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# Environmental risks to freshwater organisms from the mycotoxins deoxynivalenol and zearalenone using Species Sensitivity Distributions

Eagles, Emily

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1 Environmental risks to freshwater organisms from the mycotoxins deoxynivalenol and  
2 zearalenone using Species Sensitivity Distributions.

3 Emily J. Eagles<sup>aY</sup>, Rachel Benstead<sup>b</sup>, Susan MacDonald<sup>b</sup>,

4 Richard D. Handy<sup>c</sup> & Thomas H. Hutchinson<sup>a</sup>

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6 <sup>a</sup>School of Geography, Earth and Environmental Sciences, University of Plymouth, Drake  
7 Circus, Plymouth PL4 8AA, UK; <sup>b</sup>FERA Science Ltd., York Biotech Campus, Sand Hutton, York,  
8 YO41 1LZ, UK; <sup>c</sup>School of Biological & Marine Sciences, University of Plymouth, Drake Circus,  
9 Plymouth PL4 8AA, UK.

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15 <sup>Y</sup> Corresponding author: Emily J. Eagles

16 email [emily.eagles@plymouth.ac.uk](mailto:emily.eagles@plymouth.ac.uk)

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21 **Highlights**

- 22 • Previously mycotoxin toxicity data for freshwater invertebrates has been lacking
- 23 • The most sensitive are *Daphnia* for deoxynivalenol and snail embryos for zearalenone
- 24 • Freshwater PNEC values based on SSDs were 1.4 µg DON/L and 8.6 µg ZON/L
- 25 • Deoxynivalenol levels approach PNEC in streams subject to high agricultural run-off

26

## 27 **Abstract**

28 In this study, laboratory experiments have addressed the acute toxicity of two common  
29 mycotoxins, deoxynivalenol (DON) and zearalenone (ZON), in a range of freshwater  
30 organisms (including rotifers *Brachionus calyciflorus*, insects *Chironomus riparius* (larvae),  
31 crustaceans *Daphnia pulex* and *Thamnocephalus platyurus*, cnidarians *Hydra vulgaris*,  
32 molluscs *Lymnaea stagnalis* (embryos) and Protozoa *Tetrahymena thermophila*). Acute EC<sub>50</sub>  
33 values highlight crustaceans as the most sensitive organisms to DON, with *T. platyurus*  
34 having a 24 h EC<sub>50</sub> of 0.14 and *D. magna* having a 48 h EC<sub>50</sub> of 0.13 mg DON/L. During  
35 exposures to ZON, *H. vulgaris* and *L. stagnalis* embryos showed the highest sensitivity;  
36 mortality EC<sub>50</sub> values were 1.1 (96 h) and 0.42 mg ZON/L (7 d), respectively. Combining  
37 these novel invertebrate toxicity results, along with recent published data for freshwater  
38 plant and fish toxicity for analysis of Species Sensitivity Distributions, provides freshwater  
39 HC<sub>5</sub> values of 5.2 µg DON/L and 43 µg ZON/L, respectively. Using highest reported  
40 environmental concentrations and following REACH guidelines, risk ratios calculated here  
41 show the risk of ZON to freshwater organisms is low. In contrast, DON may periodically be  
42 cause for concern in streams subject to high agricultural run-off, likely during certain times  
43 of year where cereal crops are susceptible to higher fungal infections rates and may pose  
44 increased risks due to climate change.

45

46

## 47 **Keywords**

48 Emerging chemical, ecological risk, hazard assessment, lethal toxicity

## 49 1. Introduction

50 Deoxynivalenol (DON) and zearalenone (ZON) are two of the prominent mycotoxins which  
51 regularly contaminate food, in particular cereal products. Both these natural toxins are  
52 produced by fungi within the *Fusarium* genus commonly found in cereals grown in  
53 temperate regions of America, Asia and Europe. ZON has a distinguishing factor in its  
54 structural similarity to oestrogen and its action as an oestrogen mimic, DON is often  
55 referred to as vomitoxin due to the observed emetic response in various species and has  
56 been seen to cause neural toxicity (Pestka, 2007; Bonnet et al., 2012). Mycotoxin production  
57 can be minimized by following good farming practice. However, due to the potential health  
58 risks, the EU has established Maximum Permitted Levels (MPLs) in food of 200 - 1750 µg/kg  
59 for DON and 20-100 µg/kg for ZON, dependant on the type of foodstuff (EC, 2006a), as well  
60 as Guidance Values for various products intended for animal feed (EC, 2006b).

61 The rising interest in mycotoxins as natural chemicals of emerging concern has led to  
62 studies into the concentrations found in freshwater environments. *Fusarium* sp. are  
63 commonly found on growing cereal field crops, and to a lesser extent on harvested crops  
64 during storage, hence soil run-off to surrounding water systems would be expected to be a  
65 main route of contamination. Agricultural run-off and its accompanying mycotoxin load vary  
66 considerably; dependent on season, crop cultivation, area hydrodynamics and whether  
67 fungi are present and producing mycotoxins (Wettstein & Bucheli, 2010; Kolpin et al., 2014).  
68 Wastewater treatment plants (WWTP) are considered a widespread permanent source of  
69 mycotoxins, and studies on their mycotoxin removal efficiency show only partial removal  
70 (Wettstein & Bucheli, 2010; Gromadzka et al., 2015). Investigations have shown DON to be  
71 of higher concern in comparison to ZON, with its occurrence being more prevalent and of

72 higher concentrations. Quantification of ZON in environmental samples has shown  
73 concentrations are generally < 15 ng ZON/L with isolated periods of higher concentrations  
74 occurring infrequently across sample sites; the highest value recorded for ZON is 96 ng  
75 ZON/L at a river in an agricultural site in Iowa, USA (Gromadzka et al., 2009; Waśkiewicz et  
76 al., 2012; Kolpin et al., 2014). Whereas DON has been found at higher concentrations in  
77 numerous samples, the highest of which being 1662 ng DON/L in the same study of Iowa  
78 sites with all locations having concentrations above 48.6 ng DON/L (Kolpin et al., 2014).

79 Research into the toxicity of mycotoxins to aquatic species has been mainly focused  
80 on fish exposed via contaminated feed (Santacroce et al., 2008; Šišperová et al., 2015;  
81 Woźny et al., 2015). There are relatively few studies on waterborne exposure and toxicity in  
82 freshwater fish, with most of these reporting on effects in zebrafish. For example, DON  
83 appears to be of little concern for zebrafish embryos but ZON toxicity has been  
84 demonstrated in terms of both development and survival; 5 d development no observable  
85 effect concentration (NOEC) 25 µg ZON/L, 5 d survival EC<sub>50</sub> 890 µg ZON/L (Bakos et al., 2013;  
86 Schwartz et al., 2010; Khezri et al., 2018). There are currently no published toxicity data for  
87 invertebrates for ZON, but *Tetrahymena pyriformis* had a lowest observable effect  
88 concentration (LOEC) of 0.6 mg DON/L at 150 h (Bijl et al., 1988) for the reproduction  
89 endpoint. Phytotoxicity of both DON and ZON to microalgae and the macrophyte *Lemna*  
90 *minor* has been shown, with the former being more sensitive to ZON (72 h  
91 *Pseudokirchneriella subcapitata* growth EC<sub>50</sub> of 1.2 mg ZON/L) and the later more sensitive  
92 to DON (7 d LOEC of 0.25 mg DON/L) (Suzuki & Iwahashi, 2014; Vanhoutte et al., 2017;  
93 Eagles et al., 2019).

94 The time, space and cost involved in maintaining cultures and carrying out tests with  
95 multiple species often results in toxicity studies being focused on only one or a few model  
96 species (e.g. zebrafish, daphnids and algae). This results in a poor understanding of inter  
97 species variability in sensitivity. Species Sensitivity Distributions (SSDs) are a useful tool in  
98 assessing inter species variation in sensitivity, and SSDs have now become commonplace in  
99 assessing the risks of chemicals and setting aquatic environmental safety thresholds  
100 (Belanger et al., 2017). Briefly, SSD encompass EC<sub>50</sub> results from single species toxicity tests  
101 and based on the distribution of these we can determine hazard concentration (HCp) which  
102 will protect a defined percentage (p) of species within the distribution (frequently the HC<sub>5</sub>)  
103 (ECHA, 2008). This approach is considered to be beneficial in comparison to the alternative  
104 deterministic approach detailed in the ECHA assessment where the results of at least three  
105 studies, usually the standard test organisms algae, *Daphnia* and fish, are used with an  
106 appropriate assessment factor applied to the lowest EC<sub>50</sub>. SSDs consider a wide range of  
107 species to generate a community relevant threshold rather than one based on model or  
108 known most sensitive species (Belanger et al., 2017).

