD) (https://ec.europa.eu/environment/water/water-framework/index_en.html). Both aim to improve water quality over large areas (the GBRCA and member states of the EU), have adopted a holistic approach, acknowledge the important role that land use has on water quality, have goals or targets to be met within a certain timeframe and require action to be undertaken if these are not met. They also both consider the biological effects of chemical pollutants acting individually and in mixtures.

The water quality targets in the Reef 2050 WQIP 2017–2022 (Brodie et al., 2017; Australian Government and Queensland Government, 2018a) are based on the need to build resilience and improve ecosystem health of the Great Barrier Reef (GBR). The current pesticide target is risk-based and aims to protect at least 99% of aquatic species at the end of catchments[10] from the adverse effects of all pesticides (Brodie et al., 2017; Australian Government and Queensland Government, 2018a). This target replaced the original load-based targets (Australian Government and Queensland Government, 2009, 2013). The adoption of current risk-based target that aligned with Australian and New Zealand water quality guidelines was a fundamental change towards a more ecologically relevant approach to the way pesticides were assessed and reported.

The pesticide target of protecting at least 99% of aquatic species was selected because that is the level of protection prescribed by the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018) for *high ecological value* ecosystems such as the Great Barrier Reef. The GBR water quality guidelines (Insert ref) state that this level of protection should apply to all five waterbodies of the GBR (i.e., Enclosed coastal, Open coastal, Mid-shelf, Offshore, and the Coral Sea). The point at which the pesticide target applies is controlled by the most landward point of these five waterbodies i.e. the most landward limit of the enclosed coastal water body. The majority of fresh and estuarine waters in the GBRCA are categorised as *slightly to moderately disturbed* and therefore they should have at least 95% of aquatic species protected. This lower level of protection applies immediately upstream of the point where the pesticide target applies and creates a step-change boundary where on up-stream side 95% of species are to be protected and on the down-stream side 99% of species are to be protected and on the other side.

Another fundamental change to the pesticide target was a clear requirement that "the toxic impacts of all pesticides in the water body are considered collectively" (Brodie et al., 2017). The load-based targets in 2009 and 2013 did not specify whether the additive toxicity of pesticide mixtures should be accounted for in the reductions. Indeed, the methods for measuring progress towards the 2013 pesticide targets attempted to do this using a toxic-equivalency approach; i.e. toxicity-based pollutant loads (Smith et al., 2017a, b). At the time of measuring progress towards the 2009 and 2013 targets, only five 'high priority' photosystem II inhibiting (PSII) herbicides (i.e., ametryn, atrazine, diuron, hexazinone and tebuthiuron) were considered. However, as monitoring and analysis techniques improved, the number and types of pesticides detected increased substantially (Huggins et al. 2017), and the need to include more pesticides (with different modes of action) in measuring progress towards the target also became apparent.

With the change to the pesticide target, it is essential to establish a new starting condition – a Pesticide Risk Baseline. This baseline is an estimate of the risk that mixtures of pesticides pose at a point in time. To be effective the Pesticide Risk Baseline must use the same units as the pesticide target (i.e. the average percentage of species being protected). In order to measure progress from the Pesticide Risk Baseline (starting point) to the pesticide target of at least 99% species protection, it is necessary to estimate the toxicity due to the presence of multiple pesticides. The toxic-equivalency approach previously used for estimating the toxicity-based loads was limited to assessing mixtures of chemicals with the same mode of action. It also estimated the mixtures based on concentration equivalent units, e.g. diuron equivalent ug/L – appropriate for calculating loads but less desirable for estimating per cent species affected. A method for estimating the per cent of species affected based on the toxicity of mixtures of five and then 13 PSII herbicides was developed for the water quality risk assessment (Chapter 3) in the 2017 Scientific Consensus Statement (Waterhouse et al., 2017) to prioritise investment for land management practice change to reduce pesticide runoff, and later implemented in some of the Regional Ecosystem Health report

[10] The term "end of catchment" will be used throughout this report but technically the pesticide target applies at the most landward point in each catchment of enclosed coastal waters (Brodie et al., 2017).

cards. This earlier method was improved and expanded in the current project to include another nine pesticides, including insecticides, additional PSII herbicides, and other non-PSII herbicides such as synthetic auxins. The new method combines species sensitivity distributions using the method developed by Traas et al. (2002), the Independent Action (IA) model of joint action and multiple imputation to estimate of the risk posed by the 22 reference pesticides during the wet season; i.e., the multi-substance potentially affected fraction (msPAF) also termed the per cent of species affected. Details of these methods are provided later in this report. The combination of these methods is called the Pesticide Risk Metric. There are two key advantages of the Pesticide Risk Metric. First, it expresses the risk posed by pesticide mixtures in terms of the percentage of species that should be affected or protected at given pesticide concentrations, which is consistent with the approach of the Reef 2050 WQIP pesticide target (Australian Government and Queensland Government, 2018a) and the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC and ARMCANZ, 2000; ANZG, 2018). The second advantage is that it was developed to reflect the pulsed nature of pesticide exposure in GBR catchments, providing a more realistic assessment of pesticide risk (based on exposure and recovery) than the more standard risk assessments that only assess the risk based on upper percentile concentrations. This means the results are also less influenced by variable climate conditions (i.e. catchment discharge volume) than monitored loads, and therefore, the Pesticide Risk Metric estimated from monitoring data can be used to link change over time with land management practice, as well as being comparable between catchments.

This project will use monitored pesticide concentration data from the Great Barrier Reef Catchment Loads Monitoring Program (GBRCLMP) to estimate the Pesticide Risk Baseline. GBRCLMP monitoring data provides the best available and most current knowledge of the pesticides that are detected in GBR catchments, and therefore, what is being applied in various land uses. In order to estimate risk from pesticide toxicity, a Pesticide Risk Metric has previously been used to estimate the pesticide toxicity risk at the individual monitoring locations in the GBRCA (Lewis et al., 2013; Waterhouse et al., 2017). In this project, a modified Pesticide Risk Metric will be used to establish a Pesticide Risk baseline. In adapting the Pesticide Risk Metric to report a Pesticide Risk Baseline, the method needed to be expanded to include a larger number of pesticides with multiple modes of action, and also expanded to report pesticide risk at larger spatial scales. Pesticide monitoring is conducted for individual catchments whereas the Reef Water Quality report cards report on basins (which often contain multiple catchments), NRM regions (which contain multiple basins) and the whole GBR level (i.e. the GBRCA, which contains multiple regions). To expand from the catchment-scale monitoring data to report pesticide risk at larger spatial scales, this project aims to develop relationships between pesticide mixture toxicity and spatial, hydrological and land use variables for the monitored catchments. The resulting relationships were used to estimate the pesticide mixture toxicity for the basins, NRM regions and GBRCA that are required for reporting purposes.

It is not unreasonable to assume that there will be relationships between land use and the pesticides detected, their concentrations and their combined toxicity. This is because each type of agriculture has a suite of pesticides registered for its use. Therefore, providing pesticides are not being used off label, the various types of agriculture within a catchment will determine the pesticides that could be detected in the waterways of that catchment, and several published studies support this assumption. Schriever et al. (2007) developed an equation that predicted the annual load of a generic substance in a catchment. The equation required, amongst other variables, the relative proportion of arable land that was used for each crop. Van Gils et al. (2020) used the methodology of Sala et al. (2015) that used prescribed application rates and land use data to estimate pesticide concentrations in rivers of EU countries. Kroon et al. (2015) found a statistically significant relationship between the magnitude of vitellogenin mRNA expression (an indicator of endocrine disruption) in Barramundi collected from rivers that discharge to the GBR and the per cent of the catchment used for sugarcane production and with the concentrations of pesticides used in sugarcane. Wood et al. (2019) also found that the percentage of diatom species sensitive to herbicides present in 14 rivers that discharge to the GBR was significantly related to the combined toxicity of ametryn, atrazine, diuron, hexazinone and prometryn (using the toxic equivalency (TEQ) method developed by Smith et al., 2012). They also found that the percentage of herbicide sensitive diatom species present in rivers that discharge to the GBR decreased with increased grazing and cropping ($p \leq 0.05$) in the catchments (Wood et al., 2019). Warne et al. (2020) found that the number of pesticides sampled in rivers that discharge to the GBR increased linearly with the per cent of catchment used for sugarcane and a reduction in the per cent used for conservation. Spilsbury et al. (2020) using principal component analysis found that the percentage of each catchment used to grow sugarcane was highly correlated to the toxicity of pesticide mixtures[11] in rivers that discharge to the GBR lagoon. Munz et al. (2017) found that the frequency of detecting plant protection products was correlated to the fraction of arable land in catchments in Switzerland. Burdon et al. (2016) also found that two indices of macroinvertebrate health were related to the extent of arable land (% cropping, % pasture) in Swiss streams. In addition, Warne et al. (in prep) found that the concentration of imidacloprid in rivers that discharge to the GBR increased with an increase in the per cent of catchments used to grow sugarcane and bananas and decreased with increasing amounts of conservation and grazing. Given that relationships between land use and the presence, concentration and effects of pesticides should logically occur and have been reported (Kroon et al., 2015; Wood et al., 2019; Spilsbury et al, 2020; Warne et al., 2020; Warne et al., in prep), it was felt that the best available approach was to use pesticide mixture toxicity — land use relationships to develop a Pesticide Risk Baseline (from the Pesticide Risk Metric approach) that could be reported at the basin, regional and whole GBR scales.

Aims and Objectives

The aim of this project is to develop a method that can estimate the risk posed by pesticides (i.e., the Pesticide Risk Baseline) at multiple spatial scales (i.e., site, catchment, basin, NRM region and the entire GBRCA) and at multiple time periods (e.g. during 2015/2016 to 2017/2018 and in the future)

To achieve this aim, the project had the following objectives:

- develop a method for estimating the toxicity of pesticides mixtures (i.e., pesticide mixture toxicity) detected in GBR catchments;
- estimate the pesticide mixture toxicity for all sites with GBR catchment pesticide monitoring data for 2015/2016 to 2017/2018;
- develop, validate and ground-truth relationships able to predict the toxicity of pesticide mixtures detected in GBR catchments;
- use the relationships to predict the risk posed by pesticide mixtures (the Pesticide Risk Baseline) at 35 basins, six NRM regions and the entire GBRCA; and

[11] The toxicity of each pesticide was determined by dividing the maximum measured concentration of each pesticide by its default guideline value. These ratios were then summed for all the pesticides present in each sample. This method is referred to as the sum of the hazard quotient method - note this is termed the sum of the risk quotient method in Europe.

- present a protocol and associated excel spreadsheets and R code to permit the calculation of the Pesticide Risk Metric and Pesticide Risk Baselines for other locations and/or times.

Methods

Overview

This report has adopted the risk assessment framework that is internationally accepted by numerous countries e.g. Australia (NEPC, 2013); Canada (Government of Canada, 2012); Europe (European Chemical Bureau, 2003); United States of America (USEPA, 1992) and international organisations e.g. SETAC (2018). This consists of four main components:

- Problem formulation;
- Exposure assessment;
- Effect assessment; and
- Risk characterisation.

Characteristics of the four components are set out below.

The **problem formulation** component is where the scope of the study is defined. It defines: the stressors that will be considered and those that will not; the spatial and temporal scale of the assessment; and what organisms are to be considered. A careful problem formulation leads to a more scientifically rigorous assessment.

The **exposure assessment** component examines the spatial and temporal distribution of stressors and the extent of their co-occurrence with the ecological components of concern.

The **effects assessment** component identifies and quantifies the adverse effects caused by the defined stressors and should evaluate cause-and-effect relationships for individual species and multiple species (species sensitivity distributions).

The **risk characterisation** component uses the results from the exposure and ecological characterisation stages to evaluate the likelihood and magnitude of harmful effects associated with exposure to the defined stressors.

The four components in ecological risk assessment are followed by a **Risk Management** component, where the scientific outputs of the risk assessment are combined with societal and economic considerations to evaluate the options for managing the risk. The framework used in this report is based on, but not identical to, the ecological risk assessment framework. The differences are largely terminology, which reflects the unique problem that is being resolved. Figure 1 compares the classical ecological risk assessment framework with the framework used in this report. The key differences being, that in this report the exposure assessment, effect assessment and risk characterisation are collectively referred to as the Pesticide Risk Metric – as these are the components used to estimate the risk posed by mixtures of pesticides. The results of the Pesticide Risk Metric become the Pesticide Risk Baseline, which is used in the Risk Management.

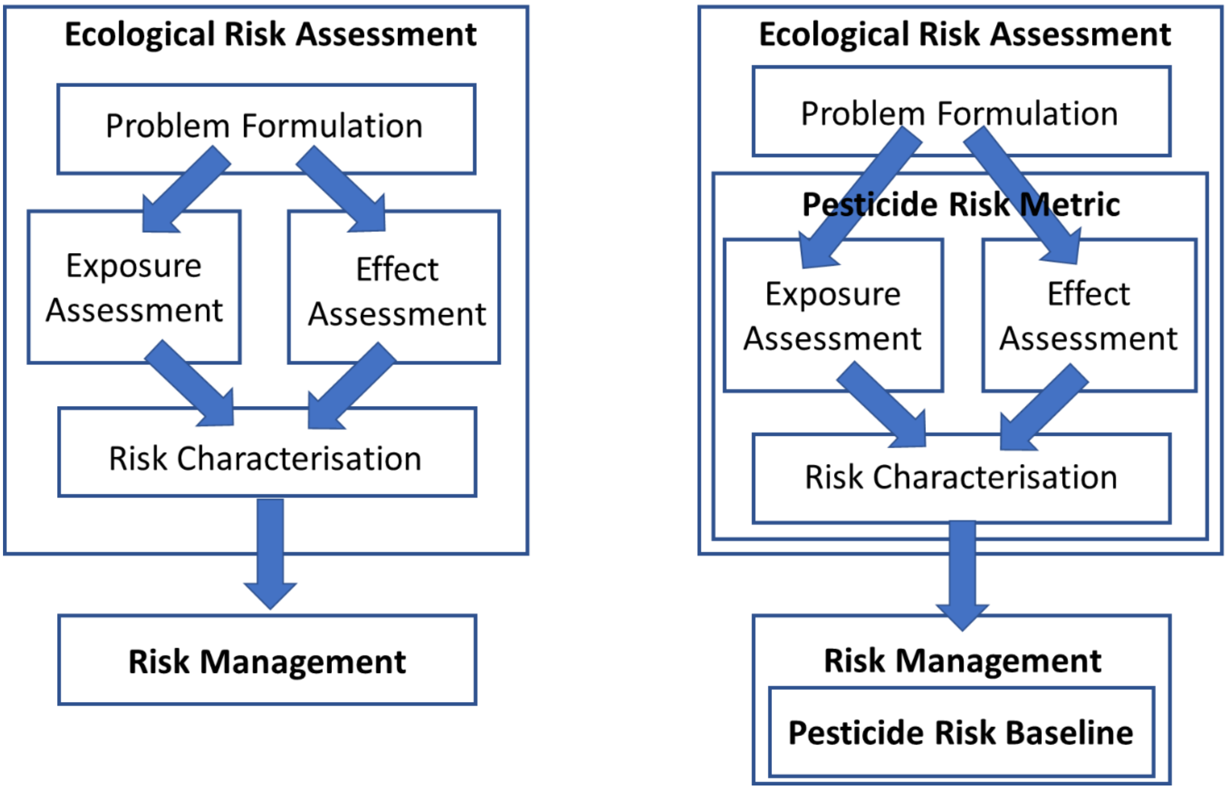

Figure 1. Comparison of the classical four component ecological risk assessment and risk management framework and the framework used in this report

Problem Formulation

Key factors to consider in the problem formulation include:

- the pesticides to be included;
- the waterways to be included (i.e. the spatial scale);
- the period of time to be covered in the pesticide risk baseline (i.e. the temporal scale); and
- the organisms to be included (i.e. the biological scope).

The Pesticides to be Included

In order for pesticides to be included in the Pesticide Risk Metric and Pesticide Risk Baseline, they needed to meet all the following criteria:

1. they are included in the Great Barrier Reef Catchment Loads Monitoring Program (GBRCLMP) (as this is the most temporally, spatially and chemically extensive pesticide monitoring program for waterways that discharge to the GBR);
2. they are, or can be, included in the Source Catchment models (because these models are used to report on progress made towards meeting the water quality targets of the Reef 2050 WQIP based on recorded land management practice changes and they take into account climatic variability);
3. they have species sensitivity distributions (SSD) for fresh and/or marine organisms (because to generate a SSD toxicity data to a diverse range of species must have been collated, reviewed and these can be used in the Effect Assessment component of the ecological risk assessment);
4. they are registered for use in Australia (there is no point including pesticides not registered as they should not be present in waterways that discharge to the GBR); and
5. they are regularly detected by the GBRCLMP or other monitoring programs (because being registered for use does not mean a pesticide will be used or that it will be transported off-site to waterways. Also the risk depends on the pesticides present.).

All the pesticides that have been detected in waters discharging to the GBR, by various projects (e.g. Lewis et al., 2009; Brodie et al., 2012; Kroon et al., 2012; Smith et al., 2012; Turner et al., 2012, 2013; Davis et al., 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 pesticides met the criteria and were included in the Pesticide Risk Metric and Pesticide Risk Baseline (Table 1).

Table 1. The 22 pesticides that met the criteria for inclusion in the Pesticide Risk Metric and Pesticide Risk Baseline

2,4-D	Ametryn	Atrazine	Chlorpyrifos
Diuron	Fipronil	Fluroxypyr	Haloxyfop
Hexazinone	Imazapic	Imidacloprid	Isoxaflutole
MCPA	Metribuzin	Metolachlor	Metsulfuron-methyl
Pendimethalin	Prometryn	Simazine	Tebuthiuron
Terbuthylazine	Triclopyr		

The Waterways to be Considered (i.e. the Spatial Scale)

The Reef 2050 WQIP pesticide target (Australian Government and Queensland Government, 2018) applies to all catchments, from the Burnett Mary to Cape York regions inclusive, that discharge to the GBR. Therefore, only waterways that discharge to the GBR will be considered in the Pesticide Risk Baseline. The Reef Water Quality Report Card (e.g. Australian Government and Queensland Government, 2019a) reports on the progress made to achieving the catchment and water quality targets at 35 basins, six Natural Resource Management (NRM) regions and the Great Barrier Reef Catchment Area as a whole. Therefore, Pesticide Risk Baseline should also generate results for each of these spatial scales. Only the spatial units reported on in the Reef Water Quality Report Card (i.e. basins, regions and the GBRCA) will be included in the Pesticide Risk Baseline. However, the method (i.e. Pesticide Risk Metric) and the regression analyses used to construct the Pesticide Risk Baseline can also be used to estimate the pesticide mixture toxicity for other waterways or at other spatial scales.

The Timescale to be Considered (i.e. the Temporal Scale)

The pesticide target, of protecting at least 99% of aquatic species at the mouth of waterways that discharge to the GBR, was adopted in 2017 (Australian Government and Queensland Government, 2018). In order to report on progress made to achieving the pesticide target a Pesticide Risk Baseline (a starting point) is required. In order to reflect conditions when the pesticide target was adopted, the Pesticide Risk Baseline must be generated for a period as close to 2017 as possible. However, because the risk is affected by climatic variability, it was considered necessary to use data for a number of years. For these reasons pesticide concentration data for 2015/2016 to 2017/2018 were used to derive the Pesticide Risk Baseline. An added benefit of using those data is that it included the greatest number of monitored waterways and more pesticides were monitored during this period than in earlier years.

The Organisms to be Considered (i.e. the Biological Scope)

The pesticide target applies to the five waterbodies of the GBR with the most landward boundary being the enclosed coastal (i.e. at the end of catchments that discharge to the GBR) (Brodie et al., 2017; Australian Government and Queensland Government, 2018). As such, all aquatic (freshwater, estuarine and marine) species were theoretically included in the Pesticide Risk Metric and Pesticide Risk Baseline, but in practice it was limited to the aquatic species for which there were ecotoxicity data for the 22 selected pesticides (Table 1).

Exposure Assessment

The exposure assessment component consists of: collecting and chemically analysing water samples; passing the concentration data through a quality assurance and quality control process that includes manipulating the data to address concentrations lower than the limit of reporting; and calculating a daily concentration for each pesticide. Details of the methods used are found in Warne et al. (2018) but a summary is presented below.

Measuring Pesticide Aqueous Concentration Values

The pesticide aqueous concentration data used in this project were generated by the Great Barrier Reef Catchment Loads Monitoring Program (GBRCLMP) for the years 2015/2016, 2016/2017 and 2017/2018. The data for 2016/2017 and 2017/2018 were not publicly available at the time of conducting this work but were provided by Water Quality and Investigations, Department of Environment and Science. The sites and years for the pesticide concentration data that were used are presented in Table 2. Details of the sites are provided in Attachment A and how the samples were collected is provided in the GBRCLMP technical loads reports (e.g. Huggins et al., 2018; Napel et al., 2019a; Napel et al., 2019b).

All pesticides analyses were conducted by the Queensland Health Forensic and Scientific Services Organics Laboratory (Coopers Plains, Queensland), which is accredited for these analyses by the National Association of Testing Authorities (NATA, Australia). Pesticides in water samples were analysed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). In all three years water samples were analysed using one or more of the following methods:

- Solid Phase Extraction followed by LC-MS/MS high analysis (used when water samples were expected to contain high pesticide concentrations e.g. during events or early in the wet season). This was the method used for the majority of samples in 2015/2016.
- Solid Phase Extraction followed by LCMS/MS low analysis (used when water samples were expected to contain low pesticide concentrations e.g. during the dry season). This method has a 10-fold lower limit of reporting (LOR) for all pesticides than the LC-MS/MS high method. This method was used for a minority of the samples in 2016/2017.
- Direct Inject LC-MS/MS analysis where a small volume of the water sample is directly injected into the LC column – this method largely replaced the LC-MS/MS high method). The limits of reporting for the direct injection method are similar to the LC-MS high method (i.e. the limits of reporting are approximately 10-times larger than for the LC-MS low method). This method was used for the majority of all water samples in 2016/2017 and all samples in 2017/2018.

Table 2. Sites and years where water samples were analysed to provide aqueous pesticide concentration data (X). Sites are arranged in alphabetical order. Full details of the sites are provided in Attachment A

Waterway	2015/2016	2016/2017	2017/2018
Baffle Creek			X
Barratta Creek	X	X	X
Barron River			X
Black River			X
Boyne River			X
Burdekin River	X	X	X
Burnett River	X (at Ben Anderson Barrage)	X (at Ben Anderson Barrage)	X (at Quay St bridge)
Calliope River			X
Comet River	X	X	X
East Barratta Creek			X
Elliot River			X
Fitzroy River	X	X	X
Haughton River	X (at Powerline)	X (at Powerline)	X (at Giru Weir)
Herbert River	X	X	X
Johnstone River	X	X	X
Kolan River			X
Mossman River			X
Mulgrave River	X	X	X
North Johnstone River	X	X	X
O'Connell River	X (at Caravan Park)	X (at Stafford's Crossing and Caravan Park)	X (at Stafford's Crossing and Caravan Park)
Pioneer River	X	X	X
Proserpine River		X	X
Russell River	X	X	X

Waterway	2015/2016	2016/2017	2017/2018
Sandy Creek	X	X	X
Styx River			X
Tinana Creek	X	X	
Tully River	X	X	X
Waterpark Creek			X

Quality Assurance and Quality Control of the Pesticide Concentration Data

The quality assurance and quality control procedures implemented by the GBRCLMP to determine the accuracy of pesticide concentrations (Huggins et al., 2017) were adopted. The one exception was how pesticide concentration values reported as lower than the limit of reporting were handled (Attachment B).

Effects Assessment

The effect assessment consists of: collating toxicity data from the literature; passing the toxicity data through a quality assurance and quality control process; calculating species sensitivity distributions and protective concentration (PCx) values. Details of the methods used are found in Warne et al. (2018) but a summary is presented below.

Collation of Toxicity Data

The toxicity data used to derive the SSDs were collated as part of the Department of Environment and Science and University of Queensland's derivation of third-party default guideline values for the current revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018). This consisted of a thorough search of the ecotoxocological literature and guidelines, standards, criteria of other jurisdictions (e.g., the USEPA, Environment Canada and the European Union). In addition, the databases of the USEPA ECOTOX (USEPA, 2015a), Office of the Pesticide Program (USEPA, 2015b), the Australasian Ecotoxicology Database (Warne et al., 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al., 2000) were searched.

Quality Assurance and Screening of Toxicity Data

The quality of the collated toxicity data were assessed using the quality assurance process used to derive the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (Warne et al., 2020), which was based on Hobbs et al. (2005). In summary, this consists of answering approximately 20 questions based on the information provided in the published study. Key features that were assessed included the: experimental design, test organism, duration and type of exposure, analytical measurement of the toxicant, calculation of the toxicity values and measures of uncertainty. The exact questions asked vary with the type of toxicant (organic or inorganic), media (fresh or marine water) and test organism (aquatic plants or animals). Depending on the answer to each question a mark is awarded and these are summed and divided by the total possible score to determine a per cent quality score. Data with quality scores of less than 50%, between 50 and 79% and equal to or greater than 80% were deemed to be unacceptable, acceptable and high quality data, respectively. Only acceptable and high quality data were used in the project.

The acceptable and high quality data were also screened to ensure that the data were suitable for the calculations to be conducted in the project. The screening followed the methods described in Warne et al. (2018).

Calculating Species Sensitivity Distributions and Protective Concentrations

Toxicity data for the 22 selected pesticides that passed the quality assurance and screening procedures were used to derive SSDs using the SSD derivation method of the Australian and New Zealand Water Quality Guidelines for Toxicants (Batley et al., 2018; Warne et al., 2018). The one exception to the methods prescribed in Warne at al. (2018) was that data for both fresh and marine species were used to derive the SSDs. The rationale for this was explained in the Problem Formulation section of this report.

The SSDs derived for this project were based on the freshwater and marine SSDs that were previously derived for ANZG (2018) guidelines or those derived by the Department of Environment and Science as third-party guidelines for the current revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (King et al., 2017a, b).

The procedure used to derive the SSDs and protective concentration (PC) values is described in Warne et al. (2018). A summary of the methods used is provided in Attachment C. This provides information on the conversion of toxicity data to estimates of chronic no observed effect concentration (NOEC) and 10% effect concentration (EC10) data, the calculation of a single toxicity value to represent each species, the order in which toxicity data were selected and how to assess and deal with the modality of the toxicity data. Details of the how the SSDs for each of the selected pesticides were calculated are presented in Attachment D.

Risk Characterisation

The risk characterisation consists of: converting each concentration datum to the corresponding proportion of species affected; calculating the joint toxicity of all pesticides in each sample; estimating the average daily proportion of species affected over the wet season for monitored catchments; developing relationships able to estimate the toxicity of pesticide mixtures at monitored catchments; scaling-up the relationships to provide estimates of the pesticide mixture toxicity for basins, regions and the Great Barrier Reef Catchment Area.

Estimating the Toxicity of Pesticide Mixtures

The SSD method was selected for estimating the toxicity of pesticide mixtures in preference to the Toxic Unit approach (see Attachment E for the rationale for this decision). The Independent Action model of joint action was selected as the model of joint action in preference to the Concentration Addition of joint action (see Attachment F for the rationale for this decision).

The IA model has two major advantages. It makes the calculations of the combined toxicity considerably simpler, and more importantly, it allows the user to estimate the contribution of individual pesticides to the combined toxicity. Because the Independent Action model of joint action consistently yields lower estimates of mixture toxicity than the Concentration Addition model (Backhaus et al., 2000; Faust et al., 2000, 2003; Dyer et al., 2010; Spilsbury et al., 2020), using the Independent Action model of joint action 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).

Calculation of the Toxicity of Mixtures of the 22 Selected Pesticides

The SSDs for the 22 selected pesticides belonged to three types of statistical distributions: Burr type III; log-logistic and inverse-Weibull (Table 3). The coefficients of the parameters for the SSD for each pesticide (Table 4 and Table 5) were inserted into the generic equations for the three distributions (Table 3). Then the concentrations of the 22 selected pesticides in each water sample were entered into the appropriate SSD equations to calculate the proportion of species affected.

Table 3. The generic equations that describe the Burr type III, Log-Logistic and Inverse-Weibull distributions

Statistical distribution	Generic equation (estimated proportion of species affected)
Burr type III	$=1/(1+(B/C)^{C})^{K}$
Log-logistic	$=1/(1+(\text{Concentration}/\text{Alpha})^{(-1*\text{Beta})})$
Inverse-Weibull	$=\text{EXP}(-1/((\text{Concentration}*\text{Beta})^{\text{Alpha}}))$

Table 4. The coefficients of the parameters for the pesticides that had a Burr type III species sensitivity distribution. Details of the SSDs are provided in Attachment D

Pesticide	B	C	K
Ametryn	5.492	1.053	1.028
Atrazine	11.428	0.684	1.745
Chlorpyrifos	3.697	0.367	1.406
Diuron	0.045	0.506	8.003
Fipronil	0.006	0.541	5.327
MCPA	28044	1.246	0.244
Metolachlor	593.6	0.749	0.548
Pendimethalin	3.260	0.715	1.540
Prometryn	6.994	1.004	1.047
Simazine	105.6	2.048	1.205
Terbuthylazine	4.852	0.921	2.080
Triclopyr	1056	5.474	0.099

Table 5. The coefficients of the parameters for the pesticides that had a log-logistic or an Inverse-Weibull species sensitivity distribution. Details of the SSDs are provided in Attachment D

Log-logistic distribution		
2,4-D	0.517	0.00718
Fluroxypyr	1323	1.874
Haloxyfop	169279	1.369
Imazapic	22.546	0.750
Tebuthiuron	53.16	1.902
Inverse-Weibull distribution		
Hexazinone	1.296	0.1712
Imidacloprid	0.502	0.841
Isoxaflutole	0.680	0.289
Metribuzin	1.711	0.204
Metsulfuron-methyl	0.259	0.439

The IA model of joint action was used in all calculations of pesticide mixture toxicity:

Pesticide mixture toxicity = $1 - [(1-F_1)(1-F_2)(1-F_3)\ldots\ldots(1-F_n)]$ (Eqn 1)

where pesticide mixture toxicity is expressed as the proportion of species affected (i.e. 0 to 1) and F is the proportion of species affected by pesticide 1, 2, 3 …n individually. The estimated percentage of affected species was obtained by multiplying the results from equation 2 by 100. Equation 1 was used to estimate the pesticide mixture toxicity of groups of pesticides:

- PSII Herbicides;
- Other Herbicides (all herbicides other than PSII herbicides);
- Insecticides; and
- Total Pesticides (all 22 pesticides included in the Pesticide Risk Metric).

Which pesticides were allocated to the above groups is shown in Table 6.

The above calculations were conducted for every sample collected as part of the pesticide monitoring conducted by the GBRCLMP for the sampling years 2015/2016, 2016/2017 and 2017/2018.

The Pesticide Risk Metric calculations were conducted using the "R" program (Strauss et al., 2019). A similar Excel spreadsheet version (Pesticide Mixture Toxicity V2.6 (Warne et al., 2019)) was also developed. Both the R code and the Excel spreadsheet versions of the calculations will be released as part of the Pesticide Risk Baseline project.

Choosing a Relevant Time Period to Estimate Pesticide Mixture Toxicity

In environmental sampling programs, it is usually recommended that large datasets are accumulated over extended periods that encompass the environmental variability (i.e. one to two years) (ANZECC and ARMCANZ, 2000; DERM, 2009). This might be appropriate for regions where the toxicant may be present throughout the year. However, rainfall in the GBRCA is highly seasonal with a distinct dry season when very little rain falls and a wet season when the vast majority of rain falls. Therefore, in the GBRCA, the wet season is the time with the greatest probability that pesticides will be transported to waterways and aquatic organisms will be exposed to pesticides. In the GBRCA, estimating the risk posed by pesticides over the entire year would dramatically underestimate the risk faced by aquatic organisms for approximately half the year. Therefore the wet season was chosen as the most relevant exposure risk period. The wet season was defined as the six-month period (182 days) following the first flush in each monitored waterway (see Attachment G for justification of the 182 day duration). The first flush was identified as the first day after July 1 of each year when river flow or height increased and there was an increase in pesticide concentrations. 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. In most cases the first flush occurred between late September and early December (Attachment H). The dates of the first flush and the end of the wet season (i.e. 182 days after the first flush) for each combination of monitored waterway and year are presented in Attachment H. If there were multiple samples collected within a 24-hour period, the pesticide mixture toxicity values were estimated for each sample and then averaged to provide a single estimate for each day (an average daily estimate of the percentage of species affected).

Choosing an Appropriate Percentage of Species Affected to Estimate Pesticide Mixture Toxicity

The preceding calculations estimate the percentage of species that should theoretically be affected by the 22 selected pesticides present in each water sample. However, a single numerical estimate of the risk posed by mixtures of up to 22 pesticides (see Table 1) at a given monitoring location is needed for several reporting instruments, including the Reef Water Quality Report Card, Regional report cards and the reports of the Great Barrier Reef Catchment Loads Monitoring Program.

The risk posed by pollutants is often estimated using the 90th or 95th percentile of pollutant concentrations (e.g., Solomon et al., 1995; Rand et al., 2010; ANZG, 2018). The 90th and 95th percentiles of pesticide concentration are amongst the higher concentrations measured (i.e., for the 90th percentile 90% of the concentration values are equal to or lower and only 10% are higher). These percentiles are used to represent a "reasonable worst case" (Viscusi et al., 1997). The use of such percentiles leads to high estimates of the risk posed by pesticides and it has been argued (e.g., Viscusi et al., 1997) that less extreme values (e.g. the average) would be more appropriate.

The main reason that the average pesticide concentration was used to estimate risk in the Pesticide Risk Metric, was to reflect the pulsed nature of exposure to pesticides and how this exerts effects on aquatic organisms. In pulse exposures, organisms are exposed to periods when pesticide concentrations are elevated and exert harmful effects and periods of no or low pesticide concentrations when the organisms can recover. The overall magnitude of the harmful effect of pulse exposure is a function of the concentration of the pesticide and the duration of the exposure period combined with the duration of the recovery period (Vallotton et al., 2008; Copin et al., 2015). To illustrate this we will use an example of the effects of herbicides on algae. When algae are exposed to a pulse of a herbicide, their population growth rate is decreased, leading to a smaller algae biomass compared to the situation if the algae were not exposed. When exposure ceases and recovery begins, the population growth rate will start to increase back to the pre- or non-exposure rate; however, although the algal biomass will also start to increase, it can never have the same algal biomass as it would if the same algae were not exposed. The more rapidly the effects of pesticides dissipate when exposure ceases the more rapidly the organisms can start to recover. Photosystem II herbicides have immediately reversible effects – that is, when exposure stops the population growth rate immediately recovers to the pre-exposure rate. Therefore, the average concentration of PSII herbicides over 182 days (see previous section) provides a better estimate of the risk during the wet season than estimating the risk using a higher percentile (e.g., 95th or 90th percentile).

Following exposure to other pesticides (i.e., not PSII herbicides) there is often a delay before the effects of exposure dissipate. Such delays increase the magnitude of the harmful effect of pesticide exposure. For example, algae exposed to such pesticides will have a delayed recovery of the population growth rate that will increase the difference in algal biomass between the exposed and non-exposed algae. Using the average concentration for such pesticides will underestimate the harmful effects of the pesticide exposure. A more detailed explanation for using the average concentration in estimating the risk posed to individual pesticides and how this is applied to estimating the risk of pesticide mixtures is provided in Attachment G.

Another reason for selecting the average rather than the 95th percentile is related to the nature of many of the SSDs used in the calculation of the Pesticide Risk Metric. Because the construction of SSDs and derivation of guideline values is dependent on having a uni-modal dataset, many of the SSDs are constructed using the most sensitive groups of organisms (see Attachment D). As a consequence, estimates of risk are more protective of ecosystems than would be the case if the SSDs represented all phylogenetic grouping in the ecosystem. To compensate for this, the average pesticide concentration is used instead of a 90th or 95th percentile pesticide concentration, which, will result in lower estimates of the risk posed by pesticides.

For the reasons stated above, the average daily estimate of the percentage of species affected over the wet season will be the value estimated in the Pesticide Risk Metric and reported in the Pesticide Risk Baseline for the 35 basins, six NRM regions and the GBRCA. These values will also be used to determine if the pesticide target has been reached at each location.

Calculation of the Average Percentage of Species Affected During the Wet Season

To provide a statistically robust estimate of the risk posed by pesticides over the wet season, an estimate of the risk was needed for all 182 days in the wet season. However, such data were not available for all 182 days at each site (it is not logistically possible to sample every day during the wet season). 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; however, it can be applied to any discipline.

In short, the multiple imputation method fitted a non-parametric Kernel Density function[12] to the distribution of pesticide mixture toxicity values for each site/year combination. In undertaking the multiple imputation calculations, 1000 imputed datasets were created for each site and year combination

[12] 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.

and the results were pooled and the average calculated. An estimated (imputed) percentage of species affected was generated for each day that did not have a risk value. The measured and imputed pesticide risk values were then combined so that there was a value for all 182 days in the wet season and the average percentage of species affected over the wet season was calculated.

The pesticide monitoring sites were mainly end of catchment sites[13] (also sometimes termed end of system sites) but there were a few sub-catchment[14] and river mouth[15] sites (Attachment A). The pesticide mixture toxicity was estimated for each monitoring site, irrespective of their location, and year for four groups of pesticides:

- PSII Herbicides;
- Other Herbicides;
- Insecticides; and
- Total Pesticides (all 22 selected pesticides)

The pesticides belonging to each of these groups are indicated in Table 6.

Table 6. The selected 22 pesticides and the group that they were allocated to for calculating pesticide mixture toxicity. All pesticides were also included in the Total Pesticides group

Name of pesticide	Pesticide Group
2,4-D	Other Herbicide
Ametryn	PSII Herbicide
Atrazine	PSII Herbicide
Chlorpyrifos	Insecticide
Diuron	PSII Herbicide
Fipronil	Insecticide
Fluroxypyr	Other Herbicide
Haloxyfop	Other Herbicide
Hexazinone	PSII Herbicide
Imazapic	Other Herbicide
Imidacloprid	Insecticide
Isoxaflutole and DKN	Other Herbicide
MCPA	Other Herbicide

[13] End of catchment sites are located at the lowest point in a river of creek where the volume of water passing that point can be accurately measured by a gauging station and typically is not subject to tidal influence close to the upper limit of the tide (Garzon-Garcia et al., 2015).
[14] Sub-catchment sites are typically well up-stream of end-of-catchment sites and at a location that permits the monitoring of the vast majority of a sub-catchment (Garzon-Garcia et al., 2015).
[15] River mouth sites are located as close as logistically possible to the mouth of rivers – as such they are under tidal influence and discharge is usually measured using a side-scanning acoustic doppler current profiler (ADCP).

Name of pesticide	Pesticide Group
Metribuzin	PSII Herbicide
Metsulfuron-methyl	Other Herbicide
Pendimethalin	Other Herbicide
Prometryn	PSII Herbicide
Simazine	PSII Herbicide
S-metolachlor (also metolachlor)	Other Herbicide
Tebuthiuron	PSII Herbicide
Terbuthylazine	PSII Herbicide
Triclopyr	Other Herbicide

Development of Pesticide Mixture Toxicity – Land Use Relationships

The preceding text explained how the pesticide mixture toxicity values were estimated. However, this is only one half of the information needed to develop the pesticide mixture toxicity vs. land-use relationships. The following text describes how the hydrological, land-use, and spatial variables were calculated.

Collation of hydrological variables

These variables were included to capture the influence of precipitation, soil moisture and run-off on pesticide mixture toxicity values. The Bureau of Meteorology provided the daily estimates of following data using the method of Frost et al. (2018) for every catchment where pesticides were monitored: rainfall, relative rainfall, relative run-off and soil moisture content. The relative values were calculated by comparing the daily values to the 10-year long-term average values for the same variable. All data that were not within the 182-day risk window for each site and year (Attachment H) were removed. The modified datasets were then used to calculate: the average relative rainfall; maximum relative rainfall; average relative runoff; maximum relative runoff; average daily rainfall; total rainfall; maximum daily rainfall; average soil moisture; total soil moisture and maximum soil moisture.

Calculation of land use variables

The most recent QLUMP (Queensland Land Use Mapping Program) dataset was extracted from QSpatial (State of Queensland, 2020b). QLUMP is part of the Australian Collaborative Land Use and Management Program (ACLUMP), coordinated by the Australian Bureau of Agricultural and Resource Economics and Sciences. The QLUMP layer is a polygon dataset with each feature having attributes describing land use classified according to the Australian Land Use and Management (ALUM) Classification Version 8 (ABARES, 2016).

To retain consistency with the approach used for the SOURCE Catchment model, grazing native vegetation (ALUM Code 2.1.0) was split into Grazing Open and Grazing Forested categories using a SLATS (Statewide Landcover and Trees Study) threshold of ≥20% groundcover, which relates to a Foliage Projected Cover (FPC) code ≥ 11. This was undertaken with the most recent publicly-available FPC imagery (2014) (State of Queensland, 2020c).

The land use data were then extracted for each monitored catchment and basin and exported to Excel. Land use types were agglomerated into twelve categories that align with the Reef Categories of the Source Catchment models (i.e. bananas, conservation, dryland cropping, forestry, forested grazing, open grazing, horticulture, irrigated crops, sugarcane, urban,[16] water, wetlands and other[17]) (Dr Melanie Shaw and Angela Pollett, *pers. comm.*). The agglomeration of QLUMP data to Reef Categories can be viewed in Waters et al. (2014). These land uses were included to examine their potential role on to pesticide pollution in line with the expansion of the focus of the Reef 2050 WQIP to sources other than agriculture. Land use was expressed as a per cent of total monitored catchment surface area.

Calculation of spatial variables

Shapefiles that delineate the catchment area above each pesticide monitoring site were derived with the assistance of the Department of Natural Resources, Mines and Energy (DNRME) and the Spatial Information Resource (SIR) Geoportal (a Queensland Government site which is not publicly available). Topographical maps, watercourses, one metre contours and imagery from the SIR Geoportal were used to digitise upstream catchment boundaries, which were then checked against local knowledge supplied by clients or subject matter experts. The surface area of each catchment's shapefile was calculated in ArcGIS Pro using the calculate geometry tool; and termed the Monitored Catchment Size (m^2).

Shapefiles for the 35 basins (reported on in the Reef 2050 WQIP and Reef Water Quality Report Card) were supplied by the Soil and Land Resources Unit within the Department of Environment and Science. The basin shapefiles were originally developed for the Great Barrier Reef (GBR) SOURCE Catchment model. Basin outlines for the SOURCE Catchment model were defined using freely available spatial data developed by the Unites States Geological Survey (USGS) that is currently hosted by Geosciences Australia (Gallant et al., 2011). The land elevation data were originally collected from NASA's Shuttle Radar Topography Mission (STRM) in 2000 and is available at 1-second arc (approximately 30 m) resolution (Gallant et al., 2011). It has been hydrologically enforced using 1:250,000 scale digital watercourse mapping to allow for the calculation of hydrological connectivity and delineation of hydrological attributes such as catchment outlines. The Soil and Land Resources Unit used these data to define internal drainage lines for GBR basins and sub-catchments when developing the SOURCE model. Some retrospective correction was necessary to infill flat coastal areas that did not yield streams of requisite magnitude for automated processing. In these cases, the placement of basin boundaries was based on supplementary data such as NRM Region boundaries, satellite imagery and local knowledge.

The natural resources management (NRM) region boundaries (shapefiles) were sourced from QSpatial (State of Queensland, 2020a) and are consistent with those used in the Reef 2050 WQIP 2017–2022 (Australian Government and Queensland Government, 2018). The surface area of each NRM region was extracted using the calculate geometry tool in ArcGIS Pro, then summed to provide an area for all six NRM Regions. The size of each monitored catchment relative to that of the entire GBRCA was then

[16] The agglomerated land use 'urban' is a combination of residential types such as urban residential, remote communities, and residential without agriculture. It also includes two types of residential land uses associated with agriculture: residential with farm infrastructure and residential with agriculture.
[17] The agglomerated land use 'other' is a combination of intensive animal production (e.g. poultry farms, feedlots), manufacturing & industrial (e.g. food processing plants, abattoirs, sawmills), residential and farm infrastructure, services (e.g. recreation, defence), utilities (e.g. water extraction, power generation), transport (e.g. roads, railways), mining, and waste management (e.g. effluent, landfill, sewage).

determined by dividing each monitored catchment surface area by the summed area of all six NRM regions. This provided the explanatory variable, Relative Monitored Catchment Size (m^2), for each of the sample sites.

Adopted Middle Thread Distance (AMTD) is the length of a waterway, in kilometres, measured along the middle of the deepest section of a watercourse from the sample site to the river mouth. Most AMTD data were extracted from the DNRME Stream Gauging Station Index 2014 (State of Queensland, 2019). AMTD was calculated manually for sites that had no entry in the Gauging Station Index using ArcGIS Pro. The AMTD was included in the development of the pesticide mixture toxicity — land use relationships to see if distance of the site from the river mouth affected pesticide mixture toxicity. Latitude and longitude of the monitoring sites were obtained as well as the adopted middle thread distance, monitored surface area of each catchment expressed as a percentage of all the catchments that discharge to the GBR, and the Natural Resource Management region in which each site was located.

Calculation of the pesticide mixture toxicity pesticide mixture — land use relationships

There was a total of 67 unique datasets (combinations of sites and years) available to be used in the derivation of the pesticide mixture toxicity — land use relationships (Table 7). The two datasets for the Mary River at Home Park (2015/2016 and 2016/2017) were removed from both the training and validation datasets as the site was an outlier in terms of AMTD (distance upstream) that impacted on the integrity of the pesticide mixture – land use relationships. The remaining 65 site/year combinations were divided into two datasets: the training set (80% of the data) used to derive the relationships; and the validation set (20% of the data) used to test the predictive accuracy of the relationships (i.e. to validate the relationships) (Table 7). Site/year combinations were randomly assigned to the training and validation sets.

The validation set was examined to ascertain how representative its data were compared to the entire dataset. It was found that it did not include any data for Sandy Creek – the site with by far the largest per cent of its catchment used for sugar cane (~45%) and the highest pesticide mixture toxicity values. As the correlation analysis indicated a strong relationship between the various pesticide mixture toxicity measurements and per cent sugar cane it was felt necessary to include one dataset for Sandy Creek in the validation set. From the three years of Sandy Creek data one was randomly selected and replaced a randomly selected dataset from those initially included as part of the validation set. The combinations of site and year allocated to the training and validation sets are presented in Table 7.

The correlation between all the variables was analysed using a Pearson's correlation test. This was done to determine which variables were strongly correlated to the various Pesticide Risk Metric measurements (PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides) and to determine if there was multi-collinearity in the explanatory variables. Some of the explanatory variables that were highly correlated with other variables were removed from the subsequent development of the pesticide mixture toxicity – land use relationships; however, they were at times substituted back into the models to check their influence on the response, and their interactions with other variables.

Table 7. Sites (in alphabetical order) and years used to provide aqueous pesticide concentration data to develop (training set) and validate (validation set) the pesticide mixture toxicity – land use relationships

Waterway	Training set			Validation set		
	2015/2016	2016/2017	2017/2018	2015/2016	2016/2017	2017/2018
Baffle Creek		-	X			
Barratta Creek	X	X	X			
Barron River		-	X			
Black River		-	X			
Boyne River		-	X			
Burdekin River	X	X		-		X
Burnett River at Ben Anderson Barrage	X				X	
Burnett River at Quay Street bridge			X			
Burrum River at Buxton Boat Ramp			X			
Calliope River			X			
Comet River	X	X	X			
East Barratta Creek			X			
Elliot River			X			
Fitzroy River	X		X		X	
Gregory River at Jarrett's Rd			X			
Haughton River at Powerline	X				X	
Haughton River at Giru Weir			X			
Herbert River	X	X	X			
Johnstone River	X		X		X	
Kolan River			X			
Mary River at Home Park		X*		X*		
Mary River at Churchill St			X			
Mossman River			X			
Mulgrave River	X	X	X			

Waterway	Training set			Validation set		
	2015/2016	2016/2017	2017/2018	2015/2016	2016/2017	2017/2018
North Johnstone		X		X		X
O'Connell at Caravan Park		X	X	X		
O'Connell at Stafford's Crossing		X				X
Pioneer River	X	X	X			
Proserpine River		X	X			
Russell River	X		X		X	
Sandy Creek	X	X				X
Styx River			X			
Tinana Creek	X	X				
Tully River		X	X	X		
Waterpark Creek			X			

* Mary River at Home Park was removed from both the training and validation datasets due to it being an outlier site in terms of AMTD (distance upstream)

Forward and backward step-wise linear regression variable selection techniques were initially conducted using non-transformed data in R. Diagnostic figures that examined the linearity and scatter of the residuals, normality (QQ plots), and leveraging, were generated for each relationship that was developed. These were conducted to assess the underlying assumptions of the multiple linear regression and to guide subsequent attempts to improve the relationships. In addition, Box-Cox plots were analysed to determine possible transformations of the response variable. Due to the predominantly right-tailed distribution of pesticide mixture data, a square root transformation of the response variable was used for all four pesticide risk measurements (PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides) when developing the pesticide mixture — land use relationships. This transform increased homogeneity of the variance for all four pesticide groups. Various polynomial transformations (e.g. quadratic and cubic) of the explanatory variables were tested individually and in combination to see if they improved the linearity of relationships (i.e. improved the diagnostic figures, adjusted R^2 values). The decisions on which y-variable transformations were to be applied were based on interpreting GAM (General Additive Model) figures. Due to the square root transformation of the response variable, the predictions from these relationships were back transformed, using ^2 transform, to obtain the predicted Pesticide Risk Metric estimates (average per cent species affected during the wet season).

Selection of the Best Pesticide Mixture Toxicity – Land Use Relationships

Multiple pesticide mixture toxicity – land use relationships were developed for each group of pesticides (PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides). The best relationship was identified for each group using the following criteria:

- how well the statistical assumptions of regression analysis were met (indicated by the diagnostic figures);
- the quality of the fit of the relationships to the pesticide mixture toxicity data in the training set (indicated by the adjusted coefficient of determination (R^2) values);
- how well the values predicted by the relationships agreed with the measured values for the sites in the validation set; and
- whether the predicted total mixture values for basins and regions were reasonable given the land use and measured pesticide mixture toxicity values of the monitored catchments.

Validation of the Pesticide Mixture Toxicity – Land Use Relationships

The best relationship for each group of pesticides (i.e. PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides) was validated by using them to predict the pesticide mixture toxicity of the sites in the validation set. The predicted pesticide mixture toxicity values were compared to the measured values and the mean absolute error (MAE) and the root mean square error (RMSE) determined. In addition, the predicted values were plotted against the measured values and compared to a 1:1 line.

Predicting the Pesticide Mixture Toxicity for 35 Basins, Six NRM Regions and the Great Barrier Reef Catchment Area

The pesticide mixture toxicity — land use relationships were developed using pesticide mixture toxicity, land use, spatial and hydrological data for three sampling years (2015/2016, 2016/2017 and 2017/2018). Therefore, where climate variables were important predictors for pesticide mixture toxicity in the catchment-level models (as is the case for the Insecticides), these were also included for prediction at the basin, NRM region and GBR scale. Yearly variation in climate data (unlike land use data for the years of this study), produced slightly different yearly predictions when the models were applied at the basin, NRM region and GBR scale. When this occurred, the average of the three yearly estimates of pesticide mixture toxicity was calculated for reporting. If the models did not include any climate variables the modelled relationships produced the same pesticide mixture toxicity prediction regardless of year, given that all other land use and spatial variables did not change.

The Reef Water Quality Report Card reports at three levels – basins (also called major catchments), NRM regions and the GBRCA. The 35 basins that are reported on in the Reef Water Quality Report Card and the regions they belong to are presented in Table 8. The pesticide mixture toxicity— land use relationships predict pesticide mixture toxicity at the sub-catchment and catchment scale depending on the location of the monitoring site. But as the hydrological, land use and spatial variables used in the relationships are for the land upstream of the monitoring site the location of the monitoring site (i.e., end of catchment, river-mouth or sub-catchment) is accounted for. The pesticide mixture toxicity predictions can be scaled up by replacing the sub-catchment and catchment-level values for the variables in the relationships with the corresponding values for basins to estimate the pesticide mixture toxicity at the basin level (Attachment I). Similarly, regional values of the variables (Attachment I) were substituted into the relationships to estimate the pesticide mixture toxicity at the regional level and GBRCA values (Attachment I) were substituted into the relationships to estimate the pesticide mixture toxicity at the GBRCA level. This scaling-up is equivalent to having a single river draining a basin, region of the entire GBRCA and discharging at a single point to the GBR lagoon and is equivalent to the approach used by the Source Catchment models for load-based calculations for total suspended solids, nutrients and previously for pesticides (David Waters, *pers. comm.*).

Ground-Truthing Predictions of the Pesticide Mixture Toxicity— Land Use Relationships

The ground-truthing is an additional quality assurance step, in addition to the validation of the pesticide mixture toxicity— land use relationships. The aim of the ground-truthing is to determine if the predicted pesticide mixture toxicity values for basins, regions and the GBRCA make sense and are reasonable. Three forms of ground-truthing were undertaken:

1. Expert elicitation;
2. Comparison of basin results to catchment results
3. Summing the predicted pesticide mixture toxicity results for PSII Herbicides, Other Herbicides and Insecticides and comparing these with the corresponding values for Total Pesticides.

Method 1. This method consists of asking appropriate experts to rank the expected pesticide mixture toxicity of the regions from lowest to highest and to rank the basins from lowest to highest without seeing the results of the Pesticide Risk Baseline. In addition, the rules of thumb that the experts use were collated.

Method 2. In this method, monitored catchments with similar land use values to each basin were identified. The pesticide mixture toxicity values of the similar catchments and basins were then compared. The logic being that (monitored) catchments and (predicted) basins with similar land use patterns should have similar pesticide mixture toxicity values. This will indicate whether the predicted pesticide mixture toxicity values are reasonable. An additional method was to compare the predicted pesticide mixture toxicity results for basins to the corresponding values from monitored catchments within the basin to determine whether the basin values are reasonable based on the differences in land use.

Method 3. Theoretically the sum of the PSII Herbicides, Other Herbicides and Insecticides mixture toxicity values should equal the Total Pesticides mixture toxicity values. However, the relationship to predict mixture toxicity for each of these groups were derived independently of each other, and therefore, the sum of the three pesticide groups mixture toxicity values is unlikely to exactly equal the Total Pesticides mixture toxicity values. Nonetheless, if the pesticide mixture toxicity— land use relationships make sensible predictions then there should be good agreement between the summed values of the three chemical groups (PSII Herbicides, Other Herbicides and Insecticides) and the Total Pesticides toxicity values. This method was only used to ground-truth the predicted mixture toxicity values for Total Pesticides.

Table 8. The 35 basins that are reported on in the Reef Water Quality Report Card and the Natural Resource Management Region that each basin belongs to

NRM Region	Basin	NRM Region	Basin
Cape York	Jacky Jacky Creek	Mackay/Whitsunday	Proserpine River
	Olive Pascoe River		O'Connell River
	Lockhart River		Pioneer River
	Stewart River		Plane Creek
	Normanby River	Fitzroy	Styx River
	Jeannie River		Shoalwater Creek
	Endeavour River		Waterpark Creek

NRM Region	Basin	NRM Region	Basin
Wet Tropics	Daintree River		Fitzroy River
	Mossman River		Calliope River
	Barron River		Boyne River
	Mulgrave-Russell River	Burnett Mary	Baffle Creek
	Johnstone River		Kolan Creek
	Tully River		Burnett River
	Murray River		Burrum River
	Herbert River		Mary River
Burdekin	Black River		
	Ross River		
	Haughton River		
	Burdekin River		
	Don River		

Qualitative Confidence Ranking of the Pesticide Risk Baseline

A multi-criteria analysis has been used to qualitatively assess the confidence, from low to high, in each indicator included in the Reef Water Quality Report Card. The approach combined expert opinion and direct measures of error for program components where available. This method (Australian Government and Queensland Government, 2019b) was used to determine the confidence ranking of the Pesticide Risk Baseline. This resulted in a three-bar confidence ranking (Figure 2)[18].

