Review of toxicity of agricultural chemicals and implications for aquatic fauna of the Keep River

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For the NWGA project "Managing water quality to enable future irrigation development in the Kimberley Region, WA", in support of the report by GHD Pty. Ltd. "Three-dimensional hydrodynamic modelling to evaluate effect of farm chemicals on the lower pools of the Keep River".

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Preface

This report was compiled for the Western Australian Department of Primary Industries and Regional Development (DPIRD) as part of the National Water Grid Authority (NWGA) project entitled "Managing water quality to enable future irrigation development in the Kimberley Region, WA". The purpose of this report is to review the toxicity of farm chemicals (herbicides and insecticides) used by farm operators in the Keep River catchment and their potential impacts on the aquatic species in the river, in particular those species that are listed under the *Environment Protection and Biodiversity Conservation Act 1999*. It is designed to complement the report entitled "Three-dimensional hydrodynamic modelling to evaluate effect of farm chemicals on the lower pools of the Keep River" compiled by GHD Pty. Ltd., and to inform management of current and future agricultural practices in the catchment.

Investigators from Murdoch University, DPIRD, GHD, The University of Queensland and Farmacist presented preliminary results of these investigations into the potential toxicity of agricultural chemicals in use on farms in the Keep River catchment and mitigation and management options to key stakeholders in Kununurra on 29-30 March 2023.

1. Introduction

The Keep River lies in the Northern Territory, its catchment extending into Western Australia, and is within a freshwater fish biogeographic province known as the Kimberley Province (Shelley et al. 2018). By Kimberley Province standards, the river is small and drains an area of ~12,000 km^2 (Midgely 1981, Pusey et al. 2017). The catchment comprises little development, consisting primarily of the Keep River National Park and several pastoral stations. The lower reaches of the Keep River receive surface water discharges from the 7400 ha Goomig Farmlands development and adjoining farms within the Ord River Irrigation Area (ORIA) (Bennett and George 2014). The Goomig Farmlands, formally named the Weaber Plain Development Project, was approved under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) in 2011, (EPBC 2010/5491), subject to conditions designed to protect the aquatic environment of downstream surface waters.

Baseline water quality monitoring in the Keep River was conducted prior to irrigation commencing and the subsequent report classified the lower Keep River as a 'Category 2' system i.e., 'slightly to moderately disturbed' due to the influence of both natural and anthropogenic factors including tides, climate variability, heavy metal mineralisation and cattle grazing. The river system is highly dynamic and water flow varies dramatically depending on seasonal rainfall and tidal influences (Bennett and George 2014).

Faunal surveys conducted in accordance with the Aquatic Fauna Management Plan for the Keep River show that it supports a high diversity of aquatic fauna. As well as providing refuge for threatened sawfishes (*Pristis* spp.), which are listed as Matters of National Environmental Significance (NES) under the EPBC *Act 1999*, the Keep River supports a diverse assemblage of fish and invertebrate species (WRM, 2013a, 2013b, 2014, 2021; Indo-Pacific Environmental 2022, 2023). In the 2022 faunal survey a total of 22 fish species were found in the Keep River system. This was considerably less than the 47 species found in 2021, however sampling effort was restricted in 2022 (Indo-Pacific Environmental 2023). The most abundant fish species found in both the 2021 and 2022 faunal surveys was the Bony Bream (*Nematalosa erebi*), which is one of Australia's most widespread freshwater fishes and the most commonly caught fish in the Kimberley's Fitzroy River (Lear et al. 2023). Other abundant species include the Diamond Mullet (*Planiliza ordensis*), Squaretail Mullet (*Ellochelon vaigiensis*), Blue Catfish (*Neoarius graeffei*), Blue Threadfin (*Eleutheronema tetradactylum*) and Barramundi (*Lates calcarifer*). Of the aquatic macroinvertebrate taxa collected, insects were the most abundant, followed by gastropods and crustaceans (malacostracans). No Red Claw Crayfish (*Cherax quadricarinatus*) (considered an invasive pest in the region (Doupé et al. 2004)) were found in the 2021 or 2022 faunal surveys (Indo-Pacific Environmental 2022, 2023). Bull sharks (*Carcharhinus leucas*) are also found in the Keep River (Indo-Pacific Environmental 2022, 2023) and although they are not listed under the EPBC Act, they are listed as 'Vulnerable' on the International Union for Conservation of Nature's Red List of Threatened Species (Rigby et al. 2021).

The potential for run-off from farmlands to deliver increased sediment, salinity, nutrients and farm chemicals to the aquatic ecosystem is of concern, especially for these EPBC listed species. Most species of fish and all macroinvertebrates found in the faunal surveys of the Keep River are commonly found throughout northern Australia (Morgan et al. 2011, Pusey et al. 2017, Shelley et al. 2018). However, these species are all important in food webs within the river system which support the threatened species (e.g., Thorburn et al. 2014). Chemical contamination from insecticides and herbicides has the potential to impact many of the aquatic species either directly or indirectly.

1.1 EPBC listed species in the Keep River

An Aquatic Fauna Management Plan was prepared and is being implemented in accordance with Condition 10 of EPBC Act Approval 2010/5491 to protect EPBC listed threatened aquatic fauna species in the Keep River. A targeted, non-lethal survey was undertaken for three years pre-development (2011 to 2013) in accordance with the approved Plan to determine species presence, populations, and to provide a baseline against which future changes could be assessed. The survey targeted the following EPBC listed aquatic fauna species:

- Critically Endangered Speartooth Shark (*Glyphis glyphis*)
- Endangered Northern River Shark (*Glyphis garricki*)
- Vulnerable Dwarf Sawfish (*Pristis clavata*)
- Vulnerable Freshwater Sawfish (*Pristis pristis*)

Surveys have been undertaken for a further three years post development (2020-2022) to assess whether the Goomig Farmland has adversely impacted those species.

Prior to the implementation of the aquatic fauna surveys in the Keep River, information relating to the aquatic fauna diversity was limited, although Dwarf Sawfish (*P. clavata*) and Freshwater Sawfish (*P. pristis*) had previously been recorded during scientific surveys (Midgely 1981, Larson 1999). The subsequent aquatic fauna surveys detected these two sawfish species, as well as an additional EPBC listed sawfish, the Green Sawfish (*Pristis zijsron*), but did not detect the Speartooth Shark (*G. glyphis*) or Northern River Shark (*G. garricki*) (WRM 2013a, 2013b, 2014, 2021; Indo-Pacific Environmental 2022, 2023). While only a single Green Sawfish (*P. zijsron*) was captured (in the Keep River estuary in 2011), in most years Dwarf Sawfish were recorded in the estuary, with Freshwater Sawfish recorded in one or more of the freshwater pools. A total of six Dwarf Sawfish and one Freshwater Sawfish were found in the Keep River during the 2022 faunal surveys (Indo-Pacific Environmental 2023). Previous aquatic surveys of the Keep River have found no evidence to suggest that the Goomig Development or adjoining farms in the ORIA have adversely impacted the aquatic fauna; although, as this development is relatively new and the fauna is intact, there is the potential for pesticides to alter natural balances within the system (Warne et al. 2022).

1.2 *Farm Chemicals in the environment*

Farm operators in the Keep River catchment provide annual farm chemical updates in accordance with the approved Stormwater and Groundwater Discharge Management Plan for the Goomig Farmlands. These updates inform the water quality monitoring program across multiple monitoring sites in the Keep River and upstream. Samples are taken and tested for the presence and concentration of pesticides as well as physicochemical stressors. The farm chemical updates list the products planned to be used in the coming year, their associated active ingredients and indicative timing of application. Specific information about application rates and timing is not provided and the presence of pesticides may be underestimated from the water sampling which represent distinct time points only (Schäfer et al. 2011). Historically, water quality monitoring did not cover all pesticides used on farms in the Goomig Farmlands, however testing is now carried out for a suite of over 100 chemicals, including most of those in use.

Australian and New Zealand Guidelines for Fresh and Marine Water Quality Testing (ANZG 2018) set out default guideline values (DGVs) for toxicants at various levels of species protection. Concentration levels designed to protect 99% of species (PC99) apply to the Keep River and exceedance of these triggers management responses. However, many pesticides used in the Goomig Farmland development do not have listed DGVs. DGVs are also listed for the active ingredients of pesticides rather than any commercial formulations (ANZG 2018), which can include surfactants and other adjuvants that increase the efficacy of the product (Annette et al. 2014). For example, there are many different commercial products that use glyphosate as an active ingredient, all with different mixtures of additives. Some of these additives can make the resulting products more toxic than the active ingredient alone (Annette et al. 2014), such as the polyethoxylated amines (POEA), which were identified as increasing the toxicity of some glyphosate-based commercial products more than 40 years ago (Folmar et al. 1979). Additionally, pesticides can degrade into chemicals related to but different from the parent pesticide, called pesticide transformation products (TPs) or metabolites, which are not considered by DGVs (ANZG 2018). Information on the toxicity of TPs is limited, although they can be more or less toxic than their parent pesticide (Romanok et al. 2021). Little is known of their presence in Australian aquatic ecosystems, and they are not monitored in the Keep River system. However, in the USA, almost 90% of 442 small streams surveyed across the country have at least one TP present (Romanok et al. 2021).