109 The first aim of this study was to test the species sensitivity of various invertebrates  
110 (rotifer *Brachionus calyciflorus*, insect *Chironomus riparius* (larvae), crustaceans *Daphnia*  
111 *pulex* and *Thamnocephalus platyurus*, cnidarian *Hydra vulgaris*, mollusc *Lymnaea stagnalis*  
112 (embryos) and Protozoan *Tetrahymena thermophila*) to DON and ZON. All organisms used  
113 were wild-type strains except for the RENILYS strain of *L. stagnalis*. For consistency, the  
114 laboratory freshwater invertebrate data are derived from acute studies, based upon  
115 measured environmental concentrations, exposure duration to high concentrations of  
116 mycotoxins is likely to be acute rather than chronic. From the invertebrate studies an SSD,  
117 along with previously reported data for freshwater plants and fish also included, can be

118 determined for each mycotoxin. Finally, HC<sub>5</sub> values with assessment factors were compared  
 119 against measured environmental concentrations of mycotoxins to assess potential  
 120 environmental risk.

121 Table 1. Details of the experimental conditions used for each toxicity test.

| Organism                        | Age                 | Time | Endpoint       | Medium                    | Temperature (°C),<br>light: dark<br>cycle (h) | Replicates,<br>volume per<br>replicate | References                       |
|---------------------------------|---------------------|------|----------------|---------------------------|---|--|----------------------------------|
| <i>Brachionus calyciflorus</i>  | < 24 h              | 24 h | Immobilisation | Artificial freshwater     | 25 ± 1, darkness                              | Six replicates of five, 0.3 ml         | MicroBioTests Inc. Gent, Belgium |
| <i>Tetrahymena thermophila</i>  | -                   | 24 h | Reproduction   | Artificial freshwater     | 30 ± 1, darkness                              | Three replicates, 2 ml                 | MicroBioTests Inc. Gent, Belgium |
| <i>Thamnocephalus platyurus</i> | < 24 h              | 24 h | Immobilisation | Artificial freshwater     | 25 ± 1, 16:8                                  | Three replicates of ten, 1 ml          | MicroBioTests Inc. Gent, Belgium |
| <i>Chironomus riparius</i>      | First instar larvae | 48 h | Immobilisation | Dechlorinated tap water   | 20 ± 1, darkness                              | Twenty replicate individuals, 2 ml     | (OECD, 2011)                     |
| <i>Daphnia magna</i>            | < 24 h              | 48 h | Immobilisation | ISO Artificial freshwater | 20 ± 1, 16:8                                  | Twenty replicate individuals, 2 ml     | (OECD, 2004)                     |
| <i>Hydra vulgaris</i>           | Nonbreeding         | 96 h | Survival       | Hydra medium              | 20 ± 1, 16:8                                  | Ten replicates with one in each, 2 ml  | (Zeeshan et al., 2016)           |
| <i>Lymnaea stagnalis</i>        | < 24 h              | 7 d  | Survival       | ISO Artificial freshwater | 20 ± 1, 16:8                                  | Twenty replicate individuals, 2 ml     | (Bandow and Weljte, 2012)        |

122 Note, *C. riparius* were provided by Fera Science Ltd. York, UK. Starter cultures of *D. magna*  
 123 and *H. vulgaris* were purchased from Blades Biological, Kent, UK, *L. stagnalis* were originally  
 124 from cultures generously provided by INRA Rennes, France. Survival of *Hydra* sp. was ranked  
 125 according to Wilby's guide for *Hydra* (1988). Details for each medium can be found in the  
 126 respective reference papers, for *L. stagnalis* the artificial freshwater water described for *D.*  
 127 *magna* was used.



128 **2. Methods**

129 **2.1. Test design**

130 Seven test organisms were used in total, and tests followed the methodology detailed in the  
131 relevant OECD test guidelines, previous literature or that provided in the purchased test kits  
132 (OECD, 2004; OECD, 2011; Bandow and Weljite, 2012; Zeeshan et al., 2016; MicroBioTests  
133 Inc. Gent, Belgium). Details on the duration, endpoint, medium, exposure conditions and  
134 replicates used for each test species are detailed in the supplementary information (Fig. S1  
135 and Table 1). In all studies immobilisation or mortality was used as an endpoint to derive  
136 EC<sub>50</sub> values, further to this in the *H. vulgaris* study the sub-lethal effects on morphology  
137 were also monitored (Wilby, 1988).

138 Zinc was used as a positive control alongside each mycotoxin study, after having first  
139 developed a suitable concentration against the zinc EC<sub>50</sub>. Details of zinc trial results, along  
140 with positive control values chosen and resulting inhibition in mycotoxin studies are  
141 provided in Table 2. The exception to this was *C. riparius* where no inhibition of greater than  
142 50 % sensitivity was seen in the exposure hence no suitable zinc concentration was found to  
143 be used as a positive control.

144 For the exposures to mycotoxins, concentrations were set in the range of 0.01, 0.032,  
145 0.1, 0.32, 1.0, 3.2 and 10 mg/L. Solvent controls of ethanol (EtOH) were used in  
146 deoxynivalenol studies and dimethyl sulfoxide (DMSO) in zearalenone. The solvent control  
147 concentration was either 3.2 or 10 µl/ml per study dependant on whether the highest test  
148 concentration in the study was 3.2 or 10 mg/L. The chemicals used were purchased from  
149 SIGMA Aldrich: zinc sulphate heptahydrate (CAS number 7446-20-0; purity ≥ 99.5%);  
150 zearalenone (CAS number 17924-92-4; purity ≥99%), deoxynivalenol (CAS number 51481-

151 10-8; purity 98%). Mycotoxin concentrations were tested at the beginning and the end of  
 152 studies with mean concentrations for the exposure period calculated, where studies were  
 153 24 h in length concentrations were based upon start concentrations only due to the small  
 154 volume of solution used across the replicates. Samples were analysed using LC-MS/MS  
 155 (Waters Acquity UPLC). Quantified deoxynivalenol concentrations (those  $\leq 1$  mg DON/L) are  
 156 supplied in the Table S1, these were stable over the exposures and within a close range to  
 157 the nominal values. ZON concentrations were not quantifiable and based on nominals.  
 158 Table 2. EC<sub>50</sub> values from zinc trial studies (based upon measured concentrations) and  
 159 measured initial concentration values for zinc positive control groups in the mycotoxin  
 160 invertebrate exposures.

| Test organism          | EC50 ± SE<br>(95 % CI)     | Positive control in DON study    |                   | Positive control in ZON study    |                   |
|------------------------|----------------------------|----------------------------------|-------------------|----------------------------------|-------------------|
|                        |                            | Measured concentration<br>(mg/L) | Inhibition<br>(%) | Measured<br>concentration (mg/L) | Inhibition<br>(%) |
| <i>B. calyciflorus</i> | 1.7 ± 0.97<br>(0 – 3.6)    | 2.14                             | 37                | 1.88                             | 37                |
| <i>T. platyurus</i>    | 0.36 ± 0.4<br>(0 - 1.3)    | 0.39                             | 100               | 0.39                             | 100               |
| <i>T. thermophila</i>  | 10.9 ± 1.6<br>(9.3 – 16.8) | 13.6                             | 24                | 11.9                             | 40                |
| <i>C. riparius</i>     | > 100                      | -                                | -                 | -                                | -                 |
| <i>D. magna</i>        | 4.1 ± 6.3<br>(0 – 16.6)    | 3.03                             | 80                | 2.76                             | 70                |
| <i>H. vulgaris</i>     | 11.3 ± 10.3<br>(0 - 32)    | 1.86                             | 100               | 1.92                             | 100               |
| <i>L. stagnalis</i>    | 0.61 ± 0.39<br>(0 - 1.4)   | 0.80                             | 10                | 2.0                              | 55                |

161 Note, Chemical analysis was performed using inductively coupled plasma emission  
 162 spectroscopy (ICP-OES, iCAP, Thermo Scientific) with a limit of detection (LOD) of 0.015 mg  
 163 Zn/L.

## 164 2.2. Statistical analysis

165 Plotting of dose response curves for each test organism were performed using R version 4.0.1  
 166 (R Core Team, 2020) and the drc-package (Ritz et al., 2015). Results of toxicity tests were  
 167 assessed using a three-parameter regression model where the distribution was assumed as

168 binary for those with survival/immobilisation as an endpoint and as normal for those with  
169 reproduction inhibition as an endpoint. The equation of the regression models:

170 
$$y = \frac{d}{1 + \left(\frac{x}{e}\right)^b}$$

171

172 Where parameters are  $b$  which describes the slope surrounding  $e$  the  $EC_{50}$  (Ritz et al., 2015)  
173 and  $d$  the upper limit of the curve. The chi-squared ( $X^2$ )/ F-value and  $P$  values from lack-of-  
174 fit/goodness-of-fit tests are provided with each model as appropriate. Further to this,  $EC_{10}$   
175 and  $EC_{20}$  values were derived from the regression model. Subsequently, SSD models were  
176 plotted using the same regression model, with proportion of species affected plotted  
177 against dose (Posthuma et al., 2002; Jiang et al., 2018). From each SSD model the  $HC_5$  was  
178 determined.

