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1 **The impact of pesticides on local waterways: a scoping review and method for identifying**  
2 **pesticides in local usage**

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10

11 **Keywords:** paraquat, bromoxynil, diquat, pesticides, waterways, agriculture

12

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## 14 **Abstract**

15 Pesticides used in agriculture are widely considered to be the most cost-effective way to reduce  
16 undesirable plants and animal pests and increase crop yields. However, these economic benefits  
17 should be evaluated against any deleterious impacts on the natural environment and human health.  
18 While a great deal of attention is paid to the impact of agricultural runoff, little is known about such  
19 impacts on local waterways. The aim of this study was to (i) develop a methodology to determine  
20 which pesticides were being used in local agriculture in the Byron Shire, Australia and (ii) search the  
21 literature for evidence of the impact of these chemicals on local waterways. After a comprehensive  
22 search involving multiple government databases, three pesticides with potentially high toxicity on the  
23 aquatic ecosystems and humans, which are used for the treatment of crops cultivated on the  
24 agricultural land in the Byron Shire, were selected for this review: bromoxynil, diquat and paraquat.  
25 In the systematic scoping review, two databases were searched (Scopus and Web of Science),  
26 including publications between January 2008 and April 2019; and from 160 obtained articles, 36  
27 papers were identified for inclusion. The evidence of harmful effects at realistic concentrations  
28 (concentrations found in the environment) was found for all selected herbicides, but not on all  
29 organisms. In aquatic environments, diquat was found to be toxic to the snail, and bromoxynil to  
30 algae. The clearest and most consistent evidence was found for paraquat. At realistic concentrations,  
31 paraquat (i) severely inhibited healthy bacterial growth (*E. coli*), (ii) distorted tropical freshwater  
32 plankton communities and (iii) increased fish kills (common carp) three times more than the weed  
33 (water hyacinth) that it was employed to control. Of particular concern is that paraquat has been  
34 banned from sale in the European Union and many countries around the world but remains available  
35 in Australia and is likely in use in the Byron Shire. Further work is required to scope the extent of its  
36 use and the effectiveness of the regulations governing its use. This study provides a methodology that  
37 can be used to identify pesticides that are likely in local use and identify evidence of any negative  
38 impacts on the health of local waterways.

## 39 1. Introduction

40 Over one-third of the world's land surface and three-quarters of its freshwater resources are dedicated  
41 to crop or livestock production (Diaz et al., 2019). The expansion of the production of food, feed,  
42 fibre and bioenergy in recent decades has afforded economic prosperity for many developed  
43 countries. However, a panel of hundreds of world scientists have warned that these advances have  
44 come at a cost to nature, particularly soil, water and air quality, climate regulation and habitat  
45 provision (Diaz et al., 2019). The Global Assessment report has identified many anthropogenic  
46 drivers of unsustainable practices, including harmful policies that favour economic activity over  
47 environmental conservation. One industry named in the report is the agricultural industry, including  
48 the use of fertilizers and pesticides to enable the over-exploitation of natural resources.

49  
50 Pesticides are a broad class of chemicals that are used to remove or reduce the presence of  
51 undesirable insects (insecticides), rodents (rodenticides), plants (herbicides), bacteria (bactericides),  
52 fungi (fungicides) and larvae (larvicides). In agriculture, herbicides are the main type of pesticides  
53 and are used to decrease the spread of weeds to achieve higher crop yields. Using pesticides is widely  
54 considered to be the most cost-effective way to achieve high crop production (Singh and Singh,  
55 2016). The agricultural industry is a strong contributor to the Australian economy providing  
56 employment for over 1.6 million Australians and returning over \$60 billion from farm production in  
57 2016-17 (by National Farmers' Federation, 2018, <https://www.nff.org.au/>). However, the agriculture  
58 industry has also been identified as the greatest contributor to water pollution in terms of nutrient  
59 enrichment, particularly from high levels of nitrogen and phosphorus detected in freshwater (Foley et  
60 al., 2005). Indeed, land-based runoff has been identified as second only to climate change for its  
61 adverse impact on the waters surrounding the Great Barrier Reef (Tan & Humphries, 2018). In  
62 addition to nutrient, pesticide residues have been detected in water resources and sediments (Allinson  
63 et al., 2015; Landis et al., 2008) and in ground water (Dabrowski et al., 2014).