Pesticides used in the Keep River catchment include those that pose a relatively high risk to aquatic ecosystems (Warne et al. 2022) such as S-metolachlor, fipronil, and atrazine. These chemicals are used to control plant and animal pest species but can have unintended toxic (lethal or sub-lethal) effects on non-target species. Depending on their chemical properties, such as soil adsorption and mobility, solubility in water and degradation rates, these chemicals differ in their likelihood of entering aquatic ecosystems through soil erosion and run-off, and in their persistence in the environment. Management of the farmlands in the Keep River catchment requires the retention of tailwater and the first 25 mm of runoff from stormwater flows, to minimise the volume of pesticides flowing into the Keep River system. While effective, this intervention does not entirely avoid discharge of chemicals to the aquatic ecosystem and some pesticides have been detected in Keep River pools by water quality monitoring, including some at levels above the PC99 DGV set by ANZG (2018) (DPIRD, unpublished data).

There is also the potential for multiple chemicals to be present in the ecosystem, with possible interactions that are difficult to predict (Hernández et al. 2017). Mixtures of pesticides can be additively or synergistically more toxic than the individual chemicals (de Souza et al. 2020). The effects on aquatic fauna of exposure to these mixtures are largely unknown, but can be substantial even at low concentrations (Relyea 2009). Chemicals can persist in aquatic ecosystems longer than expected from their published halflife (DT_{50} (water)) values, depending on many environmental factors such as temperature and pH (Lewis et al. 2016b), meaning that aquatic fauna may be exposed to chemicals for longer periods, especially during the dry season (~May-November) when the river is not flowing and pools in the river are not naturally flushed. The retention of tailwater substantially mitigates this dry season risk, although residual risk remains should pesticides persist in the Keep River after wet season stormwater run-off and river flows have ceased. The recently commenced and expanding wet season cropping of cotton in the catchment poses further risk of chemicals entering the Keep River system (Pearce 2022). Planting of cotton occurs somewhere between January and February depending on seasonal conditions.

Different pesticides have varying modes of action which work to eliminate different target species; however, they can also have unintended toxic effects on non-target species. The effects of pesticide exposure on aquatic fauna vary substantially by chemical, are highly species-dependant and can have complex effects on ecosystems. Lethal effects on some species can benefit other species through reduced competition or predation, which can change community composition and species richness (Relyea 2009). Pesticides consistently affect community composition and ecosystem functions in freshwater ecosystems (Rumschlag et al. 2020), and regional aquatic diversity decreases with increasing pesticide contamination (Stehle and Schulz 2015). For example, pesticide contamination of freshwater streams has reduced the regional biodiversity of aquatic invertebrates in Europe and Australia (Beketov et al. 2013). Even at sub-lethal concentrations, pesticides can have a range of adverse effects on exposed aquatic organisms including reduced growth rates, inhibited development, and compromised immunity (Relyea 2009).

Pesticides can accumulate in the tissues of living organisms (bioaccumulation) and be passed through food webs, becoming magnified with each successive trophic level (biomagnification) (Ali et al. 2021). Bioaccumulation of pesticides in aquatic ecosystems is seen at all trophic levels, and in many taxa, including phytoplankton, macrophytes, macroinvertebrates (Tongo et al. 2022), insects (Katagi and Tanaka 2016), amphibians (Ascoli-Morrete et al. 2022), teleost fishes (Konwick et al. 2006, Clasen et al. 2018, Rossi et al. 2020, Tongo et al. 2022) and elasmobranchs (Weijs et al. 2015). The bioaccumulation of pesticides in fish and other organisms consumed by humans poses a potential health risk (Clasen et al. 2018) and may be of concern for the Keep River catchment. Monitoring levels of pesticides in the tissues of aquatic organisms provides an additional means of assessing the contamination of the water bodies they inhabit (Schäfer et al. 2015) and could be considered in the Keep River.

1.3 Aims of the study

Sawfishes are long-lived, slow-growing and are known to occupy different habitats through different life stages, with one species occupying freshwater habitats for their first four to six years of life (Thorburn et al. 2007, Whitty et al. 2009, Morgan et al. 2011, 2017, 2021, Duly et al. 2016, Lear et al. 2019). Their strong association with coastal, estuarine and riverine habitats makes them susceptible to anthropogenic impacts (Poulakis and Seitz 2004, Whitty et al. 2014, Duly et al. 2016). While these species are threatened throughout their range by loss of habitat, as bycatch in commercial fishing and suffer from illegal hunting for their unique rostrum (saw), the threat from pesticides entering their habitats is largely unknown. The first aim of this study was to identify the relative importance of the Keep River as habitat for these threatened species in a global context, by comparing their abundance to other areas where similar data has been collected. The second aim was to collate relevant information regarding the toxicity of a suite of pesticides that may potentially be entering the Keep River environment and provide a literature review of any impact they may have with regard to aquatic fauna and food webs.

2. Methods

2.1 EPBC listed species

This report examines the catch-per-unit effort (CPUE) of EPBC listed species that were captured in the Keep River during the pre- and post-development surveys (see WRM, 2013a, 2013b, 2014, 2021; Indo-Pacific Environmental 2022, 2023). To allow for a comparison with other studies on these EPBC listed species, namely the Dwarf Sawfish (*P. clavata*) and Freshwater Sawfish (*P. pristis*), the catch data compiled in the pre- and post-development studies were compared with other relevant studies throughout their range. The CPUE was calculated as the number of sawfish captured in these studies in 20 m net.hr^{-1}. Other aspects of their life history are presented from literature reviews.

2.2 Farm chemicals in the Keep River

About 30 pesticides are listed for potential use by the various farm operators in the Keep River catchment according to annual farm chemical updates provided by farm operators (DPIRD unpublished data). At least five of these (atrazine, metolachlor, fipronil, methomyl and chlorantraniliprole) have been detected for limited time periods in the Keep River since water quality monitoring commenced in 2014 (DPIRD unpublished data, GHD 2023). A detailed literature review was conducted to compile information on known toxic effects on aquatic flora and fauna of selected pesticides in use on farms in the Keep River catchment. Searches of primary literature published in the last 5 years were conducted in Google Scholar using pesticide names, *toxicity*, and *aquatic* as keywords. Further relevant literature and other sources were obtained from citations within these publications. Information on the mode of action and the lethal and sub-lethal effects on non-target aquatic organisms of selected chemicals was collated and summarised. Pesticides were chosen as representative of a range of differing chemical classes and modes of action, and of differing aquatic risks (as identified by Warne et al. (2022)). Aquatic risk is assessed on the basis of the effect on aquatic organisms (based on inherent toxicity of active ingredients as measured by current or proposed DGVs) and the mobility and persistence of active ingredients in the environment (Warne et al. 2022).

3. Results

3.1 Comparisons of EPBC listed species abundance pre- and post-development

Dwarf Sawfish (*Pristis clavata*)

Dwarf Sawfish were recorded in all Keep River estuarine sites (EST01-03) in both 2011-2013 (n = 30) and 2020-2022 (n = 13), and in 2022 they were also found at site K1 (n = 6), presumably following large tides. During the late dry season, when the aquatic surveys of the Keep River were conducted, the mean catch-per-unit-effort (CPUE), calculated as the number of sawfish captured in 20 m net.hr^{-1} prior to development (2011-2013) was 0.139 ($\pm$0.056 SE) for *P. clavata* (Figures 1, 2). Post-development (2020-2022), the catch rate was 0.095 ($\pm$0.027 SE) Dwarf Sawfish captured in 20 m net.hr^{-1} for *P. clavata*. ANOVA revealed that there were no significant differences in the catch rates of Dwarf Sawfish between the study periods (Figure 2).