Figure 2. Representation of the confidence in the Pesticide Risk Baseline

Rationale for the confidence ranking

The confidence ranking has five components. The explanation for the score awarded to each component and the overall score is provided below.

Maturity of methods — A score of one was awarded because not all individual methods used have been reviewed, the combination of methods used have not been reviewed, and the relationships used to predict

[18] The confidence ranking was redone after just prior to release of this report as the report had been peer reviewed. This increased the score to two for maturity of the method and increased the total score to nine (from 8.5), but this did not result in change to the confidence ranking (refer to Table 9).

pesticide risk have not been reviewed. However, this component has a weighting of 0.5 (Australian Government and Queensland Government, 2019b), so the score is 0.5.

Validation — A score of two was awarded because the land use, spatial and hydrologic variables for predicting the pesticide risk (per cent of species affected), the pesticide monitoring (concentration) data, and the relationships used to predict pesticide risk were validated, but there is no validation of the per cent of species protected at the end of catchments.

Representativeness — A score of three was awarded because in 28 of the 35 basins that discharge to the Great Barrier Reef at least one catchment was monitored for pesticides. The seven basins without any pesticide monitoring are in Cape York, which should have a very low risk from pesticides (based on land use statistics).

Directness — A score of two was awarded because the assessment uses a mix of quantified assessments (i.e. catchment monitoring data, laboratory-based ecotoxicology data, remotely sensed land-use and spatial data, and modelled hydrological data) however, the per cent of species protected at the end of catchments is not directly measured.

Measurement error —A score of one was awarded because the error in the multiple data sources used and the multiple steps in the methodology is not able to be quantified at this point in time.

The scores were then summed and the confidence determined using the method set out in Table 9.

Table 9. The process for converting confidence scores to a confidence ranking (modified from Australian Government and Queensland Government, 2019b)

Confidence score categories	Ranking
≤ 6	One bar
6.5 to 8	Two bar
8.5 to 9.5	Three bar
10 to 11.5	Four bar
≥ 12	Five bar

Risk Management

The risk management consists of two components: First, comparing the Pesticide Risk Baseline values (per cent of species protected) against the pesticide target set out in the Reef 2050 WQIP (Australian Government and Queensland Government, 2018) for the 35 basins, six NRM regions and the GBRCA. Second, Pesticide Risk Baseline values are ranked in order to determine the relative risk that pesticides pose in GBR catchments, basins and NRM regions.

Classification of the Risk Posed by Pesticide Mixtures

In determining the risk classification all Pesticide Risk Metric values were rounded off to the nearest integer. Prior to then the data were rounded off to either two decimal places or four significant figures. The Pesticide Risk Metric predicted the per cent of species that would be affected by mixtures of up to 22 pesticides. These were converted to the corresponding per cent of species protected using the following equation

Per cent species protected = 100 – per cent species affected (Eqn 2)

This conversion was done to permit comparison of the results of the Pesticide Risk Metric and Pesticide Risk Baseline with the Pesticide Target which is expressed in terms of the percentage of species protected.

A system of classifying the risk posed by pesticide mixtures was developed that indicated to users the relative magnitude of the risk from 'very low' to 'very high' risk. These risk classes were based on the ecological condition classes used in the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000; ANZG, 2018) and the corresponding percentage of species to be protected i.e. 99%, 95%, 90% and 80% of species (Table 10). If at least 99% of aquatic species were estimated to be protected (the desired level for high conservation waterbodies such as those in national parks where there should be minimal to no disturbance) then pesticides were classed as posing a 'very low' risk. The level of protection to be provided for slightly to moderately disturbed waterbodies is at least 95% of species (ANZG, 2018); hence, when the percentage of species protected was estimated to be between 99 and 95% the risk posed by pesticides was classed as 'low'. For highly disturbed waterways the aim is to protect 90 or 80% of species (ANZG, 2018). Therefore, when it is estimated that less than 80% of species were protected the risk posed by pesticides was considered 'very high'. There are two bands of protection between the very high (<85% species protected) and the low risk (99–95% species protected). These were, therefore, classed as posing a moderate risk (95–90% species protected) and a high risk (90–80% species protected). The classification scheme is presented in Table 10. The risk classes should be used to indicate the relative risk in different waterways or the change in risk at a waterbody over time, and in turn would be used to confirm risk-management priorities or as a measure of the success of land management initiatives.

Table 10. The ecological condition classes and the corresponding per cent of species to be protected that are used in the Australian and New Zealand water quality guidelines (ANZG, 2018) and the risk classes allocated to these in the Pesticide Risk Baseline

Ecological condition (ANZG, 2018)	Per cent species protected	Risk class
High ecological value (minimally disturbed)	≥ 99	Very low
Slightly to moderately disturbed	95 to < 99	Low
Highly disturbed	90 to < 95	Moderate
	80 to < 90	High
	< 80	Very High

Calculation of the Relative Contribution of Individual Pesticides and Pesticide Groups to Total Pesticide Mixture Toxicity

An approximation of the contribution of each pesticide, PSII Herbicides, Other Herbicides and Insecticides to the toxicity of all 22 pesticides (Total Pesticides) was determined using the following equation:

$$\% \text{ contribution} = (\text{average } \% \text{ affected species}_{X} / \text{ average } \% \text{ affected species}_{\text{Total}}) \times 100 \quad \text{(Eqn 3)}$$

where the subscript 'x' denotes any of the individual pesticides, PSII Herbicides, Other Herbicides or Insecticides and the subscript 'Total' denotes the Total Pesticides toxicity (of all 22 pesticides).

The resulting contribution values will be useful to indicate which pesticide or group of pesticides contribute most to the total toxicity of the pesticides, and therefore, should be the focus of management actions or policy initiatives to reduce the toxicity of pesticides in discharge to the GBR.

Reporting Data

All the data used to derive the pesticide mixture toxicity – land use relationships are reported to three or four significant figures although more were used in the actual calculations. However, the final results of the pesticide risk metric calculations (per cent of species protected) are reported to the nearest whole integer. This was done to ensure consistency of reporting across programs that feed data to the Reef Water Quality Report Card. The risk categories discussed in this report are based on the rounded pesticide mixture results.

Results and Discussion

Appropriateness of the Selected Pesticides

Currently, the best estimate of the toxicity of pesticide mixtures in waterways that discharge to the GBR is that by Spilsbury et al. (2020). They assessed the average contribution of 50 individual pesticides (Table 75 and Table 76, Attachment J) to the total pesticide mixture toxicity in over 5600 water samples collected from waterways that discharge to the GBR between 2010/2011 to 2016/2017. The 50 pesticides included in Spilsbury et al. (2020) included 19 of the 22 pesticides included in the Pesticide Risk Metric and Pesticide Risk Baseline. The three pesticides included in the Pesticide Risk Metric and Pesticide Risk Baseline but not in Spilsbury et al. (2020) were fipronil, fluroxypyr and pendimethalin. The pesticides included in the Pesticide Risk Metric and Pesticide Risk Baseline account for over 99% of the total toxicity of the 50 pesticides included in Spilsbury et al. (2020). Therefore, the 22 pesticides included in the Pesticide Risk Metric and Pesticide Risk Baseline cover the vast majority of the toxicity of pesticides known to occur in GBR waterways.

However, neither Spilsbury et al. (2020) nor the Pesticide Risk Metric and Pesticide Risk Baseline include all pesticides likely to be present in GBR waterways. For example, there are over 100 active ingredients registered by the Australian Pharmaceutical and Veterinary Medicine Authority (APVMA) for use on sugar cane, mung beans, soybeans, corn and rice (APVMA, 2019). Even if they were monitored their contribution to the total toxicity could not be assessed without each pesticide having a SSD.

Species Sensitivity Distributions

Species sensitivity distributions were successfully derived for all the selected pesticides. Combining the fresh and marine ecotoxicity data in deriving the SSDs often meant that there were more of the highest preference data (i.e., chronic EC10/NOEC data) available than when SSDs were derived separately for fresh and marine species. Because the SSDs in this report use both fresh and marine data they and the resulting protective concentration values are not exactly the same as those published in ANZG (2018) or King et al. (2017a, b). Key characteristics (the number and type of data and the type of organisms used to derive the SSDs, the fit of the SSD to the data and the reliability of the SSD) of the SSDs are summarised in Table 11.

Table 11. The number and type of ecotoxicity data and the type of organisms used to derive the species sensitivity distributions (SSDs) , the fit of the SSDs to the data and the reliability of the SSD for each of the 22 selected pesticides

Pesticide	No. data	Data type[1]	Organism type	Fit of SSD	Reliability[2]
2,4-D	20	C	All available	Poor	Moderate
Ametryn	17	C	Aquatic plants only	Good	Very high
Atrazine	50	C	Aquatic plants only	Good	Very high
Chlorpyrifos	24	C	All available	Poor	Moderate
Diuron	22	C	Aquatic plants only	Good	Very high
Fipronil	24	A & C	Aquatic arthropods only	Good	Moderate
Fluroxypyr	5	C	Aquatic plants only	Good	Moderate

Pesticide	No. data	Data type[1]	Organism type	Fit of SSD	Reliability[2]
Haloxyfop	6	A & C	All available	Poor	Low
Hexazinone	8	C	Aquatic plants only	Good	High
Imazapic	7	C	Aquatic plants only	Poor	Low
Imidacloprid	23	A & C	Aquatic arthropods only	Good	Moderate
Isoxaflutole	8	C	All available	Poor	Moderate
MCPA	10	C	All available	Good	High
Metolachlor	15	C	All available	Good	Very high
Metribuzin	15	C	Aquatic plants only	Good	Very high
Metsulfuron-methyl	9	C	All available	Good	High
Pendimethalin	10	C	All available	Good	High
Prometryn	8	C	Aquatic plants only	Good	High
Simazine	8	C	Aquatic plants only	Good	High
Tebuthiuron	7	C	Aquatic plants only	Good	Moderate
Terbuthylazine	18	C	Aquatic plants only	Good	Very high
Triclopyr	18	A & C	Aquatic plants only	Good	Moderate

[1] A = acute toxicity data, C = chronic toxicity data (as defined by Warne et al., 2018). [2] The method for determining SSD reliability is described in Attachment C.

The reliability of the SSDs generated in this project range from low to very high, with the majority (20 out of 22) having at least a moderate reliability (Table 11). The reliability classification (Warne et al., 2018; Attachment C) provides a simple and transparent means of indicating the general level of confidence in a DGV. The reliability classification also indicates DGVs that would benefit from the addition of more toxicity data. The greatest benefit would be obtained by generating additional toxicity data for pesticides with low and moderate reliability (Table 11). It is not possible to know *a priori* how the inclusion of additional toxicity data would affect the DGVs, but it would improve the statistical robustness of the SSDs. An example of a study designed to address such data gaps is the National Environment Sciences Program (NESP) funded project Tropical Water Quality Hub Project 3.1.5 "Ecotoxicology of pesticides on the Great Barrier Reef for guideline development and risk assessments." (Negri et al., 2020). This project generated toxicity data for 21 pesticides to 16 tropical aquatic species. The data gaps addressed by Negri et al. (2020) had earlier been identified by the Queensland Department of Environment and Science, as part of their work to develop SSDs for a suite of pesticides used in Great Barrier Reef catchments.

The toxicity data for 14 of the 22 pesticides were bi-modal, and therefore, the SSDs were derived exclusively using the toxicity data for the most sensitive group of organisms (Table 11) in accordance with the methods for deriving the Australian and New Zealand Guidelines for Fresh and Marine Water Quality

(Warne et al., 2018). If the toxicity data for herbicides were bi-modal, the most sensitive group of organisms was always aquatic plants. Similarly, if the toxicity data for insecticides were bi-modal, the most sensitive group of organisms was always aquatic arthropods (i.e. aquatic crustaceans and insects). The SSDs for the remaining pesticides were based on toxicity data for all available species – as there was no statistically demonstrable indication that they were not uni-modal.

Species sensitivity distributions derived using toxicity data for all species (i.e. a pesticide with a uni-modal distribution of toxicity data) estimate the concentration at which a percentage of all aquatic species should be affected (e.g. at a PC95, 5% of all aquatic species should experience harmful effects). In contrast, SSDs derived using toxicity data from only the most sensitive group of organisms (i.e. a pesticide with a bi-modal distribution of toxicity data) estimate the concentration at which a certain percentage of the most sensitive group of organisms should be affected (e.g., at a PC95, 5% of aquatic plants should experience harmful effects).

This method for dealing with uni- and bi-modal toxicity data means that when the effects of each pesticide are combined (using the method described earlier), different units are being combined (e.g., x% of all species affected + y% of aquatic plant species affected + z% of aquatic arthropods affected). The only way to have a common set of units would be to either:

- always use toxicity data for all available species — the approach adopted by Posthuma et al. (2019). This would address the issue of different units; however, it would invalidate a key assumption of SSDs and the resulting DGVs; or
- always use toxicity data for one group of organisms — this would mean that for some pesticides the SSD and resulting DGVs would not be based on the most sensitive group of organisms and therefore would not provide adequate protection.

Rather than adopting either of these options it was decided to treat the percentage of all species affected, percentage of aquatic plants affected and percentage of aquatic arthropods affected as being ecologically equal. For example, 5% of affected aquatic plant species equals 5% of all aquatic species.

However, the PC values derived from any SSD based on only the most sensitive group of organisms are likely to over-estimate the percentage of species of other types of organisms that are affected. This is illustrated in Figure 3 which has separate SSDs for plants, fish and insects exposed to the same hypothetical toxicant. The size of this over-estimation will vary for different pesticides, depending on the relative difference in sensitivity of the groups of organisms.

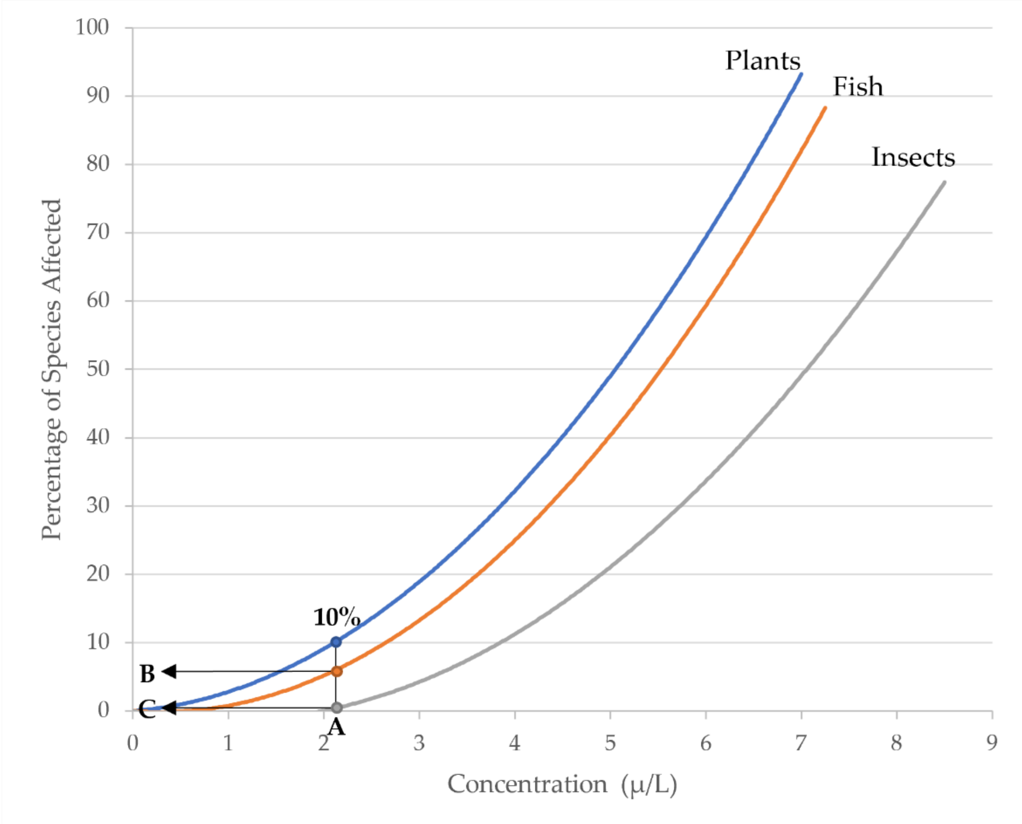

Figure 3. Three hypothetical species sensitivity distributions (SSDs) for a herbicide based on the most sensitive group of organisms (plants, blue line) and two less sensitivae groups of organisms (fish -orange line and insects – grey line). 'A' is the concentration that corresponds to 10% of the plants being affected. The same concentration (A) affects a lower percentage of fish (B) and insects (C), respectively

Although SSDs derived using only the most sensitive group of organisms will over-estimate the percentage of all aquatic species affected this is considered reasonable for the following reasons:

- There is currently no proven alternative to deriving SSDs using the most sensitive group of organisms when the toxicity data are bi-modal and this is the method used to derive the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (Warne et al., 2018);
- SSDs typically only consider the direct effects of toxicants (refer to glossary) because essentially all of the data used in deriving SSDs were generated by exposing a single species to a toxicant. The SSDs therefore do not consider the indirect effects of toxicants and thus are potentially underestimating the total effects of toxicants to other groups of organisms[19].

[19] One type of indirect effect is that reductions in the number or biomass of food organisms, by the direct effects of pesticides, can lead to population declines amongst predators. For example, Kasai and Hanazato (1995) found that exposure to the herbicide simetryn in mesocosms led to reduced phytoplankton abundance (a direct effect) but also led to reduced zooplankton abundance due to the decreased phytoplankton (an indirect effect). Boyle et al. (1996) similarly found that the insecticide diflubenzuron directly decreased zooplankton abundance and had an indirect effect of decreasing bluegill fish populations (due to reduced food (zooplankton) availability). A more recent example are the marked population declines of two commercially harvested zooplanktivorous fish (*Hypomesus nipponensis* and *Anguilla japonica)* in Shimane Prefecture, Japan following the collapse of zooplankton populations that was associated with the introduction of the insecticide imidacloprid in a rice-growing area (Yamamuro et al., 2019). Meanwhile, the populations of a planktivorous fish (*Salangichthys microdon*) in the same lake remained stable over the same time period.
Other indirect effects such as changes in community structure with certain organisms experiencing large population increases have been reported (Preston, 2002; and references therein). Preston (2002) states that this is the most

- Finally, the Pesticide Risk Metric uses the average (rather than the 95th percentile) of the percentage of species affected over the wet season (Attachment G) which will provide a lower estimate of the risk posed.

Land use, Hydrological and Spatial Explanatory Variables for the Monitored Sites

The values of the land use, hydrological and spatial variables used to derive the pesticide mixture toxicity— land use relationships are presented in Attachment I. The spatial variables will not be discussed as they are unique to each site. The three years included in the project were drier than average years with average rainfall close to the half the long-term average (ranging from 41.21 to 55.75% of the long-term average, Attachment I) but the maximum daily rainfall values at all sites were essentially the same as the long-term average of the daily maximum values with values of 97.66 to 100%. The average run-off was more variable than average rainfall with values ranging from 28.67 to 78.67% of the long-term average run-off (Attachment I). As with the maximum rainfall values, the maximum run-off values were essentially equal to the long-term maximum daily run-off values, with values of 90 to 100%.

The percentage of each monitored catchment used for growing bananas was zero for most catchments with only five catchments having more than one per cent bananas (Attachment I). The monitored catchments where bananas occurred had between 1% (Barron River) and 3.3% (Tully River) of their land used to grow bananas. The percentage of each monitored catchment that was conservation ranged from approximately 2% (Barratta Creek) to approximately 84% (Mossman River) with only 12 catchments having more than 20% of land used for conservation (Attachment I). The amount of each monitored catchment used for dryland cropping ranged from approximately 0% (Barratta Creek, Barron River, Russell River, Tully River, Herbert River, Calliope River, East Barratta Creek, Sandy Creek, O'Connell River, Boyne River, Proserpine River, Pioneer River, Waterpark Creek and Mossman River) to 10% (Comet River) with only three catchments having approximately 1% or more of dryland cropping (Attachment I). The amount of each monitored catchment devoted to forestry ranged from approximately 0% (Barratta and East Barratta creeks, Mossman River, Mulgrave River, Russell River, North Johnstone River, Johnstone River, Tully River and the Burdekin River) to approximately 60% (Tinana Creek) with 15 catchments having at least 10% forestry (Attachment I). The amount of forested grazing in the monitored catchments ranged from 0.4% (Mossman River) to approximately 68% (Black River), with only five catchments containing less than 10% forested grazing (Attachment I). The amount of open grazing ranged from 0.2% (Waterpark Creek) to approximately 40% (Styx River) with 11 monitored catchments containing more than 15% open grazing (Attachment I). The amount of each monitored catchment used for horticulture ranged from approximately 0% (Herbert River, Burdekin River, O'Connell River, Pioneer River, Styx River, Fitzroy River, Comet River, Calliope River, Boyne River and Baffle Creek) to

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common type of indirect effect. These indirect changes to community structure generally occur when one group of organisms that preys on another (e.g. zooplankton eating phytoplankton) experience population declines or are made locally extinct and as a result populations of the predated group of organisms expand. Large changes to the size of populations and community structure are in themselves changes from the normal or control states and therefore are adverse effects of pollutants.

The SSDs that do not use the toxicity data for all available species were either herbicides or insecticides. The most sensitive organisms to herbicides are aquatic plants and algae which are at the bottom of most aquatic food webs the most sensitive organisms to insecticides are insects and crustaceans which are generally towards the bottom of food webs. Direct effects of herbicides and insecticides on these types of organisms could theoretically, and have experimentally been shown to, cause population declines in organisms higher in the food chain.

approximately 8% (Elliot River) with only seven catchments containing more than 1% horticulture (Attachment I). The percentage of irrigated cropping in each monitored catchment ranged from approximately 0% (Styx River, Mulgrave River, Mossman River, Waterpark Creek, Tully River, Pioneer River and Black River) to approximately 2.5% (Barron River) with only three catchments containing more than 1% irrigated cropping (Attachment I). The amount of each monitored catchment used for "other" land uses ranged from essentially 0% (10 catchments) to 2% (Elliot River) (Attachment I). The amount of each monitored catchment used to grow sugarcane ranged from 0% (Waterpark Creek, Styx River, Baffle Creek, Fitzroy River, Boyne River, Calliope River and Black River) to 45% (Sandy Creek) with only six catchments containing more than 15% sugarcane (Attachment I). The amount of urban land use in the monitored catchments ranged from essentially 0% (Barratta Creek, East Barratta Creek, Burdekin River, Styx River and Comet River) to 5.8% (Mary River) with 19 catchments containing more than one per cent urban land use (Attachment I). The amount of land used for water in the monitored catchments ranged from less than 1% (e.g. Styx and Fitzroy rivers) to 7% (Proserpine) with 20 catchments containing less than 1% water (Attachment I). The amount of each monitored catchment that is wetlands ranged from approximately 0% (Styx River, Waterpark Creek, Calliope River and Tinana Creek) to approximately 3% (East Barratta Creek) and 12 catchments had more than 1% wetlands (Attachment I).

Correlation Coefficient Values

The relationships between all the variables collated for developing the pesticide mixture toxicity — land use relationships were assessed using Pearson's correlation coefficient (Figure 4). The major findings from this analysis were that:

- all four measures of pesticide mixture toxicity (i.e. PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides) were all strongly positively correlated to each other;
- positive correlations with pesticide mixture toxicity included % sugar cane and % water that had strong correlations, % other, % urban, % wetland and AMTD (distance of the site upstream) that had moderate and weak correlations; and
- weak or moderate negative correlations with pesticide mixture toxicity included % bananas[20], % conservation, % forested grazing, % irrigated cropping, catchment size, average rainfall and average soil moisture.
- of the variables correlated with pesticide mixture toxicity, some cross correlation (i.e. between explanatory variables) was evident. This was the case for % grazing forested and % grazing open, % dryland cropping and % irrigated cropping, % sugar cane and % grazing forested, % water, % wetland and % water.

[20] The mapping of bananas land use has focussed on the Wet Tropics and the extent of bananas in other NRM regions may not be accurate.

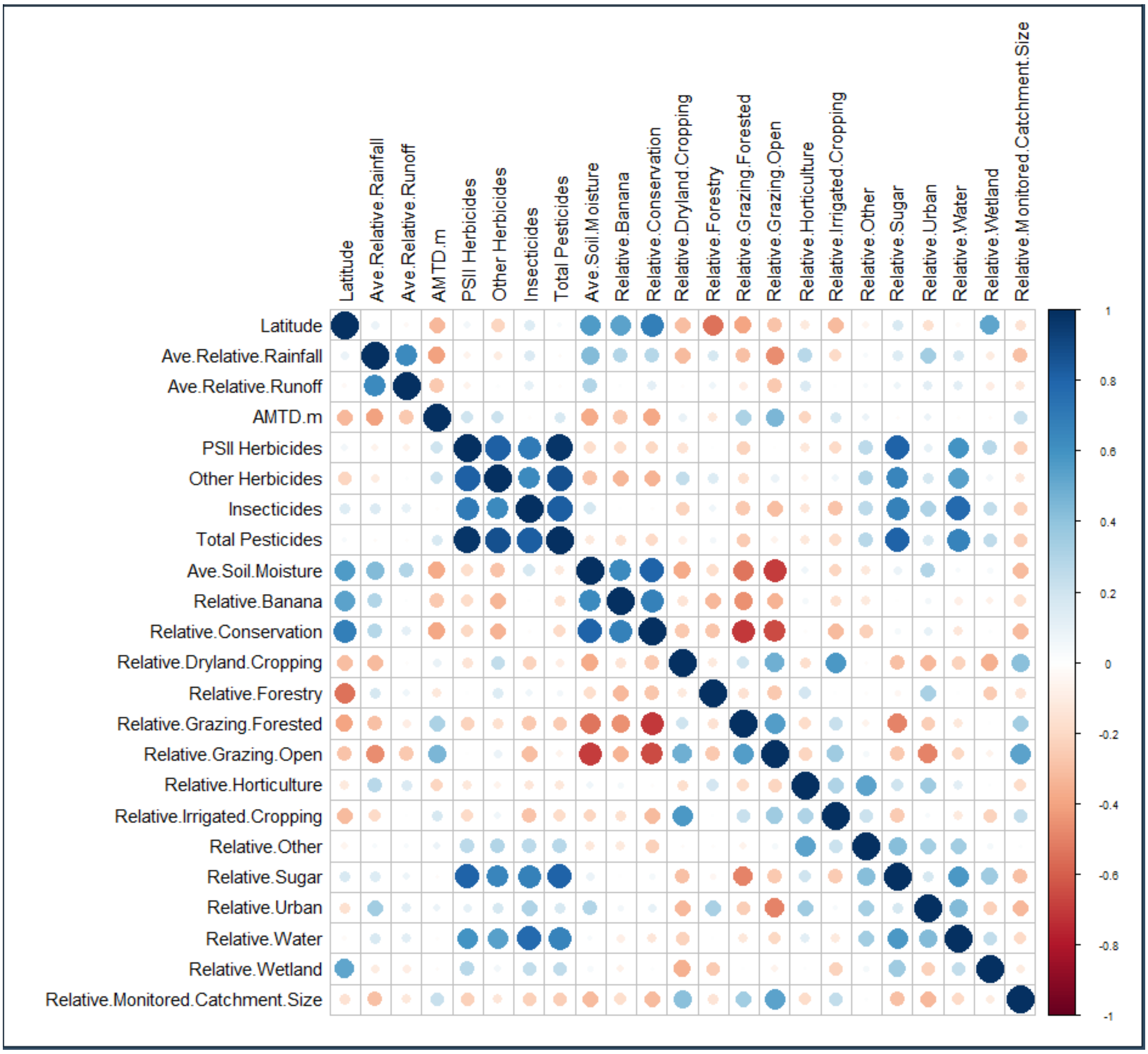

Figure 4. Correlation coefficient matrix for all the variables compiled to derive the pesticide mixture toxicity — land use relationships. The direction and magnitude of the correlation coefficient is indicated by the colour and size of the circle, respectively – the darker the colour and larger the circle the stronger the correlation. Blue circles indicate positive correlation coefficients and red circles indicate negative correlation coefficients

Pesticide Mixture Toxicity Results

The monitored Total Pesticides mixture toxicity results (expressed as average per cent of affected species during the wet season) for the 67 site and year combinations are presented in Table 12. The pesticide mixture toxicity results for PSII Herbicides, Other Herbicides and Insecticides are in Attachment K. The Total Pesticides mixture toxicity estimates were quite varied across the monitored catchments ranging from less than 0.1% (Boyne River, Calliope River, Styx River and Waterpark Creek that all occurred in 2017/2018) to approximately 42% (Sandy Creek, 2017/2018). This spatial variation was interpreted to mean that land use variables, spatial variables or hydrological variables may play a role in explaining the variation in pesticide mixture toxicity values. The Total Pesticides mixture toxicity estimates generally did not vary much at a site over the years included in the study. The largest difference between the three years was 8.51% for the Pioneer River. The average difference at catchments that had at least two years data was 3.12% and the median difference was 2.56%. This limited temporal variability at sites was interpreted to mean that it might be possible to develop relationships based on all 67 site and year combinations.

Table 12. Total Pesticides mixture toxicity values (estimated using monitoring data) for all 22 pesticides at the 67 site and year combinations

Waterway	2015–2016	2016–2017	2017–2018
Baffle Creek at Newton Road	-	-	0.14
Barratta Creek at Northcote	18.58	26.98	22.41
Barron River at Rink's Close Jetty	-	-	0.64
Black River at Bruce Highway	-	-	0.34
Boyne River at Boyne Island	-	-	0.00
Burdekin River at Home Hill	0.32	1.48	0.61
Burnett River at Ben Anderson Barrage	1.53	1.81	-
Burnett River at Quay St. Bridge	-	-	3.12
Burrum River at Buxton Boat Ramp	-	-	0.27
Calliope River at Old Bruce Highway	-	-	0.08
Comet River at Comet Weir	8.40	9.28	10.66
East Barratta Creek at Jerona Road	-	-	9.44
Elliot River at Riverview Boat Ramp	-	-	4.70
Fitzroy River at Rockhampton	1.71	1.96	2.39
Gregory River at Jarrett's Road	-	-	7.52
Haughton River at Powerline	6.51	6.06	-
Haughton River at Giru weir	-	-	2.50
Herbert River at Ingham	2.51	5.25	3.86
Johnstone River at Coquette Point	3.24	3.42	4.94
Kolan River at Booyan Boat Ramp	-	-	5.60
Mary River at Homepark	2.07	2.83	-
Mary River at Churchill St.	-	-	3.28
Mossman River at Bonnie Doon	-	-	3.22
Mulgrave River at Deeral	1.97	4.35	5.94
North Johnstone River at Old Bruce Highway	2.39	4.42	2.33
O'Connell River at Stafford's Crossing	-	12.20	8.30
O'Connell River at Caravan Park	7.72	12.39	7.92
Pioneer River at Dumbleton Weir	18.12	16.96	25.47

Waterway	2015–2016	2016–2017	2017–2018
Proserpine River at Glen Isla	-	27.01	29.28
Russell River at East Russell	2.72	4.46	6.59
Sandy Creek at Homebush	40.34	38.88	41.68
Styx River at Ogmore	-	-	0.00
Tinana Creek at Barrage	9.69	3.52	
Tully River at Euramo	4.89	7.45	6.67
Waterpark Creek at Corbett's Landing	-	-	0.00

Bioavailability

Not all of a pesticide in water is available to be absorbed by biota and hence cause toxic effects. Physical, biological and chemical processes can all modify the bioavailability of pesticides (McLaughlin and Lanno, 2013). For example, Davis et al. (2012) and Packett (2014) found that between 10% and approximately 33% of a range of pesticides were bound to suspended sediment in waters of Barratta Creek and Fitzroy River, respectively. These bound pesticides are generally not available to water column dwelling organisms (e.g. fish, algae). The bioavailable fraction can also vary both in space and time (Semple, 2004). Therefore, aqueous concentrations of pesticides were used in the Pesticide Risk Metric and Pesticide Risk Baseline calculations. This is considered appropriate as the toxicity data, which are the basis of the SSDs, the Default Guideline Values and the Pesticide Target, are all based on aqueous concentrations. It should be noted that by using the aqueous concentration the Pesticide Risk Metric and Pesticide Risk Baseline only consider the impacts to water column dwelling organisms. It does not consider the impacts exerted by pesticides to benthic organisms.

Interpretation of the Results from the Pesticide Risk Metric

The pesticide mixture toxicity values generated by the Pesticide Risk Metric are estimates of the per cent of aquatic species that should be adversely affected by pesticides in water samples (per cent species affected). They are estimates because they have been calculated from pesticide concentration data and laboratory-based pesticide toxicity data using a number of methods which all have assumptions (many of which have been discussed in this report) associated with them. As they are estimates, the per cent of species affected values should not be taken as absolute values. For example, an estimate of 5% species affected does not mean that exactly 5% of species in a waterway will be affected. We do not have the true per cent of species affected in the waterways of the GBR, nor are we ever likely to, as this would require monitoring all species within the ecosystem. Therefore, it is not possible to validate the estimates of the per cent of species affected generated by the Pesticide Risk Metric with an ecological assessment.

However, numerous field-based studies have compared the estimates of pesticide mixture toxicity and measures of ecological condition for various organism groups (e.g., Lies and Von Ohe, 2005; Posthuma and De Zwart, 2006; Beketov et al., 2009; Carafa et al., 2011; Posthuma and De Zwart, 2012; Rasmussen et al., 2013; Schäfer et al., 2013; Orlinskiy et al., 2015; Hunt et al., 2016; Kuzmanovic et al., 2016; Knillmann et al., 2018; Munz et al., 2017; Wood et al., 2019). These studies fall into two distinct groups. In the first group the pesticide mixture toxicity was estimated by combining SSDs and calculating the multiple substance potentially affected fraction (essentially, the methods used in the current project) (Posthuma and De

Zwart, 2006; Posthuma and De Zwart, 2012; Schäfer et al., 2013; Munz et al., 2017), while in the second group the Toxic Unit approach (Attachment E) was used (Lies and Von Ohe, 2005; Beketov et al., 2009; Carafa et al., 2011; Rasmussen et al., 2013; Orlinskiy et al., 2015; Hunt et al., 2016; Kuzmanovic et al., 2016; Knillmann et al., 2018). While these methods are not identical they both use either the Concentration Addition or Independent Action models of joint action and pesticide concentration data to estimate the toxicity of pesticide mixtures. With one exception, all these studies measured ecological condition using the SPEAR (SPEcies At Risk index) index[21], which is a measure of the impacts of pesticides on species composition of aquatic insects or algae in waterways. The exception was Posthuma and De Zwart (2006) who looked at fish species composition. All these studies estimated the pesticide mixture toxicity and ecological condition for waterways with differing degrees of pesticide pollution. They found statistically significant relationships between pesticide mixture toxicity increases (PAF or TU increased) and decreases in the number of species (SPEAR values or fish observed to affected ratios decreased). A similar study has also been conducted in 14 rivers of the GBRCA by Wood et al. (2019). They found there was a statistically significant negative correlation between $\text{SPEAR}_{\text{herbicides}}$[22] and the sum of TUs for herbicides. Thus, while the true per cent of species affected in waterways that correspond to a specific estimate of per cent species affected is not known, it is clear that the greater the estimate of pesticide mixture toxicity the greater the harmful effects to aquatic ecosystems. These studies were conducted in waterways in Argentina, Australia, Denmark, Finland, France, Germany, Netherlands, south-western Siberia (Russia), Spain, Switzerland, the USA indicating that these relationships are not affected by country and climate and should hold true in Queensland. Beketov and Liess (2008), using a SPEAR index modified to represent community sensitivity to organic contaminants, also found a strong relationship with the sum of TUs for organic contamination in rivers in south-western Siberia, Russia.

Posthuma and De Zwart (2006) stated that msPAF values "may have the ability to assess ecological condition of waterways and identify toxicity-impaired ecosystems". However, with over a decade of additional research being conducted, Munz et al. (2017) stated that the use of both mixture toxicity estimation methods and ecological condition methods is optimal, but that the correlation between the two methods suggests that either is useful for detecting pesticide effects in aquatic environments.

The above research shows that while the values generated by the Pesticide Risk Metric should not be used in an absolute sense, they are appropriate to establish the Pesticide Risk Baseline and to compare to the pesticide target.

Pesticide Mixture Toxicity — Land Use Relationships

The Relationship for PSII Herbicides

The best pesticide mixture toxicity — land use relationship for PSII Herbicides is presented in Table 13. The relationship has 46 degrees of freedom and an adjusted R^2 value of 0.79. The data for Mary River at Home Park in 2016/2017 were removed from the training set because this site was influencing the relationship between AMTD (adopted middle thread distance, a surrogate for distance upstream) and PSII

[21] The SPEAR index uses physiological traits of the species to assign them as sensitive or tolerant. The traits considered are: sensitivity to pesticides; generation time; whether there are aquatic lifestages; and their ability to migrate and recolonise waterways affected by pesticides (UFZ, 2020). The SPEAR index is a measure of the relative abundance of macroinvertebrate species that are sensitive to pesticides compared to tolerant species in waterways.
[22] $\text{SPEAR}_{\text{herbicides}}$ is a SPEAR index that has been modified (Wood et al., 2019) so that it classifies aquatic plants into species that are sensitive or tolerant to herbicides.

Herbicide risk. With this data removed, the adjusted R^2 values for the relationship between AMTD and PSII Herbicide mixture toxicity increased from 0.28 to 0.42 and overall model linearity improved.

Table 13. The variables and their coefficients, standard error and probability for the pesticide mixture toxicity — land use relationship for photosystem II inhibiting herbicides (PSII Herbicides)

Variable	Coefficient	Standard Error	Probability
y-intercept	2.124	0.288	2.56×10^{-9}
AMTD	5.08×10^{-6}	8.62×10^{-6}	0.558[#]
% Conservation	-1.421	0.466	0.0038
% Horticulture	-37.58	7.955	2.21×10^{-5}
% Irrigated cropping	54.60	23.55	0.0249
% Sugar cane	9.262	0.762	5.77×10^{-16}
% Sugar $cane_{poly}$[##]	-3.730	0.760	1.20×10^{-5}

[#] While AMTD was not statistically significant in the relationship for PSII herbicides it was included because it helped improve the linearity (Attachment L). [##] A quadratic (second order) polynomial function was applied to this variable.

The diagnostic figures (Attachment L) for the relationship indicate that there was a slight bunching of residuals towards the y-axis due to the predominance of left-censored data (common in environmental monitoring). However, due to the square root transformation, the residuals were otherwise evenly distributed, and linearity was acceptable. Although slightly skewed on the tails, normality was evident. There were no outliers or overly influential points. The Generalized Variance-Inflation Factors (GVIF values) for the above relationship showed that all parameters included were only moderately correlated (Attachment L) with GVIF and $GVIF^{(1/2 \times DF)}$ values ranging from 1.15 to 1.71, and therefore the interpretation is not biased by covariation amongst the variables in the regression equation. All of the parameters in the pesticide mixture toxicity – land use relationship for PSII Herbicides were therefore retained.

Validation of PSII Herbicides Relationship

The PSII Herbicides mixture — land use relationship (Table 13) was validated by predicting the pesticide mixture toxicity of the site and year combinations in the validation set. The measured and predicted pesticide mixture toxicity values for the validation set are presented in Table 14. The mean absolute error (MAE) and root mean square error (RMSE) between the measured and predicted PSII Herbicide mixture results were 1.719 and 2.652, respectively. The same values are plotted against each other and compared to a 1:1 line in Figure 5. The measured and predicted data corresponded well; however, the relationship tended to slightly under-predict larger values of PSII Herbicide mixture toxicity. Therefore, overall the PSII Herbicides mixture — land use relationship tended to underestimate the toxicity of PSIIs Herbicides slightly. The magnitude of the under-estimation will increase with PSII Herbicide mixture toxicity. Given the close agreement of the predicted and measured PSII Herbicides mixture toxicity values, the relationship was considered valid and it was decided to proceed with using this relationship to predict the PSII Herbicides mixture toxicity for the basins, regions and GBRCA.

Table 14. Measured and predicted pesticide mixture toxicity values for photosystem II inhibiting herbicides (PSII Herbicides) for the site and year combinations in the validation set

Site and year	Measured PSII Herbicides mixture toxicity (% affected species)	Predicted PSII Herbicides mixture toxicity (% affected species)
Russell 2016/2017	2.97	5.08
North Johnstone 2015/2016	0.05	0.02
North Johnstone 2017/2018	0.13	0.02
Johnstone at Coquette Pt 2016/2017	1.51	0.96
Tully 2015/2016	2.12	2.80
Burdekin 2017/2018	0.04	0.55
O'Connell at Staffords Crossing 2017/2018	4.12	3.44
O'Connell at Caravan Park 2015/2016	1.84	3.12
Sandy 2017/2018	27.91	20.36
Burnett 2016/2017	0.23	1.95
Fitzroy 2016/2017	0.07	1.72
Haughton 2016/2017	4.34	0.58

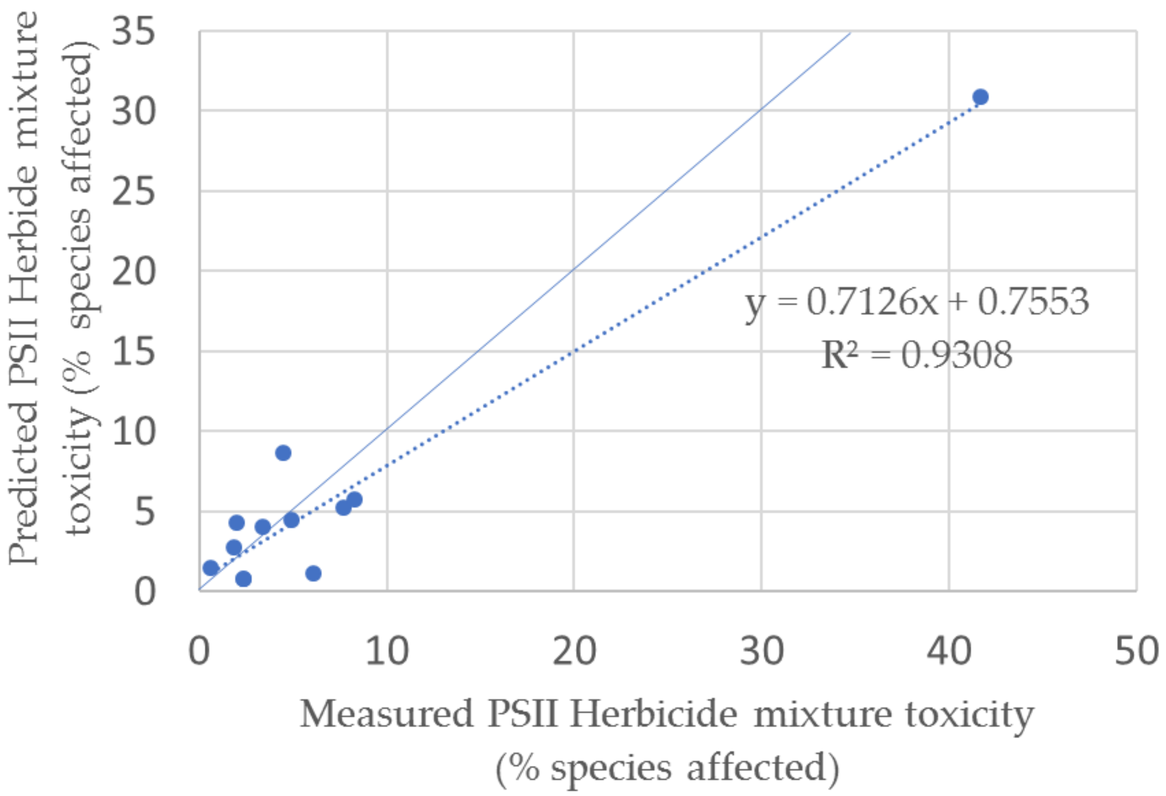

Figure 5. Plot and regression (dashed line) of measured and predicted photosystem II inhibiting herbicides (PSII Herbicides) mixture toxicity compared to a one to one line (solid line)

Ground-truthing the PSII Herbicides mixture toxicity values for basins by comparison with estimates for monitored catchments

The PSII Herbicides mixture toxicity — land use relationship (Table 13) was used to predict the pesticide mixture toxicity values for the 35 basins reported on in the Reef Water Quality Report Card (Table 14). The predicted PSII Herbicides mixture toxicity values for basins were then compared to monitoring results for catchment(s) within the basin where available. As land use variables were the strongest predictors of PSII risk, the relative toxicity of basins and catchments were interrogated using the amount and type of land use upstream and downstream of the monitoring site. Monitoring results for catchment PSII Herbicide mixture toxicity spanned three years for most sites, and therefore, monitored catchment values referred to hereafter are the mean of the values for the three monitoring years covered by this project (i.e. 2015/2016 to 2017/2018).

The Burdekin, Burnett, Burrum, Mary and Tully basins were composed almost entirely by the monitored catchments and therefore these basins and catchments should have the same or very similar PSII Herbicides mixture toxicity values. The monitored catchment of the Burdekin River had a PSII Herbicide mixture toxicity of 0.80%, while the regression model predicted the PSII Herbicide mixture toxicity to be 0.45% at the basin level. These PSII Herbicide mixture toxicity values were both less than 1%, and thus, would be allocated the same risk category (very low risk) and so the predicted value was reasonable.

The Burnett River had a predicted basin score of 1.7%, while the result for the monitored catchment at Quay Street was 0.86%. Quay Street is the most appropriate site in the Burnett to compare to the predicted basin values as it is located closest to the mouth of the river, which is where the basin values apply. However, it should be noted that there is approximately 5700 ha of sugarcane downstream of the monitoring site that would contribute pesticides, and would be expected to increase the per cent of species experiencing adverse effects at the basin level. The predicted value for the Burnett basin was therefore considered reasonable.

The Burrum basin had a predicted score of 2.03% and the monitored catchment results were 0.04%, 1.59% and 3.64% for the Burrum, Elliot and Gregory catchments, respectively. Given the relative surface areas and land use patterns of the three catchments, a weighted average of these would result in an estimated basin value of approximately 1.4%. The predicted basin value was expected to be larger than the weighted average of the three catchments because there is approximately 6000 ha of sugar cane downstream of the combined monitored area. Given the above, the predicted value for the Burrum basin, while larger, was reasonable.

The Mary basin had a predicted score of 0.62%, while the monitored catchment at Churchill Street had a score of 0.91%. The difference between the predicted and monitored values was not large. In addition, the proportion of land used to grow sugar cane is similar at the catchment (upstream of the monitoring site) and basin level (1.53% and 2.03%, respectively, Attachment I), as are those of other contributing land uses (conservation, horticulture, and irrigated cropping). Therefore, the predicted value was deemed reasonable.

The Tully basin had a predicted PSII Herbicides mixture toxicity of 3.71% while the monitored catchment had a value of 3.06%. The proportion of sugarcane in the catchment upstream of the monitoring site and in the basin is similar (11.0% and 12.8%, respectively, Attachment I), as were those of other contributing land uses (conservation, horticulture, and irrigated cropping). The difference between the predicted and monitored values was small, so the predicted value was deemed reasonable.

For the Baffle, Barron, Boyne, Calliope, Fitzroy, Herbert, Johnstone, Kolan, Mossman, Mulgrave-Russell, O'Connell, Pioneer and the Styx basins, it would be expected that the predicted basin PSII Herbicides mixture toxicity values would be larger than the monitored catchment values. This is because these basins contained additional land uses downstream of the monitored catchments that would contribute additional PSII Herbicides (Attachment I). The expectation was found to be true for the Baffle, Boyne, Calliope, Fitzroy, Herbert, Johnstone, Mossman, Mulgrave-Russell, O'Connell and the Styx (Attachment I). Therefore, the predicted basin PSII Herbicide mixture toxicity values were deemed reasonable. While the Barron, Kolan and Pioneer basins and catchments did not conform to this expectation, the differences between the predicted basin and measured catchment values were not large (Attachment I) and therefore, the predicted basin values were considered reasonable.

For the Black, Plane, Proserpine, Waterpark basins, it was expected that the predicted PSII Herbicides mixture toxicity values for basins would be smaller than the monitored catchment results as the basin contained lower percentages of land uses associated with high PSII use (Attachment I). This was the case for the Plane and Proserpine basins and catchments (Attachment I). The opposite applied to the Black and Waterpark basins and catchments (Attachment I); i.e. the predicted basins values were larger than the monitored catchment values. However, as both the monitored and predicted values for the Black and Waterpark catchments and basins were considerably less than 1%, they were deemed to be reasonable. While the Plane basin and catchment and the Proserpine basin and catchment conform to the above assumption, the differences in the values were large (i.e. ~ 10%), and therefore, warrant further explanation. The lower PSII Herbicides mixture toxicity predicted for the Plane basin compared to Sandy Creek can be explained by the three-fold increase in per cent of land used for conservation in the basin (i.e. 9.6% at the catchment scale and 31.7% at the basin scale, Attachment I) and by having approximately half as much land used for forestry (5.8% compared to 11.9%, Attachment I) and sugar cane (25.4% compared to 45%, Attachment I) compared to the Sandy Creek catchment. The markedly lower predicted PSII Herbicide mixture toxicity value for the Proserpine basin (Table 15) can be explained by it having approximately 50% more land used for conservation (29.1% compared to 20.6%, Attachment I) and approximately half the land used for sugar cane (9.6% compared to 20.8%, Attachment I) compared to the Proserpine River catchment.

No catchments were monitored within the Daintree, Don, Endeavour, Jacky Jacky, Jeannie, Lockhart, Murray, Normanby, Olive Pascoe, Ross, Shoalwater, and Stewart basins; therefore, comparisons of predicted basin and monitored constituent catchments were not possible. Of these, the Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Murray, Olive Pascoe, Shoalwater, Stewart and Normanby basins are dominated by conservation and forested grazing land uses. The per cent of the basin used for conservation and forested grazing were approximately 85% and 9%, respectively in the Daintree; 52% and 43%, respectively in the Endeavour; 81% and 9%, respectively in the Jacky Jacky; 82% and 10%, respectively in the Jeannie; 91% and 2.5%, respectively in the Lockhart; 63% and 4.6%, respectively for the Murray; 46% and 52%, respectively in the Normanby; 79% and 20% in the Olive Pascoe; 47% and 23.6%, respectively in the Shoalwater; and 94% and 2%, respectively in the Stewart basins (Attachment I). The monitored catchment that had the most similar land use composition to the above basins is Waterpark that consisted of 48% conservation and 45% forestry and less than 1% of species affected (Attachment I). Land used for conservation typically has low inputs of pesticides, while forestry does have higher levels of pesticide inputs but not as high as other, more intensive land uses. If forestry and forested grazing have similar levels of pesticide input, then it would be expected that the PSII Herbicides mixture toxicity values of the Don, Endeavour, Normanby, Olive Pascoe and Shoalwater basins would all be less than 1%. As the

Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Murray and Stewart basins have larger proportions of conservation than forested grazing, it would be expected that these basins also have PSII Herbicides mixture toxicity values less than 1%. The predicted basin PSII herbicide mixture toxicity values for the above basins were all less than 1% and agree with these expectations based on the Waterpark catchment, and therefore, the predicted basin values were considered to be reasonable.

Similarly, there are no monitored catchments within the Don, Murray and Ross basins, so comparisons to constituent catchments is not possible. Rather, the basin-level PSII Herbicide mixture toxicity values were compared to monitored catchments that had similar per cent land-usage.

The main land uses in the Don were 6% conservation, 46.5% forested grazing, 32.8% open grazing and 3.6% horticulture (Attachment I). The Burnett, Fitzroy and Haughton have similar per cent land-usage patterns. Their PSII Herbicide mixture toxicity values were 0.22% (Burnett at Ben Anderson Barrage), 0.86% (Burnett at Quay St), 0.22% (Fitzroy River at Rockhampton) and 0.92% (Haughton at Giru Weir). The predicted value for the Don (0.08%) is somewhat smaller than the catchments with similar land use patterns; however, all estimates were less than 1%, so the predicted basin value for the Don was reasonable.

The main land uses in the Murray were 63% conservation, 9.3% forestry, 4.6% forested grazing and 15% sugar cane (Attachment I). The Mossman and Russell rivers have the most similar land use patterns although they both had less than 1% of land used for forestry. It might therefore be expected that the Mossman and Russell rivers might have lower PSII Herbicides mixture toxicity values than the Murray basin. The PSII Herbicides mixture toxicity values for the Mossman and Russell catchment monitoring sites (2.3% and 2.8%, respectively) were as expected smaller than the predicted value for the Murray basin of 5.5% and was therefore considered reasonable.

The main land uses of the Ross basin are 27% conservation, 31% forested grazing and 17% open grazing (Attachment I). The catchments of the Mary (Home Park) and O'Connell (Caravan Park) monitoring sites had similar land use compositions (Attachment I) and PSII Herbicides mixture toxicity values of 0.3% and 3.3%, respectively. The Mary river catchment value was in good agreement with the predicted value for the Ross basin of 0.01% and both were less than 1%. However, the O'Connell catchment value was somewhat higher than the Ross basin result (Table 15). This larger difference is most probably due to the O'Connell catchment area having approximately 6% of its land used for sugar cane and 18% for forestry compared to the Ross basin which had no sugar cane and 2.6% forestry (Attachment I). The above comparisons indicate that the predicted PSII Herbicides mixture toxicity values for the Ross basin are reasonable.

In summary, the predicted PSII Herbicides mixture property values for all the basins appear to be reasonable given the available means of ground-truthing.

Table 15. Predicted photosystem II inhibiting (PSII) Herbicide mixture toxicity values for basins and the corresponding values for catchments within the basin (averaged when more than one year of monitoring data was available)

Basin	Predicted PSII Herbicides mixture toxicity (% species affected)	Catchment	Measured PSII Herbicides mixture toxicity (% species affected)[1]
Baffle	0.16	Baffle Creek at Newton Road	3.15×10^{-5}
Barron	0.14	Barron River at Rink's Close Jetty	0.24
Black	2.00×10^{-3}	Black River at Bruce Highway	6.83×10^{-6}
Boyne	0.25	Boyne River at Boyne Island	3.29×10^{-7}
Burdekin	0.45	Burdekin River at Home Hill	0.07
Burnett	1.72	Burnett at Ben Anderson Barrage	0.22
		Burnett at Quay St	0.86
Burrum	2.03	Burrum River at Buxton Boat Ramp	0.04
		Elliot River at Riverview Boat Ramp	1.59
		Gregory River at Jarretts Road	3.64
Calliope	0.24	Calliope River at Old Bruce HWY	9.64×10^{-6}
Daintree	6.64×10^{-3}	-	-
Don	0.08	-	-
Endeavour	5.73×10^{-3}	-	-
Fitzroy	1.12	Fitzroy River at Rockhampton	0.22
		Comet River at Comet Weir	3.83

Basin	Predicted PSII Herbicides mixture toxicity (% species affected)	Catchment	Measured PSII Herbicides mixture toxicity (% species affected) [1]
Haughton	8.85	Haughton River at Powerline	4.95
		Haughton River at Giru Weir	0.92
		Barratta Creek at Northcote	19.11
		East Barratta Ck at Jerona Road	7.28
Herbert	3.85	Herbert River at Ingham	1.20
Jacky Jacky	0.21	-	-
Jeannie	0.22	-	-
Johnstone	3.21	North Johnstone River at Old Bruce HWY	0.15
		Johnstone River at Coquette Pt	1.34
Kolan	0.80	Kolan River at Booyan Boat Ramp	4.16
Lockhart	0.36	-	-
Mary	0.62	Mary River at Homepark	0.27
		Mary River at Churchill St	0.91
		Tinana Ck at Barrage	2.75
Mossman	2.71	Mossman River at Bonnie Doon	2.31
Mulgrave – Russell	3.41	Mulgrave River at Deeral	2.20
		Russell River at East Russell	2.79

Basin	Predicted PSII Herbicides mixture toxicity (% species affected)	Catchment	Measured PSII Herbicides mixture toxicity (% species affected) [1]
Murray	5.49	-	-
Normanby	1.28×10^{-3}	-	-
O'Connell	7.72	O'Connell at Stafford's Crossing	4.29
		O'Connell at Caravan Park	3.31
Olive Pascoe	0.18	-	-
Pioneer	12.97	Pioneer River at Dumbleton Pump Station	13.37
Plane	16.16	Sandy Ck at Homebush	26.91
Proserpine	4.04	Proserpine River at Glen Isla	14.91
Ross	0.01	-	-
Shoalwater	6.49×10^{-4}	-	-
Stewart	0.41	-	-
Styx	0.38	Styx River at Ogmore	9.21×10^{-4}
Tully	3.71	Tully River at Euramo	3.06
Waterpark	0.23	Waterpark Creek at Corbett's Landing	5.53×10^{-11}

[1.] These values are the mean of the annual values for 2015/2016 to 2017/2018.