64 While much attention has focussed on the impacts of runoff on the Great Barrier Reef and the  
65 critical importance of its proper regulation (Hamman & Deane, 2018), little is known about the  
66 impact of residue pesticides and runoff on local waterways throughout regional Australia.. The aim  
67 of this systematic review was to determine whether there is any evidence in the literature of negative  
68 impacts of pesticides on local waterways.

## 69 **2. Material and methods**

### 70 **2.1. Context**

71 The study was undertaken within one ‘local’ area known as the Byron Shire, which is situated around  
72 the popular but small coastal town of Byron Bay, New South Wales (NSW), Australia. The Byron  
73 Shire Council Local Government Area (LGA) is located on the most easterly point of Australia, with  
74 tourism as its major industry, and agriculture and creative industries also major contributors to its  
75 economy (Lasky, 2019)While this review represents a case-study approach, the methodologies used  
76 in this study could readily be applied to any local area within Australia, and indeed around the world,  
77 to firstly identify what pesticides are likely to be in use in the local area, and then to find out whether  
78 there is any evidence for harmful effects on local waterways of those chemicals.

79 Located in the subtropical area, the Byron Shire has a rich agricultural industry including  
80 production of macadamias, avocados, bananas, coffee, pork, vegetables, dairy, beef, bush foods and  
81 flowers (<https://www.byron.nsw.gov.au/Business/Business-in-Byron/Agriculture>).

### 82 **2.2.Design**

83 This study consists of two parts: (1) A method of pesticide selection, which involved the  
84 development of a methodology to identify which pesticides are likely to be in use within the Bryon  
85 Shire and; (2) A systematic scoping review of the literature of the selected pesticides to identify  
86 whether there was any evidence for harmful effects of the selected pesticides on local waterways.

87

### 88 **Part 1: Pesticides selection**

89 The objective of part one of the review was to identify the most toxic agricultural pesticides used in  
90 the Byron Shire (Australia). However, information about the type and quantity of agricultural  
91 pesticides used in the local region (i.e. the Byron Shire) was not readily available or accessible in the  
92 public domain. Therefore, the following methodology, summarised in **Figure 1**, was applied to  
93 identify the pesticides most likely to be in use (based on the nature and extent of the most common of  
94 crops grown in the area):

- 95 1. The five largest (by area) agricultural land uses were identified (Figure 2);
  - 96 2. The main chemicals likely to be applied to these land uses were determined by searching the  
97 Australian Pesticides and Veterinary Medicines Authority's (APVMA) online database and  
98 identifying the chemicals with the greatest number of product registrations (Australian  
99 Pesticides and Veterinary Medicines Authority, 2018);
  - 100 3. A short list of chemicals were then subjected to two further stages of short listing using the  
101 hazard statements in the Material Safety Data Sheet. These were found using the online  
102 search tool on Safe Work Australia's website (Safe Work Australia, 2018). Selection of the  
103 pesticides was based on their Globally Harmonised System of Classification (GHS) hazard  
104 statement and their toxicity on the aquatic ecosystems and humans (**Table 1**).
- 105 3.1. In the first stage the hazard statement for aquatic toxicity containing only chemicals  
106 classified as H410 (highly toxic to marine life with long-term effects) were short listed  
107 for the next stage (highlighted red in **Table 1**).
  - 108 3.2. In the second stage, the hazard statement for human toxicity containing only the  
109 chemicals that were found to be also the most highly toxic to humans (**Table 1**) were  
110 prioritised for the current review.
  - 111 3.3. Finally, additional background information would be sourced on the selected pesticides  
112 prior to the literature search. An overview of the physiochemical properties was obtained

113 from PubChem (<https://pubchem.ncbi.nlm.nih.gov>). The World Health Organisation  
114 (WHO) was used to source information on lethal dose and toxicity rating (WHO, 2015).

## 115 ***Part 2: Scoping review***

116 Having identified a short list of chemical pesticides, the literature review was designed to address the  
117 question: are there any negative impacts on the local aquatic environments associated with their use.  
118 The literature review used the Preferred Reporting Items for Systematic Review and Meta-analysis  
119 (PRISMA) statement (Moher et al., 2009). A systematic scoping review was considered the most  
120 appropriate form of knowledge synthesis for the question, which was essentially to identify whether  
121 any such evidence exists and to examine the ‘extent, range and nature’ of the available evidence  
122 (Arksey & O'Malley, 2005). The framework for this scoping review was informed by Arksey and  
123 O'Malley (2005), with additional enhancements proposed by Levac, Colquhoun and O'Brien (Levac  
124 et al., 2010) and (Peters et al., 2015), as summarized in **Table 2**. The methodology was registered  
125 with Open Science Framework prior to the completion of the data analysis phase and can be accessed  
126 at <https://osf.io/p5z9b>.