The CPUE of *P. clavata* in the Keep River estuary is similar to that found in the late dry season in the Fitzroy River estuary in the West Kimberley; which is arguably the most important refuge for the species globally (Morgan et al. 2021). Within the Fitzroy River estuary, the CPUE is variable depending on the strength of the wet season and thus the input of freshwater flow with Dwarf Sawfish exiting the estuary during high freshwater flow events, before returning when salinity increases (Morgan et al. 2021). The mean CPUE of

Dwarf Sawfish in two adjacent estuarine pools in the dry season of 2015 in the Fitzroy River estuary was 1.82 (+0.04 SE) sawfish captured in 20 m net.hr^{-1} and 1.60 (+1.89 SE). However, between 2002 and 2016, the mean CPUE was 0.134 (+0.042) Dwarf Sawfish per 20 m net.hr^{-1}, being similar to that recorded over the six years in the Keep River. The only other comparable data is from the Gulf of Carpentaria and the northern Pilbara, with catch rates reported as <0.003 Dwarf Sawfish per 20 m net.hr^{-1} (Peverell 2005, Stevens et al. 2008), which is far less that that reported for the Keep River or the Fitzroy River.

Freshwater Sawfish (*Pristis pristis*)

No Freshwater Sawfish were captured in the estuarine pools (EST01-03) in any year of pre- or post-development studies, however, they were captured in pools K1-K2 between 2011 and 2013, and were found in pools K3 and K4 in 2020-2022. As Freshwater Sawfish are pupped either in the estuary or near the estuary mouth, each fish captured in the upstream pools must have traversed through these downstream sites to reach the nursery pools. Pupping is likely to occur at the end of the wet season (January to April), based on catches in the Fitzroy River, at a time when flows are higher (Whitty et al. 2009, Lear et al. 2019).

The number of *P. pristis* captured in 20 m net.hr^{-1} prior to development (2011-2013) was 0.024 (+0.0144 SE) (Figures 1, 2). Post-development (2020-2022), the catch rate was 0.037 (+0.0194 SE) sawfish captured in 20 m net.hr^{-1} for *P. pristis*. ANOVA revealed that there were no significant differences in the catch rates of Freshwater Sawfish between the different study periods (Figure 2). Across the six years of sampling, an average of 3 individual *P. pristis* were captured/year. Annual CPUE for Freshwater Sawfish in the Fitzroy River ranged from 0.008 to 0.45 sawfish 20 m net.hr^{-1} in freshwater pools and 0 to 0.28 in estuarine habitats (Lear et al. 2019). Peverell (2005) reports a maximum CPUE of Freshwater Sawfish from the rivers of north-eastern Gulf of Carpentaria (Queensland) to be 0.00003 sawfish captured in 20 m net.hr^{-1}; which is considerably lower than those reported above.

Pristis pristis individuals may remain in the freshwater nursey habitats for several years, and as recruitment is positively correlated to river discharge (see Lear et al. 2019), the CPUE of new recruits (young of the year (YOY)) is important to measure for comparisons between years and catchments. The highest number of new recruits of Freshwater Sawfish in the Keep River was in 2011, when five small individuals were captured (WRM 2013). As a comparison, in the Fitzroy River in 2011 there was a mass recruitment of Freshwater Sawfish following high river discharge, with >100 pups recorded (Lear et al. 2019). Indo-Pacific Environmental (2023) found a relationship between YOY and discharge in the Keep River, with higher discharge (days stage height >5.5 m) resulting in higher catches of sawfish in their first year of life. Similarly, Lear et al. (2019) reported that recruitment was minimal following poor wet seasons, and the species survival is probably linked to the periodic years where wet season discharge is high. The absence of YOY Freshwater Sawfish in the estuary sites is not surprising, particularly as the timing of the surveys occurred in the late dry season. YOY survival in estuarine sites is low, with CPUE in the Fitzroy River estuary decreasing throughout the year to be zero or near zero in the late dry season (Lear et al. 2019). In poor wet seasons, YOY Freshwater Sawfish can become trapped in the estuary sites, where predation pressure is higher from a higher density of Estuarine Crocodiles (*Crocodylus porosus*) and Bull Sharks (*C. leucas*) (Lear et al. 2019).

Figure 1: The three *Pristis* sawfishes captured in the Keep River (Photographs: D. Morgan).

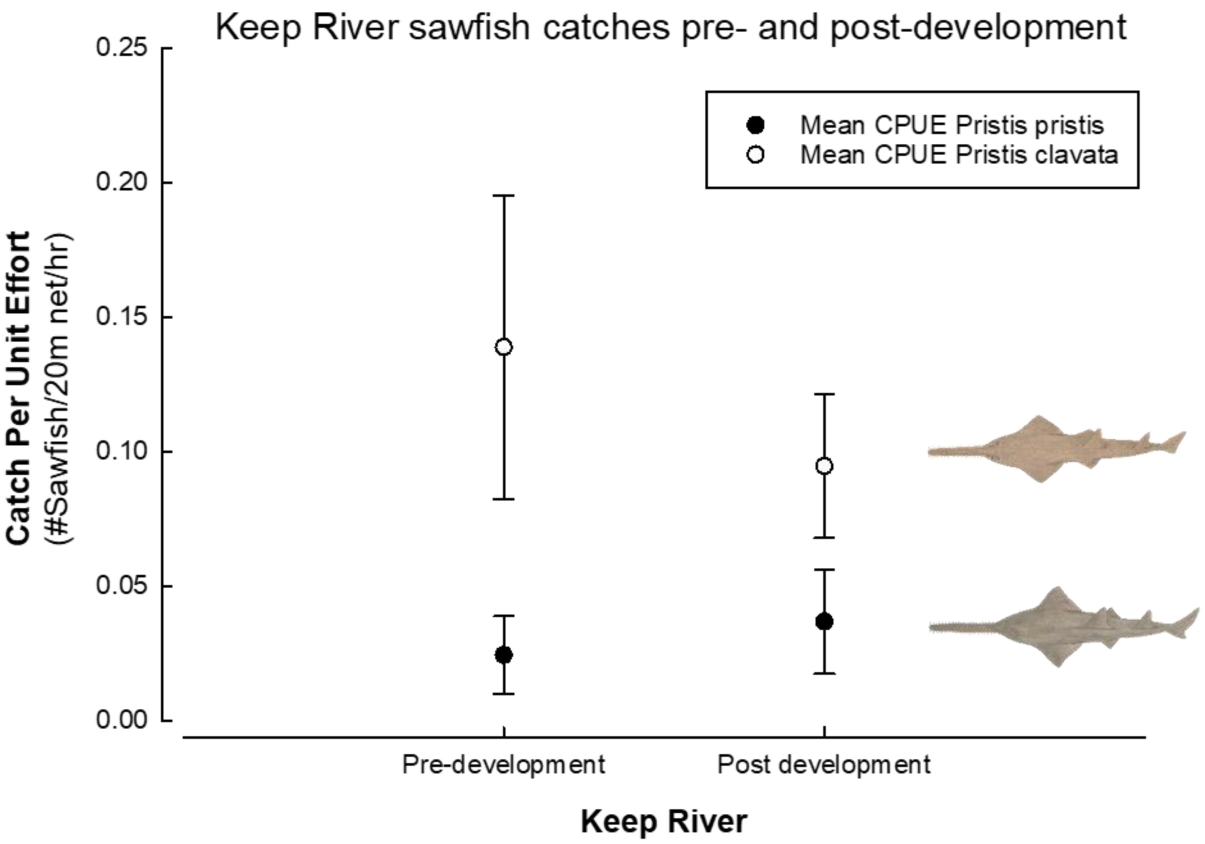

Figure 2: The catch-per-unit-effort (CPUE) of Freshwater Sawfish (*Pristis pristis*) and Dwarf Sawfish (*Pristis clavata*) in the Keep River. (Adapted from WRM, 2013a, 2013b, 2014, 2021; Indo-Pacific Environmental 2022, 2023). Artwork: K. Lear.

3.2 *Toxicity of selected pesticides in use in the catchment*

Details of the use, timing of application, mode of action, what is known about the toxic effects of selected chemicals to non-target aquatic species are presented in Table 1 and below. Pesticides chosen include those which pose relatively high risks to aquatic organisms (according to Warne et al. 2022): Fipronil, a phenylpyrazole insecticide and Metolochlor, a chloroacetanilide herbicide; intermediate risks: Atrazine, a chlorotriazine herbicide and Diuron, a phenylurea herbicide; and some with relatively low aquatic risk: Glyphosate, an organophosphorus herbicide and 2,4-D, a chlorophenoxy herbicide. Methomyl, a carbamate insecticide has not yet been assessed for aquatic risk by Warne et al. (2022) but has been detected in Keep River Pools. In addition to having DGVs for species protection determined by ANZG (2018), all these pesticides are relatively well studied with large bodies of scientific literature reporting on their effects on non-target organisms.