Ground-truthing the predicted PSII Herbicides mixture toxicity values for basins by expert elicitation

Based on the collective experience of the authors and WQI staff in monitoring pesticides in the waterways that discharge to the GBR, we would expect the toxicity of PSII Herbicides applied to various land uses would decrease in the following order:

- sugar cane;
- horticulture;
- forestry;
- open grazing;
- forested grazing; and
- conservation.

Therefore, basins dominated by conservation and forested grazing are expected to have the lowest pesticide risk and the risk will increase as the per cent of land used for open grazing and forestry increased and the risk would decrease as the per cent of forested grazing and conservation increased. It is also expected that the pesticide risk would increase further as the per cent of land used for horticulture and sugar cane increases.

The Baffle, Barron, Black, Boyne, Burdekin, Calliope, Daintree, Don, Endeavour, Fitzroy, Jacky Jacky, Jeannie, Kolan, Lockhart, Mary, Normanby, Olive Pascoe, Ross, Shoalwater, Stewart, Styx and Waterpark basins were all estimated to have less than 1% of species affected. These made sense as these basins are dominated by conservation and forested grazing, and both these land uses typically do not have high concentrations of PSII Herbicides applied. The Black, Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Shoalwater, Stewart and Waterpark basins have between 40.3% and 93.9% of the basin used for conservation. The basins with less than 40% conservation (Baffle, Barron, Boyne, Burdekin, Calliope, Don, Kolan, Mary, Ross and Styx) had forested grazing of between 24.5% to 63.5% of the basin area. The highest proportion of sugarcane in these basins was 4.8% for the Kolan basin.

The Burrum, Herbert, Johnstone, Mossman, Mulgrave-Russell, Murray, Proserpine and Tully basins, which all had pesticide mixture toxicity estimates of >1 to 5% of species affected, were associated with increases in the percentage of the basin used for sugar cane of between 7.7% and 15.0% (Attachment I). The increase in risk for the Burnett basin was associated with a large increase in the proportion of land used for Open Grazing (27.5%).

The Haughton and O'Connell basins, with pesticide mixture toxicity estimates of >5 to 10% of species affected, were predominantly associated with an increase in the per cent of the basin used for sugar cane (13.4% to 17.9%, Attachment I).

The Pioneer and Plane basins, with pesticide mixture toxicity values of >10 to 20%, were associated with further increases in the per cent of the basin devoted to sugar cane to between 20.4 and 25.4% (Attachment I).

The observed patterns of land use in the basins with different pesticide risk categories is consistent with the predictions.

Estimates of the PSII herbicides mixture toxicity for basins

The rounded, predicted PSII Herbicides mixture toxicity values over the wet season ranged from 0% affected species to 16% (Table 16). Based on the predicted values, the following basins would meet the pesticide reduction target providing no other pesticides were present: Baffle, Barron, Black, Boyne, Burdekin, Calliope, Daintree, Don, Endeavour, Fitzroy, Jacky Jacky, Jeannie, Kolan, Lockhart, Mary, Normanby, Olive Pascoe, Ross, Shoalwater, Stewart, Styx and Waterpark.

Thus 22 of the 35 basins are estimated to face a very low risk from PSII Herbicides and meet the pesticide reduction target provided there were no other pesticides present. Nine basins face a low risk from PSII Herbicides alone. Two basins face a moderate risk from PSII Herbicides alone, while two basins also face a high risk from PSII Herbicides alone.

Care needs to be taken in interpreting the predicted risk categories for the basins, as individual catchments within the basins may have different pesticide risks. This is because the predicted Pesticide Risk Baseline values were estimated as though all the land in a basin was drained by a single waterway that discharged at a single point. Therefore, although a basin meets the pesticide reduction target there may be waterways within that basin that do not meet the pesticide reduction target.

The geographical distribution of the basins and the risk category for PSII Herbicides can be viewed in Figure 6. Twenty-two of the 35 basins face a very low risk from PSII Herbicides. These are distributed throughout all NRM regions except the Mackay Whitsundays. There are nine basins facing a low risk from PSII Herbicides. Six of these are in the Wet Tropics region, followed by two in the Burnett Mary and one in the Mackay Whitsunday regions. The two basins facing a moderate risk are relatively small coastal basins, with one basin each in the Burdekin and Mackay Whitsunday regions. There are two basins with a high risk from PSII Herbicides — the Pioneer and Plane basins in the Mackay Whitsunday region. There are no basins that face a very high risk from PSII Herbicides.

All the basins in the Cape York face a very low risk from PSII Herbicides. All the basins in the Burdekin, except for the Haughton basin, faced a very low risk from PSII Herbicides. These basins have either a very low level of agricultural development (the Cape York basins) or are dominated by grazing (the Burdekin basins, except for the Haughton basin). All the basins of the Burnett-Mary and Fitzroy NRM regions face a very low or low risk from PSII Herbicides. The basins in the Wet Tropics region faced the second highest risk from PSII Herbicides having two basins facing a very low risk, five facing a low risk and one facing a moderate risk. The basins in the Mackay-Whitsunday NRM region faced the highest level of risk from PSII Herbicides – having one basin facing a low risk, one facing a moderate risk and two facing a high risk from PSII Herbicides. Thus, the risk faced by NRM regions from PSII Herbicides increased in the following order Cape York and Fitzroy, Burnett-Mary, Burdekin, Wet Tropics and finally the Mackay Whitsunday region.

Table 16. The predicted photosystem II inhibiting herbicide (PSII Herbicides) mixture toxicity values for the 35 basins and the corresponding risk category. The allocated risk categories were based solely on the presence of the selected PSII Herbicides. PSII Herbicide mixture toxicity values were rounded off to the nearest integer

Basin	Predicted PSII Herbicides mixture toxicity (% species affected)	Risk category[1]
Baffle	0	Very low
Barron	0	Very low
Black	0	Very low
Boyne	0	Very low
Burdekin	0	Very low
Burnett	2	Low
Burrum	2	Low
Calliope	0	Very low
Daintree	0	Very low
Don	0	Very low
Endeavour	0	Very low
Fitzroy	1	Very low
Haughton	9	Moderate
Herbert	4	Low
Jacky Jacky	0	Very low
Jeannie	0	Very low
Johnstone	3	Low
Kolan	1	Very low
Lockhart	0	Very low
Mary	1	Very low
Mossman	3	Low
Mulgrave–Russell	3	Low
Murray	5	Low
Normanby	0	Very low
O'Connell	8	Moderate

Basin	Predicted PSII Herbicides mixture toxicity (% species affected)	Risk category[1]
Olive Pascoe	0	**Very low**
Pioneer	13	High
Plane	16	High
Proserpine	4	Low
Ross	0	**Very low**
Shoalwater	0	**Very low**
Stewart	0	**Very low**
Styx	0	**Very low**
Tully	4	Low
Waterpark	0	**Very low**

[1] The cut-offs for the pesticide risk categories are presented in Table 10.

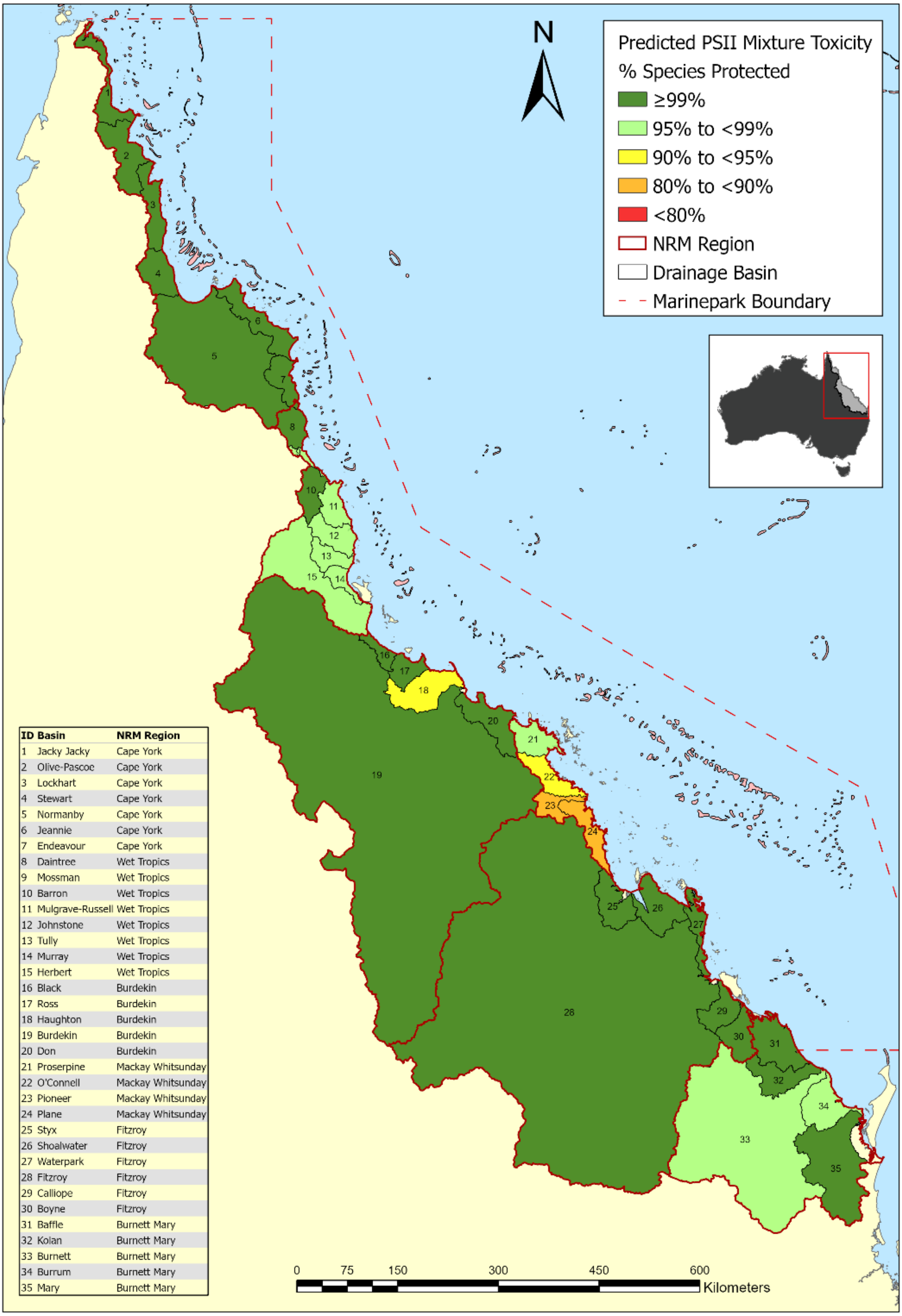

Figure 6. Map of the risk categories posed by mixtures of photosystem II inhibiting herbicides (PSII Herbicides) to 35 basins that discharge to the Great Barrier Reef lagoon . The allocated risk categories were based solely on the presence of PSII Herbicides

The Relationship for Other Herbicides

The best pesticide mixture toxicity — land use relationship for Other Herbicides is presented in Table 17. It was clearly better than any other relationship. The relationship has 42 degrees of freedom and an adjusted R^2 value of 0.76.

Table 17. The variables and their coefficients, standard error and probability for the pesticide mixture toxicity — land use relationship for Other Herbicides

Variable	Coefficient	Standard Error	Probability
Intercept	1.367	0.108	6.33×10^{-16}
% Urban	10.335	3.586	0.0062
% Conservation	-1.183	0.227	5.26×10^{-6}
% Horticulture	-1.303	0.397	0.002
% Horticulture$_{poly}$#	-0.916	0.408	0.03
% Dryland cropping	14.583	4.828	0.004
% Sugar cane	4.426	0.403	6.22×10^{-14}
% Sugar cane$_{poly}$#	-1.720	0.418	0.0002

A quadratic (second order) polynomial function was applied to this variable.

The diagnostic figures (Attachment M) for the Other Herbicides mixture toxicity — land use relationship indicate that the underlying assumptions of regression analysis were met, apart from the assumption of equivalence of variance (there was a slightly greater range of residuals for small fitted values than for larger fitted values). There was, however, a reasonably even distribution of the residuals above and below the residual equals zero line. Nonetheless residuals were reasonably linear. The data conformed to a normal distribution and there were no outliers. The Generalized Variance-Inflation Factors (GVIF values) for the above relationship showed that all the included parameters were only moderately correlated (Attachment M) with GVIF and GVIF$^{(1/2 \times DF)}$ values used in this study ranging from 1.114 to 1.478 and therefore the interpretation is not biased by covariation amongst the variables in the regression equation. All of the parameters in the pesticide mixture toxicity – land use relationship for Other Herbicides were therefore retained.

Validation of the Other Herbicides Relationship

The Other Herbicides mixture toxicity — land use relationship (Table 17) was validated by using it to predict the pesticide mixture toxicity of the site and year combinations in the validation set (Table 7).

The measured and predicted pesticide mixture toxicity values for the validation set are presented in Table 18. The mean absolute error (MAE) and root mean square error (RMSE) between the measured and predicted Other Herbicides mixture toxicity results are 0.676 and 0.822, respectively. The measured and predicted Other Herbicides mixture toxicity values were plotted against each other and compared to a 1:1 line in Figure 7. The regression equation for the measured and predicted data was similar to the 1:1 line, but had a lower gradient (Figure 7). This suggests that the Other Herbicides pesticide mixture – land use relationship should on average underestimate the toxicity of Other Herbicides. Given the close agreement of the predicted and measured Other Herbicides mixture toxicity values, indicated by the MAE and RMSE values, the relationship was considered valid and this relationship was used to predict the Other Herbicides mixture toxicity for the basins, regions and GBRCA.

Table 18. The measured and predicted mixture toxicity values for Other Herbicides to sites in the validation set

Site and year	Measured Other Herbicides mixture toxicity (% affected species)	Predicted Other Herbicides mixture toxicity (% affected species)
Russell 2016/2017	0.98	2.27
North Johnstone 2015/2016	0.082	0.32
North Johnstone 2017/2018	0.015	0.32
Johnstone at Coquette Pt 2016/2017	0.34	1.24
Tully 2015/2016	0.52	0.86
Burdekin 2017/2018	0.52	0.55
O’Connell at Stafford’s Crossing 2017/2018	2.04	1.49
O’Connell at Caravan Park 2015/2016	2.15	1.24
Sandy 2017/2018	10.35	8.88
Burnett 2016/2017	1.63	1.29
Fitzroy River at Rockhampton 2016/2017	1.67	2.07
Haughton at Powerline 2016/2017	1.97	0.63

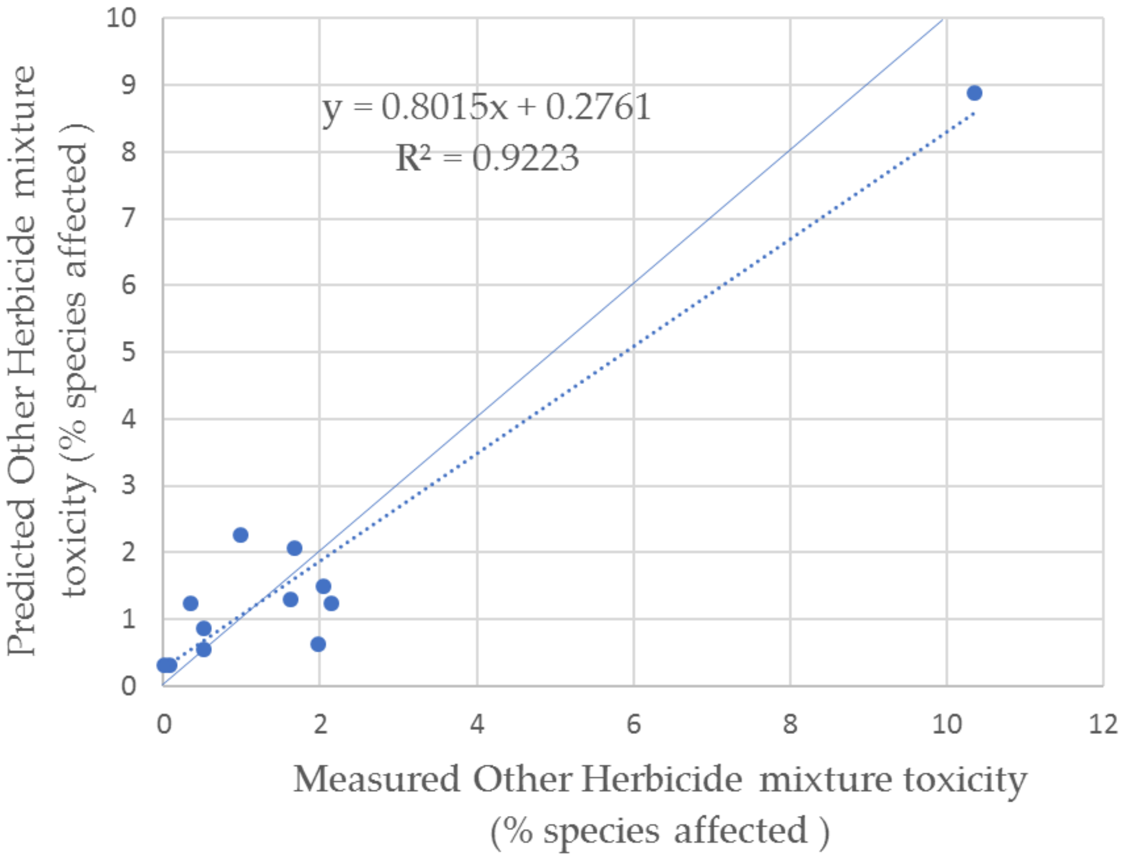

Figure 7. Plot and regression (dashed line) of measured and predicted Other Herbicides mixture toxicity values compared to a one to one line (solid line)

Ground-truthing the Other Herbicides mixture toxicity values for basins by comparison with estimates for monitored catchments

The predicted Other Herbicide mixture toxicity values for basins were assessed against monitoring results at the catchment level where available. The predicted basin and measured catchment values were compared and explained using the amount and type of land use upstream and downstream of the monitoring site. Monitoring results for Other Herbicide mixture toxicity for catchments spanned three years for most sites, and therefore, monitored catchment values referred to hereafter are the mean of the values for the three monitoring years covered by this project (i.e., 2015/2016 to 2017/2018).

The Burdekin, Burnett, Burrum, Mary and the Tully basins are composed almost entirely by the monitored constituent catchments and therefore it would be expected that these basins and catchments should have the same or very similar Other Herbicides mixture toxicity values. This assumption was found to be true, with only relatively small differences in the predicted basin and monitored catchment Other Herbicides mixture toxicity values, for all the basins except the Burrum (Table 19). In the case of the Burrum basin, the predicted Other Herbicides toxicity value (i.e., 3.59%) appears to over-estimate the monitored values (0.24%, 2.23% and 3.03% for the Burrum, Elliot and Gregory catchments, respectively). Given the relative surface areas and land use patterns of the three catchments, a weighted average of these three catchments would result in an estimated basin value of approximately 2%. However, the predicted basin value was expected to be larger than the weighted average of the three catchments because there is approximately 6000 ha of sugar cane downstream of the combined monitored area. The predicted and monitored Other Herbicides toxicity values for the Burdekin, Burnett and Mary basins are all close and would all lead to the same risk classification. In the Tully the predicted Other Herbicide mixture toxicity value is nearly double the measured values (Table 19). This is consistent with there being a considerable amount of additional high pesticide usage land uses (i.e., 5000 hectares and 400 hectares of bananas, Appendix I) downstream of the Tully Euramo site, which would increase the basin's Other Herbicide pesticide mixture toxicity value. Therefore, overall the predicted basin Other Herbicides toxicity values appear to be reasonable for the above basins.

For the Baffle, Barron, Boyne, Calliope, Fitzroy, Herbert, Johnstone, Kolan, Mossman, Mulgrave-Russell, O'Connell, Pioneer and Styx basins it would be expected that the predicted Other Herbicides mixture toxicity values would be larger than the monitored catchment values as these basins contain additional land that was not included in the monitored catchment and would contribute additional Other Herbicides. This prediction was found to be true for the Baffle, Barron, Boyne, Calliope, Herbert, Johnstone, Mossman, Mulgrave-Russell (the average of the values for the Mulgrave and the Russell catchments is 1.42), O'Connell, Pioneer and the Styx (Table 19). Given the above comparisons, the predicted results for the Baffle, Barron, Boyne, Calliope, Fitzroy, Herbert, Johnstone, Kolan, Mossman, Mulgrave-Russell, O'Connell, Pioneer and Styx basins were considered reasonable.

The predicted Other Herbicides mixture toxicity values for the Black, Plane, Proserpine and Waterpark basins would be expected to be smaller than the monitored catchment results as these basins have a higher per cent of land uses with low pesticide inputs (e.g. more conservation) and/or reduced amounts of high pesticide input land uses (e.g. sugar cane) (Attachment I). This was the case for the Plane and Proserpine basins (Table 19). The lower Other Herbicides mixture toxicity predicted for the Plane basin compared to Sandy Creek can be explained by the three-fold increase in per cent of land used for conservation and the halving of the per cent of land used for forestry and sugar cane in the basin compared to the monitored Sandy Creek catchment. The lower predicted Other Herbicides mixture toxicity value for the Proserpine basin (Table 19) can be explained by it having approximately 50% more land used for conservation and approximately half the land used for sugar cane compared to the Proserpine river catchment. However, the predicted Other Herbicides mixture toxicity value for the Black basin was approximately 50% larger than the monitored value for the Black River catchment. This result can be explained by the land use patterns in the basin and catchment. While the basin did have about four-times the per cent of land used for conservation (which would decrease the Other Herbicide mixture toxicity) it also had a large increase in the per cent of land used for sugar cane (from 0 to 14%) which would more than compensate for the increase in conservation. The Waterpark basin had a larger Other Herbicides mixture toxicity value than the Waterpark catchment (0.17 compared to 0.0022, respectively) which is most likely due to basin having double the amount of land used for horticulture. Despite the large relative difference in Other Herbicides mixture toxicity values for the Waterpark basin and catchment they are both very low values and are therefore considered reasonable. . Given the above comparisons, the predicted results for the Black, Plane, Proserpine and Waterpark basin were considered reasonable.

No catchments were monitored within the Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Ross, Shoalwater, and Stewart basins therefore comparisons of predicted basin and monitored constituent catchments were not possible. Rather, the predicted basin Other Herbicides mixture toxicity values were compared to monitored catchments that had similar per cent land-usage. The land use of the monitored Waterpark catchment is composed of 48% conservation and 45% forestry (Attachment I). Land used for conservation typically has very low inputs of pesticides, while forestry does have higher levels of pesticide inputs but not as high as other more intensive forms of agriculture. The Endeavour (52% conservation, 43% forestry grazing), Jacky Jacky (81% conservation and 8.9% forested grazing), Jeannie (82% conservation and 10% forested grazing), Lockhart (91% conservation, 2.5% forested grazing), Olive Pascoe (79% conservation, 21% forested grazing), Shoalwater (47% conservation, 23.6% forested grazing) (Attachment I) have similar land use compositions as Waterpark catchment. Assuming that forestry and forested grazing have similar levels of pesticide input), these basins would be expected to have Other Herbicides mixture toxicity values similar to that of Waterpark catchment (i.e., less than 1%). Similarly, it would be expected based on the Waterpark catchment that the Daintree (84% conservation) and Stewart (94% conservation) would have Other Herbicides mixture toxicity values less than 1%. The predicted basin Other Herbicides mixture toxicity values for the Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Stewart, and Shoalwater are all less than 1% (Table 19) and agree with the prediction based on the Waterpark catchment (Table 19) and therefore the predicted basin values are considered to be reasonable.

Similarly, there are no monitored catchments within the Don, Murray and Ross basins, so comparisons to constituent catchments were not possible. Rather, the basin Other Herbicides mixture toxicity values were compared to monitored catchments that had similar per cent land-usage. The main land uses in the Don are 6% conservation, 46.5% forested grazing, 32.8% open grazing and 3.6% horticulture (Attachment I). The Burnett, Fitzroy and Haughton catchments have similar per cent land-usage patterns (Attachment I). Their Other Herbicides mixture toxicity values were 1.48% (Burnett at Ben Anderson Barrage), 2.27% (Burnett at Quay St), 1.75% (Fitzroy River at Rockhampton) and 1.05% (Haughton at Giru Weir) (Table 19). The predicted value for the Don was 0.7%, which is in reasonable agreement with the monitored results (Table 19). The main land uses in the Murray are 63% conservation, 9.3% forested grazing, 4.6% open grazing and 15% sugar cane (Attachment I). The Mossman and Russell catchments have similar land use patterns to the Murray basin (Attachment I) and had monitored Other Herbicide mixture toxicity values of approximately 1.5% and 1.2%, respectively. These values are in reasonable agreement with the predicted value for the Murray of 2.0% (Table 19). The main land uses of the Ross are 27% conservation, 31% forested grazing and 17% open grazing (Attachment I). The Mary (Home Park) and O'Connell (Caravan Park) monitored catchments have similar land use compositions (Attachment I) except they both have approximately an additional 15% forestry. The Mary (Home Park) and O'Connell (Caravan Park) catchments had Other Herbicides mixture toxicity values of 2.18% and 2.16%, respectively while the predicted Other Herbicides mixture toxicity for the Ross basin was 1.43% (Table 19). These suggest that the predicted Other Herbicides mixture toxicity for the Ross may be an under-estimate. However, this apparent low predicted value for the Ross basin may also be due to the moderate land use (approximately 15%) for forestry in the Mary and O'Connell catchments (Attachment I). Overall, the available evidence suggests that the predicted values for the Don, Murray and Ross basins are all reasonable.

Ground-truthing the predicted Other Herbicides mixture toxicity values for basins by expert elicitation

Based on the authors and WQI staff's collective experience from monitoring pesticides in the waterways that discharge to the GBR we would expect the toxicity of Other Herbicides applied to various land uses would decrease in the following order:

- sugar cane and horticulture
- open grazing
- forestry
- forested grazing
- conservation.

Therefore, it would be expected that as the per cent of forested grazing and conservation increased that the risk would decrease and conversely as the per cent of other land uses increased so would the risk from

Other Herbicides. All the basins with a very low risk classification for Other Herbicides were either dominated by conservation (ranging from approximately 34% to 94% of the basin with an average of 47%) or forested grazing (ranging from 46% to 64% of the basin with an average of 32%) with low amounts of land used for other land uses. In comparison, the basins that faced a low risk from Other Herbicides generally had considerably lower per cent of the basin used for conservation (average of 33%), slightly more open grazing (increasing from 10% to 12%), slightly less forested grazing (average of 30%) and a modest amount of sugar cane (average of 7.6%). The basins that faced a moderate risk generally had an even lower per cent of their land used for conservation (average of 25%), a decreased per cent of land used for both forested grazing (average of 21.5%) and open grazing (average of 12%) but a marked increase in the per cent of land used for sugar cane (average of 21%).

Table 19. Predicted Other Herbicides mixture toxicity values for basins and the corresponding values for catchments within the basin (averaged when more than one year of monitoring data were available)

Basin	Predicted Other Herbicides mixture toxicity (% species affected)	Catchment	Measured Other Herbicides mixture toxicity (% species affected)
Baffle	0.49	Baffle Creek at Newton Road	0.14
Barron	0.69	Barron River at Rink's Close Jetty	0.27
Black	0.74	Black River at Bruce Highway	0.49
Boyne	0.31	Boyne River at Boyne Island	2.23×10^{-3}
Burdekin	0.56	Burdekin River at Home Hill	0.76
Burnett	1.43	Burnett River at Ben Anderson Barrage	1.48
		Burnett River at Quay St	2.27
Burrum	3.59	Burrum River at Buxton Boat Ramp	0.24
		Elliot River at Riverview Boat Ramp	2.23
		Gregory River at Jarrett's Road	3.03
Calliope	0.70	Calliope River at Old Bruce Highway	0.08
Daintree	6.75×10^{-5}	-	-
Don	0.69	-	-
Endeavour	0.045	-	-
Fitzroy	2.04	Fitzroy River at Rockhampton	1.75
		Comet River at Comet Weir	6.24
Haughton	4.97	Haughton River at Powerline	1.62

Basin	Predicted Other Herbicides mixture toxicity (% species affected)	Catchment	Measured Other Herbicides mixture toxicity (% species affected)
		Haughton River at Giru Weir	1.05
		Barratta Creek at Northcote	4.98
		East Barratta Ck at Jerona Road	2.52
Herbert	1.36	Herbert River at Ingham	0.78
Jacky Jacky	0.09	-	-
Jeannie	0.09	-	-
Johnstone	2.09	North Johnstone River at Old Bruce Highway	0.15
		Johnstone River Coquette Point	0.60
Kolan	2.30	Kolan River at Booyan Boat Ramp	1.46
Lockhart	0.18	-	-
Mary	1.83	Mary River at Homepark	2.18
		Mary River at Churchill St	2.44
		Tinana Ck at Barrage	4.02
Mossman	1.86	Mossman River at Bonnie Doon	1.46
Mulgrave–Russell	1.87	Mulgrave River at Deeral	1.66
		Russell River at East Russell	1.17
Murray	2.03	-	-
Normanby	0.02	-	-
O’Connell	3.55	O'Connell River at Stafford’s Crossing	2.38
		O'Connell River at Caravan Park	2.16
Olive Pascoe	0.08	-	
Pioneer	5.24	Pioneer River at Dumbleton Weir	3.47
Plane	6.70	Sandy Ck at Homebush	9.65
Proserpine	2.39	Proserpine River at Glen Isla	6.59
Ross	1.43	-	-

Basin	Predicted Other Herbicides mixture toxicity (% species affected)	Catchment	Measured Other Herbicides mixture toxicity (% species affected)
Shoalwater	0.01	-	-
Stewart	0.21	-	-
Styx	0.39	Styx River at Ogmore	2.23×10^{-3}
Tully	1.30	Tully River at Euramo	0.69
Waterpark	0.17	Waterpark Creek at Corbett's Landing	2.23×10^{-3}

Estimates of Other Herbicides mixture toxicity for basins

The rounded, predicted Other Herbicides mixture toxicity values ranged from 0% affected species to 7% affected species for the Plane basin (Table 20). The Baffle, Barron, Black, Boyne, Burdekin, Burnett, Calliope, Daintree, Don, Endeavour, Herbert, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Ross, Shoalwater, Stewart, Styx, Tully and Waterpark basins met the pesticide reduction target (i.e., protecting at least 99% of species) if there were no other pesticides present. Thus 22 of the 35 basins are estimated to face a very low risk from Other Herbicides alone. Twelve basins face a low risk from Other Herbicides alone (Table 20). One basin faces a moderate risk from Other Herbicides alone and no basins face a high or very high risk from exposure to Other Herbicides (Table 20).

Care needs to be taken in interpreting these predicted results as just because a basin meets the pesticide reduction target does not mean that all the waterways in that basin meet the target. This is because the predicted pesticide risk values for baselines were estimated as though all the land at the basin level was drained by a single waterway that discharged at a single point. Therefore, though a basin meets the pesticide reduction target there may be waterways within that basin that do not meet the pesticide reduction target. The geographical distribution of the basins and the risk category for Other Herbicides can be viewed in Figure 8. Seven of the 22 very low risk basins are located in the Cape York region, with five basins located in the Fitzroy, four in the Burdekin, four in the Wet Tropics and two in the Burnett Mary regions. The low risk basins are spread reasonably evenly between the Wet Tropics (4 basins), the Burnett Mary (3 basins), and the Mackay Whitsundays (3 basins), with one basin each in the Fitzroy and Burdekin regions. The basin facing a moderate risk from Other Herbicides is in the Mackay Whitsundays region. No regions contain basins which face a high or very high risk from Other Herbicides.

All of the basins in the Cape York region face a very low risk from Other Herbicides (Figure 8). The Burdekin region faces the next lowest risk as it is predominantly very low risk (Black, Burdekin and Don and the Ross basins) with only the Haughton basin facing a low risk. The Fitzroy region has the next lowest risk consisting predominantly of very low risk (Boyne, Calliope, Shoalwater, Styx and Waterpark basins) while the Fitzroy basin has a low risk from Other Herbicides. The risk for the Burnett Mary is slightly higher as basins in this region mainly face a low risk with two basins facing a very low risk and three facing a low risk. The Wet Tropics is evenly split between low and very low risk, with four basins in each category. The Mackay Whitsunday region faces the highest risk from Other Herbicides as it has one basin facing a moderate risk and three basins facing a low risk.

Table 20. Predicted mixture toxicity values (rounded to the nearest integer) for Other Herbicides to each basin and the corresponding risk category. The allocated risk categories were based solely on the presence of the selected Other Herbicides

Basin	Predicted Other Herbicides mixture toxicity (% species affected)	Risk category[1]
Baffle	0	Very low
Barron	1	Very low
Black	1	Very low
Boyne	0	Very low
Burdekin	1	Very low
Burnett	1	Very low
Burrum	4	Low
Calliope	1	Very low
Daintree	0	Very low
Don	1	Very low
Endeavour	0	Very low
Fitzroy	2	Low
Haughton	5	Low
Herbert	1	Very low
Jacky Jacky	0	Very low
Jeannie	0	Very low
Johnstone	2	Low
Kolan	2	Low
Lockhart	0	Very low
Mary	2	Low
Mossman	2	Low
Mulgrave–Russell	2	Low
Murray	2	Low

Basin	Predicted Other Herbicides mixture toxicity (% species affected)	Risk category[1]
Normanby	0	**Very low**
O'Connell	4	Low
Olive Pascoe	0	**Very low**
Pioneer	5	Low
Plane	7	Moderate
Proserpine	2	Low
Ross	1	**Very low**
Shoalwater	0	**Very low**
Stewart	0	**Very low**
Styx	0	**Very low**
Tully	1	**Very low**
Waterpark	0	**Very low**

[1.] The cut-offs for the pesticide risk categories are presented in Table 10.

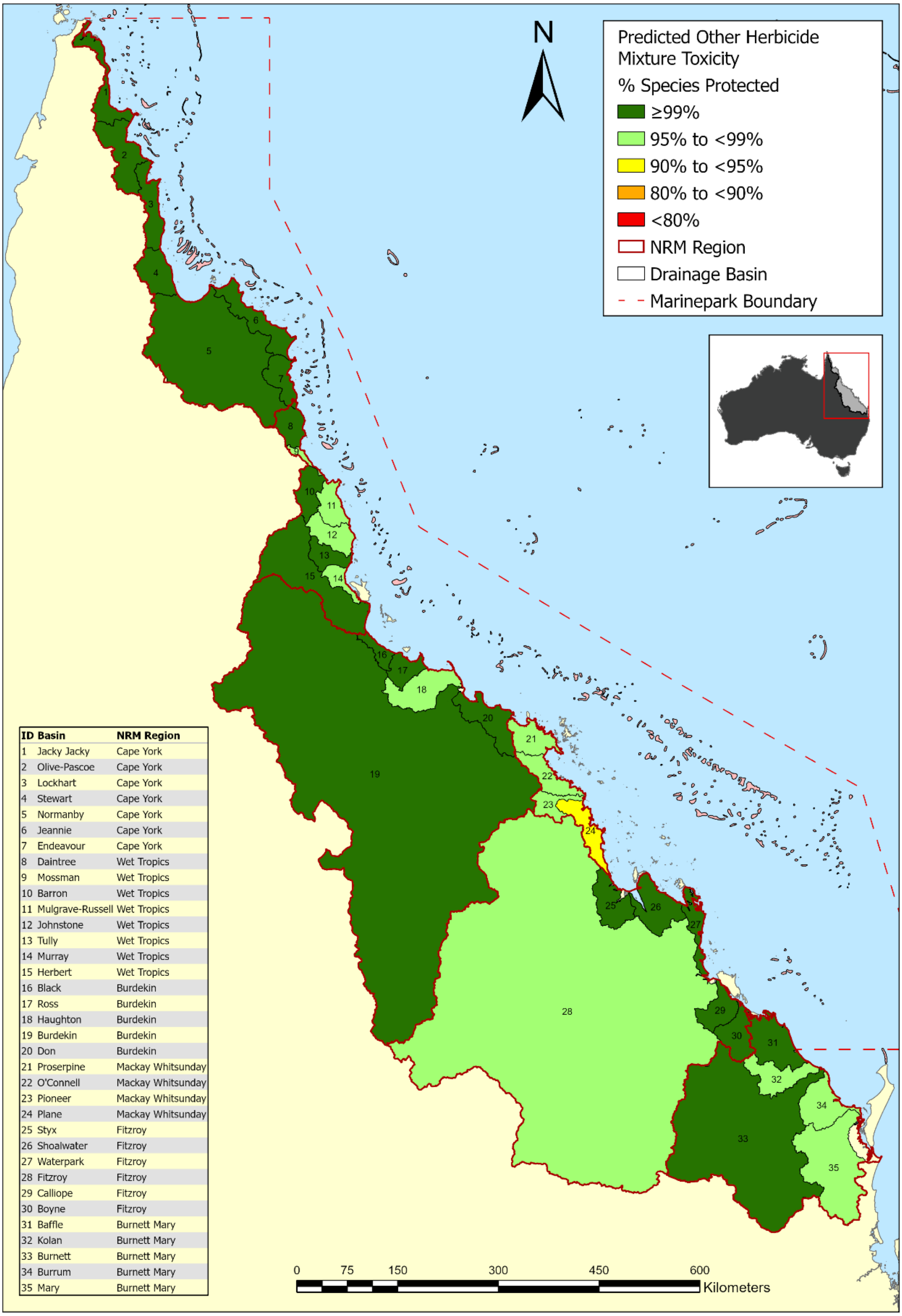

Figure 8. Map of the risk categories posed by mixtures of Other Herbicides to the basins (predicted) that discharge to the Great Barrier Reef lagoon. The allocated risk categories were based solely on the presence of Other Herbicides

The Relationship for Insecticides

The best pesticide mixture toxicity — land use relationship for Insecticides is presented in Table 21. The relationship has 47 degrees of freedom and an adjusted R^2 value of 0.68.

Table 21. The variables and their coefficients, standard error and probability for the pesticide mixture toxicity — land use relationship for Insecticides

Variable	Coefficient	Standard Error	Probability
Intercept	-1.161	0.476	0.019
Average daily rainfall	0.112	0.026	8.01×10^{-5}
% Bananas	2.220	0.732	0.004
% Forestry	29.138	7.882	0.0006
% Grazing forested	1.357	0.722	0.066
% Horticulture	-21.309	6.176	0.0012
% Sugar cane	5.233	1.167	4.72×10^{-5}

The diagnostic figures (Attachment N) for the Insecticides relationship indicate that the underlying assumptions of regression analysis were reasonably met. There was a reasonably even distribution of the residuals above and below the zero line and there was no significant shape remaining in the residuals. There is a notable diagonal 'censoring' of the residuals on the left of the residual vs fitted plot, however after some investigation this was found to be due to a large number of <LOR values for insecticide concentrations, which resulted in a large number of constant values in the data (reasonably common in environmental data). The data conformed to a normal distribution reasonably well and there were no remaining outliers or influential points. The Generalized Variance-Inflation Factors (GVIF values) for the above relationship showed that all parameters included were only moderately correlated (Attachment N) with values ranging from 1.117 to 2.786 and therefore the interpretation is not biased by covariation amongst the variables in the regression equation. All of the parameters in the pesticide mixture toxicity – land use relationship for Insecticides were therefore retained.

Validation of the Insecticides relationship

The Insecticides mixture toxicity — land use relationship (Table 21) was validated by predicting the pesticide mixture toxicity of the site and year combinations in the Validation set. The measured and predicted pesticide mixture toxicity values are presented in Table 22. The mean absolute error (MAE) and root mean square error (RMSE) between the measured and predicted Insecticides pesticide mixture results are 0.997 and 0.998, respectively. The measured and predicted Insecticides mixture toxicity values were plotted against each other and compared to a 1:1 line in Figure 9. The regression equation for the measured and predicted data has a lower gradient (0.77) than the 1:1 line. Therefore, the Insecticides mixture toxicity relationship tends to underestimate the Insecticides toxicity. Given, the close agreement of the predicted and measured Insecticides mixture toxicity values (indicated by the MAE and RMSE values) the relationship was considered valid and it was used to predict the Insecticides mixture toxicity for the basins, regions and GBRCA.

Table 22. The measured and predicted Insecticides mixture toxicity values

Site and year	Measured Insecticides mixture toxicity (% affected species)	Predicted Insecticides mixture toxicity (% affected species)
Russell 2016/2017	0.740	1.696
North Johnstone 2015/2016	2.277	0.613
North Johnstone 2017/2018	2.188	1.662
Johnstone at Coquette Pt 2016/2017	1.767	0.812
Tully 2015/2016	2.580	1.239
Burdekin 2017/2018	0.082	0.057
O'Connell at Stafford's Crossing 2017/2018	2.574	1.580
O'Connell at Caravan Park 2015/2016	4.170	1.026
Sandy 2017/2018	11.350	9.405
Burnett 2016/2017	0.002	0.071
Fitzroy River at Rockhampton 2016/2017	0.284	0.0002
Haughton at Powerline 2016/2017	0.001	0.018

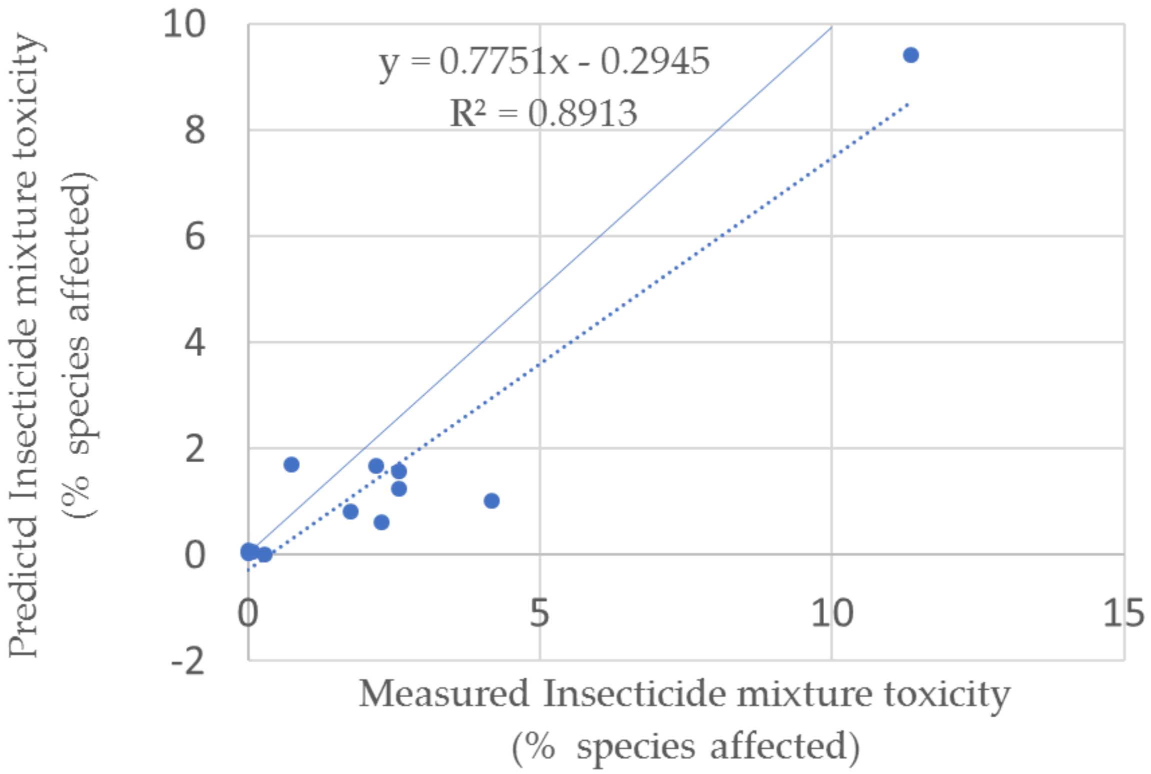

Figure 9. Plot and regression (dashed line) of measured and predicted Insecticides mixture toxicity values compared to a one to one line (solid line)

Ground-truthing the Insecticides mixture toxicity values for basins by comparison with estimates for monitored catchments

The predicted Insecticide mixture toxicity values for basins were assessed against monitoring results at the catchment level where available. The predicted basin and measured catchment values were compared and explained using the amount and type of land use upstream and downstream of the monitoring site. Monitoring results for catchment Insecticide mixture toxicity spanned three years for most sites, and therefore, monitored catchment values referred to hereafter are the mean of the values for the three monitoring years covered by this project (i.e., 2015/2016 to 2017/2018).

The Barron, Burdekin, Burnett, Fitzroy, Herbert, Kolan, Pioneer and the Tully basins are composed almost entirely by the monitored constituent catchments and therefore it would be expected that these basins and catchments should have the same or very similar Insecticides mixture toxicity values. This assumption was found to be true, with only small differences of less than 2.15 between the predicted basin and monitored catchment Insecticides mixture toxicity values, for all the basins (Table 22). Therefore, overall the predicted basin Insecticides toxicity values appear to be reasonable for the above basins.

For the Baffle, Boyne, Calliope, Herbert, Johnstone, Kolan, Mossman, Mulgrave-Russell, O'Connell, Pioneer and Styx basins it would be expected that the predicted basin Insecticides mixture toxicity values would be larger than the monitored catchment values. These basins contain additional land that was not included in the monitored catchments and would contribute additional Insecticides. This prediction was found to be true for the Baffle, Boyne, Calliope, Kolan, Mossman, Mulgrave-Russell, and the Styx (Table 22). The Herbert, Johnstone, O'Connell, and Pioneer basins do not adhere to the prediction having predicted basin values lower than the measured catchment values. However, the degree of under-prediction (of between 1 and 3) is not large, and therefore the predicted Insecticide mixture toxicity values are considered to be reasonable.

The predicted Insecticides mixture toxicity values for the Black, Plane, Proserpine and Waterpark basins would be expected to be smaller than the monitored catchment results as the basins have a higher per cent of the basin dedicated to land uses with low pesticide inputs (e.g. more conservation) and/or lower percentages of high pesticide input land uses (e.g. sugar cane). This was the case for the Plane and Proserpine basins (Table 22). For the Black and Waterpark basins the predicted basin values were larger than the monitored catchment values but as all the values were less than 1% this discrepancy is not meaningful. While the Plane basin and catchment and the Proserpine basin and catchment conform to the above prediction, the differences were sufficiently large (i.e., absolute differences of approximately 10 per cent of species being affected) to warrant further explanation. The lower Insecticides mixture toxicity predicted for the Plane basin compared to Sandy Creek can be explained by the three-fold increase in per cent of land used for conservation in the basin and having approximately half the land used for forestry and sugar cane (Attachment I). The markedly lower predicted Insecticides mixture toxicity value for the Proserpine basin (Table 22) can be explained by it having approximately 50% more land used for conservation and approximately half the land used for sugar cane compared to the Proserpine river catchment.

No catchments were monitored within the Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Olive Pascoe, Stewart, Shoalwater and Normanby basins therefore comparisons of predicted basin and monitored constituent catchments were not possible. Rather, the basin Insecticides mixture toxicity values were compared to monitored catchments that had similar per cent land-usage. The land use of Waterpark catchment is composed of 48% conservation and 45% forestry, which is similar to many of the above basins. Land used for conservation typically has very low inputs of insecticides, while forestry is likely to have higher levels of insecticide inputs but not as high as other more intensive land uses. The Endeavour (52% conservation, 43% forested grazing), Jacky Jacky (81% conservation and 8.9% forested grazing), Jeannie (82% conservation and 10% forested grazing), Lockhart (91% conservation, 2.5% forested grazing), Olive Pascoe (79% conservation, 20% forested grazing), Shoalwater (47% conservation, 23.6% forested grazing) have similar land use compositions to Waterpark, assuming that forestry and forested grazing have similar levels of pesticide input. Therefore, they would be expected to have Insecticides mixture toxicity values similar to that of Waterpark (i.e. less than 1% and thus face a very low risk from Insecticides) (Figure 10). Similarly, it would be expected, based on the Waterpark catchment, that the Daintree (84% conservation) and Stewart (94% conservation) would have Insecticide mixture toxicity values less than 1% (Attachment I). The predicted basin Insecticide mixture toxicity values for the Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Shoalwater and Stewart are all less than 1% (Attachment I, Figure 10) and agree with the prediction based on the Waterpark basin. Therefore, the predicted basin values for the Daintree, Endeavour, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Shoalwater and Stewart are considered to be reasonable.

Similarly, there are no monitored catchments within the Don, Murray and Ross basins, so comparisons to constituent catchments was not possible. Rather, the basin Insecticides mixture toxicity values were compared to monitored catchments that had similar per cent land-usage. The main land uses in the Don basin are 6% conservation, 46.5% forested grazing, 32.8% open grazing and 3.6% horticulture (Attachment I) which is similar to the Burnett and Fitzroy catchments (Attachment I). Their Insecticides mixture toxicity values were 1.16×10^{-3}% (Burnett at Burnett River at Ben Anderson Barrage), 0.12% (Burnett at Quay St) and 0.09% (Fitzroy). The predicted value for the Don basin was 0.17%. Thus, all the values are less than 1% and the estimate for the Don basin is considered reasonable. The main land uses in the Murray are 63% conservation, 9.3% forestry, 4.6% forested grazing and 15% sugar cane (Attachment I). The Mary (Churchill St), Mossman and Russell rivers all have similar land use patterns (Attachment I) and had Insecticides mixture toxicity values of 0.06%, 0.30% and 0.91%, respectively. The predicted value for the Murray basin of 1.2% slightly over-estimates the monitored catchment results but is close to the value for the Russell River. The main land uses of the Ross basin are 27% conservation, 31% forested grazing and 17% open grazing (Attachment I). The Mary (Home Park) has a similar land use composition (Attachment I) and an Insecticides mixture toxicity value of 2.45×10^{-3}%. The predicted value for the Ross basin is 0.37%. However, as both estimates are considerably less than 1% the estimate for the Ross basin is considered reasonable. The estimates of Insecticide mixture toxicity for the Don, Murray and Ross basins are therefore considered reasonable.

Table 23. Predicted Insecticides mixture toxicity values (% species affected) for basins and the corresponding values for catchments within the basin

Basin	Predicted Insecticides mixture toxicity (% species affected)	Catchment	Measured Insecticides mixture toxicity (% species affected)
Baffle	0.38	Baffle Creek at Newton Road	1.09×10^{-4}
Barron	0.17	Barron River at Rink's Close Jetty	0.20
Black	0.02	Black River at Bruce HWY	1.10×10^{-4}
Boyne	0.92	Boyne River at Boyne Island	1.09×10^{-4}
Burdekin	0.04	Burdekin River at Home Hill	0.03
Burnett	0.21	Burnett River at Ben Anderson Barrage	1.16×10^{-3}
		Burnett River at Quay St Bridge	0.12
Burrum	0.37	Burrum River at Buxton Boat Ramp	1.09×10^{-4}
		Elliot River at Riverview Boat Ramp	0.82
		Gregory River at Jarrett's Road	1.15
Calliope	0.06	Calliope River at Old Bruce HWY	1.09×10^{-4}
Daintree	0.36	-	-
Don	0.17	-	-
Endeavour	0.17	-	-
Fitzroy	2.85×10^{-3}	Fitzroy River at Rockhampton	0.09
		Comet River at Comet Weir	1.98×10^{-3}
Haughton	0.64	Haughton at Powerline	6.77×10^{-4}
		Haughton at Giru Weir	1.59
		Barratta Creek at Northcote	0.98
		East Barratta Ck at Jerona Road	0.01
Herbert	1.09	Herbert River at Ingham	2.19
Jacky Jacky	0.02	-	-
Jeannie	0.12	-	-
Johnstone	1.18	North Johnstone River at Old Bruce HWY	2.79
		Johnstone River at Coquette Pt	2.17

Basin	Predicted Insecticides mixture toxicity (% species affected)	Catchment	Measured Insecticides mixture toxicity (% species affected)
Kolan	1.04	Kolan River at Booyan Boat Ramp	0.33
Lockhart	0.19	-	-
Mary	0.22	Mary River at Homepark	2.45×10^{-3}
		Mary @ Churchill St	0.06
		Tinana Ck at Barrage	0.34
Mossman	0.56	Mossman River at Bonnie Doon	0.30
Mulgrave–Russell	1.95	Mulgrave River at Deeral	0.43
		Russell River at East Russell	0.91
Murray	1.20	-	-
Normanby	0.03	-	-
O'Connell	1.68	O'Connell River at Stafford's Crossing	4.41
		O'Connell River at Caravan Park	4.71
Olive Pascoe	0.05	-	-
Pioneer	3.81	Pioneer River at Dumbleton Pump Station	5.93
Plane	3.17	Sandy Ck at Homebush	12.71
Proserpine	2.00	Proserpine River at Glen Isla	13.81
Ross	0.37	-	-
Shoalwater	0.21	-	-
Stewart	0.3	-	-
Styx	0.01	Styx River at Ogmore	1.09×10^{-4}
Tully	1.33	Tully River at Euramo	2.90
Waterpark	0.08	Waterpark Creek at Corbett's Landing	1.09×10^{-4}

Ground-truthing the predicted Insecticides mixture toxicity values for basins by expert elicitation

Based on the authors collective experience from monitoring pesticides in the waterways that discharge to the GBR we would expect the amount of Insecticides applied to various land uses would decrease in the following order:

- dryland and irrigated cropping;
- horticulture;
- sugar cane;
- open grazing;
- forestry;
- forested grazing; and
- conservation.

All the basins have estimates of Insecticides mixture toxicity of less than 4% of species affected (Figure 10). The basins with less than 1% of species being affected were dominated by conservation (ranging from 4% to 93.92% of the basin) and/or forested grazing (ranging from 2.5% to 60% of the basin) with relatively low per cents of land used for other land uses. On average in these basins the per cent of land used for conservation and forested grazing were the same (Attachment I). In comparison, the basins where it was estimated that 1 to 4% of species would be affected had an even larger per cent of the basin used for conservation, considerably less forested grazing, open grazing and forestry but triple the per cent used for sugar cane (~10%) (Attachment I).

Estimates of Insecticides mixture toxicity for basins

The Insecticides mixture toxicity – land use relationship (Table 21) was used to predict the Insecticides mixture toxicity values for the 35 basins (Table 24) reported on in the Reef Water Quality Report Card. The rounded predicted Insecticides mixture toxicity values ranged from 0 to 4 % affected species. The Baffle, Barron, Black, Boyne, Burdekin, Burnett, Burrum, Calliope, Daintree, Don, Endeavour, Fitzroy, Haughton, Herbert, Jacky Jacky, Jeannie, Johnstone, Kolan, Lockhart, Mary, Mossman, Murray, Normanby, Olive Pascoe, Ross, Shoalwater, Stewart, Styx, Tully and Waterpark basins meet the pesticide reduction target if there were no other pesticides present. Thus 30 of the 35 basins are estimated to face a very low risk from Insecticides alone and meet the pesticide reduction target if there are no other pesticides present. Five basins face a low risk from Insecticides alone; these are the Mulgrave-Russell, O'Connell, Pioneer, Plane and Proserpine basins.