### 127 **2.3. Search strategy**

128 The literature search strategy was designed in consultation with a librarian and is detailed in **Table 2**.  
129 Each chemical was searched separately to allow counting of articles. The search using the individual  
130 chemical name as a search term was combined with AND with the key search terms for the  
131 environmental impacts: ‘toxicity’, OR ‘contamination’ OR ‘pollution’ OR ‘environment’. To focus  
132 on waterways and agriculture, the above search was combined with AND for the key search terms  
133 ‘water’ or ‘aquatic’; plus AND for the search terms ‘agriculture’ OR horticulture’ OR ‘crop’). The  
134 search was limited to title, abstract and keywords, English language and publications between  
135 January 2008 and April 2019 to ensure currency of evidence. Two databases were searched (Scopus  
136 and Web of Science), each by two independent researchers. Reference lists of all identified papers  
137 were also searched for additional studies and reviews that were relevant.

## 138 2.4. Study Selection

139 Papers that met the following criteria were included: (i) scholarly journal articles, (ii) paper included  
140 reports of bromoxynil, diquat and/or paraquat, and (iii) papers that investigated impact of at least one  
141 of the chemicals on aquatic environments at realistic (within the range of use) concentrations. Papers  
142 were initially screened by title and abstract by two independent researchers. Studies that appeared to  
143 meet the inclusion criteria were further scrutinized through an evaluation of the full text article.

144 Those studies whose full text met the study criterion were included in the review. During the process  
145 of the selection of relevant studies an iterative team approach was used, as proposed in a scoping  
146 review framework by Levac, Colquhoun and O'Brien (2010).

147

## 148 2.5. Data Extraction

149 Two reviewers independently extracted the following data into an *a priori* designed evidence table:  
150 first author's surname, year and country, title of paper, study design, feature of aquatic environment  
151 studied, location of data collection (in laboratory, in situ or other), effects on environment, and other  
152 comments/points of interest.

## 153 3. Results

### 154 3.1. Pesticide selection and toxicity

155 The three pesticides identified as having both the highest aquatic and human toxicity, in alphabetical  
156 order, were bromoxynil, diquat and paraquat (**Table 1**). Information on solubility, vapour pressure,  
157 half-life, and physical description for each of the chemicals is presented in **Table 3**. It should be  
158 noted that values varies considerably depending on environmental factors such as presence of  
159 oxygen, soil type, pH and microbial activity (Hanson et al., 2015).

160 All pesticides are characterised with a strong affinity to bind to soil particles with the half-life  
161 for soil and water presented in **Table 3** (<https://pubchem.ncbi.nlm.nih.gov>).

162 Preliminary information on toxicity for bromoxynil, diquat, and paraquat is presented in **Table 4**.

163 Lethal dose (LD<sub>50</sub>) of a chemical causes death of 50% of the animal test group. Although articles in



164 the scoping review discussed LD<sub>50</sub> of different species (Bouétard et al., 2013; Marvá et al., 2010;  
165 Thaimuangphol & Kasamesiri, 2015; Wast et al., 2016; Yarpuz-Bozdogan, 2016), none used the  
166 same species for all three chemicals. Therefore, to provide consistency between chemicals, LD<sub>50</sub> of  
167 the rat was used, consistent with values listed on the World Health Organization (WHO) website.

### 168 **3.2 Pesticide literature**

169 Before specifying the specific names of herbicides an initial generic search resulted in 1800 papers  
170 in Scopus and 1805 from the Web of Science. After inclusion of the specific names of the selected  
171 pesticides (i.e. bromoxynil, diquat and paraquat), the search produced 160 records. Following  
172 removal of duplicates, 144 papers remained (**Table 2**). A further 102 were excluded as not  
173 meeting the study criteria after screening the titles and abstracts. The remaining 42 full-text  
174 articles were further scrutinized and 6 additional papers were excluded as their focus (e.g.  
175 monitoring soil quality and microbial degradation of pesticides) was not relevant to the objective  
176 of the scoping review. Data extraction to the table was checked for consistency between reviewers,  
177 with no evidence of significant discrepancies in the extraction results. The PRISMA flow of  
178 papers through the selection process is presented in **Figure 3**.