Table 1. Summary information on selected pesticides used in the Keep River catchment

Pesticide active ingredient	ANZG (2018) default guideline value for 99% species protection	Aquatic risk[a]	Highest detected concentration in Keep River pools[b]	Example 96 hr LC_{50} value from literature	Typical soil degradation DT_{50} (days)[c]	Degradation in water DT_{50} (days)[c]
Atrazine	0.7 µg/L	8,900	6.1 µg/L on 30 May 2020	42.38 mg/L Spotted snakehead (*Channa punctata*) (Nwani et al. 2010)[d]	75	80[e]
Fipronil	0.013 µg/L	43,600	0.12 µg/L on 28 November 2019	2.64 mg/L freshwater mussels (*Unio delicatus*) (Arslan and Günal 2023)	142	54
Metolachlor	0.0084 µg/L	44,000	3.1 µg/L on 30 May 2020	51.2 mg/L[f] water flea (*Daphnia magna*) (Liu et al. 2006)	58.1	88
Methomyl	0.5 µg/L	NI	0.15 µg/L	0.425 mg/L Topmouth gudgeon (*Pseudorasbora parva*) (Li et al. 2008)	7	2.9
Diuron	0.2 µg/L	3,400	ND	14.2 mg/L Fathead minnow (*Pimephales promelas*) (Call et al. 1987)	146.6	8.8
Glyphosate	180 µg/L	2	ND	32.54 mg/L Spotted snakehead (*Channa punctata*) (Nwani et al. 2010)[d]	16.11	9.9
2,4-D	140 µg/L	9	ND	32.6 mg/L Crayfish (*Pontastacus leptodactylus*) (Benli et al. 2007)	4.4	7.7

[a] From Warne et al. (2022) – Pesticide Decision Support Tool (PDST)
[b] From DPIRD unpublished data
[c] From Lewis et al. 2016 – Pesticide Properties Database (PPDB)
[d] Commercial formulation used
[e] Water DT_{50} not available; water-sediment DT_{50} given
[f] 24 h LC_{50} value for S-metolachlor
NI = Not included in Warne et al. (2022)'s PDST
ND = Not detected in Keep River pools

Atrazine

ANZG Default guideline value (DGV) for 99% species protection: **0.7 µg/L**

Timing of use: April – May (late wet season/early dry season)

Aquatic Risk: **8,900** (Warne et al. 2022)

Note: There have been multiple detections of atrazine in Keep River pools since water quality monitoring began, including eight instances of concentrations exceeding the DGV for 99% species protection. The highest concentration detected was 6.1 µg/L on 30 May 2020 (DPIRD unpublished data).

Atrazine (6-chloro-N2-ethyl-N4-isopropyl-1,3,5-triazine-2,4-diamine) is a chlorotriazine herbicide that inhibits photosynthesis and is used to control broad-leaved weeds and grasses (Lewis et al. 2016a). It is used on farms in the Goomig Farmland development during April and May to control pest species for crops including cotton, sorghum and maize.

Atrazine works selectively on target weeds because of the different metabolic pathways and rates of metabolisation in weeds and crops (de Albuquerque et al. 2020). However, atrazine also inhibits photosynthesis in aquatic macrophytes (Graymore et al. 2001), and microalgae (Weiner et al. 2007, Sun et al. 2020), the basis of the food web in aquatic ecosystems, which has implications for organisms at higher trophic levels. Changes in freshwater zooplankton community structure after environmental exposure to atrazine is thought to be caused by reductions in their food source, rather than direct toxicity of atrazine to the zooplankton species. Aquatic gastropods and crustaceans that feed on algae or zooplankton are also likely to be indirectly affected by atrazine. Direct effects of atrazine on the survival of some crustaceans have been seen, but at high concentrations not likely to be environmentally relevant. Atrazine also affects the survival, growth and development of aquatic insects. As well as causing indirect effects on fish from disruptions to food webs, direct effects are reported including reduced growth rates, changes to swimming behaviour (attributed to effects on sensory organs and nervous system), and histological changes to gills and kidneys (Graymore et al. 2001 and references therein). Exposure to atrazine causes adverse, endocrine-related effects in all vertebrate classes, thought to be caused by alterations to the hypothalamic-pituitary-gonadal (HPG) axis (Cleary et al. 2019). Atrazine not only poses risk to exposed organisms but can also affect subsequent generations. Reproductive disfunction, including reduced sperm count and motility, and decreased fertilisation rate occur in descendants of atrazine-exposed Japanese Rice Fish (*Oryzias latipes*) (Cleary et al. 2019).

Atrazine is the most commonly detected herbicide in surface waters globally, and is still detected in Europe (along with its TPs) despite its use having been banned there since 2003 (de Souza et al. 2020). In the same year, the USA approved the continuation of atrazine usage, leading to allegations that the manufacturer of atrazine sought to influence the decision in private meetings with the U.S. Environmental Protection Agency and by producing flawed evidence of no harm (Bethsass and Colangelo 2006). Indeed, there has been some debate regarding the toxicity of atrazine in the literature, with some reviews concluding that there is little evidence of adverse effects on aquatic vertebrates (Solomon et al. 2008, Van Der Kraak et al. 2014).

However, a more recent review, which considered the effects on a broader range of aquatic organisms across different taxa and trophic levels, in studies published since 2015, found ample evidence of the toxicity of atrazine at environmentally relevant concentrations (de Albuquerque et al. 2020). For example, adverse effects on different species of algae, such as interference with photosynthesis and inhibited growth, were induced by concentrations ranging from 3 µg/L to 2 mg/L. Crustaceans *Daphnia magna* suffered oxidative stress, morphological abnormalities and reduced numbers of viable offspring; *Tigriopus japonicus* showed delayed metamorphosis because of altered gene expression; and *Faxonius virilis* suffered DNA damage causing impairments to the chemosensory system. Zebrafish (*Danio rerio*) also suffered oxidative stress, as well as neuroendocrine disruptions, altered swimming behaviours and circadian rhythm, and reduced fertility, and other fish species suffered liver and mitochondrial damage (*Cyprinus carpio*), altered sperm and reduced fertilisation success (*Oryzias latipes*), and damage or structural changes to the gills (*Rutilus frisii kutum* and *Oreochromis niloticus*). Atrazine exposure also adversely affected amphibians and reptiles, causing genotoxic and mutagenic effects in tree frogs (*Dendropsophus minutus*), morphological abnormalities in toads (*Rhinella schneideri*), and immunosuppression and malformation of embryos in freshwater turtles (*Trachemys scripta* and *Podocnemis unifilis*) (see Table 1 in de Albuquerque et al. 2020).

Fipronil

ANZG Default guideline value (DGV) for 99% species protection: **0.013 µg/L**

Timing of use: March – May (late wet season/early dry season)

Aquatic Risk: **43,600** (Warne et al. 2022)

Note: There have been two detections of fipronil concentrations in exceedance of the DGV for 99% species protection in Keep River pools. The highest concentration detected was 0.12 µg/L on 28 November 2019 (DPIRD unpublished data).

Fipronil (5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile) is a widely-used, phenylpyrazole, broad-spectrum insecticide used to control agricultural pests such as diamondback moth, aphids and locusts, as well as other insects such as mosquitos, cockroaches and termites (Tingle et al. 2003). It is used in a limited manner on farms in the Keep River catchment from March to May on cotton crops.

Fipronil's unique mode of action inhibits the gamma-aminobutyric acid (GABA)-gated chloride channel in insects, leading to hyperexcitation, convulsions, paralysis, and death (Gant et al. 1998). It is said to have selective toxicity because it has less binding affinity for the GABA-receptors of vertebrates than those of insects (Hainzl et al. 1998, Zhao et al. 2005), therefore making it safer than more toxic and persistent organochlorine insecticides. However, fipronil does have direct toxic effects on non-target organisms, as well as indirect effects via loss of prey (Gibbons et al. 2015). A review of published studies on the effects of fipronil in vertebrates found that it is highly or very highly toxic to all species of fishes studied. Acute dose median lethal concentration (LC_{50}) values range from 0.042 mg/L in Nile tilapia (*Oreochromis niloticus*) to 0.34 mg/L in Japanese carp (*Cyprinus carpio*), however, sublethal effects are seen at much lower concentrations e.g., erythrocyte damage on silver catfish (*Rhamdia quelen*) at 0.0002 mg/L (0.2 µg/L) (Gibbons et al. 2015). Negative effects on gill histology are also seen in fish at sublethal concentrations of fipronil e.g., aneurysms and necrosis in white fish (*Rutilus frisii*) (Ardeshir et al. 2017), necrosis, epithelial proliferations and haemorrhages in Nile tilapia (El-Murr et al. 2015), and disruption of cartilaginous core, degeneration of primary lamellae, aneurysm and necrosis in common carp (*Cyprinus carpio*) (Ghaffar et al. 2018). Additionally, common carp exposed to concentrations of fipronil up to 0.1 mg/L exhibited behavioural and nervous-system changes (including surface breathing, erratic swimming, and fin tremors), histopathological abnormalities in liver and kidney tissues, decreases in erythrocyte count, aberrant morphological changes to erythrocytes, and increases in urea concentration, serum creatinine and cholesterol, triglyceride, glucose and albumin, negatively impacting their physiological balance (Ghaffar et al. 2018). Acute toxic effects of fipronil are also seen in freshwater mussels (*Unio delicatus*) (96 hr LC_{50} 2.64 mg/L), as well as oxidative stress and damage to gill and digestive gland tissues at sublethal concentrations (Arslan and Günal 2023).