Care needs to be taken in interpreting these predicted results as just because a basin meets the pesticide reduction target does not mean that all the waterways in that basin meet the pesticide reduction target. This is because the predicted pesticide risk baselines were estimated as though all the land at the basin level was drained by a single waterway that discharged at a single point. Therefore, though a basin meets the pesticide reduction target there may be waterways within that basin that do not meet the pesticide reduction target.

The map of the risk categories for Insecticides for each of the 35 basins is presented in Figure 10. Thirty of the 35 basins (30) face a very low risk (<1% of species are estimated to be affected) from Insecticides. Four of the five low risk (1 to <5% of species are estimated to be affected) basins are in the Mackay Whitsunday region (O'Connell, Pioneer, Plane and Proserpine) and one is located in the Wet Tropic region (Mulgrave-Russell). There are no basins facing a moderate, high or very high risk from Insecticides.

The regions facing the lowest risk from Insecticides are the Burdekin, Burnett Mary, Cape York and the Fitzroy – each having all their basins facing a very low risk from Pesticides. The Wet Tropics region has the next lowest risk from Insecticides, while the Mackay Whitsunday region faces the greatest risk from Insecticides even though the risk for all its basins is low.

Table 24. Predicted mixture toxicity values (per cent species affected) for Insecticides and the corresponding risk category. The allocated risk categories were based solely on the presence of the selected Insecticides. Insecticide mixture toxicity values were rounded to the nearest integer

Basin	Predicted Insecticides mixture toxicity (% species affected)	Risk category[1]
Baffle	0	Very low
Barron	0	Very low
Black	0	Very low
Boyne	1	Very low
Burdekin	0	Very low
Burnett	0	Very low
Burrum	0	Very low
Calliope	0	Very low
Daintree	0	Very low
Don	0	Very low
Endeavour	0	Very low
Fitzroy	0	Very low
Haughton	1	Very low
Herbert	1	Very low
Jacky Jacky	0	Very low
Jeannie	0	Very low
Johnstone	1	Very low
Kolan	1	Very low
Lockhart	0	Very low
Mary	0	Very low
Mossman	1	Very low
Mulgrave–Russell	2	Low

Basin	Predicted Insecticides mixture toxicity (% species affected)	Risk category[1]
Murray	1	Very low
Normanby	0	Very low
O'Connell	2	Low
Olive Pascoe	0	Very low
Pioneer	4	Low
Plane	3	Low
Proserpine	2	Low
Ross	0	Very low
Shoalwater	0	Very low
Stewart	0	Very low
Styx	0	Very low
Tully	1	Very low
Waterpark	0	Very low

[1.] The cut-offs for the pesticide risk categories are presented in Table 10.

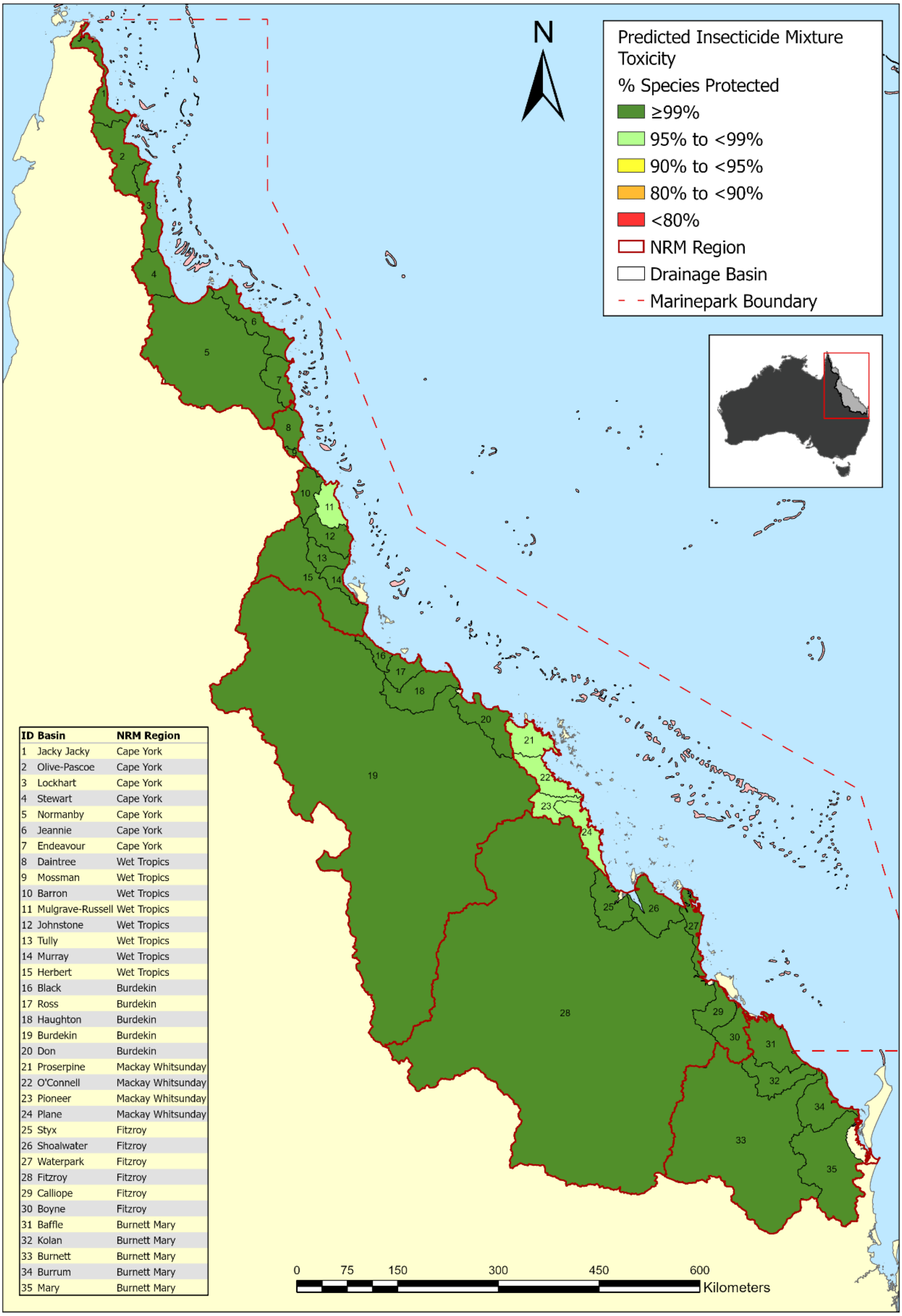

Figure 10. Map of the predicted risk categories posed by mixtures of Insecticides to the basins that discharge to the Great Barrier Reef lagoon. The allocated risk categories were based solely on the presence of Insecticides

The Relationship for Total Pesticides

The best Total Pesticide mixture toxicity — land use relationship is presented in Table 25. The relationship has 47 degrees of freedom and an adjusted R^2 value of 0.77.

Table 25. The variables and their coefficients, standard error and probability for the pesticide mixture toxicity — land use relationship for Total Pesticides

Variable	Coefficient	Standard Error	Probability
Intercept	2.685	0.208	2×10^{-16}
% Dryland cropping	18.091	4.702	0.000359
% Sugar cane	10.094	0.809	2×10^{-16}
% Sugar cane$_{poly}$#	-4.440	0.883	7.59×10^{-6}
% Conservation	-1.829	0.472	0.000327
% Horticulture	-39.553	7.855	7.46×10^{-6}
% Urban	20.476	6.823	0.00430

A quadratic (second order) polynomial function was applied to this variable

The diagnostic figures (Attachment O) for the Total Pesticides relationship indicate that the underlying assumptions of regression analysis were reasonably met. There was a reasonably even distribution of the residuals about the zero line, with acceptable linearity. The data conformed to a normal distribution although with slightly longer tails than expected. There were no outliers or highly leveraging site/year combinations. The Generalized Variance-Inflation Factors (GVIF values) for the above relationship showed that all parameters included were only moderately correlated having GVIF and $GVIF^{(1/2 \times DF)}$ values ranging from 1.115 to 1.339 (Attachment O) and therefore the interpretation is not biased by covariation amongst the variables in the regression equation. All of the parameters in the pesticide mixture toxicity – land use relationship for Total Pesticides were therefore retained.

Validation of Total Pesticides relationship

The Total Pesticide mixture toxicity — land use relationship (Table 25) was validated by predicting the pesticide mixture toxicity of the site and year combinations in the Validation set. The measured and predicted Total Pesticides mixture toxicity values are presented in Table 26. The mean absolute error (MAE) and root mean square error (RMSE) between the measured and predicted Total Pesticides mixture results are 2.782 and 3.921, respectively. The measured and predicted Total Pesticides mixture toxicity values were plotted against each other and compared to a 1:1 line in Figure 11. The regression equation for the measured and predicted data has a lower gradient (0.69) than the 1:1 line. Therefore, the Total Pesticides mixture toxicity relationship tends to underestimate the Insecticides toxicity. Despite this general underestimation, the close agreement of the predicted and measured Total Pesticides mixture toxicity values meant that the relationship was considered valid and it was used predict the Total Pesticides mixture toxicity for the basins, regions and GBRCA.

Table 26. The measured and predicted Total Pesticides mixture toxicity values

Site and year	Measured Total Pesticides mixture toxicity (% affected species)	Predicted Total Pesticides mixture toxicity (% affected species)
Russell 2016/2017	4.460	8.649
North Johnstone 2015/2016	2.394	0.776
North Johnstone 2017/2018	2.330	0.776
Johnstone at Coquette Pt 2016/2017	3.417	3.990
Tully 2015/2016	4.891	4.463
Burdekin 2017/2018	0.608	1.488
O'Connell at Staffords Crossing 2017/2018	8.305	5.702
O'Connell at Caravan Park 2015/2016	7.720	5.208
Sandy 2017/2018	41.685	30.879
Burnett 2016/2017	1.812	2.747
Fitzroy 2016/2017	1.962	4.299
Haughton at Powerline 2016/2017	6.058	1.114

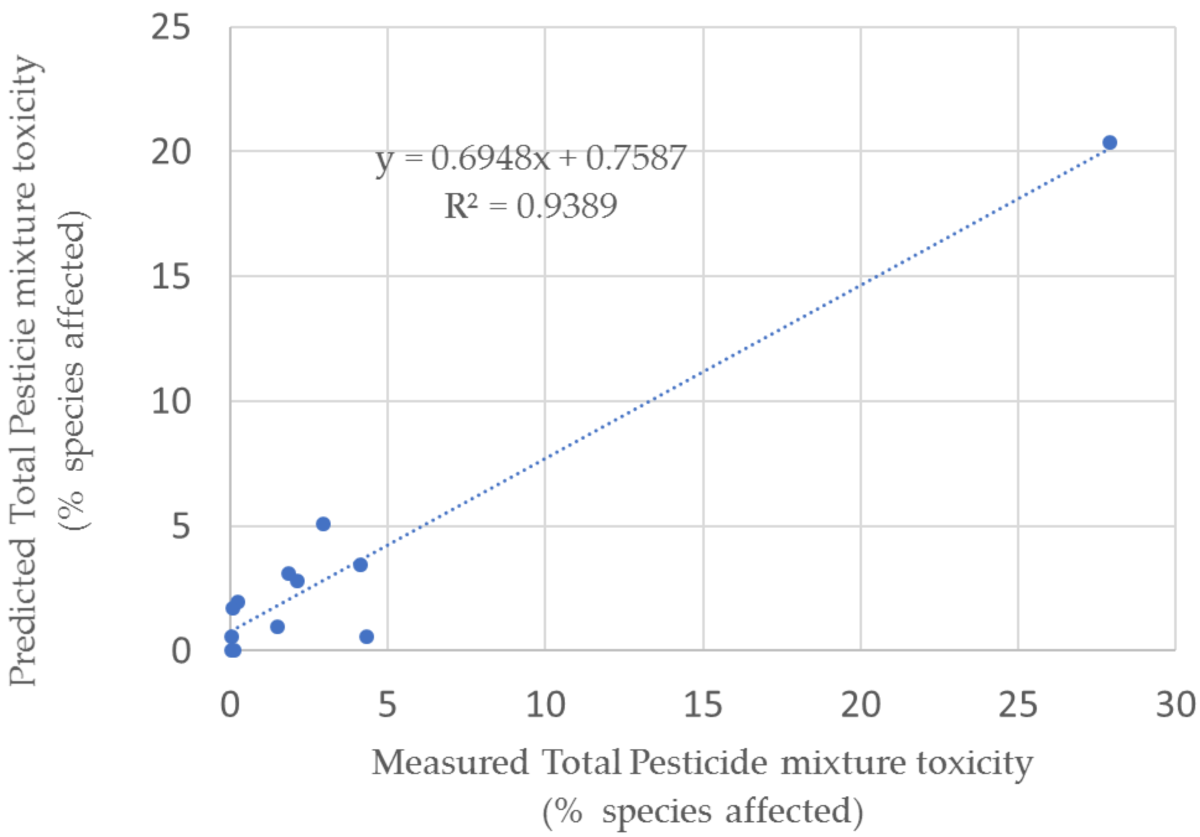

Figure 11. Plot and regression (dashed line) of measured and predicted Total Pesticides mixture toxicity compared to a one to one line (solid line)

Ground-truthing the Total Pesticides by comparison with the sum of the PSII Herbicides, Other Herbicides and Insecticides mixture toxicity values

A comparison of basin mixture toxicity values with those of the basin's constituent monitored catchments would be a repetition of the comparison already conducted for the PSII Herbicides, Other Herbicides and Insecticides. Therefore, a different form of ground-truthing was conducted for Total Pesticides. Theoretically, the sum of the mixture toxicity values for the PSII Herbicides, Other Herbicides and Insecticides should equal those for Total Pesticides. However, as the mixture toxicity values for the PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides were all predicted using independently derived regression relationships, it cannot be assumed that the summed values will equal the Total Pesticides mixture toxicity values. Rather, the summed values should approximate the Total Pesticides mixture toxicity values. The summed values and Total Pesticides mixture toxicity values are in close agreement (Table 27) thus confirming that the Total Pesticides mixture toxicity values are reasonable and sensible.

Table 27. Predicted mixture toxicity values for photosystem II inhibiting herbicide (PSII Herbicides), Other Herbicides, Insecticides, their sum and Total Pesticides (rounded to three significant figures)

Basin	Predicted mixture toxicity values (% species affected) for				
	PSII Herbicides	**Insecticides**	**Other Herbicides**	**Sum of PSII Herbicides, Other Herbicides, and Insecticides**	**Predicted Total Pesticides mixture toxicity**
Baffle	0.163	0.379	0.490	1.03	1.14
Barron	0.143	0.165	0.685	0.993	0.235
Black	0.002	0.019	0.738	0.759	1.08
Boyne	0.249	0.922	0.308	1.48	0.905
Burdekin	0.447	0.042	0.564	1.05	1.54
Burnett	1.72	0.206	1.43	3.35	3.03
Burrum	2.04	0.366	3.59	5.99	7.90
Calliope	0.244	0.059	0.702	1.00	1.64
Daintree	0.007	0.357	6.75×10^{-5}	0.363	0.072
Don	0.080	0.170	0.69	0.938	0.037
Endeavour	0.006	0.166	0.045	0.217	0.203
Fitzroy	1.12	0.003	2.04	3.16	4.247
Haughton	8.85	0.639	4.97	14.4	13.9
Herbert	3.85	1.09	1.36	6.31	6.10

Basin	Predicted mixture toxicity values (% species affected) for				
	PSII Herbicides	Insecticides	Other Herbicides	Sum of PSII Herbicides, Other Herbicides, and Insecticides	Predicted Total Pesticides mixture toxicity
Jacky Jacky	0.214	0.021	0.092	0.328	0.129
Jeannie	0.224	0.119	0.093	0.436	0.136
Johnstone	3.21	1.18	2.09	6.470	8.06
Kolan	0.801	1.04	2.30	4.13	3.75
Lockhart	0.364	0.187	0.176	0.737	0.287
Mary	0.618	0.221	1.83	2.67	4.64
Mossman	2.71	0.557	1.86	5.13	9.42
Mulgrave–Russell	3.41	1.95	1.87	7.23	8.67
Murray	5.49	1.20	2.03	8.72	9.43
Normanby	0.001	0.032	0.023	0.056	0.091
O'Connell	7.72	1.68	3.55	13.0	15.6
Olive Pascoe	0.184	0.051	0.076	0.310	0.099
Pioneer	13.0	3.81	5.24	22.0	23.8
Plane	16.2	3.18	6.70	26.0	28.9
Proserpine	4.04	2.00	2.39	8.43	9.17
Ross	0.010	0.370	1.43	1.81	2.58
Shoalwater	0.001	0.214	0.011	0.226	0.073
Stewart	0.414	0.299	0.207	0.920	0.35
Styx	0.381	0.015	0.392	0.788	1.14
Tully	3.70	1.33	1.30	6.33	6.66
Waterpark	0.226	0.082	0.167	0.477	0.268

Estimates of Total Pesticides mixture toxicity for basins

The Total Pesticides relationship (Table 25) was used to predict the Total Pesticides mixture toxicity values for the 35 basins (Table 28) reported on in the Reef Water Quality Report Card. The predicted Total Pesticides mixture toxicity values ranged from 0 to 29% affected species. Sixteen of the basins (i.e., Baffle, Barron, Black, Boyne, Daintree, Don, Endeavour, Jacky Jacky, Jeannie, Lockhart, Normanby, Olive Pascoe, Shoalwater, Stewart, Styx and Waterpark basins) currently meet the pesticide reduction target provided that only the 22 pesticides included in the Pesticide Risk Metric calculations are present. However, care needs to be taken in interpreting these predicted results because although a basin meets the pesticide reduction target it does not necessarily mean that all the waterways in that basin meet the target. The addition of more pesticides to the Pesticide Risk Metric in the future is likely to make the situation worse, although how much worse cannot be predicted. Of the 19 basins that do not meet the target, pesticides pose a low risk to seven basins, a moderate risk to eight basins, a high risk to two basins and a very high risk to two basins (Table 28).

The risk posed by Total Pesticides is, not surprisingly, greater than the risk posed by PSII Herbicides, Other Herbicides or Insecticides acting individually. This is reflected in the risk categories for individual basins and also in the NRM Regions.

Table 28. Predicted mixture toxicity values for Total Pesticides and the corresponding risk category. The allocated risk categories were based solely on the presence of the 22 selected pesticides. Total Pesticide mixture toxicity values are rounded to the nearest integer

Basin	**Predicted Total Pesticides mixture toxicity (% species affected)**	**Risk category[1]**
Baffle	1	**Very low**
Barron	0	**Very low**
Black	1	**Very low**
Boyne	1	**Very low**
Burdekin	2	Low
Burnett	3	Low
Burrum	8	Moderate
Calliope	2	Low
Daintree	0	**Very low**
Don	0	**Very low**
Endeavour	0	**Very low**
Fitzroy	4	Low
Haughton	14	High
Herbert	6	Moderate

Basin	Predicted Total Pesticides mixture toxicity (% species affected)	Risk category[1]
Jacky Jacky	0	Very low
Jeannie	0	Very low
Johnstone	8	Moderate
Kolan	4	Low
Lockhart	0	Very low
Mary	5	Low
Mossman	9	Moderate
Mulgrave–Russell	9	Moderate
Murray	9	Moderate
Normanby	0	Very low
O'Connell	16	High
Olive Pascoe	0	Very low
Pioneer	24	Very high
Plane	29	Very high
Proserpine	9	Moderate
Ross	3	Low
Shoalwater	0	Very low
Stewart	0	Very low
Styx	1	Very low
Tully	7	Moderate
Waterpark	0	Very low

1. The cut-offs for the pesticide risk categories are presented in Table 10.

The map of the risk categories for Total Pesticides for each of the 35 basins is presented in Figure 12. Of the sixteen basins facing very low risk from Total Pesticides, seven are in the Cape York NRM region, four are in the Fitzroy region, two are in both the Wet Tropics and Burdekin regions and one is in the Burnett Mary region. The low risk basins are in the Burdekin (two basins), Fitzroy (two basins) and Burnett Mary (three basins) NRM regions. There were eight basins facing a moderate risk from pesticide mixtures and these are predominantly located in the Wet Tropics (six basins) and there was one basin each in the Mackay Whitsunday and Burnett Mary NRM regions. There were only two high risk basins – one in the Mackay

Whitsunday region and one in the Burdekin region. Only two basins, both located in the Mackay Whitsunday region, face a very high risk from Total Pesticides.

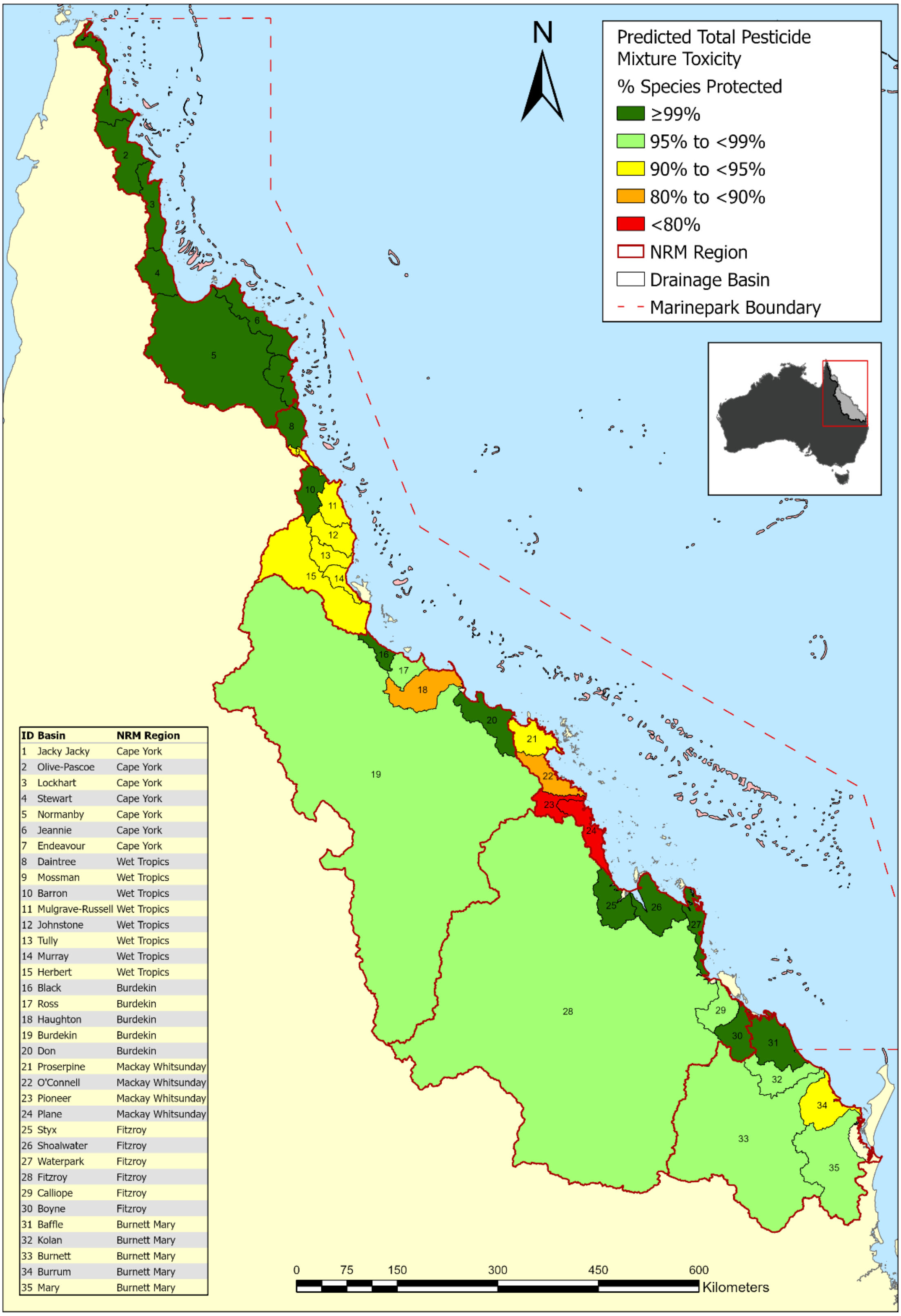

Figure 12. Map of the risk categories posed by mixtures of all 22 selected pesticides (Total Pesticides) to the basins (predicted) that discharge to the Great Barrier Reef lagoon . The allocated risk categories were based solely on the presence of the 22 selected pesticides

Predictions of Total Pesticides Mixture Toxicity for NRM Basins and the Great Barrier Reef Catchment Area

The Total Pesticides mixture toxicity vs land use relationship (Table 25) was used to predict the Total Pesticide mixture toxicity for each NRM region and for the GBRCA. The predicted Total Pesticides mixture toxicity values and the corresponding risk category for the NRM regions and the GBRCA are presented in Table 29. The same information is displayed spatially in Figure 13 and Figure 14.

Table 29. Predicted mixture toxicity values for Total Pesticides and the corresponding risk category for the NRM regions and the Great Barrier Reef Catchment Area (GBRCA). The allocated risk categories were based solely on the presence of the 22 selected pesticides

Spatial unit	**Predicted Total Pesticides mixture toxicity (% species affected)**	**Risk category[1]**
Cape York	0	**Very low**
Wet Tropics	5	Low
Burdekin	2	Low
Mackay Whitsundays	19	High
Fitzroy	4	Low
Burnett Mary	3	Low
Great Barrier Reef Catchment Area	3	Low

[1.] The cut-offs for the pesticide risk categories are presented in Table 10.

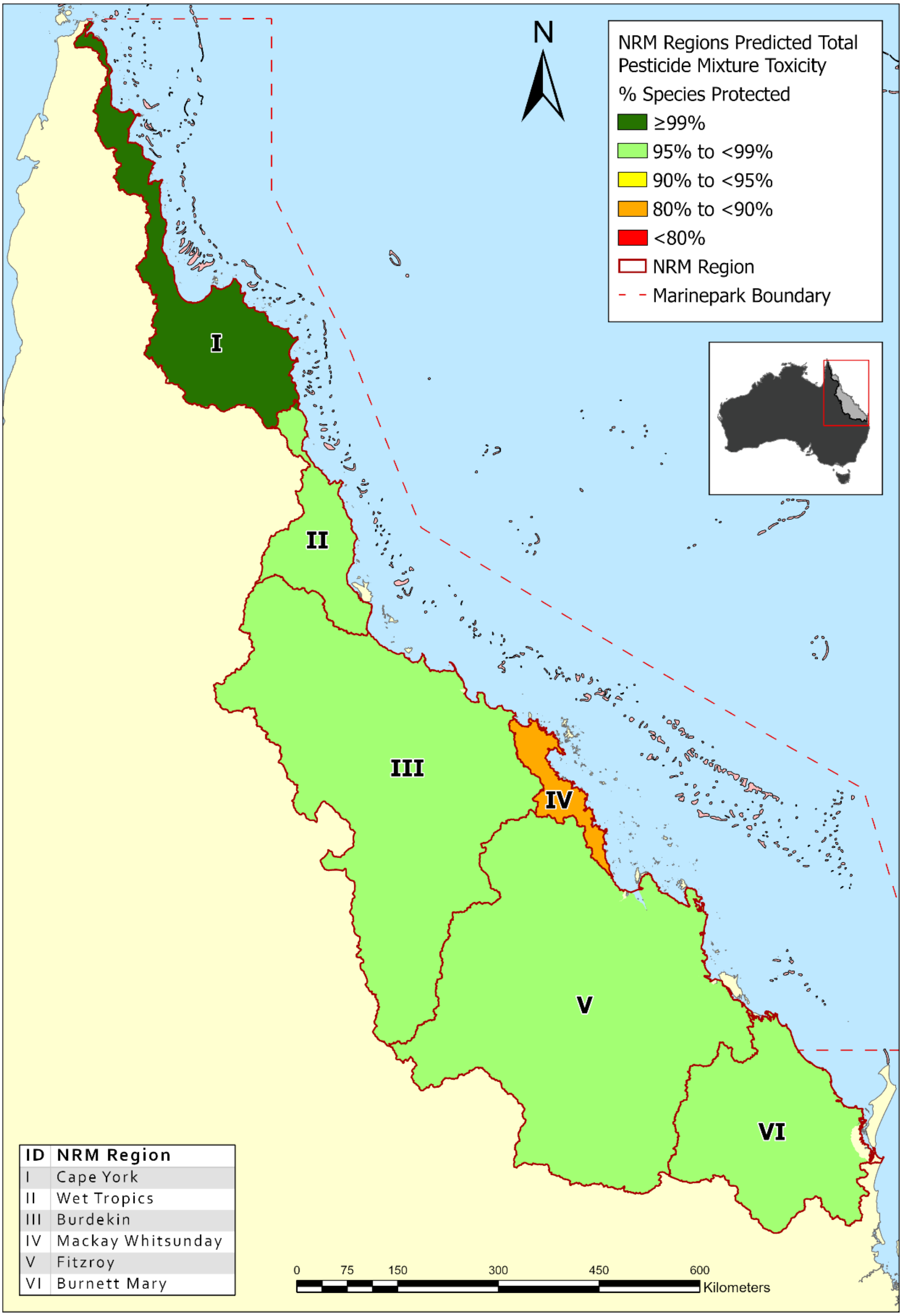

Figure 13. Map of the risk categories posed by Total Pesticides mixture toxicity for the Natural Resource Management Regions that comprise the Great Barrier Reef Catchment Area

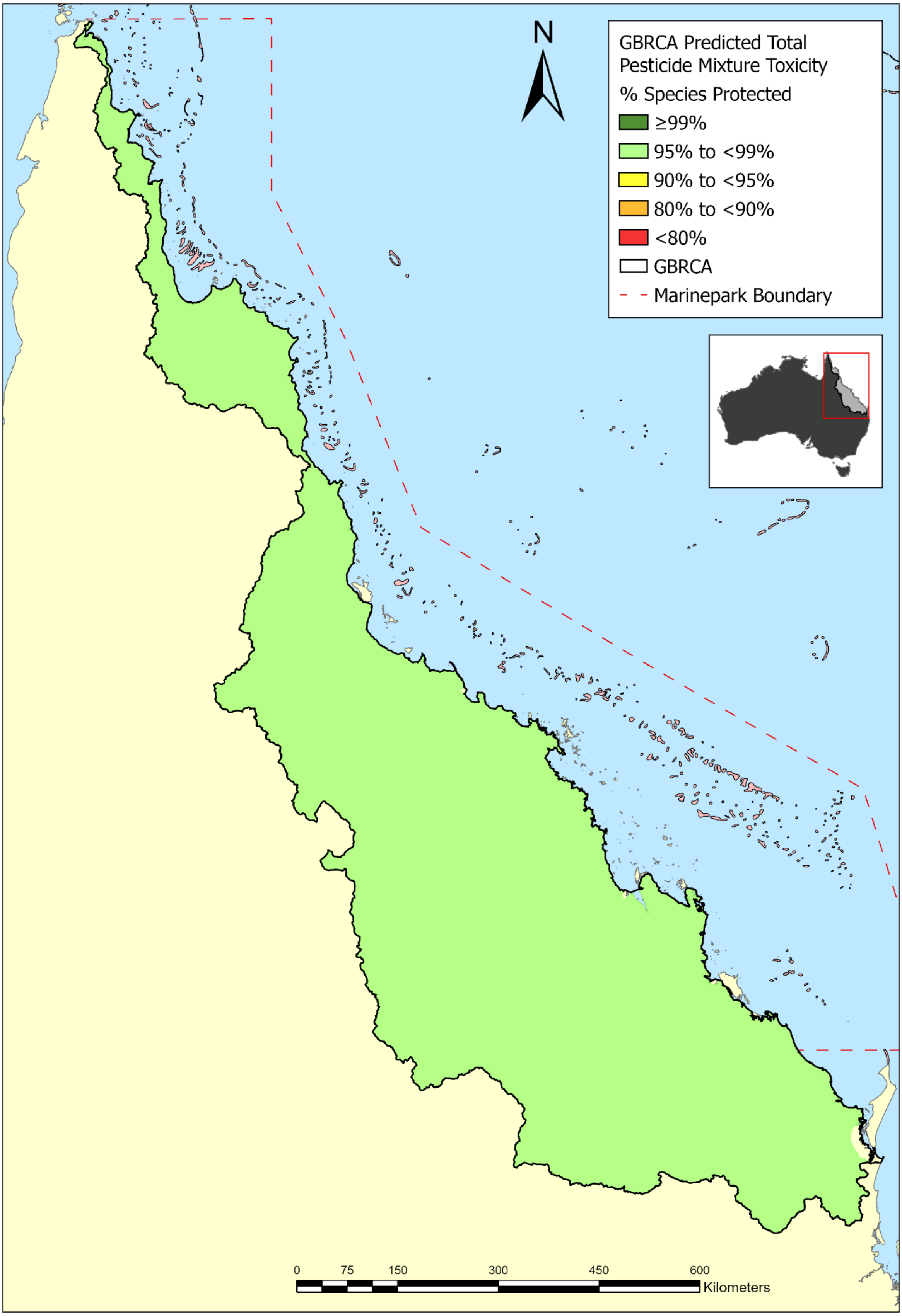

Figure 14. Map of the risk category posed by Total Pesticides mixture toxicity for the Great Barrier Reef Catchment Area

Relative Contribution of Pesticide Groups to the Total Pesticide Mixture Toxicity Estimates for NRM Regions and the Great Barrier Reef Catchment Area

In order to provide information that can guide the development of management actions and policies, the relative contribution of PSII Herbicides, Other Herbicides and Insecticides to the Total Pesticides mixture toxicity values for basins were estimated (Table 30). The relative contributions of PSII Herbicides, Other Herbicides and Insecticides are highly variable across the 35 basins reflecting the different agricultural land uses in the basins and the suite of pesticides that are permitted to be applied to each agricultural land use. The relative contribution of PSII Herbicides varies from 0 to 65%, while the relative contribution of Other Herbicides ranges from 0 to 97% and the relative contribution of Insecticides ranges from 0 to 98%.

The relative contribution of the three pesticide groups differs for basins with different risk classifications (Table 31). The main difference in the median relative contribution of the three pesticide classes that have different risk classifications is that it increases steadily for PSII Herbicides as the risk class increases. There also appears to be a weak trend for the median relative contribution of Insecticides to decrease as the risk class increases.

The relative contribution of the three pesticide groups to the Total Pesticide toxicity also differs with the NRM regions (Table 32). In Cape York PSII Herbicides contribute 88% and Insecticides contribute 11% to the Total Pesticide toxicity. The Wet Tropics and Mackay Whitsunday regions had similar relative contributions consisting of approximately 60% PSII Herbicides, 30% Other Herbicides and 12% Insecticides. The Burdekin, Burnett Mary and Fitzroy regions had similar relative contributions consisting of approximately 40% PSII Herbicides, 55% Other Herbicides and 4% Insecticides. The relative contribution to the Total Pesticide toxicity for the GBRCA was 47% for PSII Herbicides, 32% by Other Herbicides and 17% by Insecticides (Table 32). These groupings can be explained by differences in the land use of the regions – the Mackay Whitsundays and Wet Tropics have relatively large amounts of land used for sugar cane while the Burdekin, Burnett Mary and Fitzroy are dominated by cattle grazing and there is very limited amounts of agriculture in the Cape York region.

Earlier Reef Water Quality Protection Plans focussed efforts on reducing the annual loads of PSII herbicides, whereas the new Reef 2050 WQIP does not focus on any one group of pesticides, rather it considers all pesticides of equal importance. This change in emphasis is warranted given that Other Herbicides and Insecticides jointly contribute approximately 50% of the Total Pesticide toxicity at the GBRCA level. It should be noted that the relative contributions reported reflect currently available pesticide monitoring data and current pesticide usage. The relative contribution of pesticide groups could change as more pesticides are added to the Pesticide Risk Metric. For example, there are currently no fungicides included in the Pesticide Risk Metric and only three insecticides have been included. The relative contribution could also change as farmers modify their pesticide usage in response to emerging pest pressure, changes in the suite of pesticides registered for application to particular agricultural land uses and to projects aimed at minimising pesticide use, improving pesticide application and choosing pesticides that pose a lower risk to aquatic ecosystems (e.g., the Pesticide Decision Support Tool project — Warne and Neale, 2019).

Table 30. The relative contribution of Photosystem II inhibiting herbicides (PSII Herbicides), Other Herbicides and Insecticides to the Total Pesticides mixture toxicity estimates for each basin

Basin	Per cent contribution to the Total Pesticides mixture toxicity			Risk category for Total Pesticides mixture toxicity
	PSII Herbicides	Insecticides	Other Herbicides	
Baffle	16	37	47	Very low
Barron	14	17	69	Very low
Black	0	3	97	Very low
Boyne	17	62	21	Very low
Burdekin	42	4	54	Low
Burnett	51	6	43	Low
Burrum	34	6	60	Moderate
Calliope	24	6	70	Low
Daintree	2	98	0	Very low
Don	9	18	73	Very low
Endeavour	3	76	21	Very low
Fitzroy	35	0	65	Low
Haughton	61	5	34	High
Herbert	61	17	22	Moderate
Jacky Jacky	65	7	28	Very low
Jeannie	52	27	21	Very low
Johnstone	50	18	32	Moderate
Kolan	19	25	56	Low
Lockhart	50	26	24	Very low
Mary	23	8	69	Low
Mossman	53	11	36	Moderate
Mulgrave–Russell	47	27	26	Moderate
Murray	63	14	23	Moderate
Normanby	2	57	41	Very low

Basin	Per cent contribution to the Total Pesticides mixture toxicity			Risk category for Total Pesticides mixture toxicity
	PSII Herbicides	Insecticides	Other Herbicides	
O'Connell	60	13	27	High
Olive Pascoe	59	16	25	Very low
Pioneer	59	17	24	Very high
Plane	62	12	26	Very high
Proserpine	48	24	28	Moderate
Ross	1	20	79	Low
Shoalwater	0	95	5	Very low
Stewart	45	33	22	Very low
Styx	48	2	50	Very low
Tully	59	21	20	Moderate
Waterpark	48	17	35	Very low

Table 31. The median relative contribution of Photosystem II inhibiting herbicides (PSII Herbicides), Other Herbicides and Insecticides to the Total Pesticides mixture toxicity estimates for basins with different risk classifications

Risk class	Median per cent contribution to the Total Pesticides mixture toxicity[1]		
	PSII Herbicides	Insecticides	Other Herbicides
Very low	17	27	27
Low	24	6	65
Moderate	52	18	27
High	61	9	31
Very high	61	15	25

1. The sum of the values for PSII Herbicides, Other Herbicides and Insecticides do not equal 100% as they are the median contribution for each pesticide group in the region of the GBRCA.

Table 32. The relative contribution of Photosystem II inhibiting herbicides (PSII Herbicides), Other Herbicides and Insecticides to the Total Pesticides mixture toxicity estimates for each region and the Great Barrier Reef Catchment Area (GBRCA)

NRM Region	Per cent contribution to the Total Pesticides mixture toxicity		
	PSII Herbicides	Insecticides	Other Herbicides
Cape York	88	11	0
Wet Tropics	57	12	31
Burdekin	41	4	53
Mackay Whitsundays	61	12	27
Fitzroy	35	0	65
Burnett Mary	43	4	53
GBRCA[1]	47	17	32

[1] The sum of the values for PSII Herbicides, Other Herbicides and Insecticides do not equal 100% as they are the median contribution for each pesticide group in the region of the GBRCA.

Comparison to Other Studies

Posthuma et al. (2019) have conducted a study very similar to the present study. A comparison of the key features of the method used in the current project and Posthuma et al. (2019) is presented in Table 33. The key differences in the methods used were that Posthuma et al. (2019) modelled daily pesticide concentrations, they estimated risk over the entire year and used the 95th percentile concentration in assessing risk. In contrast, the current study modelled the average daily pesticide mixture toxicity, risk was assessed over 182 days (i.e., the wet season) and the average pesticide mixture toxicity was used in assessing risk.

Table 33. Key features of the current study and Posthuma et al. (2019) showing the similarities and differences

Characteristic	Current study	Posthuma et al. (2019)
No. of chemicals considered	22	1760
Pesticide concentration data	Measured	Modelled
Type of toxicity data used	NOEC, NEC or EC10	NOEC and EC50
Organisms used in SSDs	All species, only arthropods or only aquatic plants	All species
Methods of combining toxicity	SSD and Independent action	SSD and Concentration Addition
Predictions at multiple scales	Pesticide mixture toxicity values	Pesticide concentration values
Period of assessment	182 days (wet season)	365 days (entire year)

Characteristic	Current study	Posthuma et al. (2019)
Summary statistic used to determine risk	Average daily pesticide mixture toxicity	95 percentile of chemical concentration
Spatial scale of risk assessments	35 catchments, 35 basins, 6 Natural Resource Management Region and the GBRCA	>22 000 sub-catchments (average size 214 km^2)
Surface area covered	437,000 km^2	4,140,708 km^2

The risk estimates generated by Posthuma et al. (2019), based on NOEC data ranged from 0 to essentially 100% of species being affected. The majority of rivers with estimates of between 0 and 5% of species affected are located in Scandinavia and north-west Scotland. The rest of the mainland Europe generally has rivers with estimates of greater than 25% of species being affected. The highest risk group (75 to 100% of species affected) occur in southern England, northern France, Belgium, Netherlands and Germany, southern Spain, Italy and Romania.

In the current study, the estimates of the percentage of species affected in catchments ranged from 0 to 40%, with 39% of the catchments having more than 5% of species affected. Thus, the risk estimated by Posthuma et al. (2019) for a large proportion of rivers in the EU are considerably higher than in the current study. There are two possible reasons for this – that Posthuma et al. (2019) assessed the combined risk of up to 1760 chemicals if they co-occurred in water samples and that they used the 95 percentile of the chemical concentrations to assess the risk. However, Posthuma et al. (2019) also found that only 15 chemicals accounted for more than 99.5% of mixture toxicity. Thus, although they were potentially including the contributions of 1760 chemicals, only 15 routinely contributed to the toxicity. Spilsbury et al. (2020) conducted a similar analysis on GBRCA waterways. Re-analysis of the Spilsbury et al. (2020) data revealed that the pesticides included in the Pesticide Risk Metric accounted for over 99% of the total toxicity of the 50 pesticides included in the original analysis. These results indicate that the inclusion of 1760 chemicals (Posthuma et al. 2019) compared to 22 in the Pesticide Risk Metric is unlikely to have caused the greater risk estimated by Posthuma et al. (2019). Using the 95 percentile of the predicted chemical concentrations to determine the risk means that in Posthuma et al. (2019), only 18 days (5% of the 365 days in a year) would have higher daily concentrations for each chemical. In contrast, the current study used the average concentration. While the average chemical concentration does not correspond to a specific percentile it is most likely that the average of the 182 daily pesticide mixture values would correspond to a considerably lower percentile than the 95th and hence the estimate of risk would be considerably lower. This is the most likely cause that the estimates of aquatic risk are considerably larger in Posthuma et al. (2019) than those of the current study. Alternately, the GBRCA basins could have considerably lower pesticide concentrations. Posthuma (RIVM, pers. comm.) stated that if the average or median pesticide concentration had been used in Posthuma et al. (2019) that their estimates of pesticide risk would have been considerably lower. This supports the suggestion that the percentile of the data used is the major contributor to the observed differences of risk posed by pesticides

Posthuma and De Zwart (2006) estimated msPAF values (pesticide mixture toxicity values) for rivers in Ohio, USA of between 10 and 50% of fish species affected corresponded to four-orders of magnitude change in the observed to expected fish species ratio. Posthuma and De Zwart (2012) estimated msPAF values using chronic NOEC data for Dutch freshwater that ranged from 0% to ~90% which corresponded with up to 40% of macroinvertebrates species experiencing a halving of their abundance and up to 30% of macroinvertebrate species experiencing a 75% reduction in abundance. Munz et al. (2017) estimated msPAF values from 0 to 2.1% which corresponded to decreases in SPEAR index values from 50 to 15.

Usually a chemical mixture toxicity estimate (msPAF) of 5% is considered to correspond to observable ecological changes in the field. However, Smetanova et al. (2014) found that statistically significant changes in the SPEAR index (aquatic macroinvertebrate composition) happened at markedly lower msPAF values. Munz et al. (2017) also found msPAF values between 0 and 2.1% species affected caused changes to the SPEAR index values. The inference of these studies is that pesticide mixture toxicity estimates (msPAF and ΣTU) are highly correlated to adverse ecological changes in the field and that waterways with pesticide mixture toxicity values between 1 and 5% (the low risk classification) may well be experiencing marked ecological changes to species that are sensitive to pesticides (e.g. aquatic algae, crustaceans, insects and plants).

Additional Uses of the Pesticide Mixture Toxicity – Land Use Relationships

The pesticide mixture toxicity – land use relationships developed in this project have been used to generate estimates of the pesticide mixture toxicity spatial units larger than catchments (i.e., for 35 basins, 6 NRM regions and the GBRCA) for the Reef 2050 WQIP (Australian Government and Queensland Government, 2018). Since the relationships were derived using data from five of the six GBRCA regions, and a standardised land use data set, it was theorised that the relationships could be used to generate estimates of pesticide mixture toxicity for any waterbody (creek, river, lake or wetland) in the GBRCA, regardless of their size. For example, Al Ghafri (2019) used the relationships to estimate the toxicity of PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides in 170 catchments (141 in the GBRCA and 29 in South East Queensland). The relationships worked well for waterways in the GBRCA. However, the predicted Total Pesticide toxicity values for many waterways in South East Queensland were "not realistic" (Al Ghafri, 2019) and thus it appears the relationships developed for GBRCA waterways are not necessarily valid for other regions. This is not surprising as the hydrological, land use, pesticide use and spatial characteristics of waterways in South East Queensland can be different to those of the GBRCA. However, the method for deriving the pesticide mixture toxicity – land use relationships used in this project could be used to develop similar relationships for other regions. Such region-specific relationships are likely to result in realistic estimates of pesticide mixture toxicity. Similarly, pesticide mixture toxicity – land use relationships could be developed for other regions of Australia or other countries.

The relationships can also be used to estimate the toxicity of pesticide mixtures at any point or stretch in a creek or river within the GBRCA, provided the exact area of the upstream catchment is known and the relevant explanatory variables can be obtained. This is currently being done for approximately 23 000 segments of waterways (each typically being 1 to 1.5 km long) in the GBRCA (David Moffat, DES, *pers. comm.*). The calculation of pesticide mixture toxicity estimates for so many catchments and stretches of waterways will permit far more spatially explicit ecological risk assessments of pesticide impacts to be conducted in waterways of the GBRCA.

Potential Future Developments

There are four major developments that could be undertaken in the future. These are:

- expanding the pesticides included in the Pesticide Risk Metric and Pesticide Risk Baseline;
- calculating the Pesticide Risk Baseline for the next Reef WQIP;
- improving the chemical analysis; and
- including laboratory and/or *in-situ* effect-based methods.

Expanding the Pesticide Risk Metric and Baseline

The Pesticide Risk Metric and Pesticide Risk Baseline currently includes 22 pesticides, only three of which are insecticides, and no fungicides are included. It would be appropriate to consider whether additional pesticides should be added. Any new pesticides to be included would need to have a SSD already developed. The current revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality for toxicants and the default guideline values and SSDs being developed for approximately 30 pesticides by the Queensland Department of Environment and Science will greatly facilitate the inclusion of additional pesticides to the Pesticide Risk Metric and Pesticide Risk Baseline.

Calculating the Pesticide Risk Baseline for the next Reef Water Quality Improvement Plan

Irrespective of whether the Pesticide Risk Metric is expanded to include new pesticides, a new Pesticide Risk Baseline could be derived. Doing so would be consistent with the calculation of new baselines for management practice adoption, suspended solid and nutrient loads with each update of the Reef 2050 WQIP.

To derive the current Pesticide Risk Baseline toxicity data from 28 monitoring sites were collected over three years – creating 67 unique site/year datasets (there were multiple sites that were not monitored for all three years). Ideally, the same number of site/year datasets used to derive the current Pesticide Risk Baseline would be available to derive the new Pesticide Risk Baseline. However, the monitoring could be conducted over two years provided every site was monitored in both years and the number of sites was increased to 34 (thus creating 68 unique site/year datasets). The new pesticide monitoring data should be used to generate new pesticide mixture toxicity – land use relationships and then a new Pesticide Risk Baseline.

Improving the chemical analysis

The Pesticide Risk Metric and Pesticide Risk Baseline use measured chemical concentrations to estimate the biological effects (i.e., the percent of species affected or protected). This approach is inherently limited by the number of chemicals that are analysed in the water samples and further limited by requiring sufficient toxicity data to derive SSDs and DGVs. Traditionally, chemical analysis of water samples is limited to a set number of specified chemicals — this is termed targeted analysis. This limitation can be overcome using non-targeted analytical techniques using mass spectrometer instruments such as Time of Flight and Orbitraps – both of which can identify essentially any organic chemical introduced to the instrument provided they are in their library of identified chemicals. The identified chemicals can be quantified if standards are available. The electronic outputs from these analyses can be archived (rather than the water samples) and retrospective analyses can be conducted at a later date to determine if chemicals that were previously not of concern were present. If such chemicals are identified then semi-quantitative concentrations can be estimated.

Inclusion of Laboratory or *in-situ* Effect-Based Methods

Effect-based methods use the "response of whole organisms (*in vivo*) or cellular bioassays (*in vitro*) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern" (Brack et al., 2019). These are essentially toxicity tests conducted on water samples in the laboratory. The main advantages of effect-based methods is that they do not require a chemical to be identified or quantified in order to measure its biological (toxicological) effects, they measure the biological effect from all chemicals that are present (including degradation products) and they do not make assumptions about the type and magnitude of the joint toxicity (Escher and Leusch, 2012). Some effect-based methods, termed effect-directed analysis, can identify the types of chemicals that are contributing most to the measured toxicity (Brack et al., 2016; Neale et al., 2017).

In-situ effect-based methods are conducted in the actual waterways being considered and typically use local test organisms. Using local organisms that are of commercial, cultural, ecological and recreational significance (listed in alphabetical order, not order of importance) in *in-situ* methods would increase the relevance of the findings to key stakeholder groups. Involving key stakeholders in the selection of test organisms would increase the relevance of the work and is likely to lead to a greater willingness to adopt appropriate measures to minimise the impacts of pesticides (e.g., improve land management practices).

In addition, it would be preferable that potential effects are examined at multiple scales of biological complexity from sub-cellular (e.g., metabolomics, proteomics; enzyme inhibition), individual (e.g., reproduction, mobility, survival), population (e.g., population growth rate, generation time), community (e.g., community composition, SPEAR index for macroinvertebrates or algae, leaf litter breakdown, nutrient cycling, metagenomics (environmental DNA), average score per taxon, share of Ephemoptera, Plecoptera and Trichloptera, Saprobic index, Shannon diversity index).

A limitation of *in-situ* method is that they are conducted in the field and are therefore logistically more complex and difficult to run than the equivalent laboratory-based methods. They are also generally larger in scale and can be more ecologically complex than the laboratory effect-based methods. For example, the *in-situ* methods include small and moderately sized artificial ecosystems (called microcosms and mesocosms, respectively). However, these characteristics also mean that *in-situ* methods provide the most environmentally realistic estimates of the effects of toxicants on aquatic ecosystems.

The inclusion of laboratory and/or *in situ* effect-based methods with the chemistry-based methods (used in the current project) would provide multiple lines of evidence to quantify the potential impacts of pesticides on aquatic ecosystems. Importantly, the effect-based methods directly measure biological effects of exposure and thus, provide an independent means of determining the effect of toxicants on aquatic ecosystems and testing the accuracy of the estimates of pesticide mixture toxicity generated by the Pesticide Risk Metric and Pesticide Risk Baseline.

Summary

A Pesticide Risk Metric method was developed that can accurately estimate the toxicity of mixtures of up to 22 selected pesticides found in water samples. This method was used to estimate pesticide mixture toxicity values of four groups of pesticides (PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides), expressed as the average per cent of species affected during the wet season, for a total of 67 unique site and year datasets. Forward and backward step-wise regression was used to develop relationships between pesticide mixture toxicity for the four groups of pesticides and spatial, climate and land use variables. Four high quality relationships, with adjusted coefficients of determination (R^2) values between 0.68 (Insecticides) and 0.79 (PSIIs), were derived for the four groups of pesticides — one for each group of pesticides. These adjusted R^2 values mean that the explanatory variables in the relationships could explain between 68% and 79% of the observed variation in pesticide mixture toxicity data for the monitored catchments. The explanatory variables used in these relationships were a combination of spatial, climate and land use variables, although the land use variables were the most frequently used. The per cent of land in a catchment or basin that was used for horticulture and sugar cane were explanatory variables in all four relationships, conservation was an explanatory variable in three relationships, while dryland cropping and urban appeared in two relationships and irrigated cropping, bananas and forestry were each explanatory variables in one relationship. The pesticide mixture toxicity — land use relationships were validated and then used to predict pesticide mixture toxicity for the 35 basins, 6 natural resource management regions and the entire GBRCA that are reported on in the Reef Water Quality report cards. Whether the predicted pesticide mixture toxicity values were reasonable was tested using a number of different lines of evidence. The predicted toxicity mixture values were reasonable and typically differed by less than five per cent of affected species from expected values. The estimated risk values were divided into five risk classes ranging from very low to very high. Maps showing the risk classes for PSII Herbicides, Other Herbicides, Insecticides and Total Pesticides were generated for basins and maps for Total Pesticides risk classes were generated for NRM regions and the GBRCA.

Key findings from this project are:

- The toxicological risk posed by Total Pesticides was very low for all basins in the Cape York region, very low or low for all basins in the Fitzroy region and mainly very low and low in basins of the Burdekin region (although one basin had a high risk) and most basins in the Burnett Mary region had a low risk (although one basin had a very low risk and another had a moderate risk). Most of the basins in the Wet Tropics faced a moderate risk (but two had a very low risk) while two basins in the Mackay Whitsunday region faced a very high risk, one faced a high risk and another faced a moderate risk.
- The toxicological risk posed by PSII Herbicides for basins was generally very low or low. Two small coastal basins faced moderate risk (one each in the Wet Tropics and Burdekin regions), and two faced a high risk (both in the Mackay Whitsunday region).
- The toxicological risk posed by Other Herbicides was very low or low for all basins with the exception of the Plane basin (Mackay Whitsunday region) that faced a moderate risk.
- The toxicological risk posed by Insecticides was very low for all basins except for all basins in the Mackay Whitsunday regions and one basin in the Wet Tropics.
- At a regional level, Cape York faces a very low risk from Total Pesticides, while the Wet Tropics, Burdekin, Fitzroy and Burnett Mary NRM regions face a low risk. The Mackay Whitsunday NRM region faces a high risk.

- At the GBRCA level the risk faced from Total Pesticides is low.
- At the basin level the contribution of the three groups of pesticides was highly variable with no one group being dominant.
- The contribution of the three groups of pesticides was also highly variable at the regional level – with the contribution of PSII Herbicides ranging from 35 to 88%, Other Herbicides ranged from 0 to 65% and Insecticides ranged from 0 to 17%.
- At the GBRCA level the median contribution of PSII Herbicides to the Total Pesticide mixture toxicity was approximately 47% compared to 32% for Other Herbicides and 17% for Insecticides[23]. Thus, PSII Herbicides were the dominant pesticide group in terms of toxicity in the waterways discharging to the Great Barrier Reef lagoon.

