

# Do microplastics mediate the effects of chemicals on aquatic organisms?

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## Abstract

Microplastics are ubiquitous in both marine and freshwater ecosystems, where they can act as a physical contaminant, as well as interact with chemicals present in the environment. It has been suggested that chemical contaminants can sorb to microplastics, such that microplastics act as a vector for chemicals into aquatic biota and enhance their negative effects. It has been repeatedly suggested that the main factors underpinning the binding of chemicals to microplastics are hydrophobic partitioning and the size of microplastic particles. Therefore, we used the hydrophobicity of chemicals, as  $\log K_{ow}$ , as well as the size of microplastic particles to conduct a quantitative analysis of published results to evaluate the influence of microplastics on chemical toxicity. We collated data from 39 laboratory studies that assessed the effects of microplastics, chemicals and their combination on several ecotoxicological responses of freshwater and marine organisms. Each chemical was assigned the relevant octanol / water partition coefficient ( $\log K_{ow}$ ) as a measure of its hydrophobicity, and the mean size of microplastics particles used in each study was recorded. We found no effect of  $\log K_{ow}$  or the size of microplastic particles on the interaction between microplastics and chemicals with regards to any of the relevant ecotoxicological responses (behaviour, growth, survival and cellular) considered in this study. These findings are significant in showing that the effect of microplastics on the toxicity of chemicals is more complex than just considering hydrophobicity of chemicals and size of microplastics. We call for more mechanistic experiments to motivate a robust risk assessment and mitigation of microplastic toxicity in the environment.

Keywords: Aquatic organisms, chemical toxicity, microplastic pollution, multiple stressors, hydrophobicity.

## 1. INTRODUCTION

Currently, around 76% of total plastics produced have been disposed of as waste (Geyer et al., 2017) and the mass littering of plastics into aquatic ecosystems has become a major cause for concern. Microplastics, defined as particles within the size range  $1\mu\text{m} - 5\text{ mm}$  (Frias and Nash, 2019; Thompson, 2004), have been found in almost all marine (Barnes et al., 2009), freshwater (Eriksen et al., 2013; Su et al., 2016), estuarine (Sadri and Thompson, 2014) and terrestrial (Browne et al., 2011) environments. Although there is compelling evidence that microplastics can negatively impact aquatic biota (Cole et al., 2011), an overall effect remains inconclusive (Foley et al., 2018). Their physical characteristics can cause harm to organisms (e.g. blockages in the digestive tract) that can lead to reduced food intake and starvation (Galloway et al., 2017; Wright et al., 2013). Exposure of individual organisms to microplastics can also have other adverse impacts, including reduced growth, fecundity, survival and metabolism (Au et al., 2015; Kratina et al., 2019; Rist et al., 2017), hepatic stress, loss of energetic reserves, oxidative stress, genotoxicity, immunotoxicity and neurotoxicity (Barboza et al., 2018).

In addition to their independent impacts, microplastics can interact with chemical contaminants present in the environment originating from wastewater, urban runoff, and landfill leachate. Many studies have noted high concentrations of chemicals such as hydrophobic organic compounds (Hirai et al., 2011; Mato et al., 2001; Rochman et al., 2013a), pharmaceutical and personal care products (Wu et al., 2016), and heavy metals (Holmes et al., 2012) associated with microplastic particles. Microplastics also act as persistent reactive surfaces, with chemicals used as starting materials (e.g. bisphenol-A) and additives for improving plastic properties (e.g. flame retardants, heat stabilizers and plasticizers) bound to the polymer matrix (Zhang and Chen, 2014). Once ingested, chemicals sorbed to the surface of microplastics, as well as additives, can leach from the polymer matrix and negatively

73 impact aquatic biota. These chemicals may have carcinogenic and mutagenic properties  
74 (Teranishi et al., 1975), and can cause endocrine disruption by mimicking endogenous  
75 hormones (Oehlmann et al., 2009). Thus, there have been concerns that microplastics can act  
76 as a vector for the introduction of these harmful chemicals into aquatic biota (Avio et al.,  
77 2015; Oliveira et al., 2013). However, this vector effect remains poorly understood, due to a  
78 lack of consistency in effects reported in the literature.