### 179 **3. Results**

180 During the mycotoxin studies the positive control groups exposed to zinc all showed  
181 inhibition relatable to the  $EC_{50}$  (Table 2) This, accompanied by lack of inhibition in the  
182 negative controls, provides confidence that the assay procedures were working as expected,  
183 and therefore that any inhibition seen in the mycotoxin treatments could be attributed to  
184 the exposure and not artefacts in the protocols.

#### 185 **3.1. Deoxynivalenol**

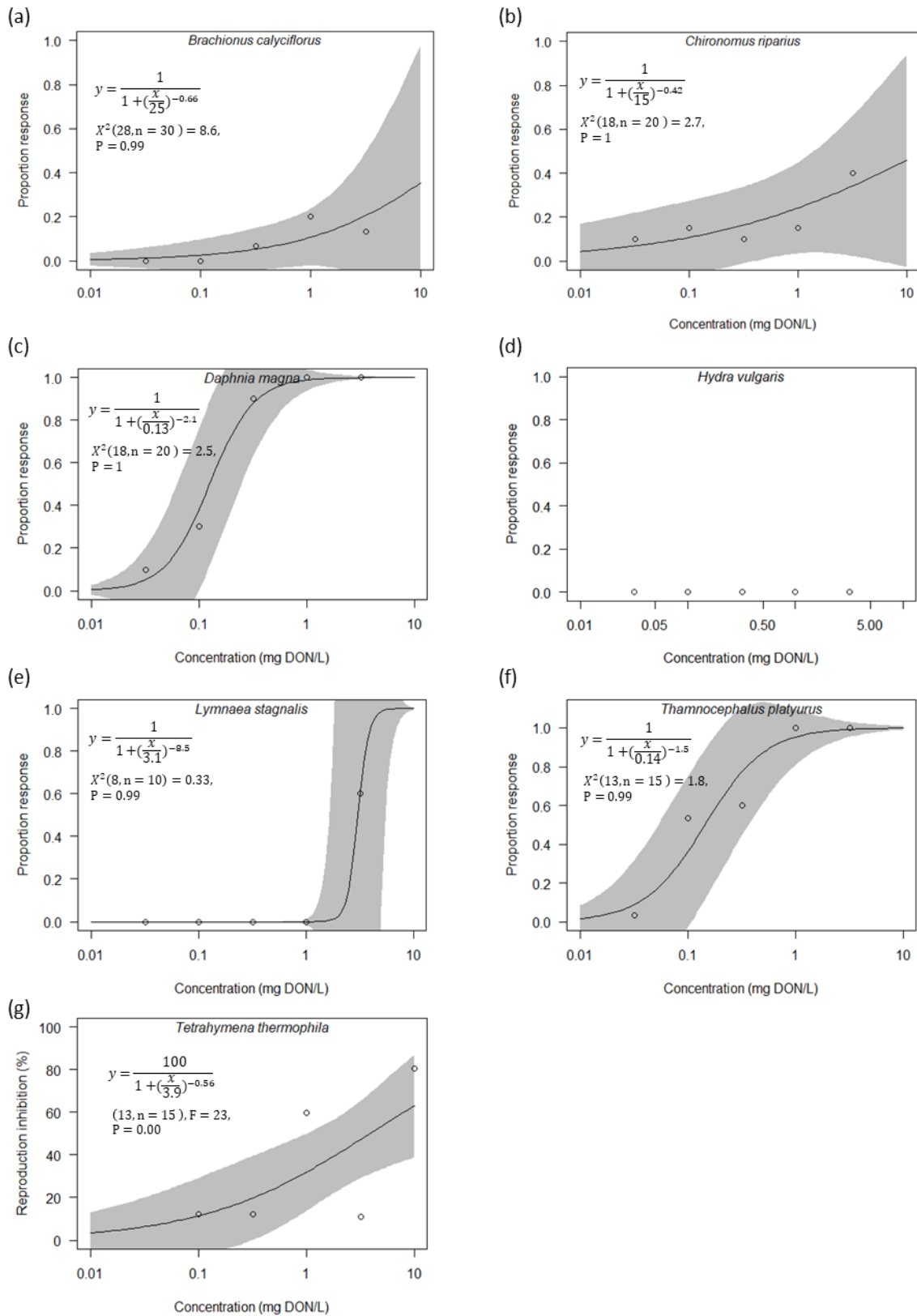
186 Results for the DON exposures are shown in Fig. 1, the concentration of test solutions in the  
187 DON studies were measured and found to be suitably accurate to the nominal values as per  
188 OECD recommendations (mean quantified concentrations varied by < 20 % of the nominal  
189 with the exception of one case at 21 %) with minimal decrease in the concentrations over

190 the period of the studies (maximum quantified variation of 6 %), the results of the LC-  
191 MS/MS measured concentrations are provided in Table S1.

192 In the 24 h studies no effect was seen in any of the solvent controls. In response to  
193 DON, the *B. calyciflorus* study showed the least sensitivity (Fig. 1) with DON treatments  
194 causing a maximum of 20 % inhibition at 0.32 and 1 mg DON/L. Both *B. calyciflorus* and *T.*  
195 *thermophila*, had EC<sub>50</sub> values > 3.2 mg DON/L.

196 During the 48 h period of the *C. riparius* study the ethanol control group had 10 %  
197 immobilisation. Those individuals exposed to DON showed some recovery between the 24  
198 and 48 h observations, with 1 mg DON/L being the only DON concentration to not have a  
199 decrease in immobilisation between the two. The lowest concentration of 0.032 mg DON/L  
200 had 20 % immobilisation at 24 h which decreased to 10 % by 48 h to match the inhibition of  
201 the solvent controls. The highest concentration had a decrease from 50 to 40 %  
202 immobilisation leading to a 48 h EC<sub>50</sub> of > 3.2 mg DON/L. Due to the solvent control  
203 inhibition percentage inhibition used in the later analysis were first normalised to this 10 %  
204 inhibition.

205 For the crustaceans, a greater effect was seen in *T. platyurus* than any of the other 24 h  
206 studies. The lowest concentration showed a minimal effect of 3 % immobilised, as  
207 concentration increased from 0.32 to 1 and 3.2 mg DON/L immobilisation increased from 53  
208 to 60 and 100 %. The EC<sub>50</sub> for *T. platyurus* was 0.14 mg DON/L. *D. magna* responded in a  
209 time dependant manner over 48 h. The solvent control had immobilisation of 0 % at 24 h  
210 which increased to 5 % at 48 h. DON immobilisation occurred at 0.32 mg DON/L (20 %  
211 immobilised) and above at 24 h and at all concentrations by 48 h. EC<sub>50</sub> values for 24 and 48  
212 h were 6.0 and 0.13 mg DON/L respectively.



213 Figure 1. Dose response curves of freshwater invertebrates exposed in acute  
 214 laboratory studies to deoxynivalenol (DON), based on nominal concentrations with shaded  
 215 bands indicating 95 % CI. Model equations along with the chi-squared ( $X^2$ )/ F-value

216 (Degrees of freedom,  $n$ ) and  $P$  value from goodness-of-fit tests are provided. Note, nominal  
217 concentrations were confirmed by measurements in the water samples.

218

219 The longer invertebrate studies with *H. vulgaris* and *L. stagnalis* embryos were less  
220 sensitive than the crustaceans. The *H. vulgaris* study with DON had no effect on survival in  
221 the solvent control or DON treatments. Sub lethal effects were noted in terms of abnormal  
222 morphology which included contraction of the body of the animals and the shortening or  
223 loss of tentacles. Abnormal morphology was seen at 48 h in the highest concentration of 3.2  
224 mg DON/L with 50 % affected, this increased to 100 % by 72 h. By 72 h some of the lower  
225 treatments also began to develop abnormalities, at 0, 0.032 and 1 mg DON/L there had 10,  
226 20 and 20 % inhibition which remained the same over the final 24 h of the study. The  $EC_{50}$   
227 was generated in terms of survival for comparison with the other test species, but lay above  
228 the concentrations tested > 3.2 mg DON/L.

229 *L. stagnalis* embryos seemed relatively insensitive to DON during a 7 d static  
230 exposure with only the highest concentration of 3.2 mg DON/L resulting in embryo mortality  
231 of 60 %. There were no visible morphological abnormalities in the remaining embryos  
232 surviving at this concentration or any of the lower concentrations. The  $EC_{50}$  value for *L.*  
233 *stagnalis* was 3.1 mg DON/L.