179  
180 Study types included 28 original research and 8 of review articles. Original research included  
181 12 laboratory experiments (Ansara-Ross et al., 2008; Botelho et al., 2012; Bouétard et al., 2013;  
182 Chusaksri et al., 2010; De Solla et al., 2012; Kim et al., 2009; Li et al., 2015; Li et al., 2017; Marvá et  
183 al., 2010; Nsibande & Forbes, 2016; Oh et al., 2014; Thaimuangphol & Kasamesiri, 2015); 11  
184 sampling studies *in situ* (de Queiroz et al., 2018; Donald et al., 2018; Ismail et al., 2011; Kathiresan &  
185 Deivasigamani, 2015; Leboulanger et al., 2011; Love et al., 2011; Messing et al., 2011; Mottes et al.,  
186 2017; Siemering et al., 2008; Wast et al., 2016; Yarpuz-Bozdogan, 2016). There was one clinical  
187 study on humans (Konthonbut et al., 2018) and one epidemiologic study (Gatto et al., 2009). Two  
188 articles were computer model risk assessment (Dabrowski et al., 2014; Henning-De Jong et al.,

189 2008). Review articles included 7 reviews (Fenner et al., 2013; Gebrehanna et al., 2014; Juraimi et  
190 al., 2013; Roberts et al., 2012; Sartori & Vidrio, 2018; Singh & Singh, 2016; Tsai, 2013) and one  
191 systematic review on the impact of pesticides on human health (Freire & Koifman, 2012). There was  
192 one case study (Tsai, 2013b). Aquatic environments from locations around the world ranged from  
193 drinking water, well water, surface water, ditches, reservoirs, ponds, wetlands, lakes, catchment  
194 areas, large watersheds and coastal ecosystems. Studies *in situ* included multiple locations in North  
195 America (De Solla et al., 2012; Donald et al., 2018; Gatto et al., 2009; Love et al., 2011; Messing et  
196 al., 2011; Mottes et al., 2017; Siemering et al., 2008), Europe (Leboulanger et al., 2011) and Asia  
197 (Ismail et al., 2011, Kathiresan & Deivasigamani, 2015, Wast et al., 2016, Yarpuz-Bozdogan, 2016).

198 Organisms explored in these studies included (Botelho, 2012), microalgae (Marvá, 2010;  
199 Siemering, 2008), an aquatic plant (Kathiresan & Deivasigamani, 2015), phytoplankton (Leboulanger  
200 et al., 2011), crustaceans (Kim et al., 2009; Thaimuangphol & Kasamesiri 2015; Yarpuz-Bozdogan,  
201 2016;), gastropods (Bouetard et al., 2013; Wast et al., 2016) and fish (Wast et al., 2016). Human  
202 studies involved a case-controlled study of farmers who had Parkinson's disease in the USA who had  
203 been exposed to pesticides, including paraquat, through contaminated drinking wells (Gatto et al.,  
204 2009), and paraquat residue found in the babies of women in Malaysia who had been exposed to  
205 pesticides through drinking wells during their pregnancy (Konthonbut et al., 2018).

206 Seven articles from 36 contained information on toxicity and effects of the pesticides at  
207 realistic concentrations on organisms found in the aquatic environment: *Escherichia coli* (Botelho et  
208 al., 2012), daphnia (Yarpuz-Bozdogan, 2016), plankton (Leboulanger et al., 2011), snails (Bouétard  
209 et al., 2013), shrimp (Thaimuangphol & Kasamesiri, 2015) and fish (Kathiresan & Deivasigamani,  
210 2015; Siemering et al., 2008) and one modelling study (Ansara-Ross et al., 2008). These results are  
211 summarised in **Table 5**.

### 212 **3.3 Bromoxynil**

213 There was one relevant paper which modelled a probabilistic risk assessment of the harmful effects to  
214 aquatic species from bromoxynil (Ansara-Ross et al., 2008). Ansara-Ross et al. (2008) set out to  
215 determine the risk to aquatic environments on a number of chemicals including bromoxynil (**Table**  
216 **5**). Bromoxynil was classified as a chemical with possible risk. Further analysis using the PERPEST  
217 (Predicting the Ecological Risks of PESTicides, van den Brink et al., 2002) model indicated no clear  
218 effects of bromoxynil on fish, crustaceans, insects, or plankton, but demonstrated a low negative  
219 effect on periphyton communities.