Bioaccumulation of fipronil and its metabolites has been seen in species ranging from *Escherichia coli* (Bhatti et al. 2019), the aquatic blackworm (*Lumbriculus variegatus*) (Wang et al. 2019), and the Chinese pond mussel (*Anodonta woodiana*) (Qu et al. 2016), to rainbow trout (*Oncorhynchus mykiss*) (Konwick et al. 2006) and fathead minnows (*Pimephales promelas*) (Baird et al. 2013), with potential for trophic transfer and biomagnification.

Metolachlor/S-Metolachlor

ANZG Default guideline value (DGV) for 99% species protection: **0.0084 µg/L** (for Metolachlor)

Aquatic Risk: **44,000** (for Metolachlor)/5,200 (for S-Metolochlor) (Warne et al. 2022)

Timing of use: April – May (late wet season/early dry season)

Note: 1) Multiple detections of metolachlor concentrations in exceedance of the DGV for 99% species protection in Keep River pools have occurred. The highest concentration detected was 3.1 µg/L on 30 May 2020. Between 12 December 2022 and 13 February 2023, metolachlor at concentrations in exceedance of PC99 was detected on five occasions in the K3 pool and once in the K2 pool of the Keep River. The highest concentration detected during this time was 0.041 µg/L on 25 January 2023 (DPIRD unpublished data).

2) After preliminary results from this report were presented to farm operators in Kununurra in March 2023, some farm operators indicated an intent to find alternatives to the use of metolachlor.

Metolachlor (2-chloro-N-(6-ethyl-o-tolyl)-N-[(1RS)-2-methoxy-1-methylethyl]acetamide) is a pre-emergence, chloroacetanilide herbicide used to control broad-leaved and grassy weeds. Metolachlor exists as the S- and R-enantiomers (Lewis et al. 2016a) and was introduced in 1976 as a racemic compound of equal amounts of these, also known as rac-metolachlor (Poiger et al. 2002). S-metolachlor is a commercial formulation introduced in 1997 that contains predominantly the S-enantiomer (~90%), which has a much higher herbicidal efficacy than the R-enantiomer, and which provides the majority (95%) of metolachlor's herbicidal action (Poiger et al. 2002). Metolachlor and S-metolachlor are both registered for use in Australia (APVMA 2023) and S-Metolachlor is used in the Keep River catchment in April and May for controlling grass weeds. It is known to have also been used in November and December by at least one farm operator.

After atrazine, metolachlor is the second most commonly detected herbicide in surface waters around the world (de Souza et al. 2020) and has been detected frequently in Australian surface waters (AATSE 2002). Like other chloroacetamides, metolachlor inhibits the formation of long chain fatty acids in plants (Böger et al. 2000). Because of this it would be expected that heterotrophic species would be less sensitive to metolachlor than phototrophic species. However, while S-metolachlor appears more toxic than metolachlor to phototrophic species (e.g., green algae, *Scenedesmus obliquus* (Liu et al. 2017) and *Chlorella pyrenoidosa* (Liu and Xiong 2009), the racemic mixture appears to have higher chronic toxicity to heterotrophs (e.g., *Daphnia magna* (Liu et al. 2006) and zebrafish *Danio rerio* (Ou-Yang et al. 2022).

Toxic effects on non-target, phototrophic, aquatic species include loss of rigidity and permeability of algal cells because of changes to the fatty acid composition of cell membranes (Böger et al. 2000), leading to inhibition of cell reproduction and growth rate (Vallotton et al. 2008). Metolachlor also reduces biomass in a range of aquatic macrophytes, but with median effect concentration (EC_{50}) values ranging from 70 µg/L to

>3000 µg/L, sensitivities of species are highly variable (Fairchild et al. 1998). In aquatic heterotrophs, varying effects of metolachlor, S-metolachlor and their metabolites have been seen in aquatic invertebrates e.g., midges (*Chironomus tentans* (Jin-Clark et al. 2008)), clams (*Scrobicularia plana* (Gutiérrez et al. 2019)), oysters (*Crassostrea gigas* (Mai et al. 2014)), cladocerans (*Daphnia longispina* (Neves et al. 2015) and *Daphnia magna* (Liu et al. 2006)), and crayfish (*Procambarus virginalis* (Stara et al. 2019, Velisek et al. 2019) and *Orconectes rusticus* (Cook and Moore 2008)), and vertebrates e.g., zebrafish (*Danio rerio* (Quintaneiro et al. 2017, Rozmánková et al. 2020, Yang et al. 2021, Ou-Yang et al. 2022)), Japanese rice fish (*Oryzias latipes* (Jin et al. 2011)) and Perez's frog (*Pelophylax perezi* (Quintaneiro et al. 2018)).

Effects are compound-specific and vary among species, life-stage and in some cases, sex. Exposure to S-metolachlor (and its TPs) increases developmental abnormalities in oysters and their sperm and reduces fertilisation success (Mai et al. 2014). It also negatively impacts *D. longispina* reproduction (age at first reproduction, number of offspring and number of broods), reducing the rate of population increase (Neves et al. 2015). Both S-metolachlor and metolachlor reduce length, longevity, number of broods per female and the rate of population increase of *D. magna*, but at lower lowest observable effect concentrations (LOEC) and no observable effect concentrations (NOEC) values for the racemic compound (Liu et al. 2006). Metolachlor disrupts the thyroid system of Japanese rice fish in a sex- and life stage-dependant manner, with female fish more sensitive to metolachlor exposure than males, and juvenile fish affected by lower concentrations than adults (Jin et al. 2011). Endocrine disruption, developmental malformations (including swim bladder and yolk sac deformities) and reduced hatching success occur in zebrafish embryos exposed to S-metolachlor (Quintaneiro et al. 2017, Rozmánková et al. 2020, Yang et al. 2021). S-metolachlor also disrupts the endocrine system of adult zebrafish, as does metolachlor (via different mechanisms), with female fish more sensitive to both compounds than males (Ou-Yang et al. 2022). Metolachlor also has deleterious effects on the liver development of zebrafish (Ou-Yang et al. 2022). S-metolachlor causes the inhibition of cholinesterase in the larvae of aquatic midges (Jin-Clark et al. 2008) and zebrafish (Quintaneiro et al. 2017), suggesting a neurotoxic effect.

To derive the DGV for metolachlor, chronic toxicity data on 21 freshwater species were used, including 12 species native to, or found in Australia and/or New Zealand (ANZG 2020). However, these 12 species are all diatoms, algae and macrophytes (ANZG 2020), highlighting the dearth of information about the effects of metolachlor and S-metolachlor on Australian aquatic fauna.

Methomyl

ANZG Default guideline value (DGV) for 99% species protection: **0.5 µg/L**

Timing of use: June – September (dry season)

Aquatic Risk: Not evaluated by Warne et al. (2022)

Note: There have been multiple detections of methomyl in Keep River Pools. The highest concentration was 0.15 µg/L in K2 on 6 June 2020.

Methomyl (S-methyl (EZ)-N-(methylcarbamoyloxy)thioacetimidate) is a carbamate insecticide that inhibits cholinesterase activity (Lewis et al. 2016a). Methomyl was first introduced in the U.S.A. in 1968 and is used to control a broad spectrum of arthropod pests. It poses a contamination risk to surface and groundwater because of its high solubility in water and weak to moderate soil adsorption (Van Scoy et al. 2013). Methomyl's mode of action works to inhibit acetylcholinesterase (AChE) activity in the synaptic junctions, causing nerve and/or tissue damage and mortality in arthropods by interrupting nerve signalling (Kuhr and Dorough 1976). The inhibition of AChE can also lead to disruption of vital functions of the peripheral nervous system in mammals, which at high enough doses can lead to death, and methomyl is considered highly toxic to humans when ingested or inhaled (Lewis et al. 2016a, Van Scoy et al. 2013).