79         Chemical contaminants can form strong and often irreversible bonds with microplastic  
80 particles. Depending on the strength of adsorption of chemicals onto microplastic surfaces,  
81 microplastic particles can make chemical contaminants more bioavailable, by acting as a  
82 vector for the introduction of chemical contaminants into aquatic organisms (Rochman et al.,  
83 2013b) and enhance the negative effects of chemicals. Microplastics can also alleviate the  
84 toxicity of chemicals on aquatic organisms and may exert a positive effect, by forming strong,  
85 irreversible bonds with chemical contaminants, rendering them less bioavailable (Rehse et al.,  
86 2018a; Yang et al., 2020). There is also evidence to suggest that microplastics do not alter the  
87 toxicity of chemicals to aquatic organisms (Gerdes et al., 2019; Magara et al., 2019). Finding  
88 no effect of microplastics on chemical toxicity could suggest that microplastics are harmful  
89 to aquatic organisms due to their physical characteristics (e.g. morphology and size) rather  
90 than their capacity to leach additives (Oliviero et al., 2019) or act as vectors for the  
91 introduction of chemicals into aquatic organisms (Ašmonaitė et al., 2018). The influence of  
92 microplastics on chemical toxicity may also differ among ecotoxicological responses, where  
93 sublethal responses such as feeding and reproduction can be more sensitive to combined  
94 microplastic and chemical exposure compared with mortality (Bartonitz et al., 2020).

95         The interaction between microplastics and chemicals is complex, likely depending on  
96 the environmental conditions, plastic characteristics, and chemical properties. For instance,  
97 ions present in seawater can interact with different surface groups of plastics and change their

overall charge (Cole and Galloway, 2015; Paul-Pont et al., 2018), altering the sorption capacity of chemicals onto microplastics (Zhao et al., 2020a; Zuo et al., 2019). This suggests that interactions between microplastics and chemicals in marine ecosystems, and thus their effect on aquatic biota, may be different from those occurring in freshwater ecosystems. The size of microplastic particles is another important consideration because it determines their uptake, retention, and effects within aquatic organisms. Smaller particles are more likely to be ingested by a wider range of aquatic organisms, and particles  $< 5\mu\text{m}$  are potentially more harmful due to their ability to enter biological tissues (Browne et al., 2008). These smaller microplastics can thus alter cellular and molecular pathways and impact immunological responses, antioxidant system, neurotoxicity, genotoxicity and gene expression (Lu et al., 2016; von Moos et al., 2012). Smaller microplastics have a larger surface area (per unit mass) to interact with environmental chemicals. The sorption capacity of chemicals onto nanoplastics is generally larger than for microplastics (Wang and Wang, 2018; Zhang et al., 2019) due to their large surface area and increased sorption sites.

Similarly, chemical properties are crucial in determining how chemicals and microplastics interact in the environment. Although there are many mechanisms that bind chemicals and microplastics together, hydrophobic partitioning is a major mechanism investigated in many published studies (Horton et al., 2018; Tourinho et al., 2019; Wang et al., 2015). During hydrophobic partitioning, chemicals partition between an aqueous phase and a hydrophobic solvent (here, microplastics), a process largely determined by the solubility of the chemical. The octanol / water partition coefficient ( $\log K_{OW}$ ) is a measure of the extent to which a chemical remains dissolved in aqueous solution (i.e. a measure of its hydrophobicity). Hydrophobic chemicals, with a higher  $\log K_{OW}$ , have low water solubility and preferentially sorb to organic particles present in the aquatic environment (Elzerman and Coates, 1987; Lee et al., 2014), subsequently enhancing the ability of microplastics to act as

vectors for these chemicals into aquatic biota (Horton et al., 2018; Koelmans et al., 2013). Chemicals with lower log  $K_{OW}$  are described as hydrophilic and rather remain dissolved in aqueous solution (Wang et al., 2018), reducing the ability of microplastics to influence chemical toxicity. It has been shown that chemicals with a higher log  $K_{OW}$  also have a higher distribution coefficient, log  $K_d$  (Šunta et al., 2020; Zhao et al., 2020b). This suggests that more hydrophobic chemicals become more strongly sorbed to microplastics, something that has been directly observed in several experiments (Bakir et al., 2012; Teuten et al., 2009).

Here, we performed a quantitative review of published studies to examine the evidence for the combined effects of microplastics and chemicals on ecotoxicological responses of growth, survival, and behaviour of aquatic organisms. We also pooled responses at the cellular level, such as gene transcription and expression, oxidative stress, neurotoxicity, immunotoxicity and genotoxicity and refer to these as “cellular responses”. Quantitative reviews allow for the combined analysis of different results from a range of studies to assess the overall effect of a given treatment (here, the influence of microplastics on chemical toxicity). Specifically, we used log  $K_{OW}$  of chemicals and the size of microplastic particles as key characteristics to determine whether and how microplastics influence chemical toxicity. We included nanoplastics in our definition of “microplastics”, even though they are smaller than 1  $\mu\text{m}$ . We focused on this broader range of particle sizes due to the growing evidence that nanoplastics can interact with and cross membranes, with significant ecotoxicological effects (Tallec et al., 2018). We hypothesized that (i) chemicals with a higher value of log  $K_{OW}$  would exert stronger negative effects on aquatic organisms than those chemicals with a lower value of log  $K_{OW}$ . We also hypothesized that (ii) smaller plastic particles would enhance the negative effects of chemical toxicity compared with larger particles. To the best of our knowledge, this is the first quantitative review investigating the two key mechanisms that underpin the interaction between microplastics and the toxicity of chemical contaminants. The