### 234 **3.2. Zearalenone**

235 The dose response curves for ZON are shown in Fig. 2. For ZON, 24 h studies identified *B.*  
236 *calyciflorus* *T. platyurus* and *T. thermophila* as relatively insensitive to ZON with an  $EC_{50}$   
237 values greater than the highest concentration tested of 10 mg ZON/L. Although *B.*

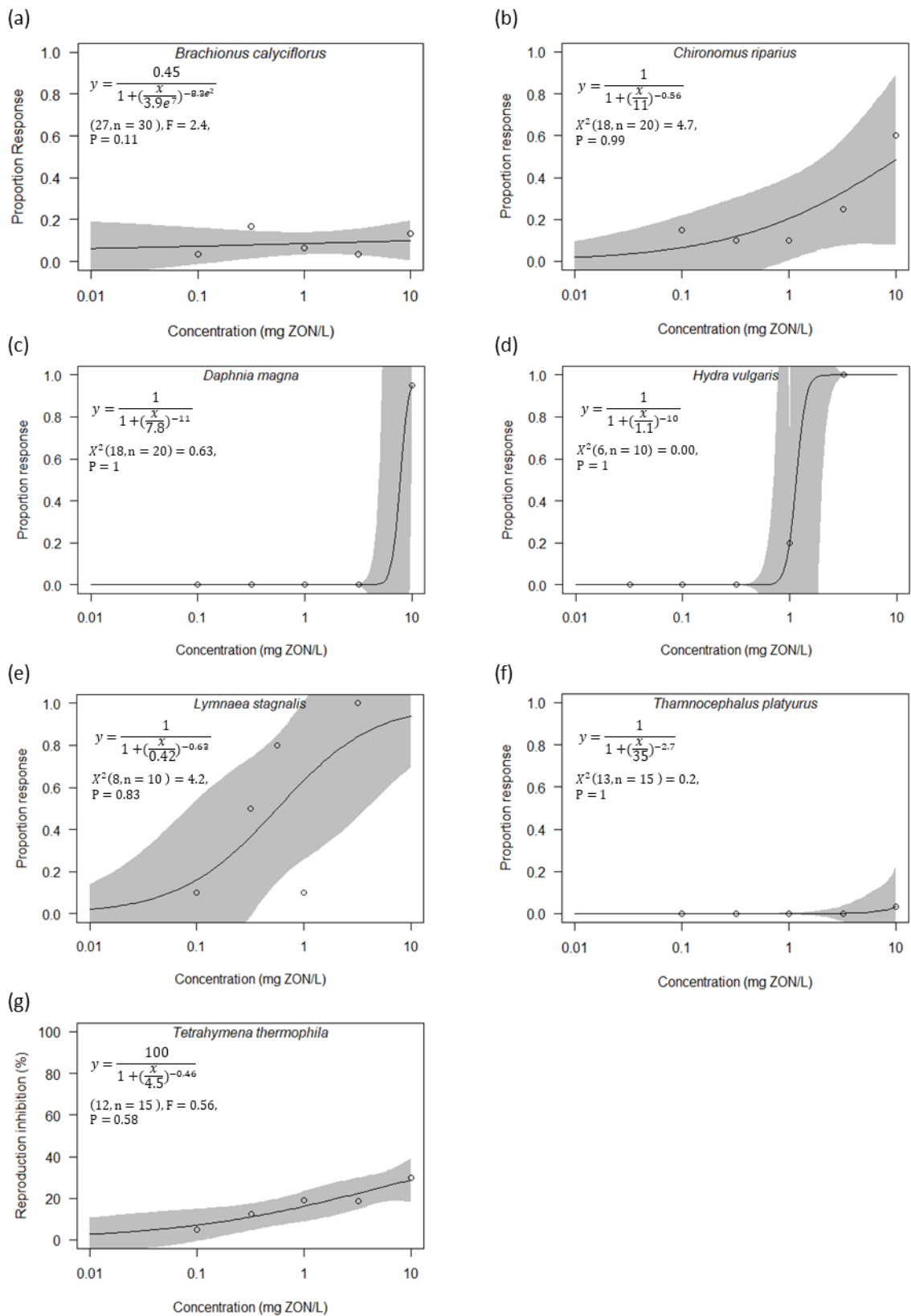
238 *calyciflorus* and *T. thermophila* showed affects at multiple concentrations, inhibition values  
239 were  $\leq 17\%$  for *B. calyciflorus* and in the dose response relationship for *T. Thermophila* only  
240 the highest concentration of 10 mg ZON/L caused significant inhibition of 30 %. These three  
241 organisms appear to be insensitive to ZON.

242 First instar larvae of *C. riparius* exposed for 48 h showed only 5 % inhibition due to  
243 the DMSO solvent control. ZON treatment showed a generally increasing toxicity with dose  
244 and time. By 48 h the lowest concentration tested had a higher effect of 15 % in comparison  
245 to the subsequent two treatments where immobilisation response was 10 %. Immobilisation  
246 increased to 25 and 60 % in the two highest treatments, 3.2 and 10 mg ZON/L, respectively.  
247 However, the regression analysis showed the  $EC_{50}$  for *C. riparius* immobilisation lay outside  
248 the test range at  $> 10$  mg ZON/L for 48 h.

249 The immobilisation of *D. magna* was also monitored over 48 h. The DMSO control  
250 had no effect on immobilisation. Only the highest ZON concentration of 10 mg ZON/L  
251 showed an effect, following a time-dependant response with 55 and 95 % mortality  
252 recorded at 24 and 48 h respectively. The limited dose response yet high effect level seen  
253 resulted in an  $EC_{50}$  of 7.8 mg ZON/L.

254 The two most sensitive species tested for ZON were *H. vulgaris* and *L. stagnalis*, both  
255 studies also allowed for sub lethal effects to be observed. *H. vulgaris* showed no inhibition  
256 in the solvent controls. Sub lethal morphological effects were seen in 20 % of those exposed  
257 to 0.32 mg ZON/L by 24 h and increased to 30 % by 96 h. At 1.0 mg ZON/L, 20 % of  
258 individuals showed mortality at 24 h, those still alive at 1.0 mg ZON/L all exhibited abnormal  
259 morphology, no further deterioration to mortality but also no recovery to normal  
260 morphology occurred over the remaining time of the study. Mortality was seen in all

261 individuals exposed to 3.2 mg ZON/L by 24 h. The 96 h mortality resulted in an EC<sub>50</sub> value of  
 262 1.1 mg ZON/L for *H. vulgaris*.





263 Figure 2. Dose response curves of freshwater invertebrates exposed in acute laboratory  
264 studies to zearalenone (ZON), based on nominal concentrations, with shaded bands  
265 indicating 95 % CI. Model equations along with the chi-squared ( $X^2$ )/ F-value (Degrees of  
266 freedom,  $n$ ) and  $P$  value from goodness-of-fit tests are provided.

267

268 *L. stagnalis* was more sensitive to mortality than *H. vulgaris*, with an EC50 of 0.42 mg  
269 ZON/L. During the 7 d study with *L. stagnalis* embryos no abnormal morphology or mortality  
270 was seen in the DMSO controls. Mortality in ZON exposed embryos generally occurred  
271 during the first few days of the study as the embryos did not develop past the initial morula  
272 development stage. Mortality occurred in all treatments and ranged from 10 to 90 %, this  
273 generally increased with dose. Apart from the 1 mg ZON/L treatment which showed a lower  
274 response than expected with only 10 % mortality.

#### 275 **4. Species sensitivity distributions**

276 In order to construct SSD models, a literature search was conducted for any previous  
277 freshwater toxicity data available. Two previous freshwater invertebrate studies with DON  
278 were found. A protozoan study with *Tetrahymena pyriformis* with a reproduction LOEC of  
279 0.6 mg DON/L at 150 h (Bijl et al., 1988). This LOEC falls in line with the results of the  
280 protozoa data generated in this study, as our result was more sensitive it was used in the  
281 SSD for *Tetrahymena* sp. A multi-generational study with the nematode *Caenorhabditis*  
282 *elegans* was also found, where acute growth effects on parental generation after 24 h  
283 provided an EC<sub>50</sub> of 372 mg DON/L, however no survival data were provided so this  
284 organism was not included in the analysis (Zhou et al., 2017).