Methomyl is also considered highly toxic in aquatic environments (Van Scoy et al. 2013), and the water flea (*Daphnia magna*) appears to be particularly susceptible to methomyl and commercial formulations which use it as an active ingredient, with 48 h LC_{50} values ranging from 0.0076 mg/L – 0.088 mg/L (IPCS 1996). Acute toxicity (96 h LC_{50}) values in fish species have been reported ranging from 0.425 mg/L in Topmouth gudgeon (*Pseudorasbora parva*) (Li et al. 2008) and 0.53 mg/L in Channel catfish (*Ictalurus punctatus*) to 2.00 mg/L in Bluegill sunfish (*Lepomis macrochirus*), with toxicity within species generally increasing at warmer water temperatures (IPCS 1996). In the Topmouth gudgeon, methomyl exposure induced changes to some hepatic enzymes (e.g., > 40% reduction in liver glutathione S-transferases (GSTs) activity) and neurotoxic effects (including a 48% decrease in AChE activity in the brain) leading to mortality (Li et al. 2008).

At sublethal concentrations, methomyl also has a range of toxic effects on fish. Methomyl exposure over a period of 21 days at a concentration of 4.3 µg/L had deleterious effects on gills and brain tissue of Nile tilapia (*Oreochromis niloticus*), including lamellar fusion, abnormal cells, inflammation, oedema, haemorrhage and necrosis, suggesting that methomyl may accumulate in these tissues. Significant decreases in AChE activity in the brain and muscle tissues were also seen and produced behavioural changes such as decreased foraging and abnormal, "jerky" movements (Habotta et al. 2022). Also in Nile tilapia, exposure to methomyl depressed immune responses, diminished swimming performance, and caused respiratory distress (Abdel-Rahman Mohamed et al. 2021), as well as oxidative damage in the testis, induced by concentrations as low as 2 µg/L (Meng et al. 2021). Exposure to sublethal concentrations (ranging from 0.5 - 23.3 mg/L) for six days showed AChE activity declining in larval zebrafish (*Danio rerio*) as methomyl concentration increased. These concentrations also negatively affected embryo hatching rates and larval morphology (smaller eyes and body size) and caused hypolocomotor activity and failure of the swim bladder to inflate (Jablonski et al. 2022). Neurotoxic effects, malformation and teratogenicity, as well as behavioural effects such as hyperactivity, extreme agitation and abnormal swimming in tadpoles of the Arabian toad (*Bufo arabicus*) have also been found (Seleem 2019).

Differences in the toxicity of methomyl as the pure active ingredient versus that of commercial formulations have also been investigated. Methomyl and a commercial formulation containing 200 g/L of methomyl were both found to be highly toxic to *D. magna* and had similar EC_{50} values (Pereira et al. 2009). However, in larval zebrafish, acute toxicity (96 h LC_{50}) values were much lower for methomyl than for a commercial formulation containing 21.5% of methomyl as its active ingredient: 59.7 mg/L (Ahmad et al. 2020) *vs*. 1200 mg/L (Jablonski et al. 2022) respectively. These toxicity values for larval zebrafish are also much higher than the range seen in adults of other species (as discussed above), suggesting that intraspecific differences such as ontogenetic stage may play a role in susceptibility to the toxic effects of methomyl. These results highlight the importance of considering the effects commercial formulations, containing other ingredients which can affect the toxicity of the active ingredients (Jablonski et al. 2022), as well as intra- and inter-specific differences in responses to exposure to farm chemicals.

Diuron

ANZG Default guideline value (DGV) for 99% species protection: **0.2 µg/L**

Timing of use: Not available

Aquatic Risk: **3,400** (Warne et al. 2022)

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a systemic, phenylurea herbicide that strongly inhibits photosynthesis at photosystem II in target weed species, but also has toxic effects on non-target, aquatic algae, plants and animals (Lewis et al. 2016a). Plants are the most sensitive to phenylurea herbicides and although the acute toxicity to mammals, birds and fish is relatively low compared to plants, algae and invertebrates, there is mounting evidence of chronic toxicity in vertebrates (Marlatt and Martyniuk 2017).

Reported LC_{50} values of diuron for juvenile/adult freshwater fishes range from 0.71 to 14.2 mg/L (Marlatt and Martyniuk 2017), and the 96 hr LC_{50} values for aquatic invertebrates, water fleas (*Daphnia pulex*), amphipods (*Hyalella azteca*), and freshwater shrimp (*Paratya australiensis*) are 17.9 mg/L, 19.4 mg/L and 8.8 mg/L respectively (Nebeker and Schuytema 1998, Kumar et al. 2010). However, adverse sub-lethal behavioural and developmental effects on aquatic fauna occur at much lower concentrations (Marlatt and Martyniuk 2017), at both the whole-organism and sub-organismal levels. For example, in zebrafish (*Danio rerio*) embryos and larvae, exposure caused behavioural changes such as reductions in spontaneous coiling movements and thigmotaxis (Velki et al. 2017a), and changes in enzyme activity and gene expression (Velki et al. 2017b). Significant inhibition of brain acetyl-cholinesterase (AChE) activity was found in juvenile goldfish (*Carassius auratus*) exposed to 500 µg/L of diuron (Bretaud et al. 2000). AChE inhibition in both blood serum and brain of Nile tilapia (*Oreochromis niloticus*) larvae occurred at 1 mg/L, along with abnormal movement behaviour and increases in liver biomarkers (El-Nahhal 2018). A significantly greater percentage of dead or grossly deformed fry immediately post-hatching, and reduced survival of juvenile fathead minnows (*Pimephales promelas*) resulted from exposure to 78 µg/L of diuron for 64 days (Call et al. 1987). Short-term exposure (24 hr) to 0.5 and 5 µg/L of diuron had significant effects on burst swimming and grouping behaviour respectively of goldfish, suggesting that diuron interferes with the chemosensory system and alters olfactory-mediated behaviours (Saglio and Trijasse 1998). Diuron exposure also decreased the swimming speed and weakened the response to stimuli of American bullfrog (*Lithobates catesbeianus*) tadpoles at 30 mg/L. An escape response was triggered at concentrations of 25 and 50 µg/L, suggesting tadpoles may be able to avoid diuron at these sub-lethal concentrations if alternative habitat is available. However, in the wild, these effects could impact foraging rates and predator avoidance, with implications for growth, development and survival (Moreira et al. 2019). There are also indications that diuron exposure could decrease fecundity of fish which could have implications for population dynamics. For example, diuron exposure at concentrations as low as 1 µg/L caused degenerative changes to ovaries and testis of Javanese rice fish (*Oryzias javanicus*), leading to a reduction in the number of oocytes and spermatozoa (Kamarudin et al. 2020).

The transformation products of diuron, especially 3,4-dichloroaniline (3,4-DCA), also have toxic effects on a wide range of organisms including protozoans, insects, crustaceans and fishes at concentrations as low as 1 µg/L (Giacomazzi and Cochet 2004). A number of diuron metabolites including 3,4-DCA significantly affected the levels of sex steroids in female Nile tilapia, suggesting disruption of estrogenic activity (Boscolo Pereira et al. 2016). Diuron and its transformation product 3,4-DCA caused disruption to thyroid function resulting in acceleration of development of tadpoles of the American bullfrog, with effects more pronounced at higher environmental temperatures (34°C vs 28°C) (Freitas et al. 2016). There is limited data on the bioaccumulation of diuron, but bioaccumulation factors reported in the literature (< 100) suggest low potential for this in fish (Marlatt and Martyniuk 2017). However, synergistic toxic effects were reported when Nile tilapia were exposed to diuron in mixtures with other pesticides (malathion and Nemacur) (El-Nahhal 2018).

Glyphosate

ANZG Default guideline value (DGV) for 99% species protection: **180 µg/L**

Timing of use: Year-round

Aquatic Risk: **2** (Warne et al. 2022)

Glyphosate (N-(phosphonomethyl)glycine) is a broad-spectrum, post-emergent, systemic organophosphorus herbicide. It is used by farm operators on maize, cotton, hay and chickpea crops in the Keep River catchment area and is applied year-round.

Historically, glyphosate has been the most widely-used herbicide in Australia, in agriculture, forestry, industrial, urban and domestic settings (AATSE 2002). There are a multitude of commercially-available, glyphosate-based products that contain various, often proprietary, surfactants and other adjuvants to increase efficacy. Most of these products are not approved for use in the aquatic environment (Annett et al. 2014), however they can enter aquatic systems in significant quantities through surface runoff, overspray or drift during application (Solomon and Thompson 2003). Glyphosate is highly soluble in water, allowing easy dispersal in aquatic environments (Lewis et al. 2016a).