220

### 221 **3.4. Diquat**

222 Two papers were identified that investigated the impacts of diquat on aquatic environments  
223 (Bouétard et al., 2013; Siemering et al., 2008) as presented in **Table 5**. A French team lead by  
224 Boutetard (Bouetard et al., 2013) investigated the toxicity pathways of exposure of ecologically  
225 realistic concentrations of diquat on the great pond snail (*Lymnaea stagnalis*). The exposure level  
226 chosen was consistent with realistic field concentrations after aerial spraying. Three snails were  
227 measured after 5, 24 and 48 hours of exposure. The results indicated that exposure significantly  
228 stimulated gene transcription in the redox-cycling pathways, inducing oxidative stress, along with  
229 other stress-responsive pathways in the haemolymph and the gonado-digestive complex. This study  
230 demonstrated that: (i) diquat is capable of inducing toxicity in a non-target animal species, (ii)  
231 oxidative stress pathways could be used as a biomarker for diquat toxicity, and (iii) there is a risk of  
232 apoptosis (cell death) in haemocytes (lymph cells) in *L. stagnalis* at high concentrations of diquat,  
233 which could seriously compromise the snail immune system.

234 Siemering et al. (2008), developed and implemented a 3-year monitoring program in the state  
235 of California to determine the extent that aquatic herbicide impacts on the environment. The program  
236 developed a risk quotient for determining regulatory limits of certain pesticides. In locations at one  
237 hour post exposure to diquat, the risk quotient for diquat's effect on green algae (*Selenastrum*),

238 duckweed (floating plants also known as bayroot) and fish (fathead minnow and delta smelt), had  
239 exceeded the Level of Concern as determined by the US Environmental Protection Agency. The  
240 number of times that risk quotient exceeded level of concern indicated that ‘more extensive risk  
241 characterizations’ are required to assess the risk of diquat to aquatic environments (Siemering et al.,  
242 2008).

### 243 **3.5. Paraquat**

244 There were four studies that reported evidence of the harmful effects of realistic  
245 concentrations of paraquat on aquatic environments (**Table 5**). In an *in vitro* laboratory study in  
246 Brazil, using a commercial formulation and recommended concentration of paraquat (200g/L),  
247 Botelho and colleagues (2012) measured the impact on aquatic micro-organisms using *E. coli* as a test  
248 organism. The study found that *E. coli* growth was completely inhibited for the first 300 minutes  
249 after exposure to paraquat. After this initial lack of growth, *E. coli* grew exponentially, but resulting in  
250 lower final concentration (based on the colony-forming units density (CFU) values) than when  
251 exposed to other herbicides. The results demonstrated extreme sensitivity of *E. coli* to paraquat  
252 exposure. This negative effect on *E. coli* growth is significant because the bacteria participate in  
253 organic decomposition, nutrient cycling and energy flow and thus the presence of *E. coli*'s is  
254 commonly used as an indicator of water quality (Botelho, 2012).

255 A study in India on controlling water hyacinth (*Eichhornia crassipes*) found that paraquat increased  
256 mortality to the common carp (Kathiresan & Deivasigamani, 2015). This study measured the impact  
257 of using paraquat-treated water on crops post-germination. Along with water hyacinth biomass, three  
258 species of fish (common carp, mrigal, rohu) mortality were measured 32 days after sowing. Water  
259 hyacinth is a weed that forms a biomass that itself reduces water quality and causes mortality to  
260 fishes (control: 14.5% carp, 50.0% mrigal, 21.7% rohu). The paraquat-treated water (at 1.50 kg/ha)  
261 substantially worsened fish mortality for the common carp (42.0% carp), but not the mrigal or rohu  
262 (50.6% and 25.0%, respectively). An examination of the tissue histopathology of the organs of

263 affected fishes found extensive tissue damage in multiple organs after herbicide exposure,  
264 particularly the liver and gills (Kathiresan & Deivasigamani, 2015).

265 A microcosm study in Mozambique (Leboulanger et al., 2011) looked at the impact of  
266 herbicides, including paraquat, on two new human constructed freshwater reservoirs. All components  
267 of tropical plankton (bacterioplankton, phytoplankton, and zooplankton) were monitored for five  
268 days as a measure of the direct and indirect effects of herbicide exposure on the health of the trophic  
269 community. Herbicide exposure had an immediate inhibitory effect. Interestingly, paraquat decreased  
270 plankton diversity and overall biomass and green microalgae became dominant. Results from this  
271 study indicated that the aquatic species of a freshwater reservoir with a simple trophic community are  
272 sensitive to the effects of paraquat exposure and an acute exposure could negatively impact the the  
273 system within days.