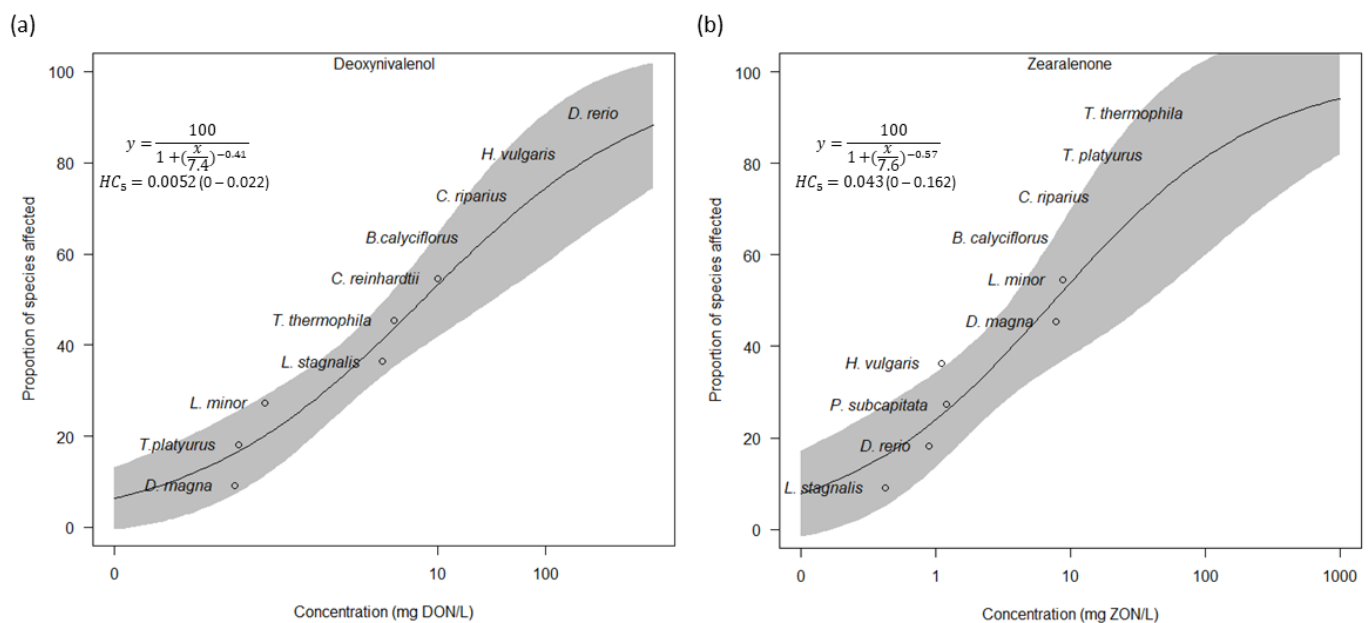
285           There is also data for freshwater plants available for DON. The microalgae  
286 *Chlamydomonas reinhardtii* had a relatively insensitive LOEC of 10 mg DON/L after a 150 h  
287 exposure (Suzuki & Iwahashi, 2014). Although EC<sub>50</sub> values were not provided for *C.*  
288 *reinhardtii*, based on the growth curves provided in the 10 mg DON/L exposure at 72 h (the  
289 length of a standard algae inhibition study, OECD, 2011), growth appeared to be at roughly  
290 40 %. The adjacent treatments of 1 and 25 mg DON/L appeared to have 0 and 100%  
291 inhibition respectively. A dose response curve was generated using these inhibition values  
292 and estimated an EC<sub>50</sub> value of 10.4 mg DON/L to be used in the SSD. A 7 d *L. minor* study  
293 had a LOEC of 0.25 mg DON/L (Vanhoutte et al., 2017) which falls in the same range as the  
294 most sensitive values reported here, at 0.5 mg DON/L inhibition was given as 41 ± 12%.  
295 Reading from the growth curves for frond number and frond area EC<sub>50</sub> values were around  
296 0.5 and 0.6 mg DON/L respectively. Hence, for the SSD an EC<sub>50</sub> value of 0.55 mg DON/L was  
297 used.

298           Zebrafish embryos have reported as resilient to DON exposures with no effects  
299 observed when embryos were exposed to aqueous levels of up to 100 mM DON (REF). Only  
300 when embryos were injected with DON were 96 hpf EC<sub>50</sub> values generated for the following  
301 endpoints: hatching 1.65 mM, deformity 1.09 mM and mortality 2.57 mM (Khezri et al.,  
302 2018). Therefore, zebrafish were included in the rank for the DON SSD but no EC<sub>50</sub> value was  
303 used in the regression.

304           For ZON no previous invertebrate data were found, but Eagles et al. (2019) reported  
305 growth EC<sub>50</sub> values for both microalgae and macrophytes. In the 7 d macrophyte study with  
306 *L. minor* the most sensitive endpoint was growth in terms of yield of frond area, with an  
307 EC<sub>50</sub> of 8.8 mg ZON/L. The reported 72 h growth EC<sub>50</sub> for the microalgae

308 *Pseudokirchneriella subcapitata* was 1.2 mg ZON/L. Unlike DON, ZON has been reported to  
 309 induce toxic effects in zebrafish embryos including sub lethal abnormalities such as oedema,  
 310 spinal curvature, pigmentation and reduced hatching success (Bakos et al., 2013). For the  
 311 SSD, the reported 5 d zebrafish mortality EC<sub>50</sub> of 0.89 mg ZON/L was used (Bakos et al.,  
 312 2013).

313 The EC<sub>50</sub> values generated in our studies along with those discussed from the literature  
 314 for DON and ZON (listed in Table 3) were used to construct SSD models, these are shown in  
 315 Fig. 3. All available species, eleven for DON and ten for ZON, were included in the rank,  
 316 however, only those with specified EC<sub>50</sub> values were considered in the regression fit. The  
 317 resulting HC<sub>5</sub> values from these regression models, which protects 95% of species (at a 50 %  
 318 effect level), are 5.2 µg DON/L and 43 µg ZON/L.



319 Figure 3. Species sensitivity distributions curves with shaded bands indicating 95 % CI.  
 320 Markers are EC<sub>50</sub> values from acute toxicity studies, those without markers had  
 321 undetermined EC<sub>50</sub> values greater than the highest concentration tested and were therefore  
 322 included in the rank but not in the SSD fit.

323 Table 3. A summary of the acute effect values from freshwater invertebrate laboratory  
 324 studies with mycotoxins deoxynivalenol and zearalenone.

| Chemical       | Duration | Organism                               | Endpoint       | Summary effect values (mg/L) |                       |                                    | References              |
|----------------|----------|--|----------------|------------------------------|-----------------------|------------------------------------|-------------------------|
|                |          |  |                | EC <sub>10</sub> ± SE        | EC <sub>20</sub> ± SE | EC <sub>50</sub> ± SE<br>(95 % CI) |                         |
| Deoxynivalenol | 24 h     | <i>Brachionus calyciflorus</i>         | Survival       | -                            | -                     | > 3.2                              | This study              |
|                | 24 h     | <i>Tetrahymena thermophila</i>         | Reproduction   | 0.07 ± 0.12                  | 0.32 ± 0.33           | 3.9 ± 2.4<br>(0 – 9.2)             | This study              |
|                | 24 h     | <i>Thamnocephalus platyurus</i>        | Immobilisation | 0.03 ± 0.03                  | 0.06 ± 0.04           | 0.14 ± 0.07<br>(0.0 – 0.29)        | This study              |
|                | 48 h     | <i>Chironomus riparius</i>             | Immobilisation | -                            | -                     | > 3.2                              | This study              |
|                | 48 h     | <i>Daphnia magna</i>                   | Immobilisation | 0.05 ± 0.03                  | 0.07 ± 0.03           | 0.13 ± 0.04<br>(0.03 – 0.22)       | This study              |
|                | 72 h     | <i>Chlamydomonas reinhardtii</i>       | Growth         | -                            | -                     | 10.4 <sup>a</sup>                  | Suzuki & Iwahashi, 2014 |
|                | 96 h     | <i>Danio rerio</i>                     | Survival       | -                            | -                     | > 10 mM                            | Khezri et al., 2018     |
|                | 96 h     | <i>Hydra vulgaris</i>                  | Survival       | -                            | -                     | > 3.2                              | This study              |
|                | 7 d      | <i>Lemna minor</i>                     | Growth         | -                            | -                     | 0.55 <sup>a</sup>                  | Vanhoutte et al., 2017  |
|                | 7d       | <i>Lymnaea stagnalis</i> (embryo)      | Survival       | 2.35 ± 5.9                   | 2.3 ± 4.5             | 3.1 ± 1.28<br>(0.5-5.6)            | This study              |
| Zearalenone    | 24 h     | <i>Brachionus calyciflorus</i>         | Survival       | -                            | -                     | > 10                               | This study              |
|                | 24 h     | <i>Tetrahymena thermophila</i>         | Reproduction   | -                            | -                     | > 10                               | This study              |
|                | 24 h     | <i>Thamnocephalus platyurus</i>        | Immobilisation | -                            | -                     | > 10                               | This study              |
|                | 48 h     | <i>Chironomus riparius</i>             | Immobilisation | -                            | -                     | > 10                               | This study              |
|                | 48 h     | <i>Daphnia magna</i>                   | Immobilisation | 6.5 ± 20                     | 7.0 ± 18              | 7.8 ± 13.8                         | This study              |
|                | 72 h     | <i>Pseudokirchneriella subcapitata</i> | Growth         | -                            | 0.19                  | 0.92                               | Eagles et al., 2019     |
|                | 96 h     | <i>Hydra vulgaris</i>                  | Survival       | 0.92 ± 0.76                  | 1.0 ± 0.18            | 1.1 ± 1.6<br>(0 – 4.2)             | This study              |
|                | 5 d      | <i>Danio rerio</i>                     | Survival       | -                            | -                     | 0.89                               | Bakos et al., 2013      |
|                | 7 d      | <i>Lemna minor</i>                     | Growth         | -                            | 3.0                   | 8.8                                | Eagles et al., 2019     |
|                | 7 d      | <i>Lymnaea stagnalis</i> (embryo)      | Survival       | 0.01 ± 0.04                  | 0.05 ± 0.1            | 0.42 ± 0.5<br>(0 – 1.4)            | This study              |

325 <sup>a</sup>EC<sub>50</sub> values estimated from response values provided

326

## 327 5. Discussion

### 328 5.1. Toxicity of DON and ZON

329 The novel data here presents the toxic impacts of DON and ZON to a variety of  
330 invertebrates, a faction of freshwater ecosystems not previously studied in detail. Based  
331 upon the summary effect values for each mycotoxin (Table 3), the DON studies identified  
332 crustaceans as the most sensitive organisms with *T. platyurus* and *D. magna* having the  
333 lowest EC<sub>50</sub> values whereas for ZON it was mollusc embryos. This high sensitivity in the  
334 mollusc embryo study was also highlighted in the SSD analysis (Fig 3.), where the zebrafish  
335 embryo study was the second lowest EC<sub>50</sub> value for ZON (Bakos et al., 2013).