Care needs to be exerted in interpreting the basin, region or GBRCA mixture toxicity values as they are aggregate or summary values and may not reflect the risk faced by particular waterways at finer spatial scales. For example, a region with a low risk could contain one or more basins and/or catchments that face a markedly higher or lower risk. This occurs in the Burdekin NRM region, which faces a low risk from Total Pesticides, but the Haughton basin faces a high risk from Total Pesticides. Similar situations occur in the Wet Tropics, Mackay Whitsundays and Burnett Mary regions.

The pesticide mixture toxicity values are not absolute values, rather, they are estimates of the risk posed. Thus, an estimate of 95% species protection should not be interpreted literally to mean that exactly 95% of species will be protected. Rather, the estimates were developed to determine if the pesticide target has been met or whether further land management change is required to reduce pesticide run-off and meet the target. Importantly these estimates of pesticide mixture toxicity can be used for relative assessments: (1) spatially to prioritise catchments, basins, or regions for on-ground improvements; and (2) temporally, to assess changes in the pesticide mixture toxicity at locations over time and improvements towards the target.

Recommendations

It is recommended that:

1. **The Pesticide Risk Metric (particularly the pesticide mixture toxicity – land use relationships) and the Pesticide Risk Baseline be periodically updated to be aligned with the updating of the Reef 2050 WQIP, and Paddock to Reef reporting**. The next update of the Reef 2050 WQIP is planned for 2022. The number of pesticide monitoring sites should be expanded from the current base level of monitoring in order to improve the pesticide mixture toxicity – land use relationships developed in the current project.
2. **Basins and regions that currently meet the pesticide target be re-evaluated with each update of the Reef 2050 Reef WQIP to ensure they continue to meet the target, or sooner if significant land use changes have occurred that might increase pesticide run-off**. This could be done by conducting on-going pesticide monitoring at appropriate sites, as well as monitoring changes to land use. But as land use data for any particular region is only updated periodically (e.g. every five years) on-going annual pesticide monitoring is likely to pick up changes more rapidly – but at

[23] The sum of the values for PSII Herbicides, Other Herbicides and Insecticides do not equal 100% as they are the median contribution for each pesticide group in the region of the GBRCA.

considerably greater expense. It is recommended that both on-going pesticide monitoring and land use monitoring are continued so that any change in risk can be periodically assessed, particularly for the basins and regions that currently meet the Pesticide Target.

3. **The information on the contribution of different pesticide groups to the total risk posed by pesticides be used to: identify which pesticide groups pose the highest risk within a catchment/basin or region and target on-ground management practice changes to reduce runoff of these higher-risk pesticides**. If an alternative active ingredient is used as a strategy to reduce the higher-risk pesticides in runoff, it is recommended to use the Pesticide Decision Support Tool (Warne and Neale, 2019) to ensure that the replacement is a lower risk to aquatic ecosystems. The Pesticide Decision Support Tool uses the same ecotoxicity information for assessing pesticide risk, as used in this report.
4. **The number and types of pesticides included in the Pesticide Risk Metric be expanded.** This should be done in a structured approach such as an audit of the use of pesticides not currently included in the Pesticide Risk Metric. Analytical methods, SSDs and Default Guideline Values (DGVs) should then be developed for pesticides identified by the audit and if needed, these pesticides should then be included in the Great Barrier Reef Catchment Loads Monitoring Program. Particular attention should be paid to including more insecticides and fungicides in the Pesticide Risk Metric as these groups are currently under-represented.

The findings of this report also have implications beyond the scope of the Reef 2050 WQIP and the Reef Water Quality report cards. Therefore it is also suggested that

1. *The Pesticide Risk Metric be used to predict the toxicity of waterways or reaches/stretches of waterways that discharge to the GBR lagoon and are not currently monitored*. This would permit the ecological risk to be determined along waterways, rather than the current situation where a single risk value is estimated for the entire waterway. This could help guide decisions on the location of future pesticide monitoring sites assist in prioritising catchments or sub-catchments where stakeholder engagement could decrease the risk posed by pesticides. It would provide data that would be extremely useful for the Regional Report Cards.
2. *Laboratory and field-based toxicity tests (effect-based methods[24]) should be added to the chemical-based methods used in this report as another line of evidence on the effects of pesticides on aquatic ecosystems*. Such techniques directly measure the effects of pesticides on aquatic species and ecosystems and provide an independent assessment, as they do not rely on pesticide concentration data. In the first instance a three to five-year project should be established that would test the accuracy of the predicted risk posed by pesticide mixture and seek to identify the effects of pesticides on aquatic species and ecosystems. The on-going or periodic inclusion of laboratory and field-based toxicity tests would provide direct evidence of whether the risk posed by pesticides was changing over time.
3. *Pesticide mixture toxicity – land use relationships be developed for all Queensland waterways.* Since land use and pesticide usage patterns vary spatially, it would be reasonable to assume that the pesticide mixture – land use relationships developed for this project may not be applicable outside of the GBRCA waterways that were used to train the models. Developing such relationships for other

[24] Effect-based methods use the "response of whole organisms (*in-vivo*) or cellular bioassays (*in-vitro*) to detect and quantify the effects of groups of chemicals on toxicological endpoints of concern" (Brack et al., 2019). These are essentially toxicity tests conducted on water samples in the laboratory or in the waterways being studied.

regions would permit the estimation of the risk that pesticides pose to other areas such as South East Queensland, the Gulf country and Central Queensland.

4. *The effects of undisturbed stream sections located in the headwaters and elsewhere in catchments on the harmful effects of pesticides in GBRCA waterways be investigated*. If undisturbed stream sections do ameliorate the effects of pesticide pollution, the extent (length or area) could be measured and included as a potential variable in new pesticide mixture toxicity – land use relationships. Their inclusion could improve the predictive capabilities of these relationships.

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Attachment A – Details of the sites used to provide aqueous pesticide concentration data

Great Barrier Reef Catchment Loads Monitoring Program monitoring sites were included in the baseline if they had been sampled adequately for pesticides within the 2015–2018 reference period.

Table 34. Aqueous concentration data were acquired for 35 GBRCLMP pesticide monitoring sites for the years 2015–2018

NRM Region	Basin	Catchment	Site ID	Site name	Pesticide monitoring start[a]	Site Type[b]	Latitude	Longitude	Monitored Area (km^2)	Catchment monitored (%)
Wet Tropics	Mossman	Mossman River	1090010	Mossman River at Bonnie Doon	2017–2018	RM	-16.445542	145.396196	197	94
	Barron	Barron River	1100023	Barron River at Rinks Close Jetty	2017–2018	EOC	-16.873157	145.733374	2132	99
	Mulgrave-Russell	Mulgrave River	1110056	Mulgrave River at Deeral	2014–2015	RM	-17.207500	145.926390	789	98
	Mulgrave-Russell	Russell River	1111019	Russell River at East Russell	2014–2015	RM	-17.267220	145.954440	521	93
	Johnstone	North Johnstone River	1120049	North Johnstone River at Old Bruce Highway Bridge (Goondi)	2006–2007	SC	-17.505944	145.991972	960	89
	Johnstone	Johnstone River	1120054	Johnstone River at Coquette Point	2015–2016	RM	-17.511190	146.060350	1635	100
	Tully	Tully River	113006A	Tully River at Euramo	2006–2007	EOC	-17.992139	145.942472	1450	93
	Herbert	Herbert River	116001F	Herbert River at Ingham	2006–2007	EOC	-18.632750	146.142670	8584	97

NRM Region	Basin	Catchment	Site ID	Site name	Pesticide monitoring start[a]	Site Type[b]	Latitude	Longitude	Monitored Area (km^2)	Catchment monitored (%)
Burdekin	Haughton	Haughton River	1190004	Haughton River at Giru Weir	2017–2018	EOC	-19.512073	147.111509	1880	92
	Haughton	East Barratta Creek	1191032	East Barratta Creek at Jerona Road	2017–2018	EOC	-19.487818	147.228359	1153	94
	Black	Black River	117002A	Black River at Bruce Highway	2010–2011	EOC	-19.237710	146.632866	256	87
	Haughton	Haughton River	119003A	Haughton River at Powerline	2013–2014	EOC	-19.633136	147.110278	1773	87
	Haughton	Barratta Creek	119101A	Barratta Creek at Northcote	2009–2010	SC	-19.690728	147.169825	759	62
	Burdekin	Burdekin River	120001A	Burdekin River at Home Hill	2006–2007	EOC	-19.642060	147.396940	129930	100
Mackay Whitsunday	O'Connell	O'Connell River	1240062	O'Connell River at Caravan Park	2007–2008	EOC	-20.566400	148.611700	825	96
	Proserpine	Proserpine River	122013A	Proserpine River at Glen Isla	2016–2017	EOC	-20.417200	148.645300	550	51
	O'Connell	O'Connell River	124001B	O'Connell River at Stafford's Crossing	2007–2008	SC	-20.652556	148.573000	340	40
	Pioneer	Pioneer River	125013A	Pioneer River at Dumbleton Pump Station	2006–2007	EOC	-21.141908	149.075839	1466	93
	Plane	Sandy Creek	126001A	Sandy Creek at Homebush	2009–2010	SC	-21.283289	149.022506	326	70

NRM Region	Basin	Catchment	Site ID	Site name	Pesticide monitoring start[a]	Site Type[b]	Latitude	Longitude	Monitored Area (km^2)	Catchment monitored (%)
Fitzroy	Styx	Styx River	1270013	Styx River at Ogmore	2017–2018	EOC	-22.592243	149.625971	1009	66
	Waterpark	Waterpark Creek	1290021	Waterpark Creek at Corbett's Landing	2017–2018	EOC	-22.886542	150.719722	387	89
	Fitzroy	Fitzroy River	1300000	Fitzroy River at Rockhampton	2007–2008	EOC	-23.381113	150.516909	139289	99
	Calliope	Calliope River	1320021	Calliope River at Old Bruce Highway	2017–2018	EOC	-23.958150	151.158528	1638	89
	Boyne	Boyne River	1330014	Boyne River at Boyne Island	2017–2018	RM	-23.946813	151.357830	2406	100
	Fitzroy	Comet River	130504B	Comet River at Comet Weir	2007–2008	SC	-23.612472	148.551389	16457	95
Burnett Mary	Baffle	Baffle Creek	1340008	Baffle Creek at Newton Road	2017–2018	EOC	-24.515097	151.974167	2374	93
	Kolan	Kolan River	1350050	Kolan River at Booyan Boat Ramp	2017–2018	EOC	-24.705437	152.188821	2576	92
	Burnett	Burnett River	1360106	Burnett River at Quay Street Bridge	2017–2018	EOC	-24.862985	152.345631	33047	100
	Burrum	Elliott River	1370005	Elliott River at Riverview Boat Ramp	2017–2018	RM	-24.929836	152.474652	370	91
	Burrum	Gregory River	1371034	Gregory River at Jarrett's Road	2017–2018	EOC	-25.157325	152.498434	818	94

NRM Region	Basin	Catchment	Site ID	Site name	Pesticide monitoring start[a]	Site Type[b]	Latitude	Longitude	Monitored Area (km^2)	Catchment monitored (%)
	Burrum	Burrum River	1373011	Burrum River at Buxton Boat Ramp	2017–2018	RM	-25.195549	152.541947	1406	100
	Mary	Mary River	1380111	Mary River at Churchill Street	2017–2018	EOC	-25.532000	152.708100	8866	93
	Burnett	Burnett River	136014A	Burnett River at Ben Anderson Barrage HW	2009–2010	EOC	-24.889636	152.292155	32841	99
	Mary	Tinana Creek	138008A	Tinana Creek at Barrage	2013–2014	EOC	-25.571961	152.717302	1284	99
	Mary	Mary River	138014A	Mary River at Home Park	2013–2014	EOC	-25.768325	152.527360	6872	72

[a] The GBR CLMP monitors from 1 July–30 June each year so commencement of monitoring follows the format of YYYY-YYYY to denote the sampling year [b] EOC refers to End of Catchment and SC refers to Sub-Catchment

Attachment B – Treatment of Concentration Values Less than the Limit of Reporting.

Assessing the risk of pesticides individually or in mixtures is complicated by the presence of non-detects or concentration measurements below the limit of reporting (LOR). Laboratories report any detection of a chemical at a concentration less than the limit of reporting simply by "< LOR" or for example "< 0.005 µg/L" if the limit of reporting was 0.005 µg/L. All that is known about the concentration of such data is that they lie somewhere between zero and the laboratories LOR. Because < LOR data are often a significant proportion of environmental monitoring data they should be considered in an appropriate manner in order to provide reasonable estimates of the toxicity and toxicological risk of individual pesticides and mixtures.

Methods to treat < LOR data fall into three main groups: statistical distribution-based estimation, distribution-free estimation and standardised substitution. The statistical estimation methods fit a distribution to the concentrations greater than the LOR and then extrapolate that distribution from the LOR to zero and thus obtain estimates to replace the < LOR values. There are also distribution free methods such as the Kaplan-Meier method that estimate a likely replacement for missing (censored) values using the 'survivor estimation' approach developed for the medical industry. Finally, the substitution methods simply replace all < LOR values by a single value which is usually zero, half the LOR or the LOR itself. The issue of which is the best method to deal with < LOR values has been a topic of interest particularly in environmental sciences where a considerable proportion of measured concentrations are less than the LOR (e.g. Liu et al., 1997; Helsel, 2005; Shaori and Dubé, 2018). These authors have criticised the substitution methods and argue that statistical methods are better. However, the guidance given by regulatory agencies on this issue is not consistent (see for example Table 2 in Shaori and Dubé, 2018). One method that is frequently recommended and widely used is the Kaplan-Meier method (Helsel, 2005; Shaori and Dubé, 2018; Spilsbury 2018).

Spilsbury (2018) compared the total risk of pesticide mixtures discharged to the GBR using three different methods of accounting for concentrations < LOR:

1. replacing with zero — a minimal risk substitution method (e.g., all <LOR values are replaced by 0);
2. replacing with the limit of reporting — a maximum risk substitution method (e.g. if the LOR was 0.005 µg/L, all < LOR values would be replaced by 0.005 µg/L); and
3. using the Kaplan-Meier statistical approach.

This analysis was conducted only using data that was suitable for the Kaplan-Meier method in order for the comparison to be valid. This meant that only 42.1% of the 3757 pesticide water samples available could be used. This analysis (Spilsbury, 2018) revealed that the estimates of risk by the three methods varied by less than one per cent with method 2 resulting in the highest toxicity, followed by method 3 and then method 1. However, the first method is highly likely to underestimate the toxicity of mixtures, while the second is highly likely to overestimate the toxicity of mixtures. Another problem with the second method is that the estimate of risk is controlled by the number of chemicals being considered. If enough chemicals are considered it is possible to have no pesticides being quantified (i.e. all < LOR values) and to still end up estimating that the combined impact of pesticides poses an environmental risk. The fact that over 50% of the samples could not be used by the Kaplan-Meier method is a severe limitation and may lead to an underestimation of the pesticide mixture toxicity. In addition, our dataset violated key assumptions of the Kaplan-Meier method, such as the proportion of censored data (too many missing values may affect reliability of estimates), patterning of missing values and independence of censoring (water quality data are typically left-censored).

Gustavsson et al. (2017) conducted a similar assessment applying the same methods to pesticide concentration data from 308 weekly samples that were analysed for between 76 and 131 pesticides and degradation products in Swedish water samples (Lindström and Kreuger, 2015). They obtained the same results as Spilsbury (2018).

Given the above results, a substitution method was develop based on the relative toxicity of the pesticides. The method of toxic proportions was developed to treat < LOR data points while accounting for relative toxicity of the 22 pesticides and allowing flexibility to adapt to changing LOR values. The steps involved in determining the substitution values were: the default guideline value for 99% protection (PC99) was converted from µg/L to µmol/L by dividing the DGV by the molecular weight of the pesticide; the relative toxicity of each pesticide was then determined by dividing the DGV (µmol/L) value of each selected pesticide by the least toxic of the selected pesticides (i.e. the pesticide with the largest PC99 (µmol/L) value. Thus, the least toxic pesticide (haloxyfop) would have a Relative Default Guideline Value of 1 and the Relative Default Guideline Values decreased with increasing toxicity of each pesticide (Table 35). Any < LOR value in the dataset was then multiplied by the appropriate Relative Default Guideline Value to determine the value to be substituted for the < LOR in each instance. Thus, the least toxic pesticide would replace its < LOR values with the limit of reporting and all other pesticides would have some fraction of their limit of reporting that decreased inversely with their relative toxicity.

The rules that were applied to deal with < LOR concentration values were:

- If no detections (i.e. concentrations larger than the LOR) of a pesticide occurred at a site for the entire wet season then all < LORs for that pesticide, site and year combination were changed to a very small value (i.e. 1 x 10-11) rather than zero as the data were logged as part of the calculations;
- All < LORs of a pesticide that occurred before the first detection of that pesticide were changed to a very small value (i.e. 1 x 10-11); and
- All < LORs of a pesticide after the first detection of the same pesticide were substituted by the product of the LOR and the relative DGV (Table 35). An example calculation of this is provided in Table 36.

Table 35. The relative default guideline values that are multiplied to the limit of reporting values to determine the values to substitute for concentrations less than the limit of reporting as per the following rules. The pesticides are presented in order of decreasing toxicity

Pesticide	Relative Default Guideline Value
Fipronil	4.7762×10^{-6}
Chlorpyrifos	9.4590×10^{-7}
Metsulfuron-methyl	1.0145×10^{-5}
Metolachlor	1.7311×10^{-5}
MCPA	2.2957×10^{-5}
Imazapic	1.0930×10^{-4}
Pendimethalin	1.1788×10^{-4}
Imidacloprid	1.3691×10^{-4}
Diuron	1.9759×10^{-4}
Ametryn	2.1341×10^{-4}
Prometryn	2.2645×10^{-4}
Triclopyr	5.2679×10^{-4}
Isoxaflutole	6.3235×10^{-4}
Atrazine	7.6874×10^{-4}
Terbuthylazine	1.3367×10^{-3}
Metribuzin	5.7315×10^{-3}
2,4-D	8.9306×10^{-3}
Tebuthiuron	1.2642×10^{-2}
Hexazinone	4.3809×10^{-3}
Fluroxypyr	2.7451×10^{-1}
Simazine	5.1769×10^{-2}
Haloxyfop	1

The very small value of 1×10^{-11} is an arbitrary value and was used instead of absolute zero due to the logging of data in subsequent calculations. It was chosen as it is so small that it would not alter, to any meaningful degree, the estimate of pesticide mixture toxicity. The value could equally be another very small value.

An R-code was created to automate the above substitution ruleset for the < LOR values to prevent human errors. The limit of reporting (LOR) values reported in Table 36 are for the direct injection analysis method conducted by the Queensland Health Forensic and Scientific Services laboratories.

Table 36. Example calculations of the relative guideline values and relative toxicity limit or reporting values. The latter were substituted for pesticide concentrations that were less than the limit of reporting (LOR)

Analyte	LOR (µg/L)	Merged PC99 DGV[1] (µg/L)	Molecular weight (g/mol)	Merged PC99% DGV[1] (µmol/L)	Relative GLV[2] multiplier	An example of the replacement value (Relative GLV x LOR)
2,4-D	0.02	3.2	220.04	1.45×10^{-2}	8.93×10^{-3}	1.79×10^{-4}
Ametryn	0.01	0.079	227.33	3.48×10^{-4}	2.13×10^{-4}	2.13×10^{-6}
Atrazine	0.02	0.27	215.68	1.25×10^{-3}	7.69×10^{-4}	1.54×10^{-5}
Chlorpyrifos	0.02	0.00054	350.59	1.54×10^{-6}	9.46×10^{-7}	2.00×10^{-8}
Diuron	0.02	0.075	233.09	3.22×10^{-4}	1.98×10^{-4}	3.95×10^{-6}
Fipronil	0.02	0.0034	437.15	7.78×10^{-6}	4.78×10^{-6}	1.00×10^{-7}
Fluroxypyr	0.05	114	255.03	4.47×10^{-1}	2.75×10^{-1}	1.37×10^{-2}
Haloxyfop (acid) *Least toxic pesticide*	0.02	589	361.70	1.63×10^{0}	1.00×10^{0}	2.00×10^{-2}
Hexazinone	0.01	1.8	252.32	7.13×10^{-3}	4.38×10^{-3}	4.38×10^{-5}
Imazapic	0.01	0.049	275.31	1.78×10^{-4}	1.09×10^{-4}	1.09×10^{-6}
Imidacloprid	0.02	0.057	255.66	2.23×10^{-4}	1.37×10^{-4}	2.74×10^{-6}
Isoxaflutole metabolite (DKN)	0.02	0.37	359.32	1.03×10^{-3}	6.32×10^{-4}	1.27×10^{-5}
MCPA	0.01	0.0075	200.62	3.74×10^{-5}	2.30×10^{-5}	2.30×10^{-7}
Metolachlor	0.01	0.008	283.80	2.82×10^{-5}	1.73×10^{-5}	1.70×10^{-7}
Metribuzin	0.02	2	214.29	9.33×10^{-3}	5.73×10^{-3}	1.15×10^{-4}
Metsulfuron methyl	0.02	0.0063	381.36	1.65×10^{-5}	1.01×10^{-5}	2.00×10^{-7}

Analyte	LOR (µg/L)	Merged PC99 DGV[1] (µg/L)	Molecular weight (g/mol)	Merged PC99% DGV[1] (µmol/L)	Relative GLV[2] multiplier	An example of the replacement value (Relative GLV x LOR)
Pendimethalin	0.02	0.054	281.31	1.92×10^{-4}	1.18×10^{-4}	2.36×10^{-6}
Prometryn	0.02	0.089	241.36	3.69×10^{-4}	2.26×10^{-4}	4.53×10^{-6}
Simazine	0.01	17	201.66	8.43×10^{-2}	5.18×10^{-2}	5.18×10^{-4}
Tebuthiuron	0.01	4.7	228.31	2.06×10^{-2}	1.26×10^{-2}	1.26×10^{-4}
Terbuthylazine	0.01	0.5	229.71	2.18×10^{-3}	1.34×10^{-3}	1.34×10^{-5}
Triclopyr	0.05	0.22	256.46	8.58×10^{-4}	5.27×10^{-4}	2.63×10^{-5}

[1.] DGV = Default guideline value. [2]. Relative GLV = Relative guideline value which is used as a multiplier for < LOR values subject to the rules stated in the quality assurance and quality control methods section.

Attachment C – Summary of Key Data Manipulations Used in Calculating the Species Sensitivity Distributions

Standardising toxicity data

Acute toxicity data were converted to chronic estimates by dividing by an acute to chronic ratio or by a default assessment factor of 2 (Warne et al., 2018). The various measures of toxicity were all converted to estimates of no effect values by dividing median effect concentration (EC50) data and median lethal concentration (LC50) data by 5, lowest observed effect concentration (LOEC) data by 2.5 and maximum acceptable threshold concentration (MATC) data by 2 (Warne et al., 2018). No effect concentration (NEC) values, no observed effect concentration (NOEC) values, no observed effect level (NOEL) values and 10% effect concentration (EC10) values, although calculated differently, were all considered to be no effect data and therefore equivalent (Warne et al., 2018).

Calculating a single toxicity value for each species

Toxicity data were sorted by species and then by, duration (acute or chronic), measure of toxicity and toxicity endpoint. If there was a single value for a duration/measure/endpoint combination then that value represented that combination. If there were multiple values for a duration/measure/endpoint combination then the geometric mean of the values was determined (details are provided in Warne et al., 2018).

Data preference rules

The data preference rules (Warne et al., 2018), which govern the order in which various types of ecotoxicity data are used, were followed to determine which ecotoxicity data for the various duration/measure/endpoint combinations for each species would be used to generate the SSD. The data preference order was:

1. chronic NEC, NOEC or EC10 data;
2. chronic LOEC, EC50 or LC50 data; and
3. acute EC50 or LC50 data.

The highest preference data for each species were selected and then from those the lowest value for all the duration/measure/endpoint combinations for a species was adopted as the single value to represent the toxicity of the species. Further details on the data manipulations and examples are provided in Warne et al. (2018). Summarising the toxicity data to a single value for each species means that each species is given equal weighting (importance) in the calculation of each chemicals SSD.

Dealing with the distribution of toxicity data

Existing methods for calculating SSDs fit a uni-modal statistical distribution to the toxicity data. If multi-modal toxicity data are used then the SSD method will fit a distribution that best fits the data and this generally results in artificially low PC values. Therefore, Warne et al. (2018) recommends that the modality of the toxicity data be assessed and if the toxicity data is multi-modal, then only the toxicity data for the most sensitive group of organisms is used to derive the SSD. Generally, the most sensitive group of organisms to pesticides is the target organisms of the pesticide. For example, for herbicides the most sensitive group is usually aquatic plants and algae, while for insecticides it is usually aquatic insects and crustaceans (which are closely related to insects). This approach is different to that proposed by Suter (2002) that separate SSDs be derived for each group of organisms. A key limitation of the Suter et al. (2002) proposal is that it does not generate a SSD that can be used to estimate 'safe' concentrations of a chemical based on toxicity data for all species, rather it generates a series of SSDs which estimate ‘safe’ concentrations for different groups of organisms. Another limitation is that it reduces the number of data used to generate the SSDs and this will reduce the statistical rigour of the SSD and resulting DGVs.

When the toxicity data for a pesticide is uni-modal the data for all available species are used to calculate the SSD. The minimum ecotoxicity data required to derive a SSD for a uni-modal pesticide is for five species that belong to at least four taxonomic groups (Warne et al., 2018). The resulting PC values should theoretically protect a certain percentage of all species (e.g. a PC95 will theoretically protect 95% of all aquatic species).

When the toxicity data for a pesticide is multi-modal only the toxicity for the most sensitive group of organisms is used to derive the SSD (Warne et al., 2018). The minimum data requirements for pesticides with multi-modal toxicity data differ from those for pesticides with uni-modal data. The most sensitive group of organisms are still required to have toxicity data for at least five species but the number of taxonomic groups can be relaxed providing the toxicity data for the complete dataset (i.e. the most and least sensitive groups of organisms) meets the minimum number of taxonomic groups. When the toxicity data for a pesticide are multi-modal then the resulting PC values should theoretically protect a certain percentage of species of the most sensitive group of organisms (e.g. if the most sensitive group of organisms was aquatic plants and algae then a PC95 will theoretically protect 95% of aquatic plants and algae species). Details of the how the SSDs for each of the selected pesticides were calculated are presented in Attachment D.

Assessing the Reliability of Species Sensitivity Distributions and Default Guideline Values

Defaults Guideline Values not derived using the SSD approach (i.e., derived using the Assessment Factor method – Warne (1998, 2001) are classed as having an unknown reliability (Warne et al., 2018). The reliability of DGVs derived using the SSD approach can range from very low, low, moderate, high to very high (Warne et al., 2018). The reliability classification provides a simple and transparent means of indicating the general level of confidence in a DGV. The reliability classification is based on three variables:

- the number of species and taxonomic groups that appropriate quality toxicity data are available for;
- whether the toxicity data are all chronic, a mixture of acute and chronic, all converted acute, or a mixture of fresh and marine toxicity data; and
- and the fit of the SSD to the toxicity data – based on a visual assessment and a grading of good or poor.

The classification scheme and how these three variables interact to determine the reliability is presented in Table 37.

Table 37. Classification scheme for assessing the reliability of guideline values derived using the SSD method (from Warne et al., 2018)

Data type	Sample size (adequacy) [b]	Adequacy of SSD model fit	Reliability
Chronic [a]	≥15 (Preferred)	Good	Very high
		Poor	Moderate
	8–14 (Good)	Good	High
		Poor	Moderate
	5–7 (Adequate)	Good	Moderate
		Poor	Low
Combined chronic and converted acute or Combined chronic fresh and chronic marine	≥15 (Preferred)	Good	Moderate
		Poor	Low
	8–14 (Good)	Good	Moderate
		Poor	Low
	5–7 (Adequate)	Good	Moderate
		Poor	Low
Converted acute	≥15 (Preferred)	Good	Moderate
		Poor	Low
	8–14 (Good)	Good	Moderate
		Poor	Low
	5–7 (Adequate)	Good	Low
		Poor	Very low

[a] This includes all types of data irrespective of whether they are chronic NEC, BEC10, EC10 and NOEC values or estimates of chronic EC10 and NOEC values that were converted from chronic LOEC, MATC or EC50 data. [b] The sample size is assumed to comprise data from at least four taxonomic groups.

Attachment D – Creation of Species Sensitivity Distributions for the Pesticide Risk Metric

2,4-D

There is no statistically significant variation in the sensitivity of different types of organisms to 2,4-D therefore ecotoxicity data for all organism types were used in the derivation of the DGVs. In freshwater there were chronic NOEC/EC10 type toxicity data (data preference 1) for eight species and there were chronic EC/LC50 type toxicity data (data preference 2) for another eight species. In marine waters there were chronic NOEC/EC10 type toxicity data (data preference 1) for one species, chronic EC/LC50 type data for three species (data preference 2) and acute EC/LC50 type data for ten species (data preference 4). There were sufficient chronic NOEC/EC10 type toxicity (preference 1) data to derive a SSD and DGVs however there were limitations with this approach. Firstly, the dataset only included a single marine species and due to the different abundance of monocot and dicot species in fresh and marine species and the difference in monocot and dicot sensitivity to 2,4-D it was felt that it would be better to include more marine species. Secondly, the resulting SSD did not fit the data well. Therefore, preference 1 and 2 toxicity data for both fresh and marine species were combined to derive the 2,4-D SSD and DGVs – giving toxicity data for 20 species.

The SSD for the combined fresh and marine 2,4-D toxicity data is presented in Figure 15. The distribution that best fitted the combined toxicity data was an Inverse Weibull distribution with the following parameter values

Inverse Weibull parameters	Parameter values
Log alpha	-0.658974562786426
Log beta	-4.93633636733866

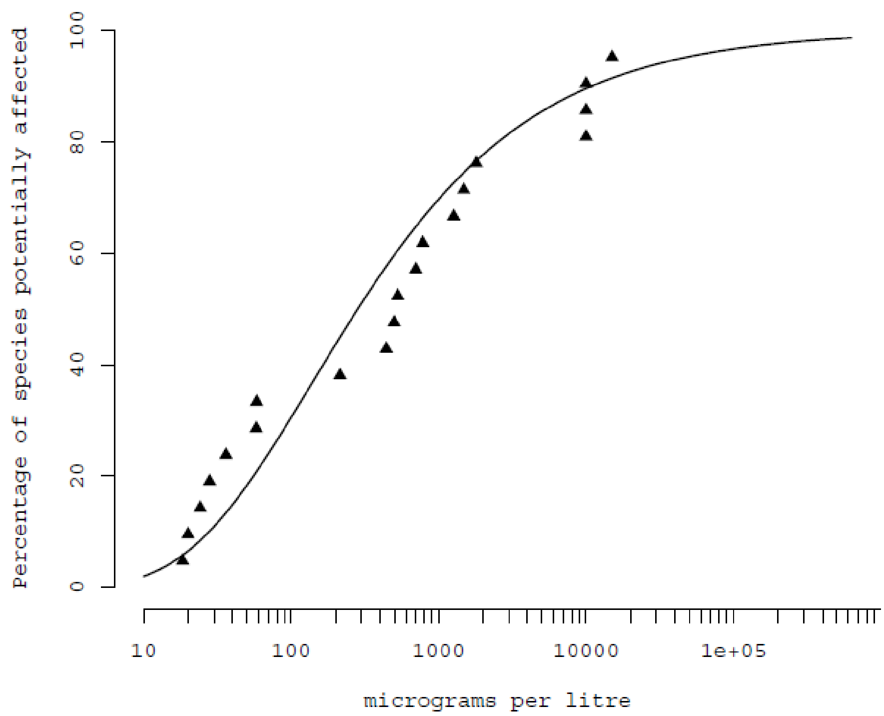

Figure 15. The SSD for the combined fresh and marine toxicity data for 2,4-D

The DGVs generated using the combined 2,4-D toxicity data are presented in Table 38.

Table 38. DGVs for the combined fresh and marine toxicity data for 2,4-D

Protection levels	PC values (µg/L)
PC99	7.3
PC95	17
PC90	28
PC80	56

Ametryn

Phototroph species are more sensitive to ametryn than heterotroph species and therefore only phototrophs were used in the derivation of the ametryn DGVs. For freshwater there were two photorophic species with chronic NOEC/EC10 (data preference score 1) and EC/LC50 type toxicity data for six phototrophs (data preference score 2), which in total belonged to only three phyla. For marine waters there were chronic NOEC/EC10 type data for one phototroph (data preference score 1) and chronic EC/LC50 type toxicity data for eight phototroph species (data preference score 2). There were not enough data preference score 1 data to derive an ametryn SSD and DGVs for the Pesticide Risk Metric. Therefore, data preference score 1 and 2 fresh and marine toxicity data were combined to derive the ametryn SSD and DGVs for the Pesticide Risk Metric.

The SSD for the combined fresh and marine ametryn toxicity data is presented in Figure 16. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	1.70321271129072
Log c	0.0517917587848087
Log k	0.0272151706126956

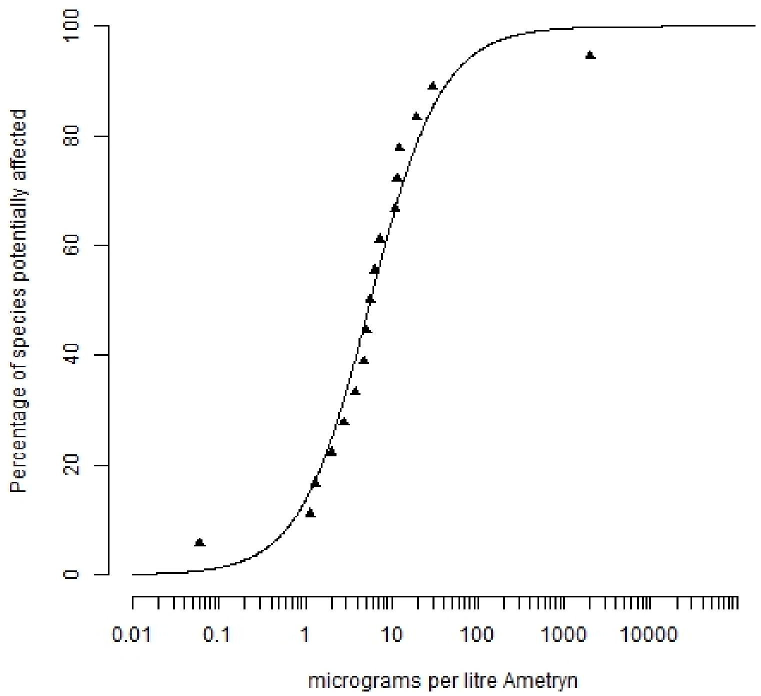

Figure 16. The SSD for the combined fresh and marine toxicity data for ametryn

The DGVs generated using the combined ametryn toxicity data are presented in Table 39.

Table 39. DGVs for the combined fresh and marine toxicity data for ametryn

Protection levels	PC values (µg/L)
PC99	0.079
PC95	0.36
PC90	0.73
PC80	1.6

Atrazine

Phototroph species are more sensitive to atrazine than heterotroph species and therefore only phototrophs were used in the derivation of the atrazine DGVs. For freshwater there were chronic NOEC/EC10 type data for 46 phototrophic species (data preference score 1). For marine waters there were NOEC/EC10 type toxicity data for four phototrophic species (data preference score 1) but there were chronic EC/LC50 type toxicity data for nine species (data preference score 2). To derive the SSD and DGVs for the Pesticide Risk Metric the 46 freshwater and four marine phototrophic species with chronic NOEC/EC10 data were combined.

The SSD for the combined fresh and marine atrazine toxicity data is presented in Figure 17. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	2.43571831275624
Log c	-0.380527520878313
Log k	0.556916028342911

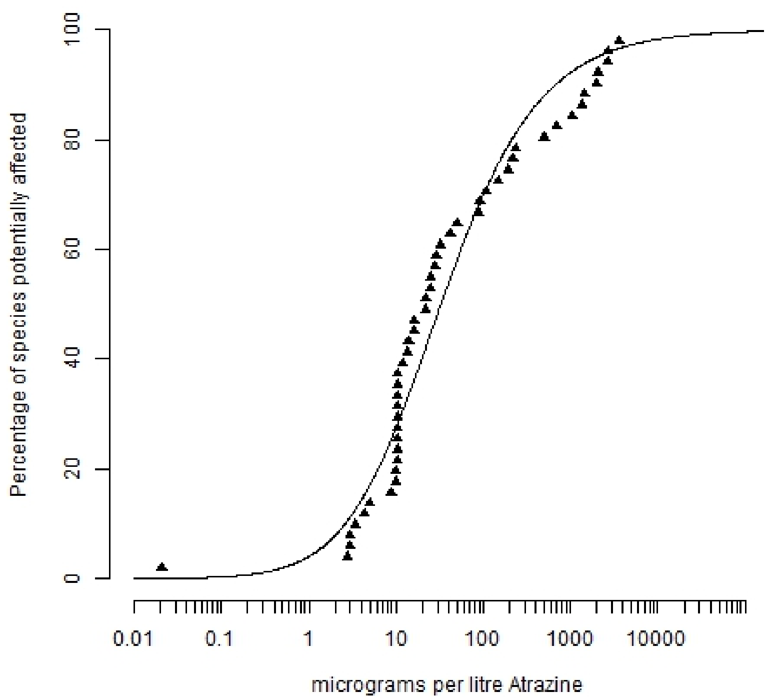

Figure 17. The SSD for the combined fresh and marine toxicity data for atrazine

There is one toxicity value which is markedly lower than the other phototrophic species (Figure 17). The paper that generated this paper was re-examined carefully to determine if there were any possible experimental causes for this difference. The study passed the normal quality assurance and quality control assessment and no reasons were found for excluding this datum.

The DGVs generated using the combined atrazine toxicity data are presented in Table 40.

Table 40. DGVs for the combined fresh and marine toxicity data for atrazine

Protection levels	**PC values (µg/L)**
PC99	0.27
PC95	1.2
PC90	2.6
PC80	6.2

Chlorpyrifos

This chemical was not part of the revision of the Australian and New Zealand WQGs nor the generation of DGVs by DES. There are high reliability Trigger Values (TVs) (therefore the data are all chronic) in the ANZECC and ARMCANZ 2000 WQGs for both fresh and marine waters. The fresh and marine data were combined and a new SSD (Figure 18) derived for the Pesticide Risk Metric.

The distribution best describing the combined chlorpyrifos toxicity data is a Burr type III equation that has the following parameter values

Burr type III parameters	Parameter values
Log b	1.30747195229139
Log c	-1.00292618436754
Log k	0.340414077657615

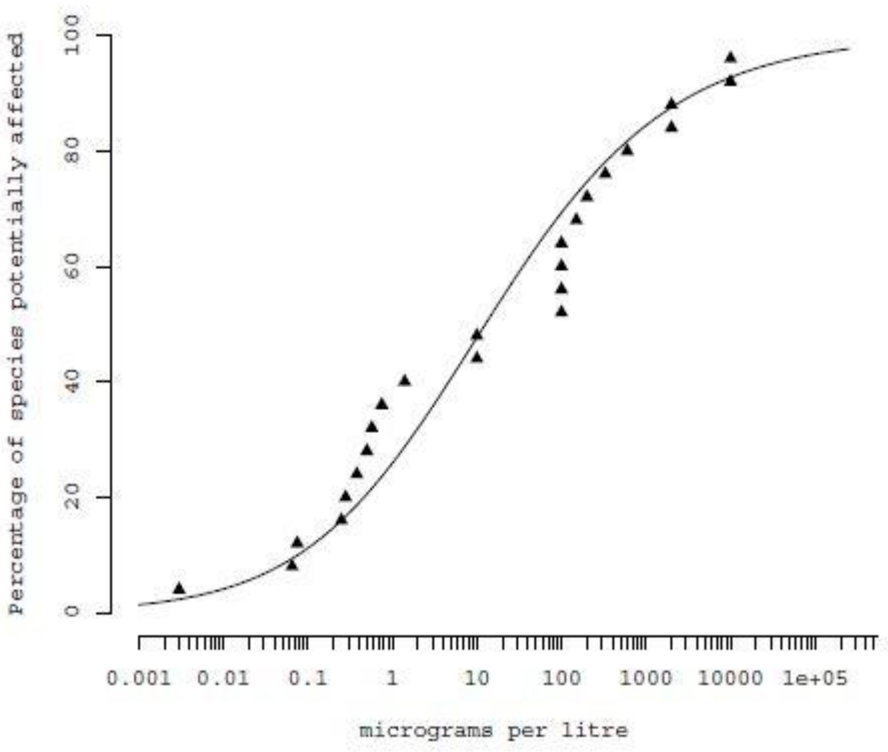

Figure 18. The SSD for the combined fresh and marine toxicity data for chlorpyrifos

The DGVs generated using the combined chlorpyrifos toxicity data are presented in Figure 18

Table 41. DGVs for the combined fresh and marine toxicity data for chlorpyrifos

Protection levels	PC values (µg/L)
PC99	0.00054
PC95	0.016
PC90	0.077
PC80	0.46

The actual toxicity data used came from ANZECC/ARMCANZ (2000) and are presented below.

Freshwater chronic NOEC data

0.57	0.075	0.065	0.5	200
10	100	100	100	10
100	330			

Marine chronic NOEC data

0.25	0.75	0.28	0.38	1.4
0.003	2000	10000	10000	600
150	2000			

Diuron

Phototroph species are more sensitive to diuron than heterotroph species and therefore only phototrophs were used in the derivation of the diuron DGVs. For freshwater there were chronic NOEC/EC10 type data for 15 phototrophic species (data preference score 1). For marine waters there were NOEC/EC10 type toxicity data for seven phototrophic species (data preference score 1). To derive the SSD and DGVs for the Pesticide Risk Metric the 15 freshwater and seven marine phototrophic species with chronic NOEC/EC10 data were combined.

The SSD for the combined fresh and marine diuron toxicity data is presented in Figure 19. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	-3.09303306233447
Log c	-0.682096599453548
Log k	2.07975712939176

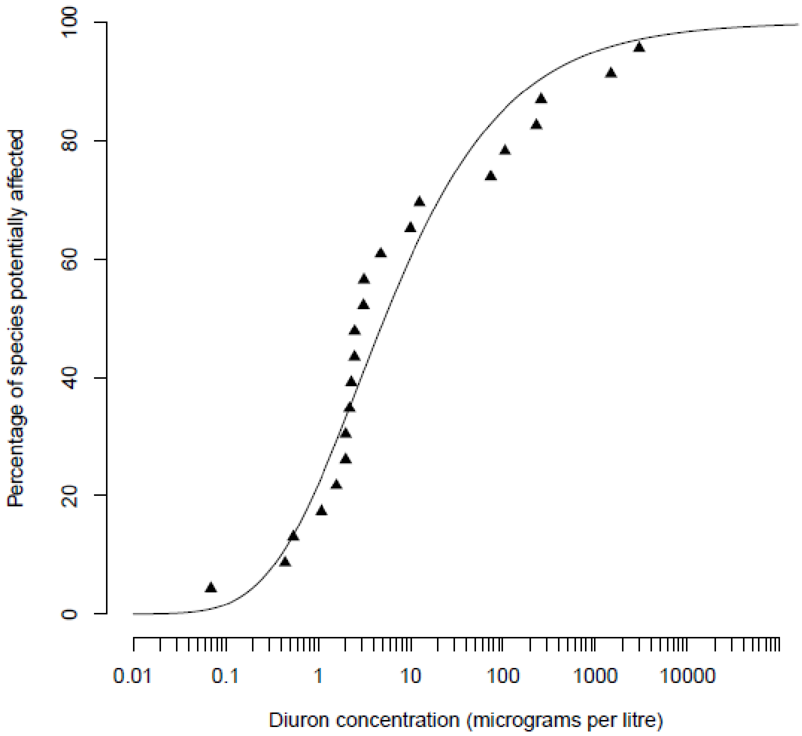

Figure 19. The SSD for the combined fresh and marine toxicity data for diuron

The DGVs generated using the combined diuron toxicity data are presented in Table 42.

Table 42. DGVs for the combined fresh and marine toxicity data for diuron

Protection levels	PC values (µg/L)
PC99	0.075
PC95	0.22
PC90	0.4
PC80	0.88

Fipronil

For fipronil arthropods are more sensitive than other organism types. Therefore, only arthropod species were used in the derivation of the DGVs for fipronil. There was only freshwater chronic NOEC/EC10 type toxicity data (data preference 1) for two species belonging to one phyla, there were no chronic EC/LC50 type data (data preference 2) but there was acute EC/LC50 type toxicity data (data preference 4) for 19 species that belonged to the same phyla as the chronic. For marine waters there was chronic NOEC/EC10 type toxicity data (data preference 1) for two species, chronic EC/LC50 type toxicity data (data preference 2) for one species all belonging one phyla. There are insufficient fresh and marine chronic NOEC/EC10 type data (data preference 1) to derive a SSD. So, the fresh and marine chronic NOEC/EC10 and EC/LC50 type toxicity data (data preference 1 & 2) were combined at first.

However, the number of toxicity data (5 species), the fit of the distribution to the data (poor) and the resulting PC values (extremely low) do not seem appropriate. It was therefore decided to merge the fresh and marine chronic NOEC/EC10, chronic EC/LC50 and acute EC/LC50 type toxicity data (data preference 1, 2 and 4), giving toxicity data for 24 species.

The SSD for the combined fresh and marine fipronil chronic NOEC/EC10, chronic EC/LC50 and acute EC/LC50 type toxicity data is presented in Figure 20. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	-5.10835439606893
Log c	-0.614167078285002
Log k	1.67280637994318

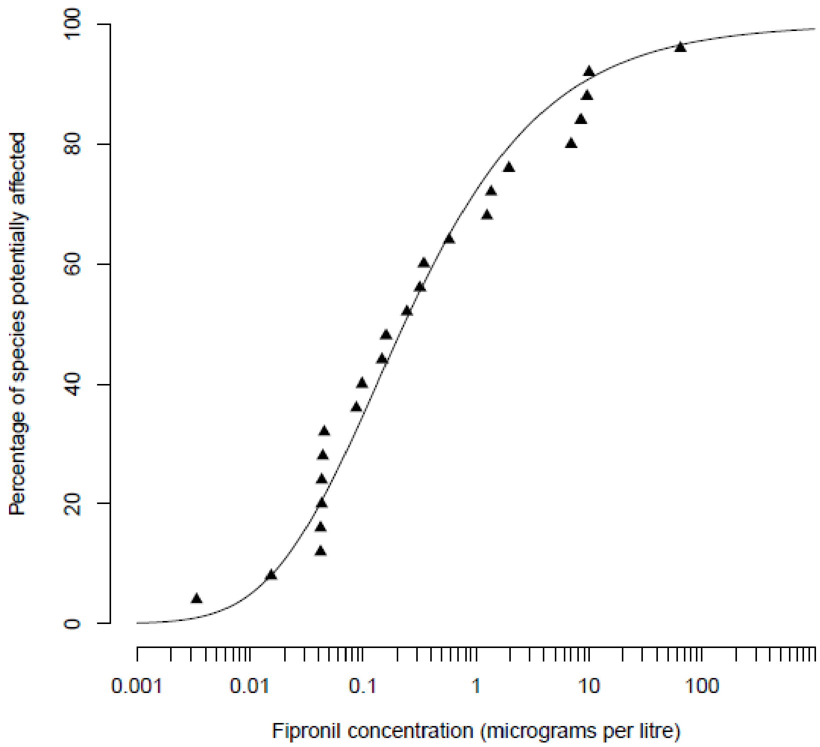

Figure 20. The SSD for the combined fresh and marine toxicity data for fipronil

The DGVs generated using the combined fipronil toxicity data are presented in Table 43.

Table 43. DGVs for the combined fresh and marine toxicity data for fipronil

Protection levels	PC values (µg/L)
PC99	0.0034
PC95	0.01
PC90	0.019
PC80	0.041

Fluroxypyr

Phototrophs were more sensitive to fluroxypyr than heterotrophs. Therefore, as per Warne et al. (2018) only toxicity data for phototroph species were used to derive the DGVs. There were chronic NOEC/EC10 type toxicity data for four freshwater species (data preference score 1) and chronic EC/LC50 type data for one freshwater species (data preference score 2). There was only chronic NOEC/EC10 type toxicity data for one marine species (data preference score 1). By combining the chronic NOEC/EC10 data for fresh and marine species there are sufficient data to derive an SSD for the Pesticide Risk Metric (i.e., there will be chronic NOEC/EC10 data for five species across four phyla). The combined fresh and marine chronic NOEC/EC10 toxicity data were used to derive the SSD and DGVs for the Pesticide Risk Metric.

The SSD for the combined fresh and marine fluroxypyr toxicity data is presented in Figure 21. The distribution that best fitted the combined toxicity data was a log-logistic distribution with the following parameter values

Log-logistic parameters	Parameter values
Log alpha	7.1872863751552
Log beta	0.628023319256836

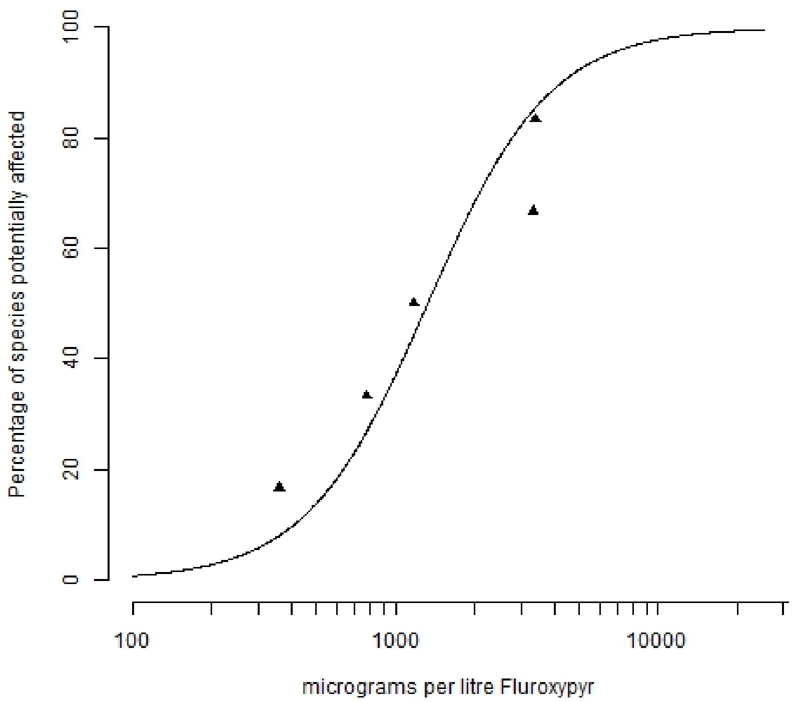

Figure 21. The SSD for the combined fresh and marine toxicity data for fluroxypyr

The DGVs generated using the combined fluroxypyr toxicity data are presented in Table 44.

Table 44. DGVs for the combined fresh and marine toxicity data for fluroxypyr

Protection levels	PC values (µg/L)
PC99	114
PC95	275
PC90	409
PC80	631

Haloxyfop

With the limited amount of toxicity data available for haloxyfop it was not possible to determine if organism types had different sensitivities. Therefore, data for all organism types were used to derive the SSD and DGVs for haloxyfop. There was insufficient toxicity data to derive DGVs separately for fresh and marine species, therefore they were combined to derive the DGVs. There was only chronic toxicity data for one freshwater species (data preference score 1) and acute toxicity data for two freshwater species (data preference score 4). There were only chronic EC/LC50 type data (data preference score 4) for three marine species (data preference score 4). There are insufficient data preference score 1, 2 or 3 data to derive a SSD. Therefore, all the available data (fresh and marine and data preference scores 1 to 4) giving a total of data for six species were combined to derive the SSDs and DGVs for the Pesticide Risk Metric.

The SSD for the combined fresh and marine haloxyfop toxicity data is presented in Figure 22. The distribution that best fitted the combined toxicity data was a log-logistic distribution with the following parameter values.

Log-logistic parameters	Parameter values
Log alpha	9.73667097309866
Log beta	0.313737396887705

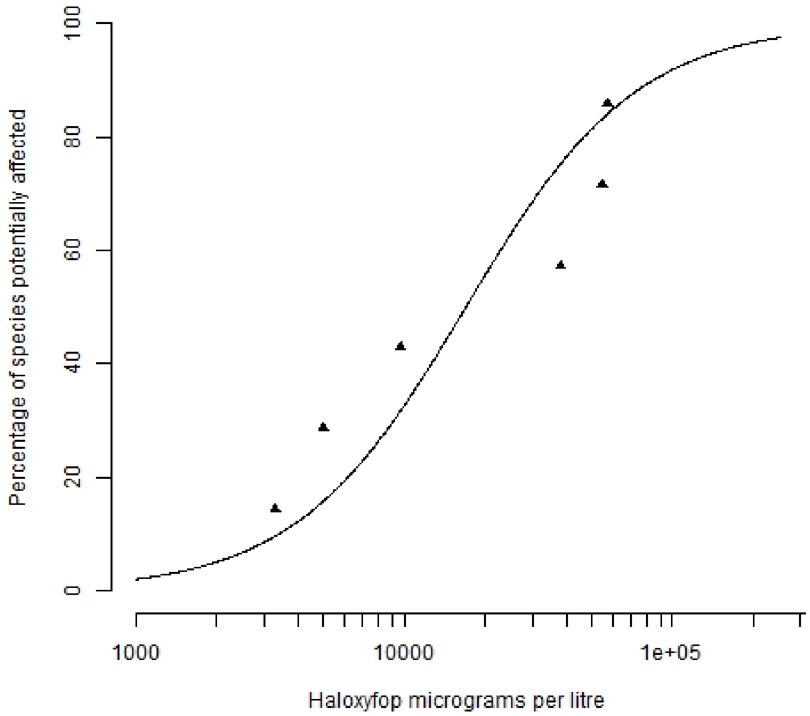

Figure 22. The SSD for the combined fresh and marine toxicity data for haloxyfop

The DGVs generated using the combined haloxyfop toxicity data are presented in Table 45.

Table 45. DGVs for the combined fresh and marine toxicity data for haloxyfop

Protection levels	PC values (µg/L)
PC99	589
PC95	1969
PC90	3399
PC80	6147

Hexazinone

The freshwater phototroph organisms were statistically more sensitive than heterotrophs and therefore only phototrophs were used to derive the fresh and marine DGVs. There were chronic NOEC/EC10 type data (data preference 1) for three freshwater phototroph species that belonged to three phyla and chronic EC/LC50 type data (data preference 2) for two freshwater phototrophs that belonged to another phyla. There were chronic NOEC/EC10 type data (data preference 1) for three marine phototroph species that belonged to one additional phyla. Initially, the three freshwater and three marine phototrophic species with chronic NOEC/EC10 data were combined to calculate the SSD and DGVs for the Pesticide Risk Metric.

However, the selected Burrlioz distribution does not fit the merged chronic NOEC/EC10 type toxicity data very well (it is a poor fit) and four of the six toxicity values are very similar (leading to a stacking of values in the SSD). Therefore, the Fresh chronic NOEC/EC10 (data preference 1) and chronic EC/LC50 type data (data preference 2) were merged with the marine chronic NOEC/EC10 data (data preference 1) – giving toxicity data for eight species. The additional data helps overcome the limitations of the SSD based on six data points. It is therefore recommended that the SSD based on 8 toxicity values is used for the Pesticide Risk Metric.

The SSD for the combined fresh and marine hexazinone toxicity data is presented in Figure 23. The distribution that best fitted the combined toxicity data was an Inverse Weibull distribution with the following parameter values.