#### 274 4. Discussion

275 There is evidence of harmful effects of three analysed herbicides on aquatic environments. At  
276 realistic concentrations, diquat was found to be toxic to the snail, and bromoxynil to algae (Siemering  
277 et al., 2008). The clearest and most consistent evidence of negative effects was found for paraquat.  
278 At realistic concentrations found in the environment (within the safety range), paraquat severely  
279 inhibited healthy bacterial growth of *E. coli* (Botelho et al., 2012), distorted tropical freshwater  
280 plankton communities (Leboulanger et al., 2011) and increased fish kills (common carp) three times  
281 more than the weed (water hyacinth) that it was employed to control (Kathiresan & Deivasigamani,  
282 2015). In non-aquatic organisms like honey bees (Cousin et al., 2013) very low concentrations of  
283 paraquat in larvae food disrupted the normal growth of honey bee eggs and colony. Critically, these  
284 low concentrations were below the detection limit by most modern analytical methods. Thus, we do  
285 not know how widespread the exposure is to honey bees, and whether this extreme sensitivity to  
286 paraquat would be exacerbated by exposure to other pesticides.

##### 287 4.1. Bromoxynil

288 Bromoxynil is a nitrite herbicide used to control broadleaf weeds by inhibiting photosynthesis. It is  
289 widely used for pests associated with maize, wheat, sorghum and onions (Ansara-Ross, 2008) and for  
290 the treatment of broad-leaved weeds (Topp et al., 1992). A number of studies have investigated the  
291 potential health effects of exposure to bromoxynil. It is also a possible human carcinogen (Toxnet  
292 Toxicology Data Network, 2012; US National Archives and Records Administration Office of  
293 Pesticides Programs - Health Effects Division Science Information Management Branch, 2006) and a  
294 moderate eye, skin and respiratory irritant (Krieger, 2001). Safety concerns have led to a number of  
295 studies on exposure, particularly of farmers who use the pesticide for their crops (Cessna & Grover,  
296 2002). The general population may also be exposed via inhalation of ambient air and dermal contact  
297 with surface water in agricultural regions where the product is used. (Semchuk et al., 2003).  
298 However, a 5-year study monitoring bromoxynil level in 7 wetlands placed on organic farms and 16  
299 wetlands located on minimum-tillage farms in Canada, have recently shown the levels below water  
300 quality guidelines (Donald et al., 2018).

#### 301 **4.2. Diquat**

302 Diquat is classified as a bipyridyl class of pesticide. It is used to protect potato, banana, vine and  
303 other seed crops. It is also used in aquatic systems for control of submersed, floating and emerging  
304 weeds. Diquat is the third most widely used herbicide in the world (Li et al., 2017). The physical  
305 properties of diquat (like paraquat) are unusual among the herbicides as it is highly absorbent in  
306 water and binds very strongly to the soil-organic matter matrix (Florencio et al., 2004). In soil, diquat  
307 becomes isolated and protected from the soil microorganisms, which can irreversibly prevent or  
308 significantly delay its cellular breakdown (Ogram et al., 1985; Weber & Coble, 1968). Hence, its  
309 biodegradation is extremely slow, but it does not translocate and consequently, diquat is rarely  
310 observed in secondary sites (Fenner et al., 2013).

311 In aquatic environments, diquat has been shown to break down quickly and completely with exposure  
312 to ultraviolet light (Florencio et al., 2004; Funderburk & Bozarth, 1967). However, caution must be

313 applied when interpreting results from lab-based studies that may not simulate natural environmental  
314 conditions. A recent review of the potential toxic effects of diquat on fish and aquatic invertebrates in  
315 natural environments concluded that there were only short term or negligible effects on fish and  
316 aquatic invertebrates, which were thought to be outweighed by the increase in the available body of  
317 water (Breckels & Kilgour, 2018).