336 The only test organisms showing low toxicity to both mycotoxins, with undefined EC<sub>50</sub>  
337 values, were *B. calyciflorus* and *C. riparius*. Within the acute DON study *C. riparius* also  
338 showed the ability to recover, with less individuals immobilised at 48 h than 24 h, suggesting  
339 potential to metabolise DON. This could be related to the induction of cytochrome P450  
340 induction seen in acute exposures of *Chironomus* sp. to various environmental pollutants,  
341 including metals, aiding in detoxification (Fisher et al., 2003; Prakash et al. 2013). Sensitivity  
342 of *Hydra* varied between the two mycotoxins for lethality but for both DON and ZON  
343 morphological effects were seen. *Hydra* are capable of recovery by reformation of the  
344 tentacles which were observed to have begun shedding in the effected individuals.  
345 Therefore, although *Hydra* were one of the more sensitive organisms to ZON and  
346 morphological effects were noted for DON, wild *Hydra* exposed to mycotoxins may recover  
347 if the exposure period is limited, ample food is available and favourable conditions return.

348 The comparative toxicity seen in the literature values, used in the SSDs, for plants and  
349 zebrafish may represent the differing toxicological mechanisms of the two mycotoxins. DON  
350 has been linked to the spread of disease in plant hosts and programmed cell death defence  
351 in plants, and higher phytotoxicity was seen in the macrophyte *Lemna minor* for DON

352 (Wagacha and Muthomi, 2007; Diamond et al., 2013; Vanhoutte et al., 2017). Whereas ZON  
353 is known as an oestrogenic compound and Bakos (2013) reported the sensitivity of zebrafish  
354 embryos to ZON may have been an indirect consequence of endocrine disruption. Overall,  
355 the range in sensitivities seen for the two mycotoxins across the invertebrate data of this  
356 study, as well as the contrasting sensitivity reported for each in literature with microalgae,  
357 *Lemna* sp. and zebrafish embryos, supports the value of the SSD approach in toxicology to  
358 encompass interspecies variation in toxicity. Particularly as DON has been seen to have no  
359 effect in the model zebrafish studies (Khezri et al., 2018), so may have received little interest  
360 in freshwater hazard assessments when compared to the lower ZON zebrafish EC<sub>50</sub> (Bakos et  
361 al., 2013), yet here DON had the lower HC<sub>5</sub> value from SSDs.

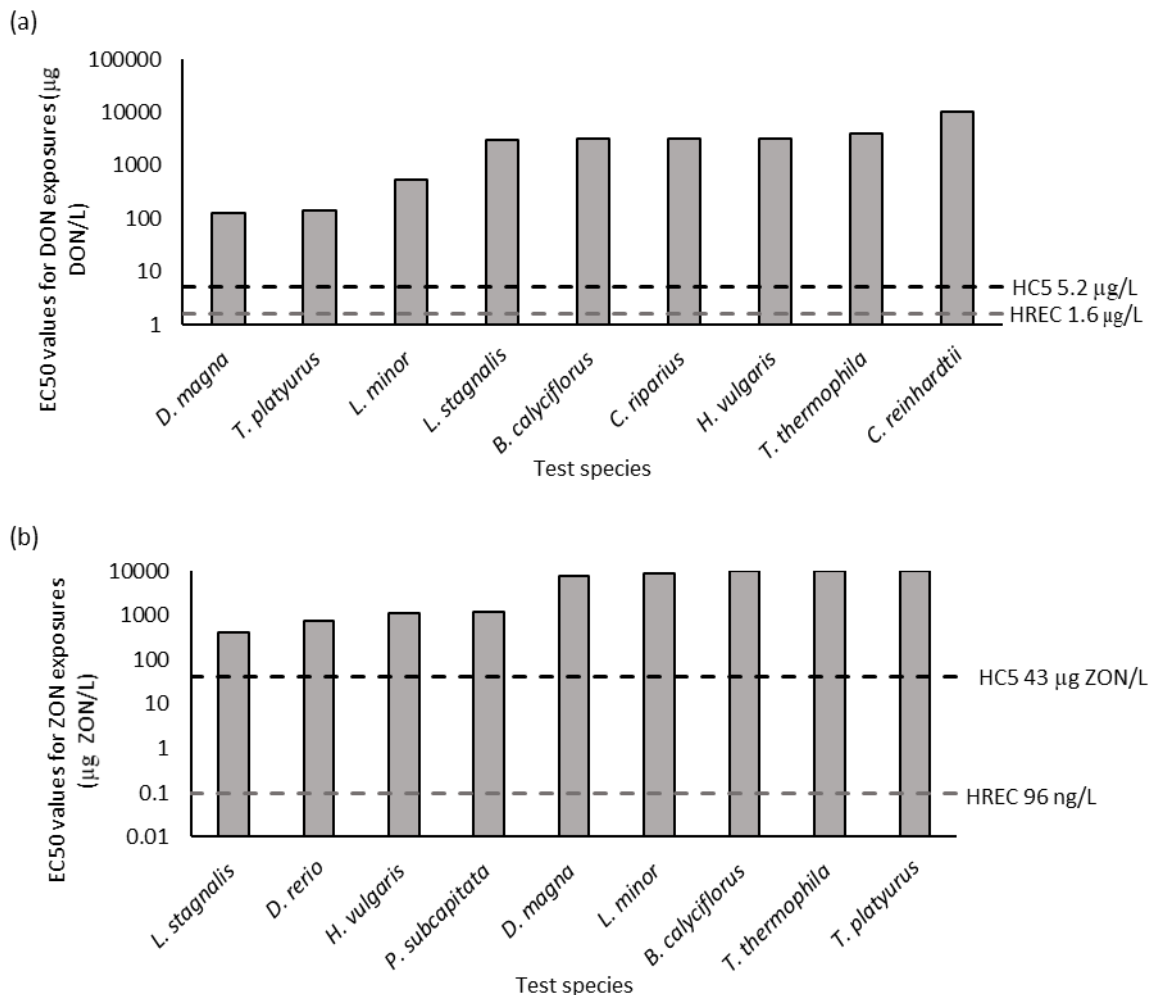
362 In the future, it would be desirable to build upon the available hazard profile of DON and  
363 ZON with a wider range of chronic studies, reflecting evidence of their widespread  
364 occurrence in the environment (Gromadzka et al., 2009; Waśkiewicz et al., 2012; Kolpin et  
365 al., 2014). From an ethical perspective, consideration should be given to plant and  
366 invertebrates where possible and not to replicate existing fish hazard assessments. For  
367 example, chronic data have been reported for the commonly studied zebrafish model with  
368 ZON, a 21 d reproduction study had a LOEC of 0.1 µg ZON/L compared with a longer life  
369 cycle study of 140 d having a LOEC of 0.32 µg ZON/L (Schwartz et al., 2010; Schwartz et al.,  
370 2013). To our knowledge, no similar fish studies have been published for DON, but with  
371 *Daphnia* being the most sensitive in the acute studies this would be a key organism to focus  
372 on in chronic studies.

## 373 **5.2. Environmental relevance and risk assessment**

374 Relevance of the effect values found here to environmental levels of DON and ZON can be  
375 evaluated using the recent studies quantifying mycotoxins in environmental samples.  
376 Commonly, measured concentrations of DON in freshwater are < 100 ng DON/L (Kolpin et  
377 al., 2014; Schenzel et al., 2012; Wettstein & Bucheli, 2010). But comparably higher  
378 concentrations of 112 - 1662 ng DON/L (Kolpin et al., 2014) were found in US streams and  
379 59.5 - 642.4 ng DON/L in Portuguese estuarine studies (Ribeiro & Tiritan, 2015; Ribeiro et al.,  
380 2016; Ribeiro et al., 2016). Levels of ZON in European freshwaters have not been seen to  
381 exceed 50 ng ZON/L (Gromadzka et al., 2009; Waśkiewicz et al., 2012). The highest recorded  
382 environmental level of ZON is in US streams at concentrations of 61.5 and 96 ng ZON/L.  
383 However, these were the only two quantifiable levels during the study from a total of 116  
384 samples (Kolpin et al., 2014). Further work is needed to understand potential seasonal  
385 trends in environmental concentrations of DON and ZON in the context of *Fusarium* sp.  
386 outbreaks in cereal crops in America, Asia and Europe. Based upon the current data  
387 available the highest recorded levels will be used, 1662 ng DON/L and 96 ng ZON/L, as a  
388 worst-case scenario environmental DON and ZON measured concentration.