Inverse Weibull parameters	Parameter values
Log alpha	0.258963617324046
Log beta	-1.7624184846681

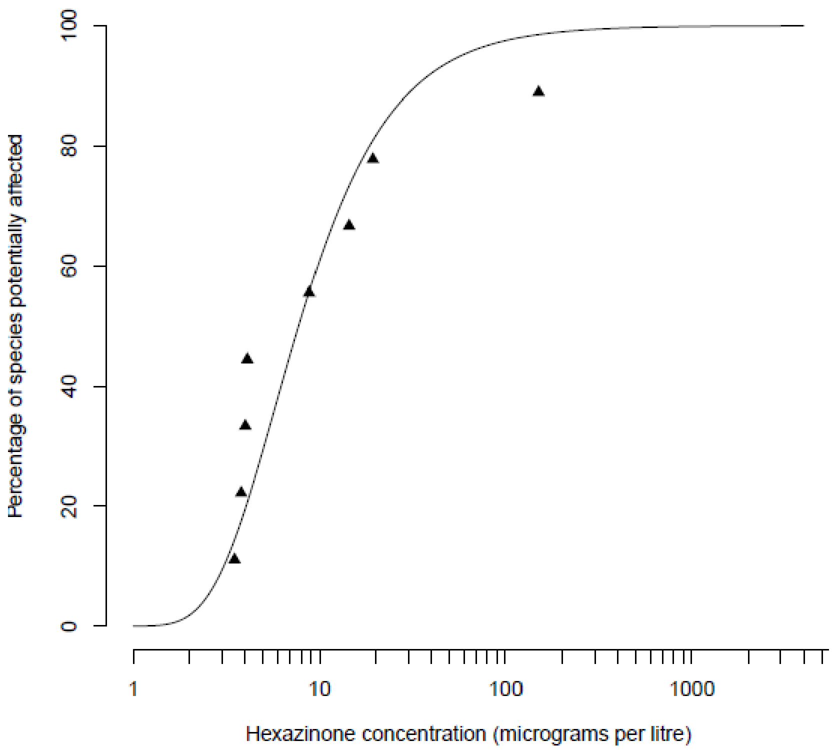

Figure 23. The SSD for the combined fresh and marine toxicity data for hexazinone

The DGVs generated using the combined hexazinone toxicity data are presented in Table 46.

Table 46. DGVs for the combined fresh and marine toxicity data for hexazinone

Protection levels	PC values (µg/L)
PC99	1.8
PC95	2.5
PC90	3.1
PC80	4.0

Imazapic

For imazapic phototrophs are more sensitive than other organism types. Therefore, only phototroph species were used in the derivation of the Fresh and Marine water DGVs. There was only one marine phototroph species that passed the QAQC and screening procedures – it was a chronic EC/LC50 type value (data preference score 2). There were chronic NOEC/EC10 type toxicity data for three freshwater phototrophs (data preference score 1) and chronic EC/LC50 type toxicity data for three freshwater phototrophs (data preference score 2). There are insufficient data with a data preference score of 1 to derive an SSD, but there are sufficient if fresh and marine data with data preference scores of 1 and 2 are combined. Therefore, the same data used to derive the marine water DGVs were used to derive SSD and DGVs of imazapic for the Pesticide Risk Metric (i.e. chronic fresh and marine data), giving a total of data for seven species across four phyla.

The SSD for the combined fresh and marine imazapic toxicity data is presented in Figure 24. The distribution that best fitted the combined toxicity data was a log-logistic distribution with the following parameter values.

Log-logistic parameters	Parameter values
Log alpha	3.11554033199658
Log beta	-0.287699081340512

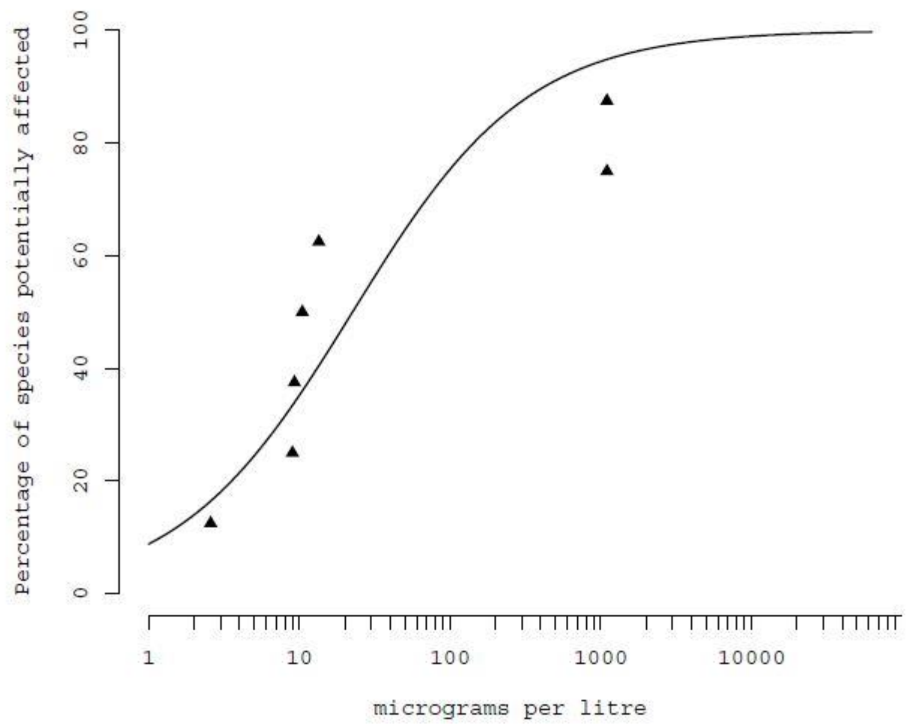

Figure 24. The SSD for the combined fresh and marine toxicity data for imazapic

The DGVs generated using the combined imazapic toxicity data are presented in Table 47.

Table 47. DGVs for the combined fresh and marine toxicity data for imazapic

Protection levels	PC values (µg/L)
PC99	0.049
PC95	0. 44
PC90	1.20
PC80	3.6

Imidacloprid

For imidacloprid, arthropods were more sensitive than other organism types and so only arthropods were used in the derivation of DGVs for both fresh and marine waters. The freshwater toxicity data consisted of chronic NOEC/EC10 type data (data preference score 1) for two arthropod species and acute LC/EC50 type data (data preference score 4) for 18 arthropod species. There were only toxicity for three marine arthropods that made it through the quality assurance and quality control and screening procedures. These were all acute EC/LC50 type data (data preference score 4). There are insufficient type 1, 2 or 3 data to derive an SSD for the Pesticide Risk Metric method. Therefore, all the freshwater and marine toxicity data (irrespective of their data preference score) were combined to calculate the SSD and DGVs for the Pesticide Risk Metric (giving toxicity data for a total of 23 species).

The SSD for the combined fresh and marine data is presented in Figure 25. The distribution that best fitted the combined imidacloprid toxicity data was an Inverse Weibull distribution with the following parameter values.

Inverse Weibull parameters	Parameter values
Log alpha	-0.689526992783055
Log beta	-0.173488965702182

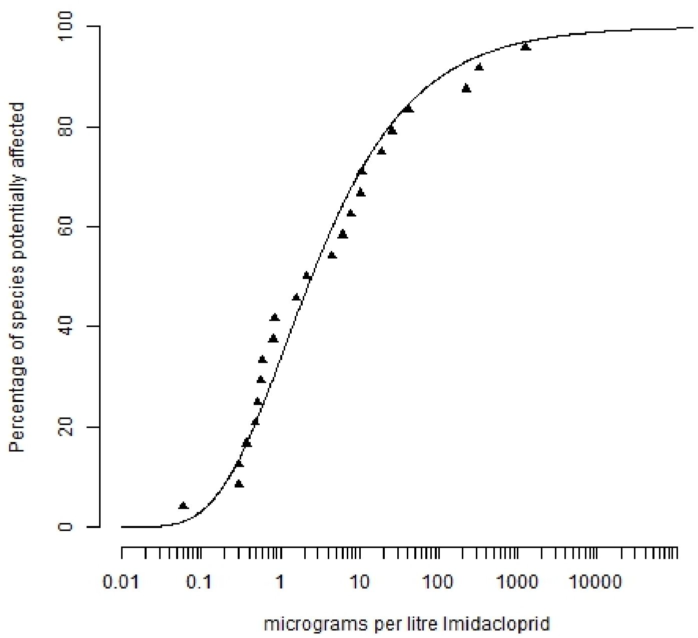

Figure 25. The SSD for the combined fresh and marine toxicity data for imidacloprid

The DGVs generated using the combined imidacloprid toxicity data are presented in Table 48.

Table 48. DGVs for the combined fresh and marine toxicity data for imidacloprid

Protection levels	PC values (µg/L)
PC99	0.057
PC95	0.13
PC90	0.23
PC80	0.46

Isoxaflutole

There was no statistically significant difference in the sensitivity of phototrophs and heterotrophs to Isoxaflutole, therefore organisms from both groups were combined in deriving the DGVs. For freshwater there was chronic NOEC/EC10 type data (data preference score 1) for six species (four phototrophs and two heterotrophs). The marine data consisted of a chronic NOEC/EC10 value (data preference score 1) for one phototroph species and one heterotroph species and acute EC/LC50 type data (data preference score 4) for one heterotroph species. When the freshwater and marine chronic NOEC/EC10 type data were combined there were data for eight species, which is sufficient to derive an SSD. Therefore, the isoxaflutole SSD and DGVs for the Pesticide Risk Metric were calculated using all the combined fresh and marine chronic NOEC/EC10 data.

The SSD for the combined fresh and marine isoxaflutole toxicity data is presented in Figure 26. The distribution that best fitted the combined toxicity data was an Inverse Weibull distribution with the following parameter values

Inverse Weibull parameters	Parameter values
Log alpha	-0.385904590901954
Log beta	-1.2420184611723

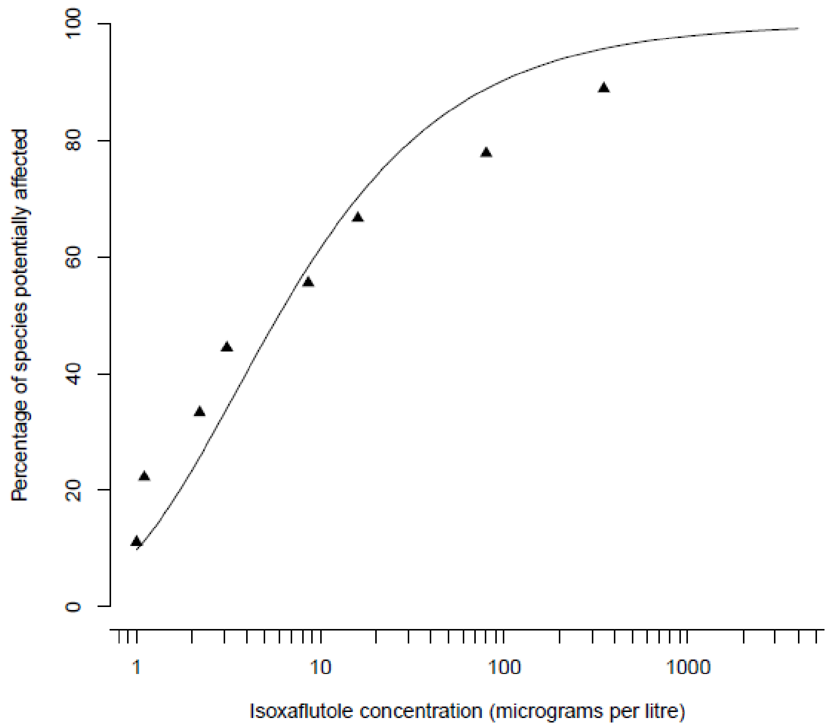

Figure 26. The SSD for the combined fresh and marine toxicity data for isoxaflutole

The DGVs generated using the combined isoxaflutole toxicity data are presented in Table 49.

Table 49. DGVs for the combined fresh and marine toxicity data for isoxaflutole

Protection levels	PC values (µg/L)
PC99	0.37
PC95	0.69
PC90	1.0
PC80	1.7

MCPA

There is no statistically significant difference in the sensitivities of phototroph and heterotroph species to MCPA. Therefore, both phototroph and heterotroph data were combined to derive the SSD and DGV for MCPA. For freshwater there were chronic NOEC/EC10 type data for seven phototrophs and one heterotroph (data preference 1) and chronic EC/LC50 type toxicity data for two phototrophs (data preference 2). The combined freshwater data belonged to five phyla. For marine waters there were chronic NOEC/EC10 type toxicity data for one phototrophic and one heterotrophic species (data preference score 1) that belonged to two phyla. There were no data preference 2 marine data but there was acute EC/LC50 type toxicity data (data preference 4) for three species belonging to two phyla. To derive the SSD and DGVs for the Pesticide Risk Metric the eight freshwater and two marine species with chronic NOEC/EC10 data were combined - giving toxicity data for ten species belonging to six phyla.

The SSD for the combined fresh and marine MCPA toxicity data is presented in Figure 27. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	10.241556415874
Log c	0.219595444359075
Log k	-1.40913347138197

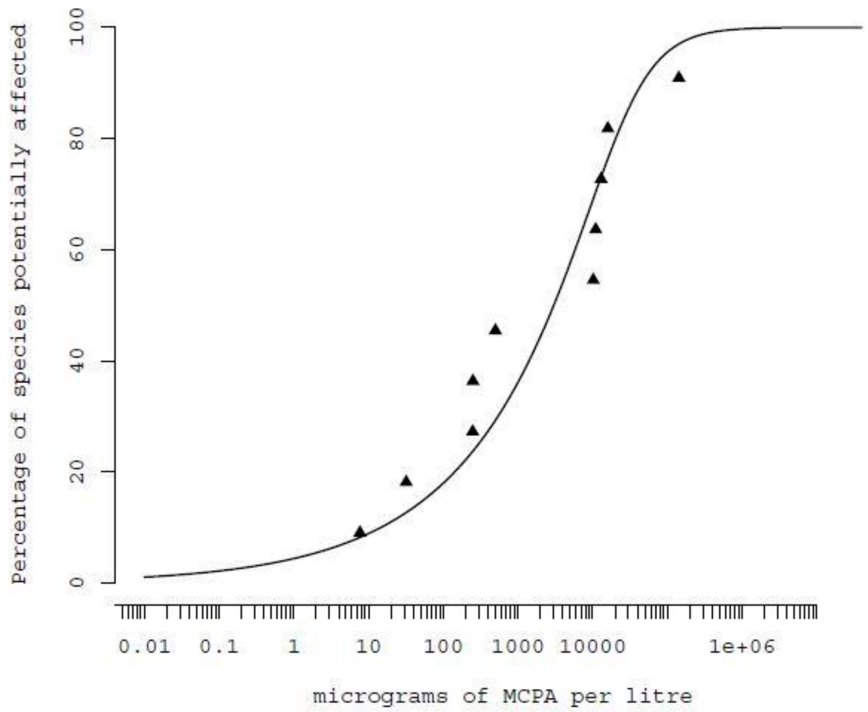

Figure 27. The SSD for the combined fresh and marine toxicity data for MCPA

The DGVs generated using the combined MCPA toxicity data are presented in Table 50.

Table 50. DGVs for the combined fresh and marine toxicity data for MCPA

Protection levels	PC values (µg/L)
PC99	0.0075
PC95	1.5
PC90	15
PC80	142

Metolachlor

There were no statistically significant differences in the sensitivity of different types of organisms to metolachlor. Therefore, the phototroph and heterotroph data were combined to derive the DGVs. There were chronic NOEC/EC10 type toxicity data for 13 freshwater species that belonged to four phyla (data preference 1) and chronic EC/LC50 type data (data preference 2) for eight species. There was only chronic NOEC/EC10 type toxicity data for two marine species (data preference 1) belonging to two additional phyla and chronic EC/LC50 data (data preference 2) for one species. To derive the SSD and DGVs for the Pesticide Risk Metric the 13 freshwater and two marine phototrophic species with chronic NOEC/EC10 data were combined – giving toxicity data for 15 species.

The SSD for the combined fresh and marine metolachlor toxicity data is presented in Figure 28. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	6.38612722873658
Log c	-0.288986308738189
Log k	-0.602319646915259

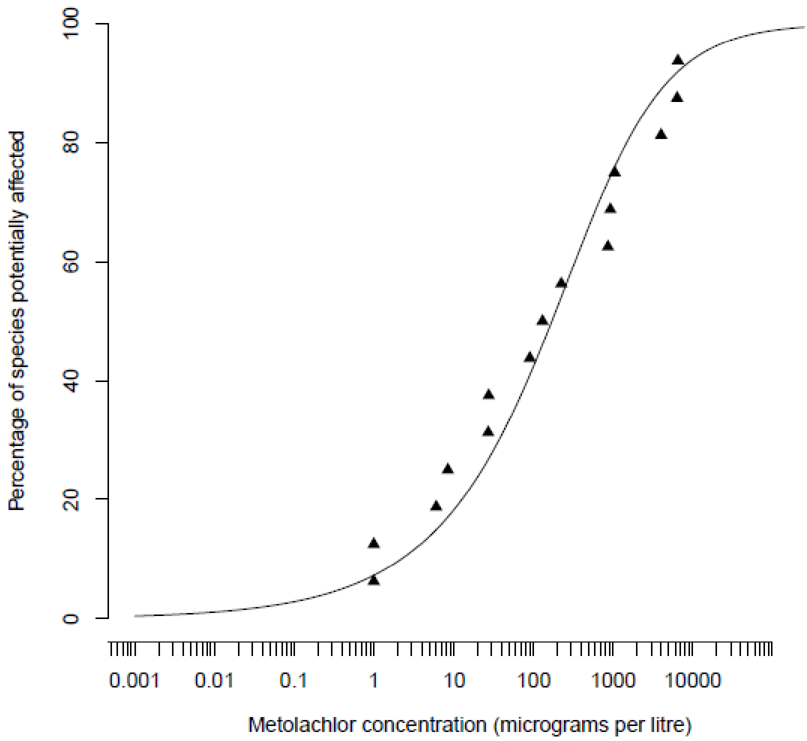

Figure 28. The SSD for the combined fresh and marine toxicity data for metolachlor

The DGVs generated using the combined metolachlor toxicity data are presented in Table 51.

Table 51. DGVs for the combined fresh and marine toxicity data for metolachlor

Protection levels	PC values (µg/L)
PC99	0.0079
PC95	0.4
PC90	2.2
PC80	13

Metribuzin

The phototroph organisms were statistically more sensitive than heterotrophs and therefore only phototrophs were used to derive the fresh and marine DGVs. In freshwater there were chronic NOEC/EC10 type toxicity data (data preference 1) for three species that belonged to three phyla and chronic EC/LC50 type toxicity data for 11 species (data preference 2). In marine waters there was only chronic EC/LC50 type toxicity data (data preference 2) for one species. There are not sufficient preference 1 data to derive an SSD. Therefore, the fresh and marine preference 1 and 2 toxicity data were combined – giving toxicity data for 15 species.

The SSD for the combined fresh and marine metribuzin toxicity data is presented in Figure 29. The distribution that best fitted the combined toxicity data was an Inverse Weibull distribution with the following parameter values

Inverse Weibull parameter	Parameter values
Log alpha	0.536922682471891
Log beta	-1.58798805319953

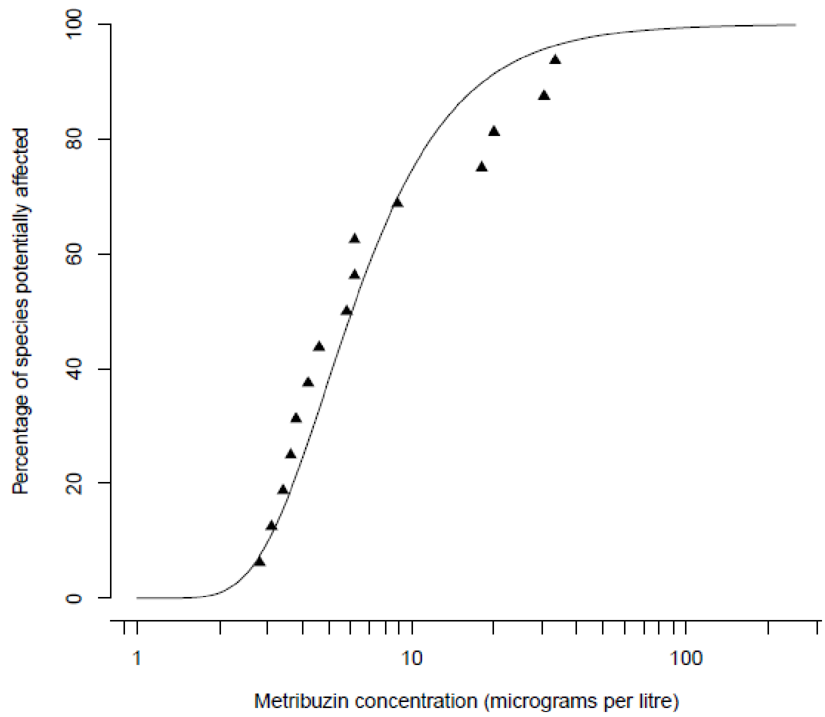

Figure 29. The SSD for the combined fresh and marine toxicity data for metribuzin

The DGVs generated using the combined metribuzin toxicity data are presented in Table 52.

Table 52. DGVs for the combined fresh and marine toxicity data for metribuzin

Protection levels	PC values (µg/L)
PC99	2.0
PC95	2.6
PC90	3.0
PC80	3.7

Metsulfuron-methyl

As there was toxicity data for only one heterotroph species it was not possible to determine if there were differences in the sensitivity. Due to this and the general paucity of toxicity data the phototroph and heterotroph data were combined. There were chronic NOEC/EC10 type toxicity data for eight freshwater species that belonged to five phyla, thus meeting the minimum data requirements to use an SSD method. There was only chronic NOEC/EC10 type toxicity data for one marine species – an alga belonging to another phyla. To derive the SSD and DGVs for the Pesticide Risk Metric the eight freshwater and one marine phototrophic species with chronic NOEC/EC10 data were combined.

The SSD for the combined fresh and marine metsulfuron-methyl toxicity data is presented in Figure 30. The distribution that best fitted the combined toxicity data was an Inverse Weibull distribution with the following parameter values

Inverse Weibull	Parameter values
Log alpha	-1.35029655831646
Log beta	-0.824160390204924

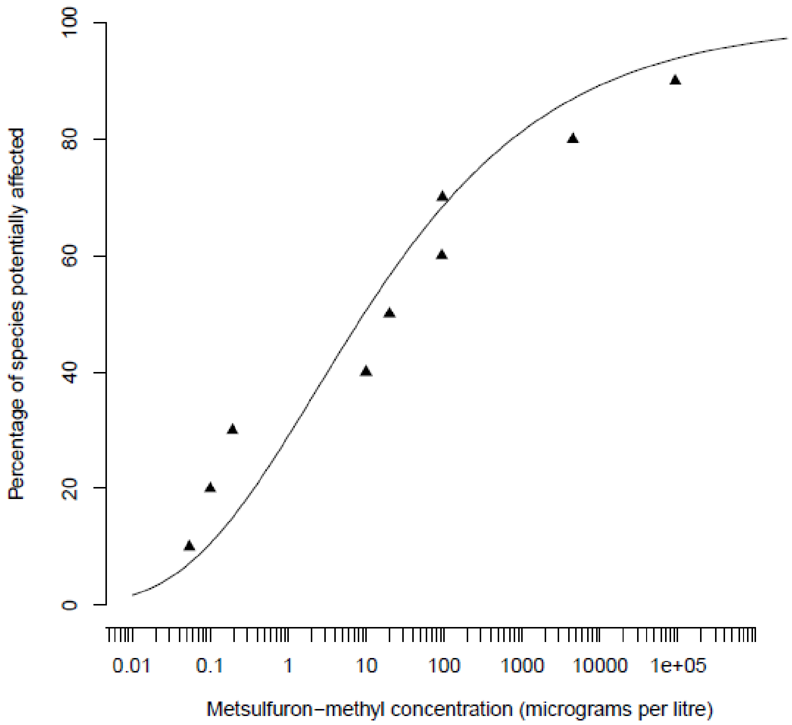

Figure 30. The SSD for the combined fresh and marine toxicity data for metsulfuron-methyl

The DGVs generated using the combined metsulfuron-methyl toxicity data are presented in Table 53.

Table 53. DGVs for the combined fresh and marine toxicity data for metsulfuron-methyl

Protection levels	PC values (µg/L)
PC99	0.0063
PC95	0.033
PC90	0.091
PC80	0.36

Pendimethalin

There was no significant difference in the sensitivity of different organism types to pendimethalin and therefore the toxicity data for all organisms were combined to derive the DGVs for pendimethalin. There were chronic NOEC/EC10 type data for nine freshwater species (data preference score 1) and chronic EC/LC50 type data for one freshwater species (data preference score 2) and acute EC/LC50 type data for four freshwater species (data preference score 4). There was chronic NOEC/EC10 type data for only one marine species (data preference score 1) and acute EC/LC50 type toxicity data for three marine species (data preference score 4). By combining the chronic NOEC/EC10 toxicity data (data preference score 1) for fresh and marine species there was toxicity data for 10 species, which is sufficient to derive a SSD using Burrlioz. The pendimethalin SSD and DGVs for the Pesticide Risk Metric were calculated using the combined fresh and marine chronic NOEC/EC10 type data (data preference score 1).

The SSD for the combined fresh and marine pendimethalin toxicity data is presented in Figure 31. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	1.1818561536147
Log c	-0.334798725966998
Log k	0.432002075945117

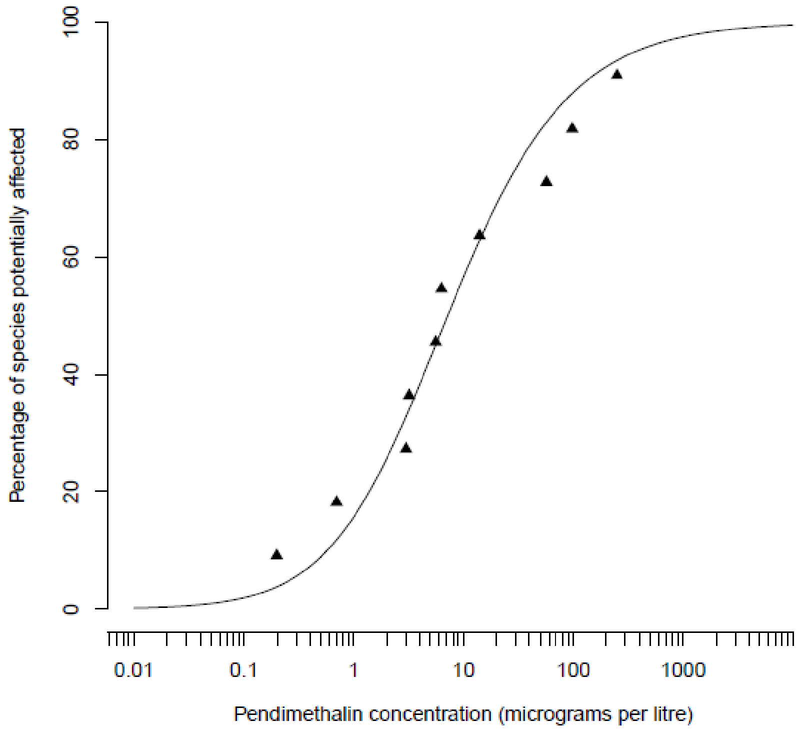

Figure 31. The SSD for the combined fresh and marine toxicity data for pendimethalin

The DGVs generated using the combined pendimethalin toxicity data are presented in Table 54.

Table 54. DGVs for the combined fresh and marine toxicity data for pendimethalin

Protection levels	PC values (μg/L)
PC99	0.054
PC95	0.27
PC90	0.58
PC80	1.4

Prometryn

The phototroph organisms were statistically more sensitive than heterotrophs and therefore only phototrophs were used to derive the fresh and marine DGVs. In freshwater there were chronic NOEC/EC10 type toxicity data (data preference 1) for seven species that belonged to six phyla – which met the minimum data requirements to derive the DGVs. In marine waters there was chronic NOEC/EC10 data for one species. To derive the SSD and DGVs for the Pesticide Risk Metric the seven freshwater and one marine phototrophic species with chronic NOEC/EC10 data were combined.

The SSD for the combined fresh and marine prometryn toxicity data is presented in Figure 32. The distribution that best fitted the combined toxicity data was a Burr type III distribution with the following parameter values

Burr Type III distribution parameters	Parameter values
Log b	1.94505495309791
Log c	0.00404321927269326
Log k	0.0463996587582432

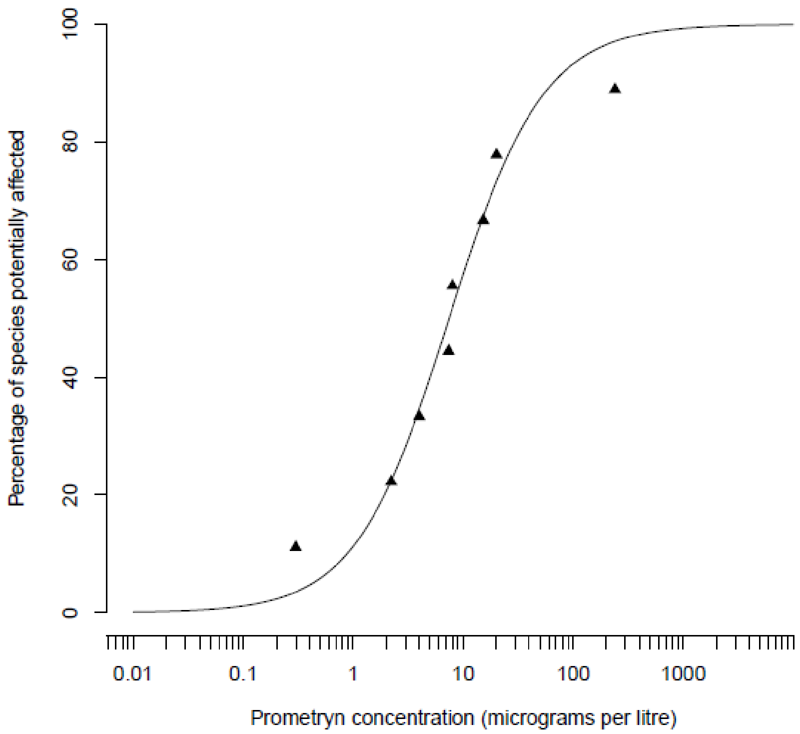

Figure 32. The SSD for the combined fresh and marine toxicity data for prometryn

The DGVs generated using the combined prometryn toxicity data are presented in Table 55.

Table 55. DGVs for the combined fresh and marine toxicity data for prometryn

Protection levels	PC values (µg/L)
PC99	0.089
PC95	0.43
PC90	0.88
PC80	1.9

Simazine

The phototroph organisms were statistically more sensitive then heterotrophs and therefore only phototrophs were used to derive the fresh and marine DGVs. For freshwater there were chronic NOEC/EC10 type data for five species that belonged to two phyla (data preference score 1) and there were chronic EC/LC50 type data for eight species that belonged to another two phyla (data preference score 2). For marine waters there were chronic NOEC/EC10 type data (data preference score 1) for three species that belonged to one phyla and there were chronic EC/LC50 type data (data preference score 2) for three species that belonged to another two phyla. To derive the SSD and DGVs for the Pesticide Risk Metric the five freshwater and three marine phototrophic species with chronic NOEC/EC10 data were combined. This was acceptable as even though only toxicity data for 8 species belonging to three phyla were used to derive the merged SSD the total dataset meets the minimum data requirements (Warne et al., 2018).

The SSD for the combined fresh and marine simazine toxicity data is presented in Figure 33. The distribution that best fitted the combined toxicity data was a Burr Type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	4.66005734676985
Log c	0.716940996280568
Log k	0.186694504803034

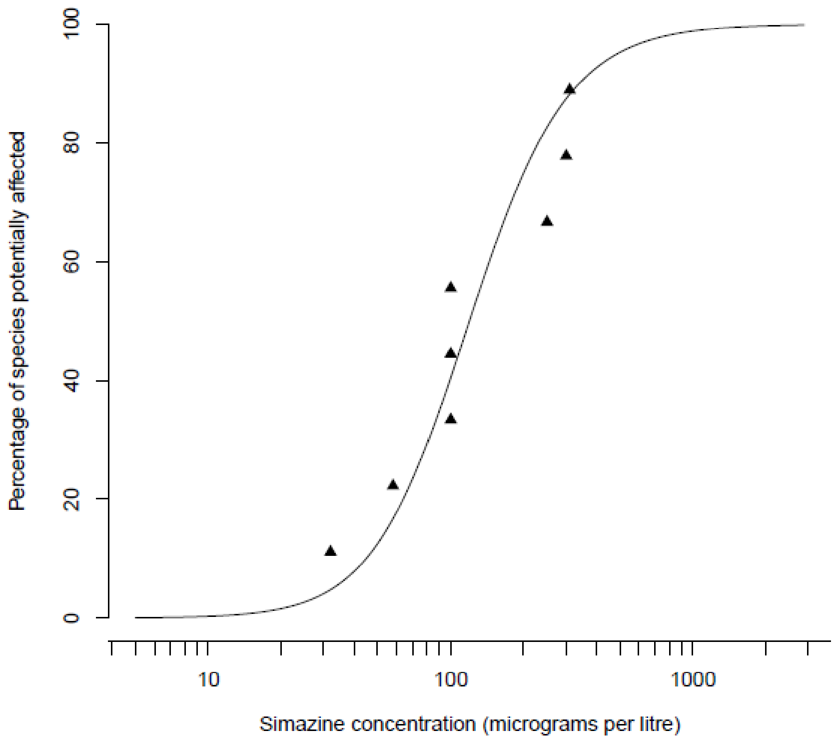

Figure 33. The SSD for the combined fresh and marine chronic toxicity data for simazine

The DGVs generated using the combined simazine toxicity data are presented in Table 56.

Table 56. DGVs for the combined fresh and marine toxicity data for simazine

Protection levels	PC values (µg/L)	Lower 95% CI (µg/L)	Upper 95% CI (µg/L)
PC99	17	5.2	71
PC95	33	22	87
PC90	45	29	99
PC80	64	38	138

Tebuthiuron

The phototroph organisms were statistically more sensitive then heterotrophs and therefore only phototrophs were used to derive the fresh and marine DGVs. In freshwater there were chronic NOEC/EC10 type toxicity data (data preference 1) for five species that belonged to four phyla which met the minimum data requirements. In marine waters there were chronic NOEC/EC10 type toxicity data (data preference 1) for only two species that belonged to two phyla. To derive the SSD and DGVs for the Pesticide Risk Metric the chronic NOEC/EC10 toxicity data for the five freshwater and two marine species were combined (giving a total of seven species that belonged to four phyla).

The SSD for the combined fresh and marine tebuthiuron toxicity data is presented in Figure 34. The distribution that best fitted the combined toxicity data was a log-logistic distribution with the following parameter values

Log-logistic parameters	Parameter values
Log alpha	3.97321874461353
Log beta	0.642836233761554

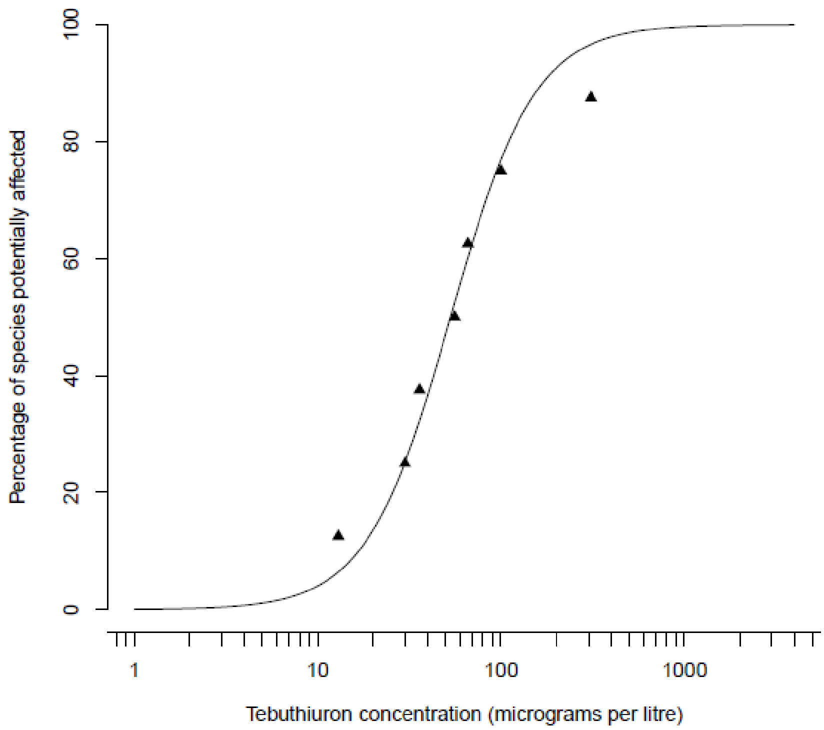

Figure 34. The SSD for the combined fresh and marine toxicity data for tebuthiuron

The DGVs generated using the combined tebuthiuron toxicity data are presented in Table 57.

Table 57. DGVs for the combined fresh and marine toxicity data for tebuthiuron

Protection levels	PC values (µg/L)
PC99	4.7
PC95	11
PC90	17
PC80	26

Terbuthylazine

The phototroph organisms were statistically more sensitive than heterotrophs and therefore only phototrophs were used to derive the fresh and marine DGVs. In freshwater there were chronic NOEC/EC10 type toxicity data (data preference 1) for 16 phototroph species that belonged to four phyla (data preference 1) and there was chronic EC/LC50 type toxicity data (data preference 2) for two species. In marine waters there was only chronic toxicity data for one phototroph species and this only had LOEC and EC50 type data (data preference 2). To derive the SSD and DGVs for the Pesticide Risk Metric the preference 1 and 2 toxicity data for the 18 freshwater and one marine species were combined. The data preference 2 data were included in the analysis because if the SSD was derived using only data preference 1 data no marine data would be included. Including the marine data preference 2 data meant that the equivalent freshwater data were included.

The SSD for the combined fresh and marine terbuthylazine toxicity data is presented in Figure 35. The distribution that best fitted the combined toxicity data was a Burr Type III distribution with the following parameter values

Burr type III parameters	Parameter values
Log b	1.57937126902431
Log c	-0.0821977271377176
Log k	0.732467232263255

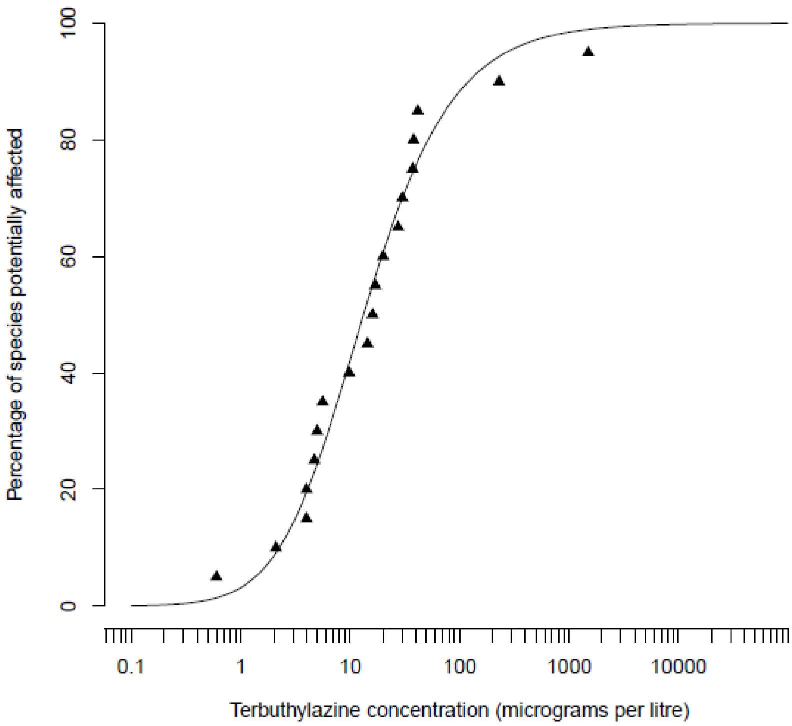

Figure 35. The SSD for the combined fresh and marine toxicity data for terbuthylazine

The DGVs generated using the combined terbuthylazine toxicity data are presented in Table 58.

Table 58. DGVs for the combined fresh and marine toxicity data for terbuthylazine

Protection levels	PC values (µg/L)
PC99	0.5
PC95	1.4
PC90	2.3
PC80	4.1

Triclopyr

The phototroph organisms were not statistically more sensitive then heterotrophs and therefore both phototrophs and heterotrophs were used to derive the fresh and marine DGVs. For freshwater there were chronic NOEC/EC10 type data (data preference 1) for five species that belonged to four phyla and acute EC/LC50 type data (data preference 4) for nine species that belonged to three phyla. For marine waters there were no chronic data and only acute EC/LC50 type toxicity data (data preference 4) for four species that belonged to four phyla. To derive the SSD and DGVs for the Pesticide Risk Metric the data preference 1 and 4 toxicity data for freshwater and marine species were combined – giving toxicity data for a total of 18 species.

The SSD for the combined fresh and marine triclopyr toxicity data is presented in Figure 36. The distribution that best fitted the combined toxicity data was a Burr Type III distribution with the following parameter values.

Burr Type III parameters	Parameter values
Log b	6.96269053743092
Log c	1.70006673073423
Log k	-2.31267762621264

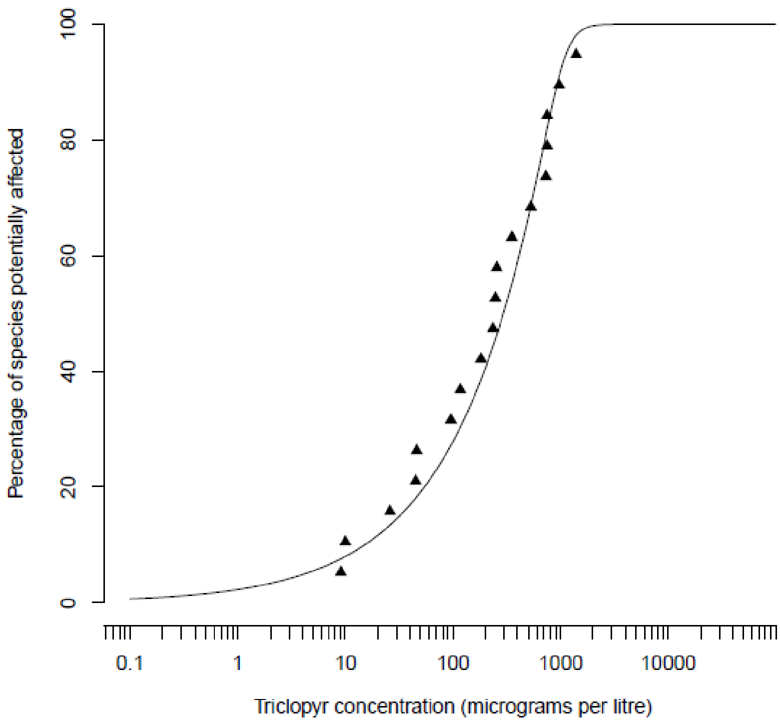

Figure 36. The SSD for the combined fresh and marine chronic toxicity data for triclopyr

The DGVs generated using the combined triclopyr toxicity data are presented in Table 59.

Table 59. DGVs for the combined fresh and marine toxicity data for triclopyr

Protection levels	PC values (µg/L)	Lower 95% CI (µg/L)	Upper 95% CI (µg/L)
PC99	0.22	0.057	21
PC95	4.2	1.8	47
PC90	15	7.8	81
PC80	54	18	162

Attachment E – Justification of Using the Species Sensitivity Distribution Based Method of Estimating the Toxicity of Pesticide Mixtures

There are two methods that can be used to estimate the toxicity of mixtures of chemicals —the Toxic Unit (TU) method and the Species Sensitivity Distribution (SSD) method. The TU method is the older of the two methods (Sprague and Ramsay, 1965) and was developed to estimate the toxicity of mixtures of chemicals to a single species using the below equation.

$$\Sigma TU = (C_1/TC_1) + (C_2/TC_2) + \ldots\ldots. (C_n/TC_n) \qquad \text{(Eqn 4)}$$

where the subscript indicates the chemical, C is the measured or predicted concentration of the chemical and TC is the toxic concentration of the chemical. When the ΣTU is less than one toxic effects to the species should not occur. Conversely, when the ΣTU is equal to or greater than one then toxic effects are expected to occur.

This method was subsequently modified to estimate the toxicity of mixtures of chemicals to multiple species. This was done by replacing the toxicity value for a single species (TC) by the water quality guideline (or equivalent) of the chemical.

$$\Sigma TU = (C_1/WQG_1) + (C_2/WQG_2) + \ldots\ldots. (C_n/WQG_n) \qquad \text{(Eqn 5)}$$

where the subscript indicates the chemical, C is the measured or predicted concentration of the chemical and WQG is the water quality guideline (or equivalent) for the chemical (that is designed to protect multiple species from harmful effects of the chemical). When the ΣTU is less than one sub-lethal toxic effects to more than five per cent of species should not occur[25]. Conversely, when the ΣTU is equal to or greater than one then sub-lethal toxic effects are expected to occur to more than five per cent of species[11].

Both versions of the TU method use the concentration addition model of joint action (as defined by Bliss (1939) and Plackett and Hewlett (1952)) to estimate the toxicity of mixtures.

The SSD method of estimating the toxicity of mixtures is calculated differently depending on the model of joint action (see next section) that is used. Irrespective of this, the SSD method converts pesticide concentration values to a percentage of species affected or protected (further details of the method can be found in the methods section).

There are two key limitations of the TU method. These are that the results are not expressed in units that are consistent with the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018) nor with the pesticide target of the Reef WQIP (Australian Government and Queensland Government, 2018) (i.e., not as a percentage of species affected by the pesticide). The second limitation is that the difference in ΣTU values relates to differences in concentration and not to differences in risk. Thus, a mixture 'p' with a ΣTU value of five has a total pesticide concentration five-times larger than a mixture 'q' with a TU of one. However, this does not mean the risk of mixture 'p' is five times greater than mixture 'q'—the risk is greater but the increase cannot be quantified. The key advantage to the TU method is the ease of calculation and comprehension.

[25] The level of impact that corresponds to the ΣTU values depends on level of protection being provided by the WQG. In Australia and New Zealand the default WQGs for slightly to moderately disturbed ecosystems is 95% of species.

The key advantage of the SSD method is that it expresses the result as an estimate of the percentage of species affected (or conversely protected) by the mixture, which is consistent with the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG, 2018) and the pesticide target of the Reef WQIP (Australian Government and Queensland Government, 2018). Differences in the results of the SSD method directly indicate the change in risk between two or more mixtures. Thus, this method overcomes the two main limitations of the TU method. The main limitation of the SSD method is that its calculation is more complex than the TU method and therefore it is not as easy to understand.

Given the above strengths and limitations, it was decided to use the SSD method to estimate the toxicity of pesticide mixtures. However, the model of joint toxicity to be used within the SSD method still needs to be determined. The justification for the chosen model is provided in Attachment F.

Attachment F – Justification of Using the Independent Action Model of Joint Action

The SSD method is a means of combining the SSDs of chemicals that permits the estimation of the risk posed by mixtures of chemicals and expresses this in terms of fraction of species that are likely to experience adverse (harmful) effects. This method was developed by Traas et al. (2002). According to the models of joint action developed by Bliss (1939) and Plackett and Hewlett (1952), the toxicity of mixtures of chemicals that have the same mode of action and do not interact with each other toxicologically (that is they do not modify the adsorption, absorption, transport, bioavailability or toxicity of each other) should conform to the concentration addition (CA) model. While chemicals with different modes of action that do not interact should conform to the independent action (IA) model of joint action.

Chemicals that exert their toxicity by the same mode of action should theoretically have SSDs that are parallel (i.e. their gradients are the same) (Traas et al., 2002). Therefore, by normalising the toxicity data for pesticides with the same mode of action it is possible to merge the SSDs for individual pesticides (henceforth termed "individual SSDs") into a single SSD (henceforth called "mixture SSDs") that can explain the toxicity of each chemical well. The normalisation process consists of dividing all the data in an individual SSD by the median toxicity value of that dataset. This converts all the toxicity data into Toxic Units (TUs), which are unitless, with the median toxicity value for each pesticide having a value of 1 TU. Thus, a plot of the individual SSDs of normalised toxicity data for chemicals with the same mode of action should all be aligned at the 50% effect level at TU = 1 on the x axis.

The 22 pesticides included in the Pesticide Risk Metric have a number of different modes of action (the means by which a toxicant exerts its toxicity at a sub-cellular level) (Table 60). The modes of action of these pesticides were determined using the classification schemes of HRAC (2010) and IRAC (2016).

Therefore, the individual SSDs of pesticides that the literature stated as having the same mode of action (Table 60) were tested to determine if they could be combined into one mixture SSD. For modes of action that included only one pesticide (Table 60) mixture SSDs were not derived, rather individual SSDs were used. There were only three modes of action that contained more than one pesticide – PSII Herbicides (nine pesticides), synthetic auxins (four pesticides) and ALS inhibitors (two pesticides) (Table 60)

Table 60. The mode of action of the 22 pesticides included in the Pesticide Risk Metric

Name of pesticide	Type	Mode of Action
Chlorpyrifos	Insecticide	AchE inhibitor[1]
Fipronil	Insecticide	GABA gated chloride channel inhibitor[2]
Haloxyfop	Herbicide	ACCase inhibitor[3]
Imazapic	Herbicide	ALS inhibitor[4]
Metsulfuron-methyl	Herbicide	
Pendimethalin	Herbicide	Microtubule synthesis inhibitor
S-metolachlor (also metolachlor)	Herbicide	VLCFA inhibitor[5]
Imidacloprid	Insecticide	Nicotinic receptor agonist

Name of pesticide	Type	Mode of Action
Ametryn	Herbicide	PSII inhibitor
Atrazine	Herbicide	
Prometryn	Herbicide	
Terbuthylazine	Herbicide	
Tebuthiuron	Herbicide	
Simazine	Herbicide	
Diuron	Herbicide	
Hexazinone	Herbicide	
Metribuzin	Herbicide	
2,4-D	Herbicide	Synthetic auxin
MCPA	Herbicide	
Fluroxypyr	Herbicide	
Triclopyr	Herbicide	
Isoxaflutole and DKN	Herbicide	4-HPPD inhibitor[6]

[1] AchE = acetylcholinesterase. [2] GABA = gamma-Aminobutyric acid. [3] ACCase = Acetyl-CoA carboxylase. [4] ALS = acetolactate synthase. [5] 4-HPPD = 4-hydroxyphenyl-pyruvate-dioxygenase. [6] VLCFA = very long chain fatty acid.

A mixture SSD was initially developed for each of these three modes of action and their appropriateness were tested in four ways. Firstly, the gradients (β) of the chemical SSDs were visually and statistically compared. An individual SSD with a different gradient to the others with the same mode of action was interpreted as breaking the assumption of parallel SSDs. Secondly, the goodness of fit of the mixture SSD was tested with, and without the chemicals with different gradients. The fit of the mixture SSD to the data for each pesticide was determined using the coefficient of determination (R^2) for each pesticide and the adjusted R^2 was used to measure the fit of the mixture SSD to the group of pesticides as a whole. If the R^2 and adjusted R^2 values were below 0.9 it was interpreted as indicating the mixture SSD was potentially not suitable for a particular pesticide or for all the grouped pesticides, respectively. The third method of determining how pesticides should be grouped was by conducting F-tests to determine whether the SSDs for each combination of pesticide belonging to a mode of action were parallel or not.

The F-test compares the residual sum of squares for the curve fit for two chemicals with no shared parameters (individual SSDs) with the residual sum of squares of a mixture curve fit (both chemicals) with shared parameters (mixture SSD), such that:

$$F = \frac{\text{SSmixture} - \text{SSchemical}}{\text{DFmixture} - \text{DFchemical}} \Big/ \frac{\text{SSchemical}}{\text{DFchemical}} \qquad \text{(Eqn 6)}$$

where SS is the sum of squares, DF is the degrees of freedom, chemical refers to the SSDs of the two pesticides being compared and mixture refers to the combined SSD for the pair of pesticides being compared.

Finally, the ability of any mixture SSD to accurately predict the PC95 and PC99 value for each pesticide included in the mixture SSD was determined. This was done using the individual SSD for each pesticide (Attachment D) included in the mixture SSD to determine the concentrations that correspond to that pesticides PC95 and PC99 values. These concentrations for each pesticide where then substituted into the mixture SSD to determine the percentage of species that would be protected. Ideally, this should result in values close to protecting 95% and 99% of species, respectively. This test was done because using a mixture SSD that does not model the individual pesticides well could lead to substantial over- or under-estimation of the toxicity of a pesticide in a mixture compared to the existing (ANZECC and ARMCANZ, 2000) or proposed Australian and New Zealand default guideline values for the same pesticide (ANZG, 2018; King et al., 2017a, b).

If the mixture SSD for a mode of action did not meet the above criteria (i.e., gradient, goodness of fit, parallelism, PC95/99 accuracy) then the next step was to group the pesticides that belonged to that mode of action according to their chemical structure. For example, within the PSII mode of action there are herbicides that belonged to four different chemical structures – triazines, triazinones, uracils and ureas.

The above analyses indicated that there were very few instances when mixture SSDs were appropriate, and when they were appropriate each mixture SSD only applied to a very limited number of pesticides (typically two). In addition, the mixture SSDs did not accurately predict the PC95 and PC99 values for individual pesticides i.e., at the bottom end of the SSD curve. Thus, it was decided to not use the Concentration Addition model or the two-step model[26].

Another reason for not using the Concentration Addition model of joint action was that it requires each chemical to be allocated to a single mode of action. While this may appear straight forward, it is in fact problematic when considering multiple species (Spilsbury et al., 2020) as is necessary in this report. This is because all pesticides have both specific and non-specific modes of action and the dominant mode of action of a pesticide can vary in different types of organisms. For example, atrazine, diuron and simazine would be classed as having a Photosystem II inhibitor mode of action in plants, but in amphibians they would be classed as having an endocrine disruptor mode of action.

Even if the above problem could be overcome there is typically not a large difference between the values generated by the Concentration Addition and Independent Action models (Backhaus et al., 2000a; Faust et al., 2000, 2003; Dyer et al., 2010) and they are usually not statistically different (Dyer et al., 2010). Additionally, Drescher and Bödeker (1995) showed theoretically that the predicted toxicity of mixtures estimated using the Concentration Addition model, Independent Action model or the Concentration Addition model followed by the Independent Action model (i.e. the two-step method – see footnote 22) were similar regardless of whether the chemicals in the mixtures conformed to the models. The difference in toxicity values estimated using the Concentration Addition and Independent Action models was assessed by Spilsbury et al. (2020) for over 3700 samples collected between 2011/2012 to 2015/2016 from

[26] The two-step method was developed by Hamers et al. (1996), Junghans (2004), Altenberger et al. (2004); De Zwart and Posthuma (2005). It was developed to estimate the toxicity of mixtures of chemicals where some chemicals had the same mode of action but others had different modes of action. The first step in the two-step method uses the Concentration Addition model of joint action (Bliss, 1939; Plackett and Hewlett, 1952; Könemann, 1981) to estimate the toxicity of chemicals that have the same mode of action. The second step uses the Independent Action model of joint action to estimate the toxicity of mixtures of chemicals that have different modes of action (Bliss, 1939; Plackett and Hewlett, 1952; Könemann, 1981). However, analyses of the SSDs derived in this project indicated that use of the Concentration Addition model of joint action would be problematic and therefore the two-step method was not used.

waterways that discharge to the GBR (a similar dataset to that used in this report to estimate the Pesticide Risk Baseline). They found that on average the Concentration Addition model estimates of mixture toxicity were 10% larger than those generated by the Independent Action model (Spilsbury et al., 2020). From this they concluded it was not necessary to use the two-step method as that method must result in estimates of pesticide mixture toxicity that lie between those estimated using the Concentration Addition and using the Independent Action models which on average only differ by 10%. They chose to use the Concentration Addition model to estimate pesticide mixture toxicity because of the small differences between the CA and IA estimates of risk, to align with OECD recommendations (OECD, 2018) and to facilitate comparison with earlier studies (Gustavsson, 2017, Gustavsson et al., 2017; Kandie et al., 2020; Markert et al., 2020).