evidence provided by this synthesis will advance our understanding of how microplastics interact with other stressors in aquatic environments.

## **2. MATERIALS AND METHODS**

### *2.1 Literature search*

We selected studies published prior to July 2020 via a search of several online databases including ISI Web of Science, Google Scholar, Science Direct, Scopus and PubMed. A combination of key words (Table S1) was used to obtain studies focused on the interactive ecotoxicological effects of microplastics and chemical contaminants on aquatic organisms. The searches identified 3449 evidence sources, of which, 39 were retained for the final analysis (Fig. 1). Sources included in the analysis were those that i) investigated how microplastics affect at least one chemical and at least one ecotoxicological response in either freshwater or marine organisms and ii) included both independent and combined treatments of microplastics and chemicals. We excluded studies that only assessed the sorption kinetics of chemicals onto plastics and those that used heavy metals as they could not be assigned a value for log  $K_{ow}$ .

Studies that investigated how microplastics affect the toxicity of chemicals encompassed a variety of different methods of introducing chemicals during the test exposure. Chemicals were either adsorbed onto microplastics prior to exposure of the study organism, added to the water during the test exposure, or the study organisms were exposed to the chemical prior to the introduction of microplastics. Some studies also assessed the toxicity of microplastic leachates on aquatic organisms. For this analysis, we did not discriminate between the various methods of exposure used. Because these different methods were not sufficiently comparable it was not possible to undertake a traditional meta – analysis. We assigned and compared the direction of effects (either positive, negative, or neutral), hereafter

referred to as outcome, to each individual record (Tourinho et al., 2019). Direction of effect was based on whether the presence of microplastics increased, decreased, or had no effect (positive, negative, or neutral, respectively) on the toxicity of the chemical as measured in the relevant ecotoxicological response. A response was considered an effect when the toxicity of chemicals was significantly different in the presence of microplastics (indicated by effective and lethal concentrations). Where effective and lethal concentrations were not reported, particularly for cellular responses, we considered a response an effect when there was a significant difference between microplastic, chemicals and their combined treatment on the ecotoxicological response (e.g. significant difference in the transcription levels of genes in the combined treatment compared with microplastics and chemical contaminants considered independently).

From each study, we extracted information about ecosystem type (freshwater or marine), the study organisms, properties of the microplastic used in the experiment (polymer type, mean size and morphology, such as bead, mixture, flake, or fibre), the chemical used and the measured ecotoxicological response. These responses included feeding rate, individual growth (change in weight and/or length), reproduction (e.g., number of offspring, fecundity), survival (number of organisms alive at the end of the experiment), metabolism (e.g. disturbed metabolism of lipids analysed via biomarker responses) and behavioural changes, such as inhibition of mobility or impairment of swimming ability. For each chemical tested we obtained information on their octanol / water partition coefficient ( $\log K_{OW}$ ) either from direct reporting within evidence sources, or from the PubChem website (<https://pubchem.ncbi.nlm.nih.gov>). Some studies used multiple treatments to assess the effect of microplastics on chemical toxicity to aquatic organisms (e.g., the response was analysed over more than one size of microplastic particles, or more than one chemical). For these studies we created individual records. Some studies also measured the effect of

microplastics on chemical toxicity to aquatic organisms using more than one ecotoxicological response (e.g. growth and metabolism) and individual records were also included from each experiment.

## *2.2 Reliability scoring*

To ensure that this review was based on robust evidence, the reliability of the evidence provided from each study was assessed using the CRED (Criteria for Reporting and evaluating Ecotoxicity Data) method (Moermond et al., 2016). A full list of the criteria and descriptors can be found in the supplementary material (Table S2). In brief, a quality assessment of each source obtained for the final analysis was carried out. Each source was assessed on fifteen criteria that cover various aspects of the experimental design (e.g. validity criteria, adequate controls, appropriate statistical methods) and reporting of the results (e.g. identity and source of test organisms, duration of test clearly stated). For each criterion, the source under review