318         The toxicity of diquat may vary depend on aquatic species tested. Snails (Bouétard et al.,  
319 2013) and daphnia (Siemering et al., 2008) were more sensitive to negative impacts of diquat  
320 exposure compared with, for example, some fish species (Siemering et al., 2008). However a study  
321 on continuous lethal doses of diquat on microalgae reported concerning findings. In a Spanish study  
322 on herbicide resistance in herbicide-polluted waters, Marva et al (2010) looked at the impact on the  
323 natural evolution of a species of freshwater microalgae after exposure to lethal doses of diquat  
324 (*Scenedesmus intermedius*). Specifically, they used a very rapid evolution model to investigate: (i)  
325 whether adaptation occurred at lethal doses, and (ii) whether this occurred through physiological  
326 adaptation (acclimatization) due to post-adaptive mutation after herbicide exposure; or from (iii)  
327 adaptation arising through rare spontaneous mutations before subsequent exposures. The result of this  
328 study, demonstrated that rare, pre-selective spontaneous mutations of the microalgae appeared before  
329 exposure to subsequent lethal doses, ensuring survival of the species but with unpredictable  
330 consequences. They concluded that human manufactured pollutants could cause the ‘emergence of  
331 evolutionary novelties in aquatic environments’. These findings should be followed up due to  
332 potential mutagenic effects with long-term consequences on the aquatic environment.

### 333 **4.3. Paraquat**

334 Paraquat is a bipyridylium herbicide used to control weeds around rice crops (Ismail, 2011), cotton,  
335 tomatoes, beans, soybeans, potatoes, sunflowers, sugar cane (Tsai, 2013a), fruit orchards and  
336 mulberry forests (Kim et al., 2009). It is a chemical with characteristically strong adsorption to  
337 organic matter due to its binding to negatively-charged components in the soil (**Table 3**). This greatly

338 reduces its mobility in soil through leaching or other processes, and it is not readily chemically or  
339 microbiologically degraded (Smith & Mayfield, 1978). In water, the persistence of paraquat seems to  
340 depend upon the quantity and quality of sediment present. In one study, 80% of applied paraquat  
341 persisted in river water 56 days post-application (Wang et al., 1994), while U.S. Environmental  
342 Protection Agency reported approximate half life of 160 days (<https://nepis.epa.gov>).

343 In an early study in New Zealand (Burnet, 1972), the recommended concentration of paraquat  
344 was added to the Waimakariri River, Canterbury, to control aquatic weeds. This resulted in a massive  
345 kill of drift invertebrate fauna (amphipods) to 5% of pre-treatment levels within one month,  
346 demonstrating a profound immediate and prolonged toxic effect. Interestingly, paraquat exposure on  
347 six day old water flea (*Daphnia magna*) in the three different types of water-sediment systems  
348 (system A water-alone control; system B simulating a spray drift exposure and system C simulating  
349 the irrigation scenario), did not lead to acute affect on *D. magna*. This was explained by a quick  
350 reduction of paraquat in water after the introduction of sediment, which quickly adsorbs to it in a  
351 dose-dependent manner (80 - 90%) within 48 hours (Kim et al., 2009).

352 At very low concentrations, as low as ng/kg, paraquat was shown to induce damages in honey  
353 bees (Cousin et al., 2013). In a French study (Cousin et al., 2013), researchers randomly selected a  
354 sample of healthy hives to insert an empty comb, returning 24 hours later to collect the lava. This was  
355 taken back to the laboratory and exposed to graded concentrations of paraquat 0 (control), 0.001,  
356 0.01, 0.1 and 1 µg/kg in the water used as part of their food preparation, along with royal jelly.  
357 Within 48 hours, paraquat had substantially and significantly decreased the size of the oenocyte cells,  
358 cytoplasm and nucleus in a dose-dependent manner. The most remarkable thing was that disruption  
359 occurred at every concentration level, with the lowest concentration being lower than the detection  
360 limit of the most mass spectrometry analysis. This suggests that the honey bees, and their oenocytes  
361 in particular, are highly sensitive to paraquat (Cousin et al., 2013). A recent review by Sartori and



362 Vidrio (2018) provides an overview of research studies demonstrating high toxicity of paraquat to  
363 honeybees, fish, and other aquatic species including teratogenic effects in birds and amphibians.

#### 364 **4.4. Risk to humans**

365 Of these three agricultural chemicals, paraquat appears to pose the most imminent threat to  
366 humans. Paraquat is classified by WHO as a class II pesticide or moderately hazardous to human  
367 health (**Table 4**). Due to its high toxicity and deleterious effects, this pesticide has been reportedly  
368 banned from use in over 50 countries including China, the 27 countries of the European Union,  
369 Cambodia, and Vietnam (Konthonbut et al., 2018).

370 The early literature documents many cases of children and young people being severely poisoned  
371 from accidentally swallowing paraquat from unlabeled containers (Haley, 1979). Paraquat was found  
372 to be easily absorbed via skin, lungs and the gastrointestinal tract (Huang et al., 2012), with a high  
373 number of cases of harmful effects on humans particularly in Asia where this chemical was used for  
374 suicides especially in rural communities (Wu et al., 2013).