Because the Concentration Addition model of joint action was determined to be inappropriate for the SSDs for the 22 selected pesticides in the Pesticide Risk Metric, it was decided to assume all the pesticides had different modes of action and to estimate their combined toxicity using the Independent Action model of joint action. The use of the Independent Action model of joint action will, on average, lead to slightly lower estimates (≈ 10%) of the risk that pesticides pose compared to the Concentration Addition model.

Attachment G – Justification for using the average PAF and 182-day period in calculating the pesticide risk metric

The pesticide risk metric is based on estimating the average potentially affected fraction (PAF) of species affected by mixtures of pesticides, estimated over a standardised 182 day period. Here we outline the justification for using the average PAF and 182 day period.

Justification for Using the Average PAF

Pesticide monitoring in GBR catchments demonstrate that pulse exposure of pesticides occur during the wet season where pesticide concentrations and pulse duration vary between pulses, catchments and years (Smith et al., 2012; Devlin et al., 2015; Davis et al., 2017). Pesticide runoff in GBR catchments is highly dependent on the hydrological regime of the catchment, where the summer wet season coincides with the end of the spring application period and pesticide residues mobilise with surface water runoff. Event duration (and time between events) varies depending on the time of year, the catchment's climate and inter-annual climate variability – smaller and more frequent events can often be seen in smaller coastal catchments, at the start of the wet season and during drier years, whereas longer and larger events can occur later in the wet season corresponding with monsoonal rains, in larger inland catchments and in wetter years (Devlin et al., 2015, Davis et al., 2017).

Concentrations of pesticides detected in GBR catchments generally follow an exponential decay pattern with concentrations highest at the start of the wet season, decreasing with increasing volume of flow, i.e. with each subsequent event (Smith et al., 2011). Additional peaks in concentration are common after the 'first flush', and generally coincide with second and third events early in the wet season (Devlin et al., 2015 and references therein). Larger catchments, such as the Fitzroy River, show different concentration dynamics, often with lower less variable concentrations over longer periods of consistent exposure (Smith et al., 2012; Devlin et al., 2015). Wetter than average years often have higher pesticide loads but lower pesticide concentrations than drier years (Devlin et al., 2015), likely an effect of the increased runoff occurring earlier in the wet season mobilising more pesticide residues but also increasing the level of dilution – size and number of events and overall longer wet season periods. Pulsed pesticide exposure occurs quite commonly in river runoff and, therefore, how to account for it in risk assessments has been the focus of a number of studies (Reinert et al., 2002; Vallotton et al., 2008; Boxall et al., 2013; Copin et al., 2015).

As toxicity of a pesticide is both concentration and time dependent, ecosystems exposed to these different exposure regimes will face different toxicity risks. For example, in some instances a long pulse of low pesticide concentration causes greater phototrophic growth inhibition than short pulses of high concentration (Vallotton et al., 2008; Copin et al., 2015 and references therein). Additionally, recovery from pesticide impact can occur after the pulsed exposure has ended (e.g., Vallotton et al., 2008; Copin et al., 2015); noting that this kind of response is compound and mode-of-action specific (Copin et al., 2016). How the pesticide risk for these catchments is quantified should therefore reflect these pulse regimes (Devlin et al., 2015; Vallotton et al., 2008; Copin et al., 2015; Copin et al., 2016), particularly when comparing risk between catchments and over time. To do so, requires mathematical models that account for inhibition caused by fluctuating concentrations, varying temporal exposure and recovery periods.

Copin et al. (2015) developed a model to estimate total growth inhibition of the microalga, *Scenedesmus vacuolatus*, from pulse exposures of the PSII herbicide, isoproturon. The model included key characteristics of the inhibition response of phototrophs to PSII herbicides, that is:

1. recovery is immediate after the pulse ends;
2. inhibition is cumulative with sequential pulse events, and;
3. sensitivity of the organism to the PSII herbicide does not change with sequential events, i.e. the inhibition response is constant irrespective of the number of pulsed exposures that have previously occurred.

The model (as demonstrated in Figure 37) essentially determines the proportion of the species' production (i.e., growth) compared to that under control conditions.

That is,

$$P_{Loss} = \frac{P_{inhib}}{P_{control}} \times 100 \qquad \text{(Eqn 7)}$$

and,

$$P_{inhib} = P_{control} - P_{pulse+recovery} \qquad \text{(Eqn 8)}$$

where P_{Loss} is the per cent loss in production relative to an appropriate control, $P_{control}$ and $P_{pulse+recovery}$ is production during pulse exposure and recovery conditions. Production is calculated as the growth rate under the exposure conditions (i.e., control, pulse exposed or recovery conditions) multiplied by the duration of the exposure (T).

When there are multiple pulse/recovery events (as represented in Figure 37), the total loss in production is the aggregate of the reduced production during the pulsed exposure events ($p_1 + p_2$) plus the production during the recovery periods ($r_2 + r_1$) as a proportion of the total growth under control conditions ($P_{control}$) that would have occurred over the whole period, that is

$$P_{pulse+recovery} = p_1 + r_1 + p_2 + r_2 \qquad \text{(Eqn 9)}$$

where, p_1 and p_2 are the production that has occurred during the first and second pulses and r_1 and r_2 are the production that has occurred during first and second recovery periods. For all pulse and recovery periods the production is calculated as the product of growth rate at the pesticide concentration during the period and the duration of the period.

As the sensitivity of aquatic plants to PSII herbicides does not change with repeated exposure, the order that p_1 and r_1 is calculated is inconsequential and theoretically the duration of the pulsed exposures could be aggregated if all were the same PSII herbicide concentration. Equally, if a pulsed exposure had fluctuating concentrations within the one event, the duration of the event could be divided up based on the fluctuations, with the inhibition in production calculated separately for each change in concentration before being aggregated. The important aspect here is to ensure the exposure duration to each concentration is always calculated as a proportion of the total time period (pulse + recovery).

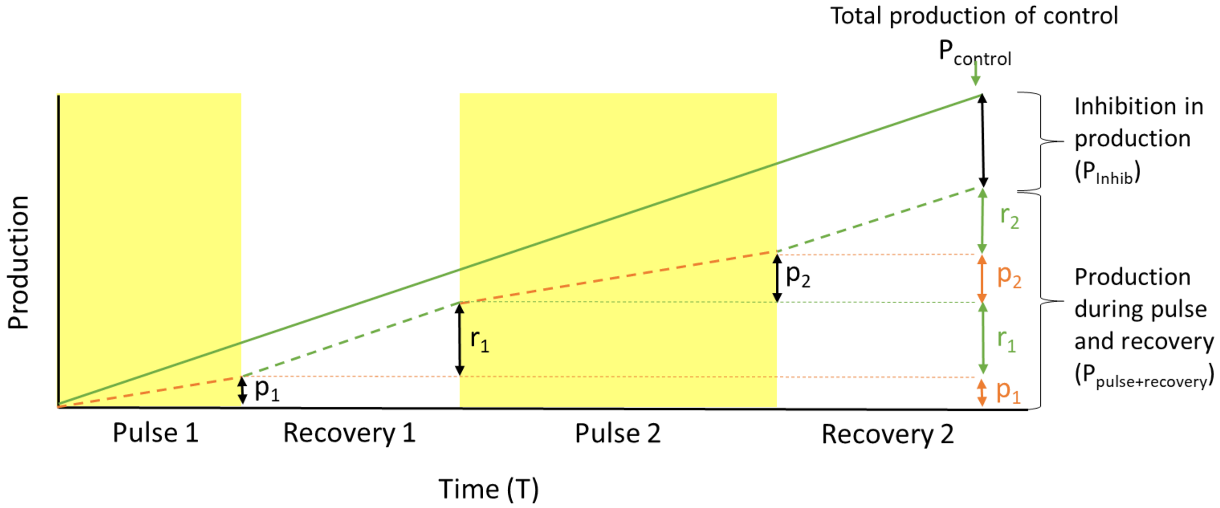

Figure 37. Inhibition of production as a result of pulse exposure and recovery scenarios adapted from Copin et al. (2015) . The model shows that the rate of production recovers immediately after exposure ends – as indicated by the parallel solid and dashed green lines during Recovery 1 and Recovery 2. However, the amount of production that has occurred over time does not recover to control levels during recovery events, rather the loss in production is cumulative with each pulsed exposure and dependent on the concentration of the PSII herbicide and the duration of the pulse

The inhibition in production during pulse and recovery events is concentration dependent. Copin et al. (2015) used a concentration-response relationship, measured from a microalgal species exposed to a PSII herbicide, to estimate the levels of production they would expect to see based on the concentration and duration of multiple pulsed events the algae were exposed to. If we assumed that all phototrophic species exhibited the same response to pulses of PSII herbicides, we could upscale the model to multiple species using species sensitivity distributions (SSDs), as these are also based on a concentration-response relationship.

If we consider the data used to generate PSII herbicide SSDs (see King et al., 2017a, b for PSII herbicide SSDs), each data point used in a SSD is derived from a concentration-response relationship for a single species to a PSII herbicide. These data are the concentration of a PSII herbicide that inhibits production in a species (whether it is growth, reproduction, biomass etc.) by approximately 10% over a chronic time period of ≥ 24 hours; i.e., the No Observed Effect Concentration (NOEC) or the Effective Concentration that causes 10% inhibition (EC10) (King et al., 2017a, b). The SSD distributes these values for multiple species over a concentration range in order to estimate what percentage of species would be affected from a given concentration of the PSII herbicide and is therefore a concentration-response relationship as well (Figure 38). Species occurring in the lower percentiles of the y-axis are more sensitive than those that occur in the higher percentiles, as exposure to a lower concentration will cause the same toxic effect to occur (~10% inhibition in production). In the example presented in Figure 38, concentration X of a PSII herbicide is estimated to affect 50% of species. In addition, the species that has the median sensitivity (i.e. that corresponds to the 50% point on the y-axis) would experience a ~10% reduction in production. However, more sensitive species would experience a higher reduction in production. Some of the more tolerant species (i.e., the species above 50% on the y-axis) will still experience toxic effects but these will be less than a 10% inhibition of production.

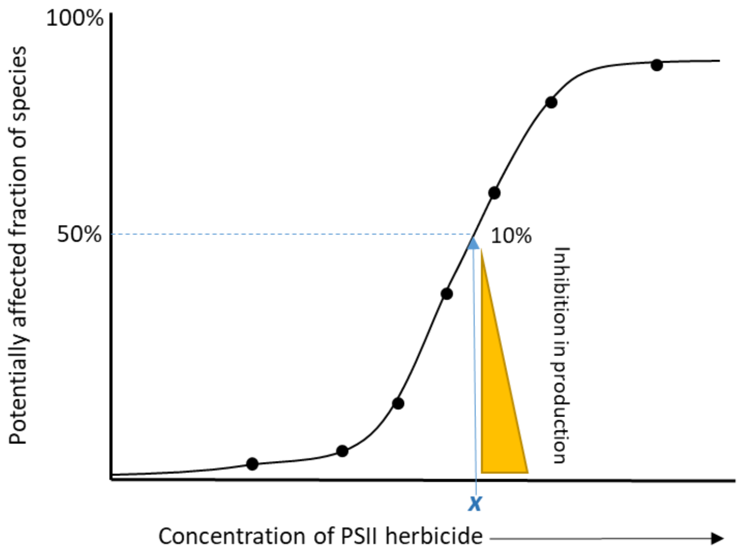

Figure 38. An example of a species sensitivity distribution (SSD) for a PSII herbicide to aquatic plants . Black dots represent single-species data used to generate the SSD, each represents the NOEC or EC10 concentration at which production is inhibited in a phototrophic species by ~10%. From those data, we fit a statistical distribution (black curved line) to estimate the percentage of species affected (y-axis) for any concentration of the PSII herbicide (x-axis). At concentration X, 50% of species are estimated to be affected. Production in species at or close to the 50th percentile (blue dotted line) is inhibited by ~10%, inhibition in production (yellow shape) increases for species at lower percentiles, i.e. as species sensitivity increases

From the SSD, we can expand on the Copin et al. (2015) model (Figure 37) and estimate P_{LOSS} for multiple species. The potentially affected fraction (PAF) of species can be determined for any concentration of a PSII herbicide with an SSD. We know that for the PAF of species, the inhibition in production would be at least 10% inhibition as this corresponds to the toxicity data used (i.e., NOEC/EC10) and the minimum time period that we can measure that at is 24 hours[27]. To estimate the inhibition in production of all species within the PAF would require concentration-response distribution models for each of the species the SSD was generated from, which are often not available for data sourced from the peer-reviewed literature. All PAF species would experience some degree of reduced production (irrespective of where they lie on the SSD). For simplicity, all species within the PAF are treated the same – they have been adversely affected irrespective of their degree of reduced production. Support for the more sensitive species experiencing greater effects at a given pesticide concentration is provided by Wood et al. (2019). They examined diatoms in 14 rivers that discharge to the GBR and assigned them as being either sensitive or tolerant to herbicides based on species traits. Subsequent monitoring of diatoms at the 14 rivers revealed that the number of sensitive diatoms decreased with increasing herbicide toxicity at sites (Wood et al., 2019).

It was also decided to use the minimum possible time step[14] in calculating the toxicological effect, i.e., calculating a daily average estimate of toxicity (% species affected) rather than a 3, 7 or 21 day average value. Using a daily time step has advantages for scenarios where pesticide concentrations and therefore the toxic effects can vary over a 24 hour period, like those observed in many GBR catchments. Fluctuating concentrations from one day to the next can mean a species may be affected one day, but the next day

[27] Chronic test periods for ecotoxicity tests range from 24 hours up to ≥ 21 days; therefore, the data that generates the SSDs could be based on a range of exposure periods but always ≥ 24 hours (Warne et al., 2018).

concentrations drop to a concentration where the effect becomes negligible and recovery can start. Assessing the PAF of species from pulse recovery scenarios ($PAF_{pulse+recovery}$) on a daily basis means that the early initiation of recovery for less sensitive species can be considered.

The data used to generate SSDs are the % inhibition relative to a control and therefore don't require a measure of production under control conditions (as required in Equation 5). Instead, we can estimate $PAF_{pulse+recovery}$ from the aggregated PAF for each day as a proportion of the total time period, as if each day was an individual pulse or recovery period (depending on the PSII concentration). Thus, the $PAF_{pulse+recovery}$ can be estimated by

$$\mathrm{PAF}_{\mathrm{pulse+recovery}} = \frac{\sum \text{daily PAF}}{\text{Total no of days}} \times 100 \qquad \text{(Eqn 10)}$$

For example, if 50% of species production was inhibited for 1 day and 30% inhibited on another day followed by two recovery days with 0% of species inhibited, then an average of 20% of species would be affected by having their production inhibited over the 4 days (Eqn 11).

$$\mathrm{PAF}_{\mathrm{pulse+recovery}} = \frac{0.5+0.3+0+0}{4} \times 100 = 20\% \qquad \text{(Eqn 11)}$$

While the justification for estimating the loss in production for multiple species under pulsed scenarios is quite complex, the resulting method is simply a calculation of the daily average of percentage of species affected, i.e. the average PAF.

The Copin et al. (2015) model is specific to the response characteristics of phototrophs to pulses of PSII herbicides. Nonetheless, this model was chosen to model the toxic effect of all 22 pesticides included in the Pesticide Risk Metric as PSII herbicides are the dominant pesticides detected in GBR catchments, in terms of:

1. frequency of detection —e.g., diuron and atrazine are detected in almost 70% of samples (Spilsbury et al., 2020);
2. contribution to toxicity — PSII herbicides contribute almost 70% to pesticide mixture toxicity (Spilsbury et al., 2020), and;
3. contribution to annual loads — PSII herbicides contribute an average of ~80% of the total load (Devlin et al., 2015).

However, not all the pesticides included in the Pesticide Risk Metric are PSII herbicides nor do they necessarily conform to the three key characteristics of the Copin et al. (2015) model (stated earlier). For example, algae exposed to metsulfuron-methyl for only 24 hours took at least four days for the growth rate to return to that of the control and the greater the concentration the longer the time needed to recover (Rosenkrantz et al., 2013). Exposure of an alga (*Scenedesmus vacuolatus*) to a pulse of S-metolachlor led to delays of 20 and 29 hours (Copin et al., 2016 and Vallotton et al., 2008, respectively) before growth rates returned to pre-exposure rates. In addition, Copin et al. (2016) found that the sensitivity of the alga decreased approximately 20-fold if it had previously been exposed to S-metolachlor. Cedergreen et al. (2005) exposed the aquatic plant *Lemna minor* for three hours separately to imazamox, metsulfuron-methyl (both ALS inhibiting herbicides), propyzamide and pendimethalin (both microtubule assembly inhibiting herbicides) and found that each led to the growth rate taking 4-days to recover to pre-exposure rates and predicted that the recovery of growth rate would take longer following longer exposure periods. It is important to note, as shown in the Copin et al. (2016) model that while the growth rate of exposed algae may recover, the total biomass of algae does not recover and remains suppressed. Alexander et al. (2007)

exposed mayflies and oligochaetes to a 24-h pulse of imidacloprid and found that feeding rate was suppressed for at least four days after exposure ceased and that these effects occurred at concentrations that occur in rivers and creeks. Hayasaka et al. (2012) applied imidacloprid and fipronil, via treated rice seedlings, at commercial rates in two successive years to rice paddy mesocosms. They found that soil concentrations increased between the two years and that the aqueous concentrations remained essentially the same, but the changes in aquatic community composition became larger and persisted for longer in the second year of application. These delays in recovery could be due to the persistence of the pesticide in the organism and prolonging toxic effects. The increased sensitivity following previous exposure could be due the persistence of the pesticide in organisms such that lower aqueous concentrations are required to exert toxic effects. In such cases the frequency of the pulsed exposure will be an important factor with more frequent pulses potentially leading to increased sensitivity. For example, if the toxic effects caused by pesticides are irreversible (e.g. because the pesticide binds irreversibly to a target site or because the effect itself is not reversible) then repeated pulse exposure will increase the magnitude of the toxic effect. For these types of pesticides, the Copin et al. (2015) model would underestimate the biological effect and the potential harm caused to aquatic ecosystems. It was considered that this was likely to be off-set by the environmentally conservative assumption made in merging the Copin et al. (2015) model and SSDs that treated all species in the PAF equally, irrespective of where they were located in the SSD.

Justification for Using a 182-Day Period

As stated earlier, the majority of the pesticide load and the highest concentrations of pesticides generally occurs in the wet season. However, the duration of the wet season can vary both spatially and temporally. For example, in periods of drought the wet season can be shorter than normal and in wet years the wet season can be longer than normal. It was therefore important to determine a typical duration of the 'primary exposure window'.

A key parameter in the Copin et al. (2015) model is the total time period that is considered (i.e. of control, pulse exposure and recovery, which equates to the 'primary exposure window'). The effect of changing the primary exposure window can be illustrated using the data in equation 11. If the primary exposure window was expanded from the current four days to 10 or 20 days and there were no further pulse exposure days then the pesticide mixture toxicity would decrease to 8% (i.e. (0.5 + 0.3 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0/10) or 4% (i.e., (0.5 + 0.3 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0 + 0)/20), respectively. In order to permit valid comparisons of the estimated average PAF at a site from year to year and to allow comparison between sites at the same year the primary exposure period must be constant, irrespective of the flow regime and duration of pesticide exposure.

Standardising the primary exposure window would also help to alleviate variations observed from inter-annual climate variability. The estimates of pesticide mixture toxicity would be similar for a drier year with higher concentrations and shorter pulsed exposures, and a wetter year with lower concentrations and longer exposure periods (Figure 39). While this approach would seem to 'dampen' the estimates of risk (compared to only looking at the highest concentrations as often done in risk assessments), this is a better reflection of the exposure conditions, particularly when evaluating between catchments with such different hydrological regimes.

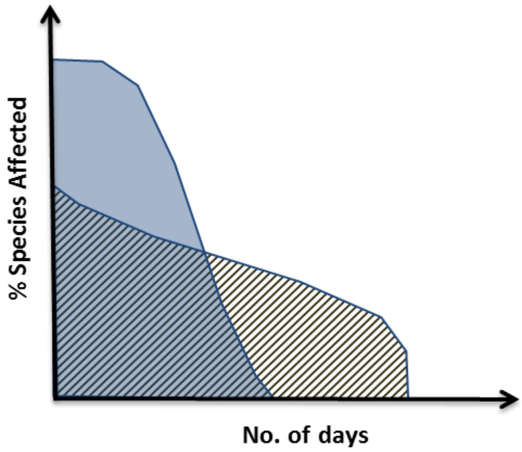

Figure 39. Two pulsed exposure scenarios demonstrating a high concentration with a short exposure period (blue shaded area) vs a lower concentration with a longer exposure period (striped area) . The two areas are equal in shape and size

To determine a standard primary exposure window, hydrological data for seven selected catchments from 2011 to 2014 were used to determine the number of days between the first day after September when a marked increase in river flow (termed an event) and pesticide concentrations occurred and the end of the event when pesticide concentrations have returned to below the limit of reporting (Figure 39). This revealed that the duration of the primary exposure window varied between catchments but was always less than six months (Figure 40). Small events can occur outside the primary exposure window (Figure 40) but these are typically associated with low aqueous concentrations of pesticides, shorter exposure and are considered to pose a lower risk. Therefore, it was decided to use six months (i.e., 182 days) as the standard duration of the primary exposure window to cover the vast majority of the period when organisms are exposed to elevated pesticide concentrations.

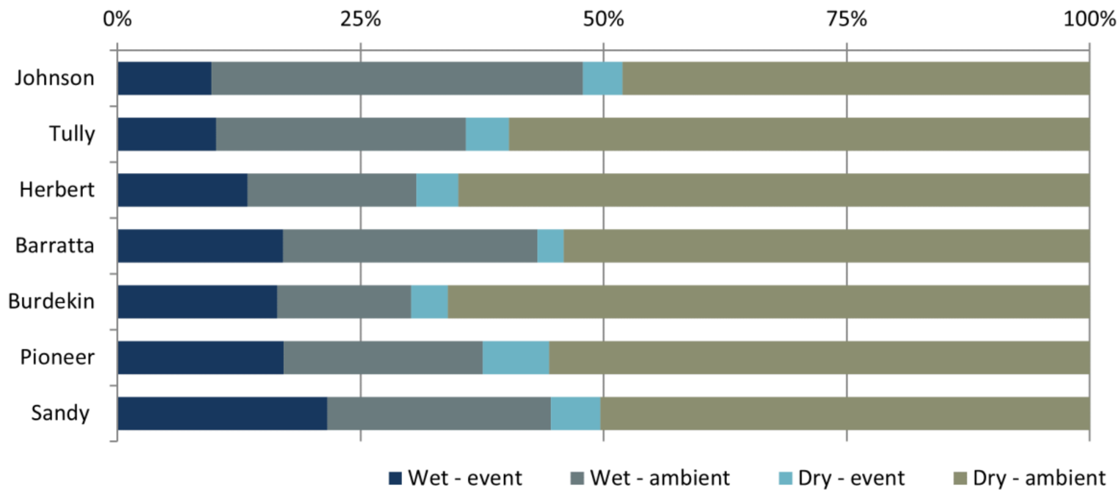

Figure 40. The percentage of a year in the wet season (event and ambient) and the dry season based on discharge data for 2011 – 2014 for selected catchments that discharge to the Great Barrier Reef

Attachment H – Identification of the date of the first flush and 182-day risk window

Table 61. Summary of the date for the first flush and the subsequent 182 days of the Wet Season

Site Name	2015–2016	2016–2017	2017–2018	Flow Notes
Back Creek at Bells Rd			14/10/2017 – 13/04/2018	
Baffle Creek at Newton Road			16/10/2017 - 15/04/2018	Using surrogate site for flow - 134001B & 134002A.
Barratta Creek at Northcote	9/11/2015 – 8/05/2016	30/09/2016 – 31/03/2017	18/10/2017 – 17/04/2018	
Barron River at Rink's Close Jetty			18/10/2017 – 17/04/2018	Using surrogate site for flow - 110001D.
Black River at Bruce Highway			21/02/2018–21/08/2018	Late first flush, data should go until 22 August 2018 (into following sampling year).
Boyne River at Boyne Island			18/10/2017 – 17/04/2018	Used combination of tidal site and nearest rainfall station as well as days where multiple samples were taken (indicating event).
Burdekin River at Home Hill	30/12/2015 – 29/06/2016	6/01/2017 – 6/07/2017	2/12/2017 – 1/06/2018	
Burnett River at Ben Anderson Barrage	28/10/2015 – 27/04/2016	20/12/2016 – 20/06/2017	Only 3 samples. DO NOT USE	Site moved to Quay Street Bridge 2017–2018
Burnett River at Quay Street Bridge			2/10/2017 – 1/04/2018	Used tidal site to mark out first flush.
Burrum River at Buxton Boat Ramp			03/10/2017 – 2/04/2018	

Site Name	2015–2016	2016–2017	2017–2018	Flow Notes
Calliope River at Old Bruce Highway			16/10/2017 – 15/04/2018	
Comet River at Comet Weir	16/11/2015 – 16/05/2016	19/01/2017 – 19/07/2017	14/10/2017 – 13/04/2018	
East Barratta Creek at Jerona Road			18/10/2017 – 17/04/2018	Used surrogate site for flow - 119101A Barratta Creek at Northcote.
Elliott River at Riverview Boat Ramp			3/10/2017 – 2/04/2018	Used surrogate Elliott BOM Rainfall Station 039128 (KMZ file) - No data available.
Fitzroy River at Rockhampton	3/02/2015 – 4/08/2016	10/01/2017 – 11/07/2017	17/10/2017 – 16/04/2018	
Gregory River at Jarrett's Road			2/10/2017 – 01/04/2018	
Haughton River at Powerline	9/11/2015 – 9/05/2016	6/01/2017 – 6/07/2017	Only 2 samples. Data not used	Site moved to Giru Weir 2017–2018
Haughton River at Giru Weir (TW)			20/10/2017 – 19/04/2018	Used surrogate site for flow - 119003A.
Herbert River at Ingham	29/12/2015 – 28/6/2016	15/10/2016 – 15/04/2017	18/10/2017 – 17/04/2018	
Johnstone River at Coquette Point	21/12/2015 – 20/06/2016	14/12/2016 – 14/06/2017	18/10/2017 – 17/04/2018	Used surrogate site for flow - 1120053 Johnstone at Innisfail for flow.
Kolan River at Booyan Boat Ramp			2/10/2017 – 1/04/2018	
Mary River at Home Park	14/11/2015 – 14/05/2016	27/12/2016 – 27/06/2017	Only 3 samples. Data not used	Site moved to Churchill St 2017–2018

Site Name	2015–2016	2016–2017	2017–2018	Flow Notes
Mary River at Churchill Street			16/10/2017 – 15/04/2018	Used surrogate site Mary River at Home Park flow (also referred to Tinana).
Mossman River at Bonnie Doon			18/10/2017 – 17/04/2018	
Mulgrave River at Deeral	21/12/2015 – 20/06/2016	11/12/2016 – 11/06/2017	19/09/2017 – 19/03/2018	
North Johnstone River at Old Bruce Hwy Bridge (Goondi)	23/12/2015 – 22/06/2016	12/12/2016 – 12/06/2017	18/10/2017 – 17/04/2018	
O'Connell River at Stafford’s Crossing		16/12/2016 – 16/06/2017	18/10/2017 – 17/04/2018	
O'Connell River at Caravan Park	18/01/2016 – 18/07/2016	16/12/2016 – 16/06/2017	18/10/2017 – 17/04/2018	
Pioneer River at Dumbleton Pump Station (HW)	9/01/2016 – 9/07/2016	13/12/2016 – 13/06/2017	2/12/2017 – 2/06/2018	
Proserpine River at Glen Isla		3/01/2017 – 4/07/2017	16/10/2017 – 15/04/2018	
Ross River at Rooney's Bridge			16/10/2017 – 15/04/2018	Tidal data missing for beginning of year. Used nearby Townsville rainfall station 23040 to inform decision.
Russell River at East Russell	20/12/2015 – 19/06/2016	12/12/2016 – 12/06/2016	18/09/2017 – 18/03/2018	
Sandy Creek at Homebush	17/11/2015 – 17/05/2016	12/12/2016 – 12/06/2017	18/10/2017 – 17/04/2018	

Site Name	2015–2016	2016–2017	2017–2018	Flow Notes
Styx River at Ogmore			2/12/2017 – 1/06/2018	First flush identified by Rohan Wallace, looking at depth logger data. Tricky site for flow - highly influenced by tides, no other gauging stations upstream.
Tinana Creek at Barrage (Mary River)	27/10/2015 – 26/04/2016	26/11/2016 – 27/05/2017	Only 3 samples. Data not used	
Tully River at Euramo	23/12/2015 – 22/06/2016	4/01/2017 – 5/07/2017	18/10/2017 – 17/04/2018	
Waterpark Creek at Corbett's Landing			17/10/2017 – 16/04/2018	

Files with the hydrographs and pesticide concentrations can be provided upon request

Attachment I – Land use and spatial variables used in developing the pesticide mixture toxicity vs land use relationships and to estimate pesticide mixture toxicity estimates.

Table 62. The latitude, longitude, adopted middle thread distance (AMTD) of each site and the relative monitored catchment size upstream of each site and year

Monitoring site	Latitude (degrees)	Longitude (degrees)	AMTD (km)	Monitored Catchment size (m^2)	Relative monitored catchment size[1] (proportion)
Mossman River at Bonnie Doon	-16.4455	145.3962	2.1	1.98×10^{8}	4.66×10^{-4}
Barron River at Rink's Close Jetty	-16.8732	145.7334	6.96	2.21×10^{9}	5.20×10^{-3}
Mulgrave River at Deeral	-17.2075	145.9264	8.93	7.90×10^{8}	1.86×10^{-3}
Russell River at East Russell	-17.2672	145.9544	7.59	5.25×10^{8}	1.24×10^{-3}
North Johnstone River at Old Bruce Hwy Bridge (Goondi)	-17.5059	145.992	18	9.63×10^{8}	2.27×10^{-3}
Johnstone River at Coquette Point	-17.5112	146.0604	2.2	1.64×10^{9}	3.87×10^{-3}
Tully River at Euramo	-17.9921	145.9425	17.5	1.45×10^{9}	3.42×10^{-3}
Herbert River at Ingham	-18.6328	146.1427	30.5	8.59×10^{9}	2.02×10^{-2}
Black River at Bruce Highway	-19.2377	146.6329	8.9	2.57×10^{8}	6.06×10^{-4}
Haughton River at Giru Weir tailwater	-19.5121	147.1115	22.2	1.90×10^{9}	4.49×10^{-3}
Haughton River at Powerline	-19.6331	147.1103	32.5	1.78×10^{9}	4.19×10^{-3}
Barratta Creek at Northcote	-19.6907	147.1698	51.3	7.59×10^{8}	1.79×10^{-3}
East Barratta Creek at Jerona Road	-19.4878	147.2284	17.9	1.16×10^{9}	2.73×10^{-3}
Burdekin River at Home Hill	-19.6421	147.3969	17.4	1.30×10^{11}	3.06×10^{-1}
Proserpine River at Glen Isla	-20.4172	148.6453	20.4	5.50×10^{8}	1.30×10^{-3}

Monitoring site	Latitude (degrees)	Longitude (degrees)	AMTD (km)	Monitored Catchment size (m^2)	Relative monitored catchment size[1] (proportion)
O'Connell at Stafford's Crossing	-20.6526	148.573	19.5	3.40×10^{8}	8.02×10^{-4}
O'Connell River at Caravan Park	-20.5664	148.6117	8.51	8.26×10^{8}	1.95×10^{-3}
Pioneer River at Dumbleton Pump Station	-21.1419	149.0758	16.7	1.47×10^{9}	3.46×10^{-3}
Sandy Creek at Homebush	-21.2833	149.0225	32.7	3.26×10^{9}	7.69×10^{-4}
Styx River at Ogmore	-22.5922	149.6260	32.8	1.01×10^{9}	2.38×10^{-3}
Waterpark Creek at Corbett's Landing	-22.8865	150.7197	14.4	3.88×10^{8}	9.13×10^{-4}
Fitzroy River at Rockhampton	-23.3811	150.5169	48.0	1.39×10^{11}	3.28×10^{-1}
Comet River at Comet Weir	-23.6125	148.5514	17.2	1.65×10^{10}	3.88×10^{-2}
Calliope River at Old Bruce Highway	-23.9582	151.1585	21.7	1.64×10^{9}	3.86×10^{-3}
Boyne River at Boyne Island	-23.9468	151.3578	4.17	2.41×10^{9}	5.67×10^{-3}
Baffle Creek at Newton Road	-24.5151	151.9742	11.5	2.37×10^{9}	5.59×10^{-3}
Kolan River at Booyan Boat Ramp	-24.7054	152.1888	13.9	2.58×10^{9}	6.09×10^{-3}
Burnett River at Quay Street Bridge	-24.8630	152.3456	18.3	3.31×10^{10}	7.79×10^{-2}
Burnett River at Ben Anderson Barrage	-24.8892	152.2912	25.8	3.29×10^{10}	7.74×10^{-2}
Elliott River at Riverview Boat Ramp	-24.9298	152.4747	2.31	3.73×10^{8}	8.79×10^{-4}
Gregory River at Jarretts Road	-25.1573	152.4984	23.2	8.29×10^{8}	1.95×10^{-3}
Burrum River at Buxton Boat Ramp	-25.1955	152.5419	13.0	1.41×10^{9}	3.32×10^{-3}
Tinana Creek at Barrage	-25.5720	152.7173	1.7	1.28×10^{9}	3.02×10^{-3}

Monitoring site	Latitude (degrees)	Longitude (degrees)	AMTD (km)	Monitored Catchment size (m^2)	Relative monitored catchment size[1] (proportion)
Mary River at Churchill Street	-25.5320	152.7081	37.9	8.87×10^9	2.09×10^{-2}
Mary River at Home Park	-25.7671	152.5283	90.8	6.88×10^9	1.62×10^{-2}

[1.] Relative catchment size is the surface area monitored by each site expressed as a proportion of the entire Great Barrier Reef catchment area (i.e. the total surface area of all catchments that discharge to the GBR).

Table 63. The average relative rainfall, average relative runoff, maximum relative rainfall and maximum relative runoff for each site and year

Monitoring site	Sampling year	Average relative rainfall (proportion)[1]	Maximum relative rainfall (proportion)[2]	Average relative runoff (proportion)[3]	Maximum relative runoff (proportion)[4]
Mossman River at Bonnie Doon	2017–2018	0.50	1.00	0.66	1.00
Barron River at Rink's Close Jetty	2017–2018	0.51	1.00	0.64	0.99
Mulgrave River at Deeral	2015–2016	0.47	1.00	0.51	0.99
	2016–2017	0.51	0.98	0.49	0.95
	2017–2018	0.48	1.00	0.63	0.99
Russell River at East Russell	2015–2016	0.47	1.00	0.51	0.99
	2016–2017	0.51	0.98	0.50	0.96
	2017–2018	0.49	1.00	0.64	0.99
North Johnstone River at Old Bruce Hwy Bridge (Goondi)	2015–2016	0.48	1.00	0.48	0.99
	2016–2017	0.51	0.99	0.46	0.99
	2017–2018	0.52	1.00	0.59	0.99
Johnstone River at Coquette Point	2015–2016	0.49	1.00	0.47	0.99
	2016–2017	0.51	0.99	0.46	0.99
	2017–2018	0.53	1.00	0.58	0.99
Tully River at Euramo	2015–2016	0.49	0.99	0.46	0.99
	2016–2017	0.50	0.99	0.48	0.96
	2017–2018	0.52	1.00	0.59	1.00

Monitoring site	Sampling year	Average relative rainfall (proportion)[1]	Maximum relative rainfall (proportion)[2]	Average relative runoff (proportion)[3]	Maximum relative runoff (proportion)[4]
Herbert River at Ingham	2015–2016	0.46	0.99	0.42	0.98
	2016–2017	0.46	0.99	0.52	0.93
	2017–2018	0.49	1.00	0.53	0.99
Black River at Bruce Highway	2017–2018	0.46	0.99	0.53	0.99
Haughton River at Giru Weir tailwater	2017–2018	0.49	0.99	0.49	0.99
	2015–2016	0.42	0.99	0.29	0.96
	2016–2017	0.47	1.00	0.32	0.99
Barratta Creek at Northcote	2015–2016	0.41	0.99	0.45	0.96
	2016–2017	0.47	0.99	0.44	0.98
	2017–2018	0.47	0.99	0.51	0.99
East Barratta Creek at Jerona Road	2017–2018	0.49	0.99	0.48	0.99
Burdekin River at Home Hill	2015–2016	0.45	0.99	0.44	0.94
	2016–2017	0.50	0.99	0.52	0.99
	2017–2018	0.44	0.99	0.42	0.99
Proserpine River at Glen Isla	2016–2017	0.48	1.00	0.61	1.00
	2017–2018	0.52	0.99	0.54	0.99
O'Connell at Stafford's Crossing	2016–2017	0.51	1.00	0.62	1.00
	2017–2018	0.50	0.99	0.53	0.99

Monitoring site	Sampling year	Average relative rainfall (proportion)[1]	Maximum relative rainfall (proportion)[2]	Average relative runoff (proportion)[3]	Maximum relative runoff (proportion)[4]
O'Connell River at Caravan Park	2015–2016	0.44	1.00	0.38	0.99
	2016–2017	0.50	1.00	0.67	1.00
	2017–2018	0.51	0.99	0.59	0.99
Pioneer River at Dumbleton Pump Station	2015–2016	0.44	0.99	0.46	0.99
	2016–2017	0.52	0.99	0.71	1.00
	2017–2018	0.43	0.98	0.33	0.95
Sandy Creek at Homebush	2015–2016	0.45	0.99	0.36	0.98
	2016–2017	0.54	1.00	0.66	1.00
	2017–2018	0.50	0.98	0.54	0.99
Styx River at Ogmore	2017–2018	0.48	0.99	0.47	0.97
Waterpark Creek at Corbett's Landing	2017–2018	0.51	1.00	0.47	1.00
Fitzroy River at Rockhampton	2015–2016	0.47	0.99	0.63	0.99
	2016–2017	0.43	0.99	0.48	1.00
	2017–2018	0.46	1.00	0.40	1.00
Comet River at Comet Weir	2015–2016	0.44	0.99	0.56	0.95
	2016–2017	0.45	1.00	0.60	0.99
	2017–2018	0.47	0.99	0.45	0.98
Calliope River at Old Bruce Highway	2017–2018	0.48	1.00	0.54	1.00

Monitoring site	Sampling year	Average relative rainfall (proportion)[1]	Maximum relative rainfall (proportion)[2]	Average relative runoff (proportion)[3]	Maximum relative runoff (proportion)[4]
Boyne River at Boyne Island	2017–2018	0.48	1.00	0.49	1.00
Baffle Creek at Newton Road	2017–2018	0.53	1.00	0.65	1.00
Kolan River at Booyan Boat Ramp	2017–2018	0.52	1.00	0.64	1.00
Burnett River at Quay Street Bridge	2017–2018	0.50	1.00	0.63	1.00
Burnett at Ben Anderson Barrage	2015–2016	0.45	1.00	0.42	0.96
	2016–2017	0.43	0.99	0.39	0.99
Elliott River at Riverview Boat Ramp	2017–2018	0.51	1.00	0.59	1.00
Gregory River at Jarretts Road	2017–2018	0.52	1.00	0.69	1.00
Burrum River at Buxton Boat Ramp	2017–2018	0.51	1.00	0.79	1.00
Tinana Creek at Barrage	2015–2016	0.49	0.99	0.48	0.95
	2016–2017	0.50	0.99	0.33	0.90
Mary River at Churchill Street	2017–2018	0.56	1.00	0.67	1.00
Mary River at Homepark	2015–2016	0.45	1.00	0.46	0.98
	2016–2017	0.45	0.99	0.36	0.97

[1.] The average rainfall for the year expressed as a proportion of the long-term average rainfall for that site.[2.] The maximum rainfall for the year expressed as a proportion of the long-term maximum rainfall for that site.[3.] The average runoff for the year expressed as a proportion of the long-term average run-off for that site.[4.] The maximum runoff for the year expressed as a proportion of the long-term maximum run-off for that site.

Table 64. The relative surface area of bananas, conservation, dryland cropping, forestry and forested grazing land uses in each monitored catchment

Monitoring site	Relative banana (proportion)[1]	Relative conservation (proportion)[1]	Relative dryland cropping (proportion)[1]	Relative forestry (proportion)[1]	Relative grazing forested (proportion)[1]
Mossman River at Bonnie Doon	0.000	0.840	0.000	0.000	0.004
Barron River at Rink's Close Jetty	0.010	0.345	0.000	0.108	0.252
Mulgrave River at Deeral	0.000	0.819	0.002	0.006	0.037
Russell River at East Russell	0.018	0.684	0.000	0.003	0.043
North Johnstone River at Old Bruce Hwy Bridge (Goondi)	0.019	0.519	0.006	0.001	0.302
Johnstone River at Coquette Point	0.025	0.549	0.004	0.002	0.211
Tully River at Euramo	0.033	0.761	0.000	0.000	0.043
Herbert River at Ingham	0.000	0.285	0.000	0.046	0.569
Black River at Bruce Highway	0.000	0.114	0.001	0.050	0.684
Haughton River at Giru Weir tailwater	0.000	0.135	0.003	0.017	0.471
Haughton River at Powerline	0.000	0.137	0.003	0.018	0.493
Barratta Creek at Northcote	0.000	0.023	0.000	0.000	0.371
East Barratta Creek at Jerona Road	0.000	0.049	0.000	0.000	0.269
Burdekin River at Home Hill	0.000	0.061	0.010	0.006	0.581
Proserpine River at Glen Isla	0.000	0.207	0.000	0.069	0.280
O'Connell at Stafford's Crossing	0.000	0.132	0.000	0.372	0.266

Monitoring site	Relative banana (proportion)[1]	Relative conservation (proportion)[1]	Relative dryland cropping (proportion)[1]	Relative forestry (proportion)[1]	Relative grazing forested (proportion)[1]
O'Connell River at Caravan Park	0.000	0.207	0.000	0.179	0.357
Pioneer River at Dumbleton Pump Station	0.000	0.312	0.000	0.203	0.207
Sandy Creek at Homebush	0.000	0.096	0.000	0.119	0.171
Styx River at Ogmore	0.000	0.069	0.001	0.020	0.509
Waterpark Creek at Corbett's Landing	0.000	0.484	0.000	0.456	0.014
Fitzroy River at Rockhampton	0.000	0.059	0.057	0.064	0.422
Comet River at Comet Weir	0.000	0.093	0.103	0.056	0.418
Calliope River at Old Bruce Highway	0.000	0.041	0.000	0.028	0.559
Boyne River at Boyne Island	0.000	0.158	0.000	0.056	0.649
Baffle Creek at Newton Road	0.000	0.114	0.000	0.107	0.658
Kolan River at Booyan Boat Ramp	0.000	0.075	0.001	0.101	0.643
Burnett River at Quay Street Bridge	0.000	0.040	0.024	0.124	0.497
Burnett at Ben Anderson Barrage	0.000	0.040	0.025	0.125	0.498
Elliott River at Riverview Boat Ramp	0.000	0.159	0.000	0.142	0.109
Gregory River at Jarretts Road	0.000	0.083	0.002	0.163	0.482
Burrum River at Buxton Boat Ramp	0.000	0.164	0.000	0.404	0.344

Monitoring site	Relative banana (proportion)[1]	Relative conservation (proportion)[1]	Relative dryland cropping (proportion)[1]	Relative forestry (proportion)[1]	Relative grazing forested (proportion)[1]
Tinana Creek at Barrage	0.000	0.103	0.000	0.607	0.124
Mary River at Churchill Street	0.000	0.168	0.000	0.213	0.381
Mary River at Home Park	0.000	0.191	0.000	0.131	0.429

1. The surface area of the stated land use expressed as a proportion of the surface area of the monitored catchment upstream of each site.

Table 65. The relative surface area of open grazing, horticulture, irrigated cropping, other land uses and sugar cane land uses in each monitored catchment

Monitoring site	Relative grazing open (proportion) [1]	Relative horticulture (proportion) [1]	Relative irrigated cropping (proportion) [1]	Relative "other" (proportion) [1, 2]	Relative sugar (proportion) [1]
Mossman River at Bonnie Doon	0.004	0.003	0.000	0.006	0.121
Barron River at Rink's Close Jetty	0.069	0.056	0.026	0.012	0.037
Mulgrave River at Deeral	0.007	0.002	0.000	0.001	0.098
Russell River at East Russell	0.045	0.011	0.000	0.003	0.163
North Johnstone River at Old Bruce Hwy Bridge (Goondi)	0.101	0.005	0.001	0.003	0.011
Johnstone River at Coquette Point	0.087	0.008	0.001	0.006	0.063
Tully River at Euramo	0.018	0.004	0.000	0.002	0.110
Herbert River at Ingham	0.035	0.001	0.003	0.001	0.027
Black River at Bruce Highway	0.070	0.009	0.000	0.015	0.000
Haughton River at Giru Weir tailwater	0.278	0.020	0.003	0.007	0.035
Haughton River at Powerline	0.293	0.007	0.002	0.007	0.011
Barratta Creek at Northcote	0.389	0.001	0.002	0.004	0.182
East Barratta Creek at Jerona Road	0.281	0.013	0.002	0.005	0.339
Burdekin River at Home Hill	0.319	0.000	0.001	0.002	0.001
Proserpine River at Glen Isla	0.088	0.002	0.000	0.006	0.208
O'Connell at Stafford's Crossing	0.133	0.002	0.000	0.006	0.063

Monitoring site	Relative grazing open (proportion) [1]	Relative horticulture (proportion) [1]	Relative irrigated cropping (proportion) [1]	Relative "other" (proportion) [1, 2]	Relative sugar (proportion) [1]
O'Connell River at Caravan Park	0.162	0.001	0.001	0.004	0.063
Pioneer River at Dumbleton Pump Station	0.041	0.001	0.000	0.003	0.191
Sandy Creek at Homebush	0.073	0.004	0.001	0.011	0.450
Styx River at Ogmore	0.399	0.000	0.000	0.001	0.000
Waterpark Creek at Corbett's Landing	0.002	0.004	0.000	0.000	0.000
Fitzroy River at Rockhampton	0.375	0.000	0.009	0.008	0.000
Comet River at Comet Weir	0.311	0.000	0.011	0.003	0.000
Calliope River at Old Bruce Highway	0.359	0.001	0.000	0.006	0.000
Boyne River at Boyne Island	0.105	0.001	0.001	0.003	0.000
Baffle Creek at Newton Road	0.102	0.000	0.001	0.001	0.000
Kolan River at Booyan Boat Ramp	0.092	0.005	0.002	0.001	0.020
Burnett River at Quay Street Bridge	0.276	0.003	0.012	0.002	0.005
Burnett at Ben Anderson Barrage	0.277	0.003	0.012	0.002	0.003
Elliott River at Riverview Boat Ramp	0.071	0.081	0.007	0.020	0.341
Gregory River at Jarretts Road	0.054	0.046	0.001	0.003	0.131
Burrum River at Buxton Boat Ramp	0.031	0.004	0.000	0.002	0.009

Monitoring site	Relative grazing open (proportion) [1]	Relative horticulture (proportion) [1]	Relative irrigated cropping (proportion) [1]	Relative "other" (proportion) [1, 2]	Relative sugar (proportion) [1]
Tinana Creek at Barrage	0.041	0.023	0.003	0.003	0.047
Mary River at Churchill Street	0.139	0.009	0.004	0.005	0.015
Mary River at Homepark	0.164	0.006	0.005	0.005	0.002

[1.] The surface area of the stated land use expressed as a proportion of the surface area of the monitored catchment upstream of each site. [2.] Land use types were agglomerated into 11–13 categories that align with the Reef Categories of the Source Catchment models (Waters et al., 2014). The agglomerated landuse 'Other' is a combination of intensive animal production (e.g. poultry farms, feedlots), manufacturing & industrial (e.g. food processing plants, abattoirs, sawmills), residential and farm infrastructure, services (e.g. recreation, defence), utilities (e.g. water extraction, power generation), transport (e.g. roads, railways), mining, and waste management (e.g. effluent, landfill, sewage).

Table 66. The relative surface area of urban, water and wetland land uses in each monitored catchment

Monitoring site	Relative urban (proportion)[1, 2]	Relative water (proportion)[1]	Relative wetland (proportion)[1]
Mossman River at Bonnie Doon	0.018	0.000	0.003
Barron River at Rink's Close Jetty	0.055	0.020	0.011
Mulgrave River at Deeral	0.015	0.005	0.008
Russell River at East Russell	0.013	0.007	0.010
North Johnstone River at Old Bruce Hwy Bridge (Goondi)	0.025	0.006	0.001
Johnstone River at Coquette Point	0.031	0.008	0.005
Tully River at Euramo	0.007	0.013	0.008
Herbert River at Ingham	0.005	0.004	0.024
Black River at Bruce Highway	0.042	0.006	0.008
Haughton River at Giru Weir tailwater	0.007	0.012	0.013
Haughton River at Powerline	0.006	0.011	0.012
Barratta Creek at Northcote	0.000	0.013	0.015
East Barratta Creek at Jerona Road	0.001	0.013	0.030
Burdekin River at Home Hill	0.001	0.007	0.012
Proserpine River at Glen Isla	0.054	0.070	0.016
O'Connell at Stafford's Crossing	0.005	0.010	0.011
O'Connell River at Caravan Park	0.004	0.011	0.012
Pioneer River at Dumbleton Pump Station	0.017	0.017	0.008

Monitoring site	Relative urban (proportion)[1, 2]	Relative water (proportion)[1]	Relative wetland (proportion)[1]
Sandy Creek at Homebush	0.034	0.033	0.007
Styx River at Ogmore	0.000	0.000	0.000
Waterpark Creek at Corbett's Landing	0.039	0.000	0.000
Fitzroy River at Rockhampton	0.002	0.004	0.000
Comet River at Comet Weir	0.000	0.004	0.000
Calliope River at Old Bruce Highway	0.005	0.001	0.000
Boyne River at Boyne Island	0.004	0.024	0.000
Baffle Creek at Newton Road	0.011	0.006	0.000
Kolan River at Booyan Boat Ramp	0.032	0.029	0.000
Burnett River at Quay Street Bridge	0.010	0.005	0.000
Burnett at Ben Anderson Barrage	0.010	0.005	0.000
Elliott River at Riverview Boat Ramp	0.030	0.029	0.011
Gregory River at Jarretts Road	0.023	0.011	0.001
Burrum River at Buxton Boat Ramp	0.026	0.011	0.003
Tinana Creek at Barrage	0.041	0.007	0.000
Mary River at Churchill Street	0.057	0.008	0.000
Mary River at Home Park	0.058	0.008	0.000

[1.] The surface area of the stated land use expressed as a proportion of the surface area of the monitored catchment upstream of each site.[2.] The agglomerated land use 'urban' is a combination of residential types such as urban residential, remote communities, and residential without agriculture. It also includes two types of residential land uses associated with agriculture: residential with farm infrastructure and residential with agriculture.

Table 67. The average relative rainfall and run-off and the relative surface area of each basin and year for bananas and conservation land uses

Basin	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion)[2]	Relative bananas (proportion)[3]	Relative conservation (proportion)[3]
Baffle	2015–2016	0.47	0.42	0.000	0.19
Baffle	2016-2017	0.48	0.44	0.000	0.19
Baffle	2017–2018	0.53	0.66	0.000	0.19
Barron	2015–2016	0.49	0.54	0.010	0.34
Barron	2016–2017	0.47	0.48	0.010	0.34
Barron	2017–2018	0.51	0.63	0.010	0.34
Black	2015–2016	0.46	0.33	0.000	0.40
Black	2016–2017	0.46	0.39	0.000	0.40
Black	2017–2018	0.50	0.55	0.000	0.40
Boyne	2015–2016	0.50	0.51	0.000	0.16
Boyne	2016–2017	0.48	0.42	0.000	0.16
Boyne	2017–2018	0.51	0.52	0.000	0.16
Burdekin	2015–2016	0.50	0.37	0.000	0.06
Burdekin	2016–2017	0.54	0.49	0.000	0.06
Burdekin	2017–2018	0.50	0.48	0.000	0.06
Burnett	2015–2016	0.49	0.50	0.000	0.04
Burnett	2016–2017	0.47	0.38	0.000	0.04
Burnett	2017–2018	0.52	0.63	0.000	0.04

Basin	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion)[2]	Relative bananas (proportion)[3]	Relative conservation (proportion)[3]
Burrum	2015–2016	0.47	0.42	0.000	0.21
Burrum	2016–2017	0.42	0.30	0.000	0.21
Burrum	2017–2018	0.51	0.73	0.000	0.21
Calliope	2015–2016	0.46	0.53	0.000	0.05
Calliope	2016–2017	0.45	0.50	0.000	0.05
Calliope	2017–2018	0.47	0.56	0.000	0.05
Daintree	2015–2016	0.51	0.58	0.000	0.85
Daintree	2016–2017	0.49	0.53	0.000	0.85
Daintree	2017–2018	0.51	0.61	0.000	0.85
Don	2015–2016	0.43	0.37	0.000	0.060
Don	2016–2017	0.48	0.60	0.000	0.060
Don	2017–2018	0.49	0.50	0.000	0.060
Endeavour	2015–2016	0.50	0.52	0.000	0.52
Endeavour	2016–2017	0.48	0.47	0.000	0.52
Endeavour	2017–2018	0.51	0.55	0.000	0.52
Fitzroy	2015–2016	0.47	0.37	0.000	0.06
Fitzroy	2016–2017	0.46	0.38	0.000	0.06
Fitzroy	2017–2018	0.48	0.44	0.000	0.06
Haughton	2015–2016	0.49	0.38	0.000	0.14

Basin	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion)[2]	Relative bananas (proportion)[3]	Relative conservation (proportion)[3]
Haughton	2016–2017	0.51	0.49	0.000	0.14
Haughton	2017–2018	0.49	0.50	0.000	0.14
Herbert	2015–2016	0.46	0.32	0.000	0.29
Herbert	2016–2017	0.49	0.56	0.000	0.29
Herbert	2017–2018	0.50	0.53	0.000	0.29
Jacky Jacky	2015–2016	0.42	0.22	0.000	0.81
Jacky Jacky	2016–2017	0.48	0.48	0.000	0.81
Jacky Jacky	2017–2018	0.47	0.50	0.000	0.81
Jeannie	2015–2016	0.47	0.47	0.000	0.82
Jeannie	2016–2017	0.44	0.35	0.000	0.82
Jeannie	2017–2018	0.47	0.40	0.000	0.82
Johnstone	2015–2016	0.49	0.47	0.023	0.56
Johnstone	2016–2017	0.49	0.46	0.023	0.56
Johnstone	2017–2018	0.53	0.59	0.023	0.56
Kolan	2015–2016	0.47	0.40	0.000	0.08
Kolan	2016–2017	0.45	0.41	0.000	0.08
Kolan	2017–2018	0.51	0.64	0.000	0.08
Lockhart	2015–2016	0.44	0.44	0.000	0.91
Lockhart	2016–2017	0.47	0.24	0.000	0.91

Basin	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion)[2]	Relative bananas (proportion)[3]	Relative conservation (proportion)[3]
Lockhart	2017–2018	0.49	0.55	0.000	0.91
Mary	2015–2016	0.52	0.46	0.000	0.18
Mary	2016–2017	0.47	0.26	0.000	0.18
Mary	2017–2018	0.57	0.66	0.000	0.18
Mossman	2015–2016	0.52	0.40	0.000	0.75
Mossman	2016–2017	0.50	0.68	0.000	0.75
Mossman	2017–2018	0.51	0.65	0.000	0.75
Mulgrave-Russell	2015–2016	0.48	0.54	0.005	0.71
Mulgrave-Russell	2016–2017	0.48	0.48	0.005	0.71
Mulgrave-Russell	2017–2018	0.51	0.64	0.005	0.71
Murray	2015–2016	0.48	0.37	0.005	0.63
Murray	2016–2017	0.47	0.54	0.005	0.63
Murray	2017–2018	0.51	0.61	0.005	0.63
Normanby	2015–2016	0.45	0.49	0.000	0.46
Normanby	2016–2017	0.44	0.36	0.000	0.46
Normanby	2017–2018	0.43	0.48	0.000	0.46
O’Connell	2015–2016	0.46	0.44	0.000	0.27
O’Connell	2016–2017	0.52	0.57	0.000	0.27
O’Connell	2017–2018	0.51	0.49	0.000	0.27

Basin	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion)[2]	Relative bananas (proportion)[3]	Relative conservation (proportion)[3]
Olive Pascoe	2015–2016	0.46	0.43	0.000	0.79
Olive Pascoe	2016–2017	0.50	0.29	0.000	0.79
Olive Pascoe	2017–2018	0.51	0.56	0.000	0.79
Pioneer	2015–2016	0.48	0.46	0.000	0.29
Pioneer	2016–2017	0.48	0.61	0.000	0.29
Pioneer	2017–2018	0.48	0.43	0.000	0.29
Plane	2015–2016	0.50	0.46	0.000	0.32
Plane	2016–2017	0.53	0.61	0.000	0.32
Plane	2017–2018	0.51	0.45	0.000	0.32
Proserpine	2015–2016	0.47	0.41	0.000	0.29
Proserpine	2016–2017	0.51	0.54	0.000	0.29
Proserpine	2017–2018	0.51	0.46	0.000	0.29
Ross	2015–2016	0.49	0.44	0.000	0.27
Ross	2016–2017	0.49	0.43	0.000	0.27
Ross	2017–2018	0.50	0.51	0.000	0.27
Shoalwater	2015–2016	0.45	0.44	0.000	0.47
Shoalwater	2016–2017	0.48	0.47	0.000	0.47
Shoalwater	2017–2018	0.49	0.50	0.000	0.47
Stewart	2015–2016	0.46	0.44	0.000	0.94

Basin	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion)[2]	Relative bananas (proportion)[3]	Relative conservation (proportion)[3]
Stewart	2016–2017	0.46	0.31	0.000	0.94
Stewart	2017–2018	0.45	0.48	0.000	0.94
Styx	2015–2016	0.47	0.40	0.000	0.05
Styx	2016–2017	0.50	0.37	0.000	0.05
Styx	2017–2018	0.51	0.52	0.000	0.05
Tully	2015–2016	0.48	0.31	0.031	0.74
Tully	2016–2017	0.47	0.48	0.031	0.74
Tully	2017–2018	0.52	0.61	0.031	0.74
Waterpark	2015–2016	0.43	0.35	0.000	0.63
Waterpark	2016–2017	0.46	0.39	0.000	0.63
Waterpark	2017–2018	0.50	0.48	0.000	0.63

[1]. The average rainfall for the year expressed as a proportion of the long-term average rainfall for that basin. [2]. The average runoff for the year expressed as a proportion of the long-term average run-off for that basin. [3]. The surface area of the stated land use expressed as a proportion of the surface area of the basin.