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase found in plants, preventing the synthesis of aromatic amino acids that produce a range of essential hormones, vitamins and metabolites (Myers et al. 2016). This suggests that its toxicity to animal species should be limited. However, acute and chronic toxicity have been reported in a number of vertebrate and invertebrate species. Toxicity is highly species-specific and also dependent on timing, dose and method of exposure (Annett et al. 2014, Tresnakova et al. 2021). Additionally, environmental factors such as temperature and pH can alter the toxicity of glyphosate (Annett et al. 2014). Commercial formulations can potentially have higher toxicity than glyphosate alone, due to the addition of surfactants such as polyethoxylated amines (POEA), which were identified as key factors in the toxicity of some commercial products more than 40 years ago (Folmar et al. 1979). Commercially-available products each contain complex combinations of adjuvants, many of which are proprietary, and different concentrations of glyphosate, producing large variation in their toxicity and making attributing toxicity to individual components difficult (Annett et al. 2014). Because of these differences in formulations, glyphosate DGVs may be over- or under-protective in certain settings.

Because glyphosate targets a biosynthetic pathway found in plants and algae, these phototrophic species are generally more susceptible to it than heterotrophic species. However, glyphosate toxicity has been seen in a number of animal species including crustaceans, molluscs, insects, cnidaria, amphibians and fish (ANZG 2021, Tresnakova et al. 2021). The mechanisms of toxicity in animals, which lack the enzyme EPSP, are still poorly understood and the majority of studies focus on acute toxicity. Consequently, there is limited information on the chronic toxicity of glyphosate and its long-term effects on individuals, populations and ecosystems (Annett et al. 2014).

Acute toxic effects of glyphosate and glyphosate-based products on fish, amphibians and invertebrates include changes to growth and development, oxidative stress, genotoxicity and histopathologic changes in the gills, liver and kidneys. Acute toxicity (LC_{50}) values reported at 24-, 48- and 96-hours exposure range from 0.295 to 645 mg/L for fishes, 6.5 to 115 mg/L for amphibians, and 35 to 461.54 mg/L for invertebrates (Tresnakova et al. 2021 and references therein). However, non-lethal deleterious effects are seen in vertebrate and invertebrate species at concentrations much lower than these LC_{50} values (ANZG 2021, Tresnakova et al. 2021).

The toxicity values for 15 species which were used to derive the ANZG DGVs for glyphosate in freshwater range from 316 to 65,000 µg/L (see Table 1 of ANZG 2021), however none of these were vertebrate species (because studies on vertebrates either did not include information on chronic toxicity or did not report negligible effects data (e.g., NOEC, EC10) and therefore were not suitable for inclusion). Six of the 15 species used (two crustaceans and four autotrophic species (macrophytes, diatoms and algae)) are native to or distributed in Australia (ANZG 2021). Despite the majority of studies on glyphosate toxicity to vertebrates being conducted on fish and amphibians, few studies have included native Australian species and knowledge of the effects of glyphosate on endemic Australian species is particularly lacking (Brovini et al. 2021). Reductions in, or even bans on the use of glyphosate have occurred in some countries, because of its detrimental effects on aquatic organisms and ecosystems, and glyphosate concentrations in Australia represent a "High risk" to aquatic organisms (Brovini et al. 2021).

(Image: Sharks and Rays Australia)

2,4-D

ANZG Default guideline value (DGV) for 99% species protection: **140 µg/L**

Timing of use: September – January (late dry season/mid wet season)

Aquatic Risk: **9** (Warne et al. 2022)

2,4-D ((2,4-dichlorophenoxy)acetic acid) is one of the most commonly used herbicides in the world, with more than 1500 commercial products using it as an active ingredient (Islam et al. 2018). 2,4-D produces uncoordinated cell growth and increases cell-wall plasticity in plants, damaging conductive vessels and causing leaf, stem and root malformations (Zuanazzi et al. 2020). It is used in the Keep River catchment for cotton volunteer control from September through to January (end of the dry season into the wet season).

High solubility in water and low soil adsorption mean 2,4-D is often detected in ground and surface waters (Islam et al. 2018). Toxic effects on aquatic organisms across different taxa and life stages have been reported for both 2,4-D itself and commercial formulations which use it as an active ingredient. Exposure to low, environmentally relevant concentrations (0.50 and 2.00 ppm) of 2,4-D and commercial formulations significantly decreased the larval survival of fathead minnows (*Pimephales promelas*) by similar amounts, suggesting that it is the active ingredient, 2,4-D that is causing these decreases (Dehnert et al. 2018). AChE activity in the brain and muscle of piavia (*Leporinus obtusidens*) was reduced by 2,4-D concentrations of 10 mg/L and 1 mg/L respectively, impacting the nervous system of the fish (da Fonseca et al. 2008). Exposure to 2,4-D decreased survival (LC_{50} = 46.71 mg/L) and hatching success (IC_{50} = 46.26 mg/L), and caused cardiac malformation and oxidative stress in zebrafish (*Danio rerio*) embryos (Li et al. 2017). Crayfish (*Orconectes rusticus*) exposed to sublethal concentrations of 2,4-D for 96 hours were unable to forage as effectively as unexposed animals (Browne and Moore 2014). Embryonic toads (*Rhinella arenarum*) suffered adverse teratogenic effects including reduced body size and microcephaly from exposure to both 2,4-D and a commercial formulation (Aronzon et al. 2011), and adult toads of the same species exposed to 20 mg/L of 2,4-D for 48 hours suffered oxidative DNA damage and depressed immune response (Lajmanovich et al. 2015).

4. Discussion

4.1 EPBC listed species in the Keep River

The aquatic fauna studies between 2011 and 2022 revealed that all three of Australia's threatened *Pristis* spp. have been recorded in the Keep River estuary, with one species, the Freshwater Sawfish (*P. pristis*) recorded in the first major non-tidal freshwater pool (K4) (2020 and 2021) upstream of the discharge point at the confluence of Border Creek and in the permanent tidal pools (EST01-03) downstream of Border Creek (see GHD 2023). Dwarf Sawfish (*P. clavata*), which were regularly captured in the estuarine pools (EST01-03) in all studies, were recorded in K1 in 2022 for the first time, at a time of higher salinites. The explanation for the Dwarf Sawfish (n = 6) being found in K1 for the first time relates to their movement patterns being strongly related to tidal influence (see Morgan et al. 2021). Only a single Green Sawfish (*P. zijsron*) was recorded in the estuary during all surveys (in 2011); a species which is usually associated with the mouths of rivers and tidal creeks (see Morgan et al. 2015, 2017). All Freshwater Sawfish found in sites K1-K4 at some point migrate from their estuarine pupping site seeking refuge pools where predators are fewer and prey is abundant, the strength of their upstream migration directly linked to river discharge and tidal influence (see Whitty et al. 2009, Lear et al. 2019). Poor wet seasons (i.e. reduced rainfall and water flow in rivers) result in the pups becoming trapped in the estuary where they are subjected to higher predation levels (Lear et al. 2019).

None of the aquatic studies between 2011 and 2022 recorded either of the river sharks (*Glyphis* spp.), however, based on other surveys within adjacent catchments of the region, both are likely to be present, possibly downstream of the survey sites. Within northern Australia, different river catchments support distinct populations of Speartooth Shark (*Glyphis glyphis*), which in Australia occurs from the Ord River in the west to the Wenlock River in the east, and has been recorded from the Adelaide, Wildman, West Alligator, South Alligator, East Alligator, Bizant and Ducie rivers (Compago et al. 2008; Feutry et al. 2014; Morgan 2018; Pillans et al. 2022). More recently, the species, together with the congeneric *G. garricki* was found in the Victoria River which is adjacent to the Keep River (R. Pillans unpublished data). *Glyphis garricki* was only described in 2008 (Compagno et al. 2008) and ranges from King Sound in the West Kimberley to Van Diemen Gulf in the Northern Territory, with recent genetic work identifying four distinct populations (Fuetry et al. 2020). As both species of *Glyphis* are known from rivers that flow into the Cambridge Gulf, together with the recent discovery of both in the Victoria River, it is likely that these species may occur further downstream in the Keep River estuary but were not detected during the surveys.

When these *Pristis* spp. and *Glyphis* spp. are present in the estuary sites (EST01-03), exposure to chemical residues is likely to be lessened by tidal flushing where tidal ranges of 3-7 m are common. However, within the pools K1-K3, tidal influence is far less influential and chemical exposure is likely to be greater (see GHD 2023). Currently, while *P. pristis* reside in pool K4 they are not directly exposed to farm chemicals in discharge from existing irrigated agricultural development in the Keep River catchment. However, the future expansion of irrigated agriculture on the Knox Plains may change this.