375 A review by the American Academy of Paediatrics listed paraquat and diquat as highly toxic  
376 chemicals (Roberts et al., 2012). Children are considered to be very vulnerable to adverse effects of  
377 pesticides because of developmental, dietary, and physiologic factors. However, ongoing exposure  
378 remains a part of life for many children. A longitudinal study from agricultural areas of Thailand  
379 where paraquat is known to be used (Konthonbut et al., 2018), showed that drinking from a  
380 community well during pregnancy was associated with significantly higher paraquat in the meconium  
381 of the neonate than those who did not drink from the well (geometric mean (standard deviation):  
382 124.9 (3.35) ng/mL exposed versus 26.99 (4.37) ng/mL not exposed,  $p = 0.012$ ). Similarly, living  
383 next to farmland where pesticides were sprayed (46.62 (4.79) exposed versus 19.70 (3.77) ng/mL,  
384 non-exposed,  $p = 0.031$ ), and having household family members who are agriculturalists were  
385 associated with significantly higher paraquat in meconium (42.09 (5.10) versus non-agriculturalists  
386 household 16.81(2.47) ng/mL,  $p = 0.016$ ). This study confirmed that paraquat can pass the placenta

387 barrier and 55% of the newborn meconium samples had measurable paraquat concentrations.  
388 Although this study does not link increased levels of paraquat in biological tissue with direct harmful  
389 effects, it is very concerning in the context of all that is now known about paraquat toxicity.  
390 Another study investigated whether consumption of water from private wells located in areas with  
391 documented historical agricultural pesticide use was associated with an increased risk of Parkinson's  
392 disease (PD) among residents of the Central Valley of California (Gatto et al., 2009). While the  
393 authors concluded that the consumption of well water potentially contaminated with pesticides may  
394 play a role in the incidence of PD, no direct link could be made for exposure to paraquat alone as  
395 paraquate was not detected in well water. The authors did note that they were not expecting to detect  
396 paraquat, as it does not usually enter wells through ground water (Gatto et al., 2009), although recent  
397 findings confirmed increased risk for contamination of surface waters and groundwater when  
398 pesticides used were used in local coffee plantations (de Queiroz et al., 2018).

#### 399 ***4. 5. Limitations***

400 The search strategy used in this study included only papers published in English within the previous  
401 10 years. The publication dates limits was to ensure currency of information. However, there were  
402 highly relevant earlier studies on these chemicals that were not identified in the initial search but  
403 were found on searches of the references from papers identified in the initial search. The most  
404 relevant of these papers were then included in the discussion.

405 Environmental research outcomes vary according to the methodologies used, potential biases,  
406 seasonal variability in environmental conditions, the characteristics of analysed species and other  
407 contributing biotic and abiotic factors. The scope of our literature review was to assess whether or not  
408 there was evidence of harmful effects of chemicals used in agriculture entering the local waterways.  
409 Questions about how frequent and the concentrations of the chemicals that are likely to be in use  
410 were beyond the scope of the current project but should be addressed in future research. Also, an

411 assessment on the propensity of the chemicals to persist in soil and water was also beyond the scope  
412 of the current study.

413  
414 Our findings provide information about three agricultural herbicides identified as likely to be in  
415 current use in the Byron Shire (Australia). In this scoping review, we have collated the available  
416 scientific evidence on the toxicity of three agricultural pesticides acquired from laboratory controlled  
417 conditions, via *in situ* monitoring, computer modelling studies, risk assessments and epidemiological  
418 studies conducted in a number of countries around the globe (USA, Canada, Brazil, China, Korea,  
419 Malaysia, Taiwan, India, South Africa, Netherlands and France). This review found evidence of  
420 harmful effects on aquatic environments from all the three herbicides investigated: bromoxynil,  
421 diquat and paraquat, even when used at realistic (recommended) concentrations.

## 422 5. *Conclusions*

423 This scoping review identified bromoxynil, diquat and paraquat as three chemicals most likely to be  
424 in common use in the Byron Shire that were most toxic to aquatic ecosystems in the local waterways.  
425 Paraquat has been banned from sale in many countries because of its high toxicity potential but  
426 remains available in Australia. Further research is urgently required to scope the extent of its use and  
427 the effectiveness of regulations governing its use. This study provides a methodology that can be  
428 used in regional areas to identify pesticides that are likely in local use and identify evidence of any  
429 negative impact on the health of local waterways.

430

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