389 Comparison of the EC<sub>50</sub> and HC<sub>5</sub> values to highest recorded DON and ZON  
390 concentrations are shown in Fig. 4. The confidence intervals around the derived parameters  
391 shown on the mycotoxin SSDs in Fig. 3 reflect the need for further data and do not allow for  
392 lower bound values to be considered. However, we can make a provisional assessment of  
393 risk, acknowledging the potential uncertainty associated with the derived HC<sub>5</sub> values,  
394 following the REACH guidance on chemical safety assessment for freshwater compartments.  
395 Considering the absence of confidence intervals, the use of acute values and no available  
396 mesocosm/field data for comparison to the laboratory derived values, the maximum  
397 recommended assessment factor for SSD derived PNEC values of 5 was used to account for

398 the uncertainty around the HC<sub>5</sub> values (ECHA, 2008). This gave PNEC values of 1.4 µg DON/L  
 399 and 8.6 µg ZON/L. Using HREC as predicted environmental concentration (PEC), the  
 400 PEC/PNEC ratio value generated for DON was 1.6 and 0.01 for ZON.



401 Figure 4. EC<sub>50</sub> values from freshwater toxicity tests performed in this study and those  
 402 available or calculated from literature for deoxynivalenol and zearalenone. The highest  
 403 concentration tested is plotted for those which had EC<sub>50</sub> values greater than HC<sub>5</sub> values  
 404 calculated from SSD models and highest recorded environmental concentrations (HREC) also  
 405 shown.

406



407 Based upon these, the risk to freshwater ecosystems from ZON is expected to be  
408 low. Furthermore, the peaks in ZON concentrations seem to be minimal in environmental  
409 surveys (Gromadzka et al., 2009; Kolpin et al., 2014), and only occurring at single collection  
410 points attributed to a likely high presence of producing *Fusarium* sp. or favourable weather  
411 conditions increasing surface run-off. As with ZON, peaks in DON concentrations are  
412 similarly often confined to single time points but the concentrations reached can be  
413 considerably higher. This reflects the maximum concentrations of DON found in crops being  
414 higher than that of ZON (Gruber-Dorninger et al., 2019; Vogelgsang et al., 2017) and the  
415 higher water solubility of DON (Schenzel et al., 2012). In the US study (Kolpin et al., 2014)  
416 multiple samples had concentrations > 100 ug/L approaching the PNEC, but only the highest  
417 reported concentration used in the ratio calculation exceeded the PNEC. The risk ratio  
418 calculated here to be above 1 does warrant further consideration (ECHA, 2002), but any  
419 concern is limited to exceptional concentrations of DON, with the majority of environmental  
420 concentrations < 100 ng DON/L.

## 421 **6. Conclusions and knowledge gaps**

422 This study is one of the first to develop a comprehensive assessment of mycotoxin risks to  
423 aquatic life in freshwater. Based upon the experimental data for freshwater invertebrates,  
424 acute toxicity studies suggest that DON poses the greater toxic hazard to crustaceans *D.*  
425 *magna* and *T. platyurus*, whereas ZON was most toxic to mollusc embryos and cnidarians.  
426 Utilising all the experimental and published data for freshwater algae, macrophytes,  
427 invertebrates and fish (where available) allowed the successful use of the Species Sensitivity  
428 Distribution approach to derive HC<sub>5</sub> values of 5.2 µg DON/L and 43 µg ZON/L. Based on  
429 currently available data, the ecotoxicological impact of exposure to individual mycotoxins at

430 low levels (generally < 100 ng/L: Gromadzka et al., 2009; Waśkiewicz et al., 2012; Kolpin et  
431 al., 2014) would appear to be low risk.

432 However, exposure to comparatively high levels of mycotoxins may occur over short  
433 time periods during the year and cumulative exposures to mixtures of mycotoxins should  
434 also be considered in future studies. For DON, with a calculated PEC/PNEC ratio of 1.6,  
435 there is a concern, but also data gaps on exposure profiles. Data on the fluctuations in  
436 freshwater concentrations of DON and ZON (and other mycotoxins) are needed to  
437 understand peak exposure durations and effectively assess acute and chronic risks  
438 associated with their cumulative exposure. Additional acute or chronic toxicity data will  
439 reduce uncertainty in any risk assessment of DON and ZON for freshwater; but risks to  
440 marine organisms also should be assessed. Climate change is increasing the incidence of  
441 *Fusarium* sp. fungal diseases in European cereal crops (Moretti et al., 2019), and so any risk  
442 assessment conducted now would be a baseline for future hazards of these emerging  
443 contaminants.

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450 **References**

- 451 Bakos, K., Kovacs, R., Staszny, A., Sipos, D. K., Urbanyi, B., Muller, F., Csenki, Z. and Kovacs, B.  
452 2013. Developmental toxicity and estrogenic potency of zearalenone in zebrafish (*Danio*  
453 *rerio*). *Aquat. Toxicol.* 136-137: 13-21
- 454 Bandow, C. & Weltje, L. 2012. Development of an embryo toxicity test with the pond snail  
455 *Lymnaea stagnalis* using the model substance tributyltin and common solvents. *Sci.*  
456 *Total Environ.* 435-436: 90-95
- 457 Belanger, S., Barron, M., Craig, P., Dyer, S., Galay-Burgos, M., Hamer, M., Marshall, S.,  
458 Posthuma, L., Raimondo, S. & Whitehouse, P. 2016. Future Needs and  
459 Recommendations in the Development of Species Sensitivity Distributions: Estimating  
460 Toxicity Thresholds for Aquatic Ecological Communities and Assessing Impacts of  
461 Chemical Exposures. *Integr. Environ. Asses.* 13: 664-674
- 462 Bijl, J.P.; Rousseau, D.M.; Dive, D.G.; Vanpeteghem, C.H. 1988. Potentials of a synchronized  
463 culture of *Tetrahymena pyriformis* for toxicity studies of mycotoxins. *J. Assoc. Off. Anal.*  
464 *Chem.* 71: 282–285.
- 465 Bonnet, M.S., Roux, J., Mounien, L., Dallaporta, M. & Troadec, J.D. 2012. Advances in  
466 Deoxynivalenol Toxicity Mechanisms: The Brain as a Target. *Toxins.* 4: 1120-1138
- 467 Diamond, M., Reape, T.J., Rocha, O., Doyle, S.M., Kacprzyk, J., Doohan, F.M. & McCabe, P.F.  
468 2013. The *Fusarium* Mycotoxin Deoxynivalenol Can Inhibit Plant Apoptosis-Like  
469 Programmed Cell Death. *PloS one* 8(7): e69542. doi: 10.1371/journal.pone.0069542
- 470 Eagles, E.J., Benstead, R., MacDonald, S., Handy, R. & Hutchinson, T.H. 2019. Impacts of the  
471 mycotoxin zearalenone on growth and photosynthetic responses in laboratory

472 populations of freshwater macrophytes (*Lemna minor*) and microalgae  
473 (*Pseudokirchneriella subcapitata*). *Ecotoxicol. Environ. Saf.* 169: 225-231

474 ECHA. 2008. Guidance on information requirements and chemical safety assessment  
475 Chapter R.10: Characterisation of dose [concentration]-response for environment. [Online]  
476 Available at:  
477 [https://echa.europa.eu/documents/10162/13632/information\\_requirements\\_r10\\_en.p](https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf/bb902be7-a503-4ab7-9036-d866b8ddce69)  
478 [df/bb902be7-a503-4ab7-9036-d866b8ddce69](https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf/bb902be7-a503-4ab7-9036-d866b8ddce69)

479 ECHA. 2002. Technical guidance document on risk assessment. Part 1. Part 2. [Online]  
480 Available at:  
481 [https://echa.europa.eu/documents/10162/16960216/tgdpart2\\_2ed\\_en.pdf](https://echa.europa.eu/documents/10162/16960216/tgdpart2_2ed_en.pdf)

482 EC, 2006a. Regulation setting maximum levels for certain contaminants in foodstuffs.  
483 European Commission regulation number 1881/2006 on 19<sup>th</sup> December 2006. [Online]  
484 Available at: [https://eur-](https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)  
485 [lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF](https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF)

486 EC, 2006b. Commission Recommendation of 17 August 2006 on the presence of  
487 deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products  
488 intended for animal feeding. [Online] Available at: [https://eur-](https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF)  
489 [lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF](https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF)

490 EC, 2010. European Union Risk Assessment Report CAS: 7440-66-6 EINECS No: 231-175-3  
491 ZINC METAL. [Online] Available at:  
492 <https://echa.europa.eu/documents/10162/d7248de0-eb5b-4a9b-83b9-042c4fd66998>

493 Environment Agency, 2007. The direct toxicity assessment of aqueous environmental  
494 samples using the juvenile *Daphnia magna* immobilisation test. *Methods for the*  
495 *Examination of Waters and Associated Materials*. UK Environment Agency, Bristol, UK