4.2 Implications of farm chemicals in the Keep River

Depending on the concentration and length of exposure, agricultural pesticides that are in use in the Keep River catchment may have a wide range of toxic effects on aquatic organisms and ecosystems. As well as potential lethality, effects on aquatic organisms at sub-lethal concentrations include oxidative stress, morphological/developmental abnormalities, reduced fertility and/or fecundity, reproductive problems and behavioural changes. Even if some organisms do not suffer direct toxic effects, they may be indirectly affected by impacts of pesticides on their prey, predator and/or competitive species. The effects of pesticides could negatively impact growth, fitness and survival of individuals with implications for species richness and community composition in aquatic ecosystems.

However, although a large body of literature exists documenting numerous effects both lethal and sublethal, of many pesticides, there are a number of knowledge gaps that hinder our understanding of the potential impacts that the chemicals used locally may have on the flora and fauna of the Keep River if they enter the aquatic ecosystem. Indeed, there is a paucity of knowledge on the effects of pesticides in the tropics in general and a lack of data on the ecotoxicological threat they pose to tropical organisms and ecosystems (Lewis et al. 2016b).

Many studies on the toxicity of pesticides use model organisms because of the ease of access to animals, extensive knowledge about the species and the supposed applicability of results to other species. These studies provide invaluable information on the effects of pesticides on aquatic organisms and can be used for risk assessment guidelines (e.g., ANZG 2018). However, the broad variation in toxicity values and sublethal effects seen between species, and indeed, even within species (at different life-stages and the different sexes), precludes the direct translation of results from tested species to those which have not been studied. This suggests that the effects of pesticides on more specific species need to be tested in order to elucidate common effects within taxa. Studies on the effects of chemicals used in Australia on native Australian aquatic fauna, which are currently lacking, would help to fill this knowledge gap and provide definitive data for the management of our aquatic ecosystems.

The environmental relevance of the concentrations of pesticides tested in many studies is debated, with mortality and deleterious sub-lethal effects only seen at high concentrations of some chemicals that are unlikely to occur under natural conditions, except in the case of a spill. From monitoring of water quality in the Keep River system it appears relatively rare that pesticides enter the river, at least at detectable concentrations. However, these tests represent discrete timepoints and may not provide the full picture of the occurrence of pesticides in Keep River waters. Pesticides found in the tissues of organisms (bioaccumulation) may provide an additional monitoring tool for assessing the levels of chemicals entering the ecosystem.

The Kimberley region of Western Australia experiences wet and dry seasons and consequently the Keep River system is highly variable in terms of water flow throughout the year. During the wet season, the risk of run-off carrying pesticides to the river may be high, but the volume of water flowing through the system may dilute the concentration of chemicals and flush them rapidly away. However, during the dry season, the river flow is greatly reduced and isolated pools form along the Keep River. If pesticides persist in the pools following the wet season, or in the unlikely event that pesticides enter these pools in the dry season because of accidental tailwater release, they would not be flushed out and could become more concentrated through evaporation, potentially exposing organisms to higher concentrations of chemicals for longer periods. Additionally, many studies of toxicity are conducted under laboratory conditions, at specific temperatures and pH levels. However, temperature, pH and other environmental factors can affect the toxicity of chemicals and influence the sensitivity of organisms to them. Conditions specific to the Keep River should be considered when evaluating the likely toxicity of pesticides to the system's organisms.

Commercial formulations of pesticides are varying mixtures of the active ingredient and other ingredients that are supposedly inactive, but which can have their own toxic effects and/or increase the toxicity of the mixture to aquatic organisms e.g., the polyethoxylated amines (POEA) that are added as surfactants to some glyphosate-based commercial pesticides (Folmar et al. 1979). Tests conducted using only the active ingredient may provide different results to tests that use commercial formulations (Annett et al. 2014). Additionally, pesticide transformation products (TPs) may have differing effects to their parent compound, and organisms may be exposed to multiple pesticides and/or TPs at once, with the potential for additive or synergistic effects.

We therefore propose studies be performed to determine the specific effects of chemicals that are used locally (and possibly combinations of chemicals) on the aquatic fauna of the Keep River, and whether there is any bioaccumulation of pesticides in their bodies. Ethical considerations would preclude the testing of chemicals directly on threatened fauna such as the elasmobranch species found in the Keep River, however, it may be possible to use one or more widespread and abundant teleost species. Potential model teleost species include three tropical species used by Patra et al. (2015) to test the interaction of temperature and toxicity of three agricultural pesticides: silver perch (*Bidyanus bidyanus*, family Terapontidae), rainbowfish (*Melanotaenia duboulayi*, family Melanotaeniidae), western carp gudgeon (*Hypseleotris klunzingeri*, family Eleotridae). Other common species found in faunal surveys of the Keep River could also potentially be used to elucidate the effects of pesticides in use in the catchment e.g., bony bream (*Nematalosa erebi*, family Clupeidae), the most abundant species found in the Keep River in the latest faunal surveys, diamond mullet (*Planiliza ordensis*, family Mugilidae), blue catfish (*Neoarius graeffei*, family Ariidae), Spangled Perch (*Leiopotherapon unicolor*, family Terapontidae), Western rainbowfish (*Melanotaenia australis*, family Melanotaeniidae), Northwest Glassfish (*Ambassis* sp. 1, family Ambassidae) and barramundi (*Lates calcarifer*, family Latidae).

Apart from determining median lethal effects (LC_{50}) values for pesticides that have been detected in Keep River pools for Australian species under environmental conditions relevant to the Keep River, testing to determine median effect concentrations (EC_{50}) could also be performed. Sub-lethal endpoints such as loss of equilibrium, erratic swimming behaviour, surface breathing and increased operculum movement, damage to reproductive system resulting in decreased fecundity, and inhibition of AChE activity could be used following methods described by Patra et al. (2015), El-Nahhal (2018), Ghaffar et al. (2018), Kamarudin et al. (2020).

Hydrodynamic modelling carried out by GHD (2023) as part of this project to evaluate risks to the Keep River from pesticides in the catchment provides information on the likely transport and dispersion of chemicals from farms to the Keep River system, considering rainfall, river flows, tides and currents. This modelling could be used to inform concentration levels of chemicals used in future investigations into the toxic effects of pesticides on Keep River fauna.

4.3 Conclusions

The Keep River is a dynamic, tropical river in the Kimberley Province, with highly variable water flow between wet and dry seasons. The lower reaches are subject to tidal influence and also receive surface water discharges from farms within the Ord River Irrigation Area. It supports a high diversity of aquatic organisms including species listed as Matters of National Environmental Significance under the EPBC Act. Two species of EPBC listed vulnerable sawfish, the Dwarf Sawfish (*Pristis clavata*) and Freshwater Sawfish (*Pristis pristis*) are regularly found in faunal surveys of the Keep River and one specimen of a third vulnerable sawfish, the Green Sawfish (*Pristis zijsron*) was found in the estuary in 2011.

The importance of the Keep River as habitat for these EPBC listed species is highlighted by the fact that catches (per unit effort) of the Dwarf and Freshwater Sawfishes in the Keep River are comparable to those in the West Kimberley's Fitzroy River, which is a global refuge for these species. Importantly, any impacts to these species from chemical exposure is likely to be reduced when they reside in the estuary due to the high degree of tidal flushing (see GHD 2023). Similarly, species residing in pools upstream of the Border Creek chemical discharge point are at least risk of direct exposure, however this may change depending on the risks associated with chemical releases from the future Knox Plains development (see GHD 2023). In the case of chemical release into the Keep River, a series of mitigation scenarios are described in GHD (2023). Consideration should be given to the impact of artificial flows on the life history of these species, many aspects of which are driven by flow (e.g., Lear et al. 2019, Morgan et al. 2021).

Water quality monitoring of the Keep River has found concentrations of farm chemicals above the default guideline values (DGVs) for the protection of 99% of species set by Australian and New Zealand guidelines (ANZG 2018). Although relatively rare, these findings indicate the potential for further farm chemicals to enter the Keep River and are of concern for the organisms it supports. Despite the dearth of information on toxicity of farm chemicals to tropical species in general, and Australian tropical species in particular, this report highlights the potential risks to the vulnerable species of the Keep River and the food webs which support them if pesticides enter the aquatic ecosystem. Testing the toxicity of specific pesticides in use in the Keep River catchment on local species and under realistic environmental conditions would provide invaluable data for accurate assessment of the risks posed by agricultural developments in the region. We also advocate engagement with local farm operators and use of tools such as the Pesticide Decision Support Tool developed by Warne at al. (2022), to encourage the use of pesticides with less toxic effects on aquatic organisms and higher environmental degradation rates.

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