Table 68. The relative surface area of each basin for dryland cropping, forestry, forested grazing and open grazing land uses

Basin	Relative dryland cropping (proportion)[1]	Relative forestry (proportion)[1]	Relative grazing forested (proportion)[1]	Relative grazing open (proportion)[1]
Baffle	0.000	0.069	0.585	0.085
Barron	0.000	0.106	0.245	0.068
Black	0.000	0.074	0.368	0.040
Boyne	0.000	0.054	0.635	0.103
Burdekin	0.010	0.006	0.580	0.319
Burnett	0.024	0.123	0.495	0.275
Burrum	0.001	0.227	0.322	0.050
Calliope	0.000	0.059	0.526	0.289
Daintree	0.000	0.000	0.086	0.004
Don	0.000	0.000	0.465	0.328
Endeavour	0.001	0.009	0.430	0.009
Fitzroy	0.056	0.063	0.423	0.374
Haughton	0.002	0.008	0.309	0.244
Herbert	0.000	0.040	0.503	0.034
Jacky Jacky	0.000	0.000	0.089	0.000
Jeannie	0.000	0.006	0.104	0.002
Johnstone	0.003	0.002	0.158	0.079
Kolan	0.000	0.090	0.602	0.089

Basin	Relative dryland cropping (proportion)[1]	Relative forestry (proportion)[1]	Relative grazing forested (proportion)[1]	Relative grazing open (proportion)[1]
Lockhart	0.000	0.000	0.025	0.000
Mary	0.000	0.207	0.370	0.134
Mossman	0.000	0.000	0.007	0.009
Mulgrave-Russell	0.001	0.004	0.032	0.018
Murray	0.000	0.093	0.046	0.028
Normanby	0.002	0.000	0.517	0.012
O'Connell	0.000	0.066	0.287	0.122
Olive Pascoe	0.000	0.000	0.208	0.000
Pioneer	0.000	0.190	0.196	0.040
Plane	0.000	0.058	0.142	0.080
Proserpine	0.000	0.054	0.316	0.133
Ross	0.001	0.026	0.310	0.172
Shoalwater	0.000	0.003	0.236	0.170
Stewart	0.000	0.000	0.023	0.000
Styx	0.002	0.025	0.466	0.336
Tully	0.000	0.000	0.041	0.020
Waterpark	0.000	0.105	0.117	0.038

[1] The surface area of the stated land use expressed as a proportion of the surface area of the basin.

Table 69. The relative surface area of each basin for horticulture, irrigated cropping, sugar cane and "other" land uses

Basin	Relative horticulture (proportion) [1]	Relative irrigated cropping (proportion) [1]	Relative "other" (proportion) [1,2]	Relative sugar (proportion) [1]
Baffle	0.004	0.001	0.002	0.003
Barron	0.055	0.025	0.016	0.040
Black	0.013	0.001	0.021	0.014
Boyne	0.001	0.001	0.014	0.000
Burdekin	0.000	0.001	0.002	0.002
Burnett	0.004	0.012	0.003	0.006
Burrum	0.024	0.002	0.010	0.092
Calliope	0.004	0.000	0.015	0.000
Daintree	0.001	0.000	0.002	0.021
Don	0.036	0.001	0.007	0.018
Endeavour	0.001	0.000	0.003	0.000
Fitzroy	0.000	0.008	0.008	0.000
Haughton	0.019	0.002	0.009	0.179
Herbert	0.001	0.003	0.002	0.077
Jacky Jacky	0.000	0.000	0.000	0.000
Jeannie	0.0001	0.000	0.001	0.000
Johnstone	0.007	0.001	0.007	0.108
Kolan	0.022	0.003	0.001	0.048

Basin	Relative horticulture (proportion) [1]	Relative irrigated cropping (proportion) [1]	Relative “other” (proportion) [1,2]	Relative sugar (proportion) [1]
Lockhart	0.000	0.000	0.000	0.000
Mary	0.009	0.004	0.007	0.020
Mossman	0.002	0.000	0.022	0.109
Mulgrave-Russell	0.004	0.000	0.014	0.123
Murray	0.004	0.000	0.005	0.150
Normanby	0.001	0.000	0.000	0.000
O'Connell	0.002	0.001	0.010	0.134
Olive Pascoe	0.000	0.000	0.000	0.000
Pioneer	0.001	0.000	0.013	0.204
Plane	0.002	0.002	0.019	0.254
Proserpine	0.005	0.000	0.010	0.096
Ross	0.013	0.002	0.054	0.000
Shoalwater	0.000	0.000	0.001	0.000
Stewart	0.000	0.000	0.000	0.000

Basin	Relative horticulture (proportion) [1]	Relative irrigated cropping (proportion) [1]	Relative "other" (proportion) [1,2]	Relative sugar (proportion) [1]
Styx	0.000	0.000	0.001	0.000
Tully	0.003	0.000	0.003	0.128
Waterpark	0.007	0.000	0.004	0.000

[1.] The surface area of the stated land use expressed as a proportion of the surface area of the basin. [2] Land use types were agglomerated into 11–13 categories that align with the Reef Categories of the Source Catchment models (Waters et al., 2014). The agglomerated landuse 'Other' is a combination of intensive animal production (e.g. poultry farms, feedlots), manufacturing & industrial (e.g. food processing plants, abattoirs, sawmills), residential and farm infrastructure, services (e.g. recreation, defence), utilities (e.g. water extraction, power generation), transport (e.g. roads, railways), mining, and waste management (e.g. effluent, landfill, sewage).

Table 70. Relative surface area of each basin for urban, water, wetland land uses, the adopted middle thread distance (AMTD) and the relative monitored catchment size

Basin	Relative urban (proportion)[1, 2]	Relative water (proportion)[1]	Relative wetland (proportion)[1]	AMTD (m)[1, 3]	Relative basin size (proportion)[4]
Baffle	0.018	0.009	0.031	0	0.0096
Barron	0.057	0.020	0.014	0	0.0053
Black	0.040	0.005	0.021	0	0.0025
Boyne	0.007	0.024	0.006	0	0.0059
Burdekin	0.0006	0.007	0.012	0	0.3066
Burnett	0.011	0.005	0.0005	0	0.0782
Burrum	0.045	0.013	0.007	0	0.0080
Calliope	0.019	0.005	0.034	0	0.0053
Daintree	0.011	0.006	0.022	0	0.0045
Don	0.007	0.013	0.064	0	0.0088
Endeavour	0.014	0.003	0.009	0	0.0051
Fitzroy	0.002	0.004	0.002	0	0.3359
Haughton	0.008	0.020	0.057	0	0.0096
Herbert	0.007	0.004	0.038	0	0.0232
Jacky Jacky	0.000	0.013	0.086	0	0.0068
Jeannie	0.000	0.005	0.064	0	0.0086
Johnstone	0.029	0.008	0.014	0	0.0055
Kolan	0.034	0.027	0.003	0	0.0069

Basin	Relative urban (proportion)[1, 2]	Relative water (proportion)[1]	Relative wetland (proportion) [1]	AMTD (m)[1, 3]	Relative basin size (proportion)[4]
Lockhart	0.000	0.005	0.058	0	0.0068
Mary	0.058	0.009	0.004	0	0.0223
Mossman	0.051	0.003	0.045	0	0.0010
Mulgrave-Russell	0.033	0.009	0.044	0	0.0047
Murray	0.012	0.004	0.020	0	0.0026
Normanby	0.000	0.001	0.008	0	0.0574
O'Connell	0.029	0.012	0.065	0	0.0056
Olive Pascoe	0.000	0.003	0.000	0	0.0098
Pioneer	0.029	0.019	0.015	0	0.0037
Plane	0.038	0.015	0.074	0	0.0060
Proserpine	0.024	0.023	0.049	0	0.0059
Ross	0.072	0.038	0.040	0	0.0040
Shoalwater	0.000	0.002	0.119	0	0.0085
Stewart	0.000	0.001	0.037	0	0.0064
Styx	0.000	0.006	0.116	0	0.0071

Basin	Relative urban (proportion)[1, 2]	Relative water (proportion)[1]	Relative wetland (proportion) [1]	AMTD (m)[1, 3]	Relative basin size (proportion)[4]
Tully	0.012	0.014	0.012	0	0.0040
Waterpark	0.041	0.004	0.050	0	0.0043

[1] The surface area of the stated land use expressed as a proportion of the surface area of the basin. [2] The agglomerated land use ‘urban’ is a combination of residential types such as urban residential, remote communities, and residential without agriculture. It also includes two types of residential land uses associated with agriculture: residential with farm infrastructure and residential with agriculture. [3] The AMTD values for all basins are zero because the discharge point is at the mouth of the waterway. [4] Relative basin size is the surface area monitored by each site expressed as a proportion of the entire Great Barrier Reef catchment area (i.e. the total surface area of all catchments that discharge to the GBR).

Table 71. The average relative rainfall, average relative runoff, and average daily rainfall for each Natural Resource Management Region and year and for the Great Barrier Reef Catchment Area and each year

NRM region	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion) [2]	Average daily rainfall (mm)
Burdekin	2015–2016	0.473233	0.3786	3.34623
	2016–2017	0.496868	0.481182	3.722473
	2017–2018	0.4972	0.506341	4.722527
Burnett Mary	2015–2016	0.483219	0.440371	3.503898
	2016–2017	0.457726	0.357604	3.714579
	2017–2018	0.529638	0.6631	6.790659
Cape York	2015–2016	0.456125	0.422461	4.67969
	2016–2017	0.473658	0.358197	5.651374
	2017–2018	0.475219	0.520813	7.159524
Fitzroy	2015–2016	0.462874	0.433059	3.155647
	2016–2017	0.471144	0.422394	4.755586
	2017–2018	0.491807	0.501555	3.556777
Mackay Whitsunday	2015–2016	0.477972	0.442989	6.417714
	2016–2017	0.50865	0.583294	11.79329
	2017–2018	0.501802	0.457883	4.861081
Wet Tropics	2015–2016	0.488353	0.441929	8.50765
	2016–2017	0.482364	0.52578	9.466003

NRM region	Sampling year	Average relative rainfall (proportion)[1]	Average relative runoff (proportion) [2]	Average daily rainfall (mm)
	2017–2018	0.512002	0.607123	14.64141
Great Barrier Reef Catchment Area	2015–2016	0.473958	0.428625	5.161192
	2016–2017	0.479425	0.450617	6.451669
	2017–2018	0.50024	0.544721	7.532067

[1.] The average rainfall for the year expressed as a proportion of the long-term average rainfall for that basin [2.] The average runoff for the year expressed as a proportion of the long-term average run-off for that basin

Table 72. The relative surface area of bananas, conservation, dryland cropping, forestry and forested grazing land uses expressed as a proportion of the total surface area of the appropriate Natural Resource Management (NRM) Region or the Great Barrier Reef Catchment Area (GBRCA) for each combination of site and year

NRM region	Sampling year	Relative bananas (proportion)	Relative conservation (proportion)	Relative dryland cropping (proportion)	Relative forestry (proportion)	Relative grazing forested (proportion)
Burdekin	2015–2016	2.59×10^{-7}	0.068285	0.00894	0.00659	0.564195
	2016–2017	2.59×10^{-7}	0.068285	0.00894	0.00659	0.564195
	2017–2018	2.59×10^{-7}	0.068285	0.00894	0.00659	0.564195
Burnett Mary	2015–2016	0	0.089386	0.015439	0.138754	0.474651
	2016–2017	0	0.089386	0.015439	0.138754	0.474651
	2017–2018	0	0.089386	0.015439	0.138754	0.474651
Cape York	2015–2016	0	0.589655	0.001321	0.000544	0.377554
	2016–2017	0	0.589655	0.001321	0.000544	0.377554
	2017–2018	0	0.589655	0.001321	0.000544	0.377554
Fitzroy	2015–2016	1.25×10^{-6}	0.075796	0.051012	0.061297	0.420764
	2016–2017	1.25×10^{-6}	0.075796	0.051012	0.061297	0.420764
	2017–2018	1.25×10^{-6}	0.075796	0.051012	0.061297	0.420764
Mackay Whitsunday	2015–2016	2.06×10^{-5}	0.293984	0	0.082097	0.238348
	2016–2017	2.06×10^{-5}	0.293984	0	0.082097	0.238348
	2017–2018	2.06×10^{-5}	0.293984	0	0.082097	0.238348
Wet Tropics	2015–2016	0.006764	0.475844	0.00039	0.034775	0.288626

NRM region	Sampling year	Relative bananas (proportion)	Relative conservation (proportion)	Relative dryland cropping (proportion)	Relative forestry (proportion)	Relative grazing forested (proportion)
	2016–2017	0.006764	0.475844	0.00039	0.034775	0.288626
	2017–2018	0.006764	0.475844	0.00039	0.034775	0.288626
Great Barrier Reef Catchment Area	2015–2016	0.000345	0.153973	0.023844	0.045787	0.457951
	2016–2017	0.000345	0.153973	0.023844	0.045787	0.457951
	2017–2018	0.000345	0.153973	0.023844	0.045787	0.457951

Table 73. The relative surface area of open grazing, horticulture, irrigated cropping, “other” and sugar cane land uses expressed as a proportion of the total surface area of the appropriate Natural Resource Management (NRM) Region or the Great Barrier Reef Catchment Area (GBRCA) for each combination of site and year

NRM region	Sampling year	Relative grazing open (proportion)	Relative horticulture (proportion)	Relative irrigated cropping (proportion)	Relative “other” (proportion)[1]	Relative sugar cane (proportion)
Burdekin	2015–2016	0.31331	0.00183	0.000583	0.003153	0.007362
	2016–2017	0.31331	0.00183	0.000583	0.003153	0.007362
	2017–2018	0.31331	0.00183	0.000583	0.003153	0.007362
Burnett Mary	2015–2016	0.210542	0.006917	0.00878	0.003799	0.01634
	2016–2017	0.210542	0.006917	0.00878	0.003799	0.01634
	2017–2018	0.210542	0.006917	0.00878	0.003799	0.01634
Cape York	2015–2016	0.007768	0.000397	0.000206	0.000403	0
	2016–2017	0.007768	0.000397	0.000206	0.000403	0
	2017–2018	0.007768	0.000397	0.000206	0.000403	0
Fitzroy	2015–2016	0.359059	0.000463	0.007793	0.007961	2.13×10^{-5}
	2016–2017	0.359059	0.000463	0.007793	0.007961	2.13×10^{-5}
	2017–2018	0.359059	0.000463	0.007793	0.007961	2.13×10^{-5}
Mackay Whitsunday	2015–2016	0.098684	0.002455	0.000721	0.013046	0.169711
	2016–2017	0.098684	0.002455	0.000721	0.013046	0.169711
	2017–2018	0.098684	0.002455	0.000721	0.013046	0.169711
Wet Tropics	2015–2016	0.03635	0.007935	0.003921	0.005526	0.084297
	2016–2017	0.03635	0.007935	0.003921	0.005526	0.084297

NRM region	Sampling year	Relative grazing open (proportion)	Relative horticulture (proportion)	Relative irrigated cropping (proportion)	Relative "other" (proportion)[1]	Relative sugar cane (proportion)
	2017–2018	0.03635	0.007935	0.003921	0.005526	0.084297
Great Barrier Reef Catchment Area	2015–2016	0.267631	0.002141	0.004399	0.005065	0.012401
	2016–2017	0.267631	0.002141	0.004399	0.005065	0.012401
	2017–2018	0.267631	0.002141	0.004399	0.005065	0.012401

[1.] Land use types were agglomerated into 11–13 categories that align with the Reef Categories of the Source Catchment models (Waters et al., 2014). The agglomerated land use 'Other' is a combination of intensive animal production (e.g. poultry farms, feedlots), manufacturing & industrial (e.g. food processing plants, abattoirs, sawmills), residential and farm infrastructure, services (e.g. recreation, defence), utilities (e.g. water extraction, power generation), transport (e.g. roads, railways), mining, and waste management (e.g. effluent, landfill, sewage)

Table 74. The relative surface area of urban, water, wetland land uses expressed as a proportion of the total surface area of the appropriate Natural Resource Management (NRM) Region or the Great Barrier Reef Catchment Area (GBRCA) for each combination of site and year

NRM region	Sampling year	Relative urban (proportion)[1]	Relative water (proportion)	Relative wetland (proportion)
Burdekin	2015–2016	0.002131	0.008137	0.015484
	2016–2017	0.002131	0.008137	0.015484
	2017–2018	0.002131	0.008137	0.015484
Burnett Mary	2015–2016	0.023435	0.008008	0.003949
	2016–2017	0.023435	0.008008	0.003949
	2017–2018	0.023435	0.008008	0.003949
Cape York	2015–2016	0.000823	0.002635	0.018693
	2016–2017	0.000823	0.002635	0.018693
	2017–2018	0.000823	0.002635	0.018693
Fitzroy	2015–2016	0.002986	0.004506	0.008341
	2016–2017	0.002986	0.004506	0.008341
	2017–2018	0.002986	0.004506	0.008341
Mackay Whitsunday	2015–2016	0.029879	0.016917	0.054138
	2016–2017	0.029879	0.016917	0.054138
	2017–2018	0.029879	0.016917	0.054138
Wet Tropics	2015–2016	0.018662	0.007793	0.029118
	2016–2017	0.018662	0.007793	0.029118
	2017–2018	0.018662	0.007793	0.029118
Great Barrier Reef Catchment Area	2015–2016	0.006409	0.006419	0.013635
	2016–2017	0.006409	0.006419	0.013635
	2017–2018	0.006409	0.006419	0.013635

[1]. The agglomerated land use 'urban' is a combination of residential types such as urban residential, remote communities, and residential without agriculture. It also includes two types of residential land uses associated with agriculture: residential with farm infrastructure and residential with agriculture

Attachment J – Data used by Spilsbury et al. (2020)

Table 75. The 50 pesticides included in the determination of the total toxicity of pesticides by Spilsbury et al. (2020)

2,4-D	Fluroxypyr	Metsulfuron-methyl
2,4-DB	Flusilazole	Napropamide
3,4-Dichloroaniline	Glyphosate	Prometryn
Acetamiprid	Haloxyfop	Propachlor
Acifluorfen	Hexazinone	Propazine
Ametryn	Imazapic	Sethoxydim
AMPA	Imazapyr	Simazine
Atrazine	Imazethapyr	Sulfosulfuron
Bromacil	Imidacloprid	Tebuthiuron
Clomazone	Isoxaflutole	Terbuthylazine
Clothianidin	MCPA	Terbuthylazine desethyl
Cyanazine	MCPB	Terbutryn
Desethyl Atrazine	Mecoprop	Thiacloprid
Desisopropyl Atrazine	Mesosulfuron methyl	Thiamethoxam
Diuron	Methoxyfenozide	Triclopyr
Ethametsulfuron methyl	Metolachlor	Trifloxysulfuron
Fluometuron	Metribuzin	

Table 76. The average percent of the total pesticide toxicity contributed by each pesticide included in the Pesticide Risk Metric and Pesticide Risk Baseline. Percentages obtained from Spilsbury et al. (2020). The 28 pesticides included in Table 75 but not included here contributed less than 0.01% of the total pesticide toxicity

Pesticide	**% contribution to total pesticide toxicity**	**Pesticide**	**% contribution to total pesticide toxicity**
2,4-D	1.2	Isoxaflutole	1.38
Ametryn	1.31	MCPA	0.32
Atrazine	14.5	Metolachlor	3.49
Chlorpyrifos	NI	Metribuzin	0.65
Diuron	45.7	Metsulfuron-methyl	0.83
Fipronil	NI	Pendimethalin	NI
Fluroxypyr	<0.01	Prometryn	0.01
Haloxyfop	<0.01	Simazine	<0.01
Hexazinone	2.3	Tebuthiuron	<0.01
Imazapic	1.52	Terbuthylazine	0.01
Imidacloprid	26.1	Triclopyr	<0.01

NI = Not included in Spilsbury et al. (2020)

Attachment K – Pesticide mixture toxicity estimates for PSII Herbicides, Other Herbicides and Insecticides

Table 77. Estimated per cent of aquatic species affected by PSII Herbicides at the sites used in this study

Monitoring site	Estimated per cent of species affected		
	2015–2016	**2016–2017**	**2017–2018**
Baffle Creek at Newton Road	-	-	3.15×10^{-5}
Barratta Creek at Northcote	14.28	23.20	19.87
Barron River at Rink's Close Jetty	-	-	0.24
Black River at Bruce Highway	-	-	6.83×10^{-6}
Boyne River at Boyne Island	-	-	3.29×10^{-7}
Burdekin River at Home Hill	0.08	0.09	0.04
Burnett River at Ben Anderson Barrage	0.21	0.23	-
Burnett River at Quay St. Bridge	-	-	0.86
Burrum River at Buxton Boat Ramp	-	-	0.04
Calliope River at Old Bruce Highway	-	-	9.64×10^{-6}
Comet River at Comet Weir	2.22	3.52	5.76
East Barratta Creek at Jerona Road	-	-	7.28
Elliot River at Riverview Boat Ramp	-	-	1.60
Fitzroy River at Rockhampton	0.24	0.07	0.36
Gregory River at Jarrett's Road	-	-	3.65
Haughton River at Powerline	5.57	4.34	-
Haughton River at Giru Weir tailwater	-	-	0.92
Herbert River at Ingham	0.67	1.99	0.93
Johnstone River at Coquette Point	0.69	1.51	1.83
Kolan River at Booyan Boat Ramp	-	-	4.16
Mary River at Home Park	0.21	0.33	-
Mary River at Churchill St.	-	-	0.91
Mossman River at Bonnie Doon	-	-	2.31
Mulgrave River at Deeral	1.08	2.52	2.99
North Johnstone River at Old Brice Highway (Goondi)	0.05	0.27	0.13

Monitoring site	Estimated per cent of species affected		
	2015–2016	2016–2017	2017–2018
O'Connell River at Stafford's Crossing	-	4.47	4.12
O'Connell River at Caravan Park	1.84	4.22	3.88
Pioneer River at Dumbleton Pump Station	11.01	9.94	19.17
Proserpine River at Glen Isla	-	13.47	16.34
Russell River at East Russell	1.68	2.98	3.71
Sandy Creek at Homebush	27.05	25.78	27.91
Styx River at Ogmore	-	-	9.21×10^{-4}
Tinana Creek at Barrage	4.09	1.41	-
Tully River at Euramo	2.12	3.37	3.70
Waterpark Creek	-	-	5.53×10^{-11}

Table 78. Estimated per cent of aquatic species affected by Other Herbicides for the sites used in this project

Monitoring site	Estimated per cent of species affected		
	2015–2016	2016–2017	2017–2018
Baffle Creek at Newton Road	-	-	0.14
Barratta Creek at Northcote	5.87	4.99	4.09
Barron River at Rink's Close Jetty	-	-	0.27
Black River at Bruce Highway	-	-	0.49
Boyne River at Boyne Island	-	-	2.23×10^{-3}
Burdekin River at Home Hill	0.36	1.39	0.52
Burnett River at Ben Anderson Barrage	1.32	1.63	-
Burnett River at Quay St. Bridge	-	-	2.27
Burrum River at Buxton Boat Ramp	-	-	0.24
Calliope River at Old Bruce Highway	-	-	0.08
Comet River at Comet Weir	6.36	6.33	6.02
East Barratta Creek at Jerona Road	-	-	2.52
Elliot River at Riverview Boat Ramp	-	-	2.23
Fitzroy River at Rockhampton	1.52	1.67	2.06
Gregory River at Jarrett's Road	-	-	3.03
Haughton River at Powerline	1.27	1.97	-
Haughton River at Giru Weir tailwater	-	-	1.05
Herbert River at Ingham	0.26	1.39	0.68
Johnstone River at Coquette Point	0.49	0.34	0.96
Kolan River at Booyan Boat Ramp	-	-	1.46
Mary River at Home Park	1.87	2.50	-
Mary River at Churchill St.	-	-	2.44
Mossman River at Bonnie Doon	-	-	1.46
Mulgrave River at Deeral	0.88	1.68	2.42
North Johnstone River at Old Bruce Highway (Goondi)	0.08	0.35	0.02
O'Connell River at Stafford's Crossing	-	2.72	2.04
O'Connell River at Caravan Park	2.15	2.23	2.09

Monitoring site	Estimated per cent of species affected		
	2015–2016	**2016–2017**	**2017–2018**
Pioneer River at Dumbleton Pump Station	3.35	3.27	3.79
Proserpine River at Glen Isla	-	7.01	6.17
Russell River at East Russell	0.48	0.98	2.04
Sandy Creek at Homebush	9.13	9.47	10.35
Styx River at Ogmore	-	-	2.23×10^{-3}
Tinana Creek at Barrage	5.73	2.30	-
Tully River at Euramo	0.52	1.01	0.53
Waterpark Creek at Corbetts Landing	-	-	2.23×10^{-3}

Table 79. Estimated per cent of aquatic species affected by Insecticides for the sites used in this project

Monitoring site	Estimated per cent of species affected		
	2015–2016	2016–2017	2017–2018
Baffle Creek at Newton Road	-	-	1.0×10^{-4}
Barratta Creek at Northcote	1.96	0.74	0.23
Barron River at Rinks Close Jetty	-	-	0.20
Black River at Bruce Highway	-	-	1.0×10^{-4}
Boyne River at Boyne Island	-	-	1.09×10^{-4}
Burdekin River at Home Hill	1.00×10^{-11}	1.15×10^{-3}	0.08
Burnett River at Ben Anderson Barrage	1.00×10^{-11}	2.32×10^{-3}	-
Burnett River at Quay St. Bridge	-	-	0.12
Burrum River at Buxton Boat Ramp	-	-	1.09×10^{-4}
Calliope River at Old Bruce Highway	-	-	1.09×10^{-4}
Comet River at Comet Weir	3.26×10^{-10}	0.01	1.10×10^{-4}
East Barratta Creek at Jerona Road	-	-	0.01
Elliot River at Riverview Boat Ramp	-	-	0.82
Fitzroy River at Rockhampton	1.01×10^{-11}	0.28	1.10×10^{-4}
Gregory River at Jarretts Road	-	-	1.15
Haughton River at Powerline	1.02×10^{-11}	1.35×10^{-3}	-
Haughton River at Giru Weir tailwater	-	-	1.59
Herbert River at Ingham	1.66	2.31	2.59
Johnstone River at Coquette Point	2.15	1.77	2.58
Kolan River at Booyan Boat Ramp	-	-	0.33
Mary River at Home Park	1.01×10^{-11}	4.91×10^{-3}	-
Mary River at Churchill St.	-	-	0.06
Mossman River at Bonnie Doon	-	-	0.30
Mulgrave River at Deeral	0.12	0.30	0.87
North Johnstone River at Old Bruce Highway (Goondi)	2.28	3.90	2.19
O'Connell River at Stafford's Crossing	-	2.57	6.24
O'Connell River at Caravan Park	4.17	7.35	2.62

Monitoring site	Estimated per cent of species affected		
	2015–2016	2016–2017	2017–2018
Pioneer River at Dumbleton Pump Station	5.99	6.33	5.48
Proserpine River at Glen Isla	-	13.85	13.78
Russell River at East Russell	0.72	0.74	1.27
Sandy Creek at Homebush	13.39	13.39	11.35
Styx River at Ogmore	-	-	1.09×10^{-4}
Tinana Creek at Barrage	0.53	0.15	-
Tully River at Euramo	2.58	3.40	2.71
Waterpark Creek	-	-	1.09×10^{-4}

Attachment L – Diagnostic figures for the PSII Herbicide relationship

The diagnostic diagrams used to assess the underlying assumptions of regression analysis are presented in Figure 41. The top left figure shows the distribution of the residuals versus fitted values (ideally the residuals should be equally spaced above and below the residual = 0 line and evenly spaced along the x axis). The top right figure shows the normality of the data (perfectly normal data would lie on the dashed line). The bottom right figure uses Cooks distance to indicate outliers (data with Cooks distance values of greater than 0.4 were deemed to be outliers and values to the extreme right were deemed to be influential sites). The bottom figure shows the actual Cooks distance values for each site/year combination.

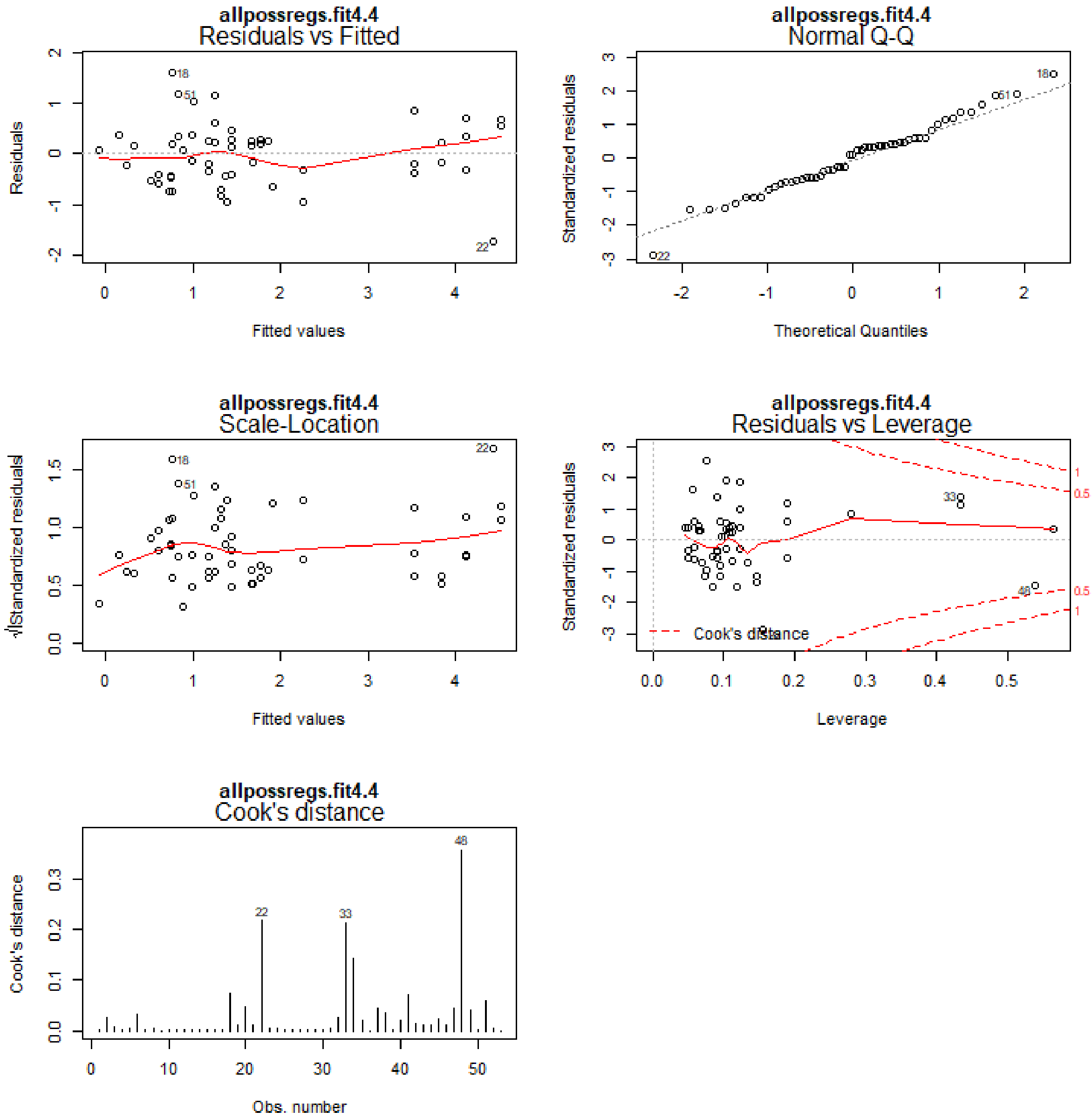

Figure 41. Diagnostic figures of the assumptions of regression analysis for the photosystem II inhibiting herbicides

The GVIF and GVIF$^{(1/2 \times DF)}$ values for the PSII relationship are presented in Table 80. The second (middle) column of GVIF values are the Generalised Variance Inflation Factors that refer to the increase in the variance of that coefficient due to its collinearity with the other variables, accounting for the correlation introduced due to the polynomial terms. The third (right) column of GVIF$^{(1/2 \times DF)}$ are the standardised GVIF values which are comparable across a different number of parameters (Fox and Monette, 1992). Whether a parameter is highly correlated to another parameter in a regression model can be determined by squaring the GVIF$^{(1/2 \times DF)}$ values and comparing the resulting value to a cut-off value of 4. If the squared GVIF$^{(1/2 \times DF)}$ value for a parameter is less than four it is considered to not be highly correlated to the other parameters. A squared GVIF$^{(1/2 \times DF)}$ value greater than four indicates the parameter is highly correlated to another parameters and should be investigated and possibly removed from the regression model. This cut-off value of four is the lowest (most restrictive) of the cut-off values (O'Brien, 2007) commonly used to assess the collinearity of parameters and thus makes it more difficult to accept the use of parameters. As the square of the GVIF$^{(1/2 \times DF)}$ values were all less than four they were classed as not being highly correlated and were retained in the pesticide mixture toxicity – land use relationship for PSII Herbicides.

Table 80. The generalized variance-inflation factors for the photosystem II herbicides . The bold values are the values that were used to evaluate collinearity

Variable	GVIF	GVIF$^{(1/2 \times DF)}$ [#]
AMTD	1.6471	**1.2834**
% Conservation	1.7098	**1.3076**
% Horticulture	1.6654	**1.2905**
% Irrigated cropping	1.5250	**1.2349**
% Sugar cane$_{poly}$[##]	1.7691	**1.1533**

[#] values of less than two are not highly correlated as the squared value will not exceed four. [##] A quadratic (second order) polynomial function was applied to this variable

Attachment M – Diagnostic figures for the Other Herbicide relationship

The diagnostic diagrams used to assess the underlying assumptions of regression analysis for the Other Herbicides relationship are presented in Figure 42. The top left figure shows the distribution of the residuals versus fitted values (ideally the residuals should be equally spaced above and below the residual = 0 line and evenly spaced along the x axis). The top right figure shows the normality of the data (perfectly normal data would lie on the dashed line). The bottom right figure uses Cooks distance to indicate outliers (data with Cooks distance values of greater than 0.4 were deemed to be outliers and values to the extreme right were deemed to be influential sites). The bottom figure shows the actual Cooks distance values for each site/year combination.

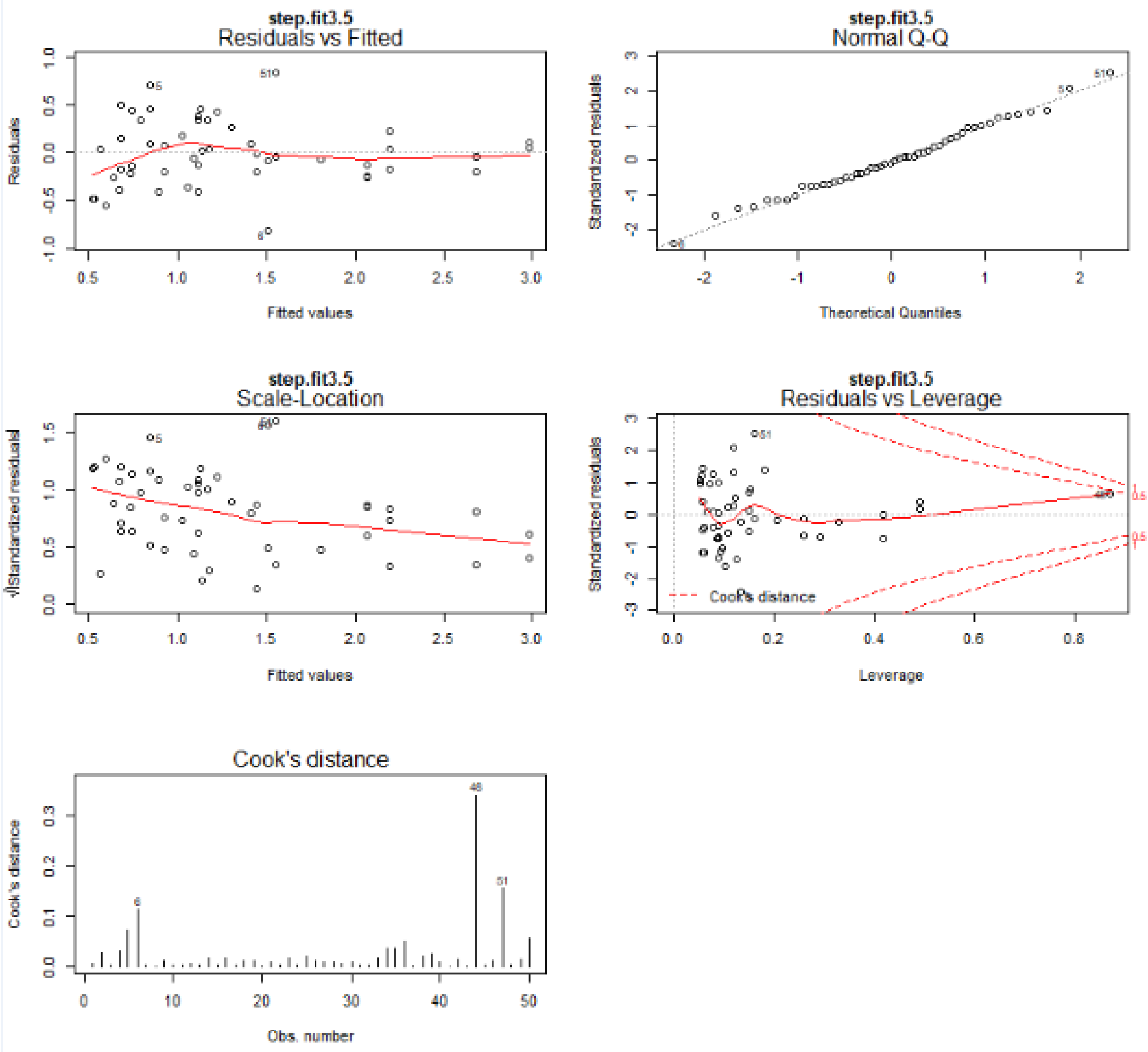

Figure 42. Diagnostic figures of the assumptions of regression analysis for the Other Herbicides relationship

The GVIF and GVIF$^{(1/2 \times DF)}$ values for the PSII relationship are presented in Table 81. The second (middle) column of GVIF values are the Generalised Variance Inflation Factors that refer to the increase in the variance of that coefficient due to its collinearity with the other variables, accounting for the correlation introduced due to the polynomial terms. The third (right) column of GVIF$^{(1/2 \times DF)}$ are the standardised GVIF values which are comparable across a different number of parameters (Fox and Monette, 1992). Whether a parameter is highly correlated to another parameter in a regression model can be determined by squaring the GVIF$^{(1/2 \times DF)}$ values and comparing the resulting value to a cut-off value of 4. If the squared GVIF$^{(1/2 \times DF)}$ value for a parameter is less than four it is considered to not be highly correlated to the other parameters. A squared GVIF$^{(1/2 \times DF)}$ value greater than four indicates the parameter is highly correlated to another parameters and should be investigated and possibly removed from the regression model. This cut-off value of four is the lowest (most restrictive) of the cut-off values (O'Brien, 2007) commonly used to assess the collinearity of parameters and thus makes it more difficult to accept the use of parameters. As the square of the GVIF$^{(1/2 \times DF)}$ values were all less than four they were classed as not being highly correlated and were retained in the pesticide mixture toxicity – land use relationship for other Other Herbicides.

Table 81. The generalized variance-inflation factors for the Other Herbicides. The bold values are the values that were used to evaluate collinearity

Variable	GVIF	GVIF$^{(1/2 \times DF)}$ #
% Urban	1.478	**1.216**
% Conservation	1.287	**1.134**
% Horticulture$_{poly}$ ##	1.538	**1.114**
% Dryland cropping	1.262	**1.123**
% Sugar cane$_{poly}$ ##	1.676	**1.138**

values of less than two are not highly correlated as the squared value will not exceed four. ## A quadratic (second order) polynomial function was applied to this variable

Attachment N – Diagnostic figures for the Insecticide relationship

The diagnostic diagrams used to assess the underlying assumptions of regression analysis for the Insecticide relationship are presented in Figure 43. The top left figure shows the distribution of the residuals versus fitted values (ideally the residuals should be equally spaced above and below the residual = 0 line and evenly spaced along the x axis). The top right figure shows the normality of the data (perfectly normal data would lie on the dashed line). The bottom right figure uses Cooks distance to indicate outliers (data with Cooks distance values of greater than 0.4 were deemed to be outliers and values to the extreme right were deemed to be influential sites). The bottom figure shows the actual Cooks distance values for each site/year combination.

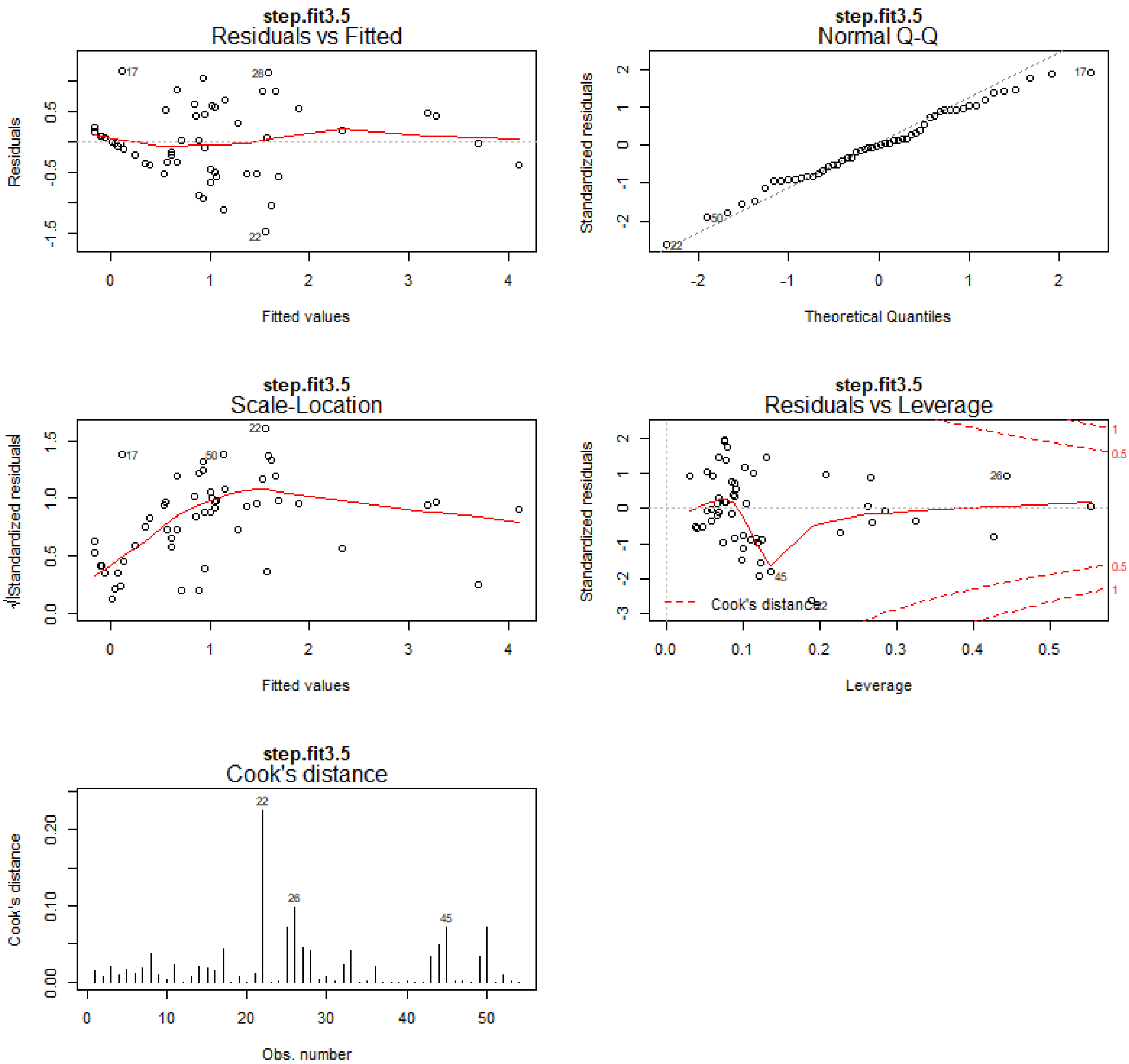

Figure 43. Diagnostic figures of the assumptions of regression analysis for the Insecticide relationship

The GVIF and GVIF$^{(1/2 \times DF)}$ values for the PSII relationship are presented in Table 82. The second (middle) column of GVIF values are the Generalised Variance Inflation Factors that refer to the increase in the variance of that coefficient due to its collinearity with the other variables, accounting for the correlation introduced due to the polynomial terms. The third (right) column of GVIF$^{(1/2 \times DF)}$ are the standardised GVIF values which are comparable across a different number of parameters (Fox and Monette, 1992). Whether a parameter is highly correlated to another parameter in a regression model can be determined by squaring the GVIF$^{(1/2 \times DF)}$ values and comparing the resulting value to a cut-off value of 4. If the squared GVIF$^{(1/2 \times DF)}$ value for a parameter is less than four it is considered to not be highly correlated to the other parameters. A squared GVIF$^{(1/2 \times DF)}$ value greater than four indicates the parameter is highly correlated to another parameters and should be investigated and possibly removed from the regression model. This cut-off value of four is the lowest (most restrictive) of the cut-off values (O'Brien, 2007) commonly used to assess the collinearity of parameters and thus makes it more difficult to accept the use of parameters. As the square of the GVIF$^{(1/2 \times DF)}$ values were all less than four they were classed as not being highly correlated and were retained in the pesticide mixture toxicity – land use relationship for other Insecticides.

Table 82. Generalized variance-inflation factors for the variables in the Insecticide relationship . The bold values are the GVIF values that were used to evaluate collinearity

Variable	GVIF	GVIF$^{(1/2 \times DF)}$ #
Average rainfall	2.055	**1.434**
% Forestry	1.502	**1.226**
% Water	1.629	**1.276**
% Relative grazing forested	2.787	**1.669**
% Horticulture	1.117	**1.056**
% Sugar cane	2.340	**1.530**

values of less than two are not highly correlated as the squared value will not exceed four

Attachment O – Diagnostic figures for the Total Pesticide mixture relationship

The diagnostic diagrams used to assess the underlying assumptions of regression analysis for the Total Pesticide mixture relationship are presented in Figure 44. The top left figure shows the distribution of the residuals versus fitted values (ideally the residuals should be equally spaced above and below the residual = 0 line and evenly spaced along the x axis). The top right figure shows the normality of the data (perfectly normal data would lie on the dashed line). The bottom right figure uses Cooks distance to indicate outliers (data with Cooks distance values of greater than 0.4 were deemed to be outliers and values to the extreme right were deemed to be influential sites). The bottom figure shows the actual Cooks distance values for each site/year combination.

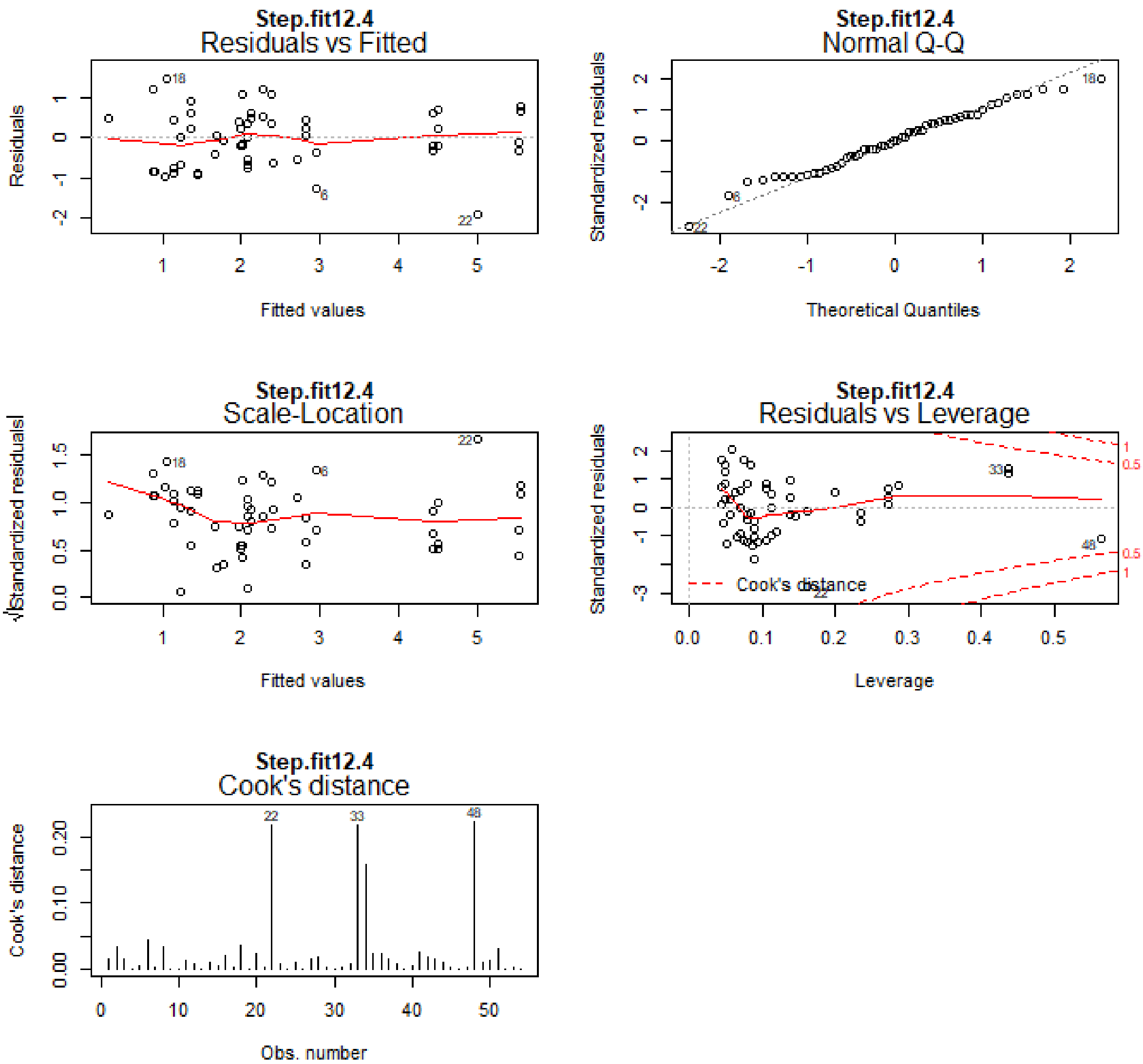

Figure 44. Diagnostic figures of the assumptions of regression analysis for the Total Pesticide mixture relationship

The GVIF and GVIF$^{(1/2 \times DF)}$ values for the PSII relationship are presented in Table 83. The second (middle) column of GVIF values are the Generalised Variance Inflation Factors that refer to the increase in the variance of that coefficient due to its collinearity with the other variables, accounting for the correlation introduced due to the polynomial terms. The third (right) column of GVIF$^{(1/2 \times DF)}$ are the standardised GVIF values which are comparable across a different number of parameters (Fox and Monette, 1992). Whether a parameter is highly correlated to another parameter in a regression model can be determined by squaring the GVIF$^{(1/2 \times DF)}$ values and comparing the resulting value to a cut-off value of 4. If the squared GVIF$^{(1/2 \times DF)}$ value for a parameter is less than four it is considered to not be highly correlated to the other parameters. A squared GVIF$^{(1/2 \times DF)}$ value greater than four indicates the parameter is highly correlated to another parameters and should be investigated and possibly removed from the regression model. This cut-off value of four is the lowest (most restrictive) of the cut-off values (O'Brien, 2007) commonly used to assess the collinearity of parameters and thus makes it more difficult to accept the use of parameters. As the square of the GVIF$^{(1/2 \times DF)}$ values were all less than four they were classed as not being highly correlated and were retained in the pesticide mixture toxicity – land use relationship for Total Pesticides.

Table 83. Generalized variance-inflation factors for the variables in the Total Pesticide mixture relationship . The bold values are the GVIF values that that were used to evaluate collinearity

Variable	GVIF	GVIF$^{(1/2 \times DF)}$ #
% Dryland cropping	1.339	**1.157**
% Sugar cane$_{poly}$##	1.547	**1.115**
% Conservation	1.318	**1.148**
% Horticulture	1.223	**1.106**
% Urban	1.320	**1.149**

values of less than two are not highly correlated as the squared value will not exceed four. ## A quadratic (second order) polynomial function was applied to this variable