496 Fisher, T., Crane, M. & Callaghan, A. 2003. Induction of cytochrome P-450 activity in  
497 individual *Chironomus riparius* Meigen larvae exposed to xenobiotics. *Ecotoxicol.*  
498 *Environ. Saf.* 54: 1-6

499 Gromadzka, K., Waśkiewicz, A., Goliński, P. and Świetlik, J. 2009. Occurrence of estrogenic  
500 mycotoxin - Zearalenone in aqueous environmental samples with various NOM content.  
501 *Water Res.* 43: 1051-1059

502 Gruber-Dorninger, C., Jenkins, T. & Schatzmayer, G. 2019. Global Mycotoxin Occurrence in  
503 Feed: A Ten-Year Survey. *Toxins*. 11: 375

504 Jiang, X., Hansen, H.C.B., Strobel., B.W. & Cedergreen, N. 2018. What is the aquatic toxicity  
505 of saponin-rich plant extracts used as biopesticides? *Environ. Pollut.* 236: 416-424

506 Khezri, A., Herranz-Jusdado, J.G., Ropstad, E. & Fraser, T. WK. 2018. Mycotoxins induce  
507 developmental toxicity and behavioural aberrations in zebrafish larvae. *Environ. Pollut.*  
508 242: 500-506

509 Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience  
510 Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol*  
511 8(6): e1000412. doi:10.1371/ journal.pbio.1000412

512 Kolpin, D.W., Schenzel, J., Meyer, M.T., Phillips, P.J., Hubbard, L.E., Scott, T.M., Bucheli, T.D.  
513 2014. Mycotoxins: Diffuse and point source contributions of natural contaminants of  
514 emerging concern to streams. *Sci. Total Environ.* 470 - 471: 669-676

515 Moretti A., Pascale M., Logrieco A.F. 2019. Mycotoxin risks under a climate change  
516 scenario in Europe. *Trends. Food Sci. Tech.* 84: 38-40

517 OECD, 2004. Daphnia sp. Acute Immobilisation Test. Organisation for Economic Cooperation  
518 and Development Guideline for Testing of Chemicals. Test Guideline 202. OECD, Paris.

519 OECD, 2011. Test Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition  
520 Test. Organisation for Economic Cooperation and Development Guideline for Testing of  
521 Chemicals. OECD Paris.

522 OECD, 2011. Test Guideline 235: Chironomus sp., Acute Immobilisation Test. Organisation  
523 for Economic Cooperation and Development Guideline for Testing of Chemicals. OECD  
524 Paris.

525 Pestka, J.J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. *Anim. Feed*  
526 *Sci. Technol.* 137: 283-298

527 Posthuma II, Leo, Glenn, W., Suter Traas, T.P., 2002. *Species Sensitivity Distributions in*  
528 *Ecotoxicology*. Lewis Publishers, Boca Raton, Florida.

529 Prakash, M., Nair, G., Park, S.Y. & Choi, J. 2013. Characterization and expression of  
530 cytochrome p450 cDNA (CYP9AT2) in *Chironomus riparius* fourth instar larvae exposed  
531 to multiple xenobiotics. *Environ. Toxicol. Pharmacol.* 36: 1133 - 1140

532 R Core Team (2020). R: A language and environment for statistical computing. R Foundation  
533 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

534 Ribeiro, C. & Tiritan, M.E. 2015. Development and validation of a gas chromatography mass  
535 spectrometry method for the analysis of phytoestrogens, phytosterols and mycotoxins  
536 in estuarine water samples. *Int. J. Environ. An. Ch.* 95: 187-202

- 537 Ribeiro, C.M.R., Maia, A.S., Ribeiro, A.R. & Couto, C., Almeida, A.A., Santos, M. & Tiritan,  
538 M.E. 2016. Anthropogenic pressure in a Portuguese river: Endocrine-disrupting  
539 compounds, trace elements and nutrients. *J. Environ. Sci. Heal. A, Part A*. 51: 1043-1052
- 540 Ribeiro, A.R., Maia, A., Mariana, S., Tiritan, M.E., Ribeiro, C.M.R. 2016. Occurrence of Natural  
541 Contaminants of Emerging Concern in the Douro River Estuary, Portugal. *Arch. Environ.*  
542 *Con. Tox.* 70: 361 - 371
- 543 Ritz, C., Baty, F., Streibig, J. C. & Gerhard, D. 2015. Dose-Response Analysis Using R PLOS  
544 ONE, 10(12), e0146021
- 545 Santacroce, M.P., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G. &  
546 Crescenzo, G. 2008. Aflatoxins in aquatic species: metabolism, toxicity and perspectives.  
547 *Rev. Fish. Biol. Fisher.* 18: 99-130
- 548 Schenzel, J., Hungerbühler, K. & Bucheli, T.D. 2012. Mycotoxins in the Environment: II.  
549 Occurrence and Origin in Swiss River Waters. *Environ. Sci. Technol.* 46: 13076–13084
- 550 Schwartz, P., Thorpe, K.L., Bucheli, T.D., Wettstein, F.E. and Burkhardt-Holm, P. 2010. Short-  
551 term exposure to the environmentally relevant estrogenic mycotoxin zearalenone  
552 impairs reproduction in fish. *Sci. Total Environ.* 409: 326–333
- 553 Schwartz, P., Bucheli, T.D., Wettstein, F.E. and Burkhardt-Holm, P. 2013. Life-cycle exposure  
554 to the estrogenic mycotoxin zearalenone affects zebrafish (*Danio rerio*) development  
555 and reproduction. *Environ. Toxicol.* 28: 276-289
- 556 Šišperová, E., Modrá, H., Ziková, A., Kloas, W., Blahová, J., Matejová, I., Živná, D. &  
557 Svobodová, Z. 2015. The effect of mycotoxin deoxynivalenol (DON) on the oxidative

558 stress markers in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792). *J. Appl.*  
559 *Ichthyol.* 31: 855-861

560 Suzuki, T. & Iwahashi, Y. 2014. Phytotoxicity evaluation of Type B Trichothecenes using a  
561 *Chlamydomonas reinhardtii* model system. *Toxins.* 6: 453-463

562 United States Environmental Protection Agency. 2009. National Recommended Water  
563 Quality Criteria. <http://www.epa.gov/ost/criteria/wqctable/>

564 Vanhoutte, I., Mets, L.D., Bouvre, M.D., Uka, V., Mavungu, J.D.D., Saeger, S.D., Gelder, L.D. &  
565 Audenaert, K. 2017. Microbial detoxification of deoxynivalenol (DON), assessed via a  
566 *Lemna minor* L. bioassay, through biotransformation to 3-epi-DON and 3-epi-DOM-1.  
567 *Toxins.* 9: 63

568 Vogelgsang, S., Musa, T., Bänziger, I., Kägi, A., Bucheli, T.D., Wettstein, F.E., Pasquali, M. &  
569 Forrer, H. 2017. *Fusarium* mycotoxins in Swiss wheat: a survey of growers' samples  
570 between 2007 and 2014 shows strong year and minor geographic effects. *Toxins.* 9: 246

571 Wagacha, J.M. and Muthomi, J.W. *Fusarium culmorum*: Infection process, mechanisms of  
572 mycotoxin production and their role in pathogenesis in wheat. *Crop Prot.* 26: 877-885

573 Waśkiewicz, A., Gromadzka, K., Bocianowski, J., Pluta, P. and Goliński, P. 2012. Zearalenone  
574 contamination of the aquatic environment as a result of its presence in crops. *Arh. Hig.*  
575 *Rada. Toksikol.* 63: 429-435

576 Wettstein, F.E. & Bucheli, T.D. 2010. Poor elimination rates in wastewater treatment plants  
577 lead to continuous emission of deoxynivalenol into the aquatic environment. *Water*  
578 *Res.* 44: 4137-4142



- 579 Wilby, O. K. 1988. The Hydra regeneration assay. *Proceedings of Workshop Organised by*  
580 *Association Francaise de Teratologie* 108–124
- 581 Woźny, M., Doboszb, S., Obremskic, K., Hliwad, P., Gomułkad, P., Łakomiaka, A., Różyńskib,  
582 R., Zalewskib, T. & Brzuzana, P. 2015. Feed-borne exposure to zearalenone leads to  
583 advanced ovarian development and limited histopathological changes in the liver of  
584 premarket size rainbow trout. *Aquaculture*. 448: 71-81
- 585 Zeeshan, M., Murugadas, A., Ghaskadbi, S., Rajendran, R.B. & Abdulkader Akbarsha M.A.  
586 2016. ROS dependent copper toxicity in Hydra-biochemical and molecular study. *Comp.*  
587 *Biochem. Phys. Part C*. 185-186: 1-12
- 588 Zhou, H., Tang, L., Xue, K.S., Qian, H., Sun, X., Williams, P.L. & Wang, J.S. 2018. Trans-/multi-  
589 generational effects of deoxynivalenol on *Caenorhabditis elegans*. *Chemosphere*. 201:  
590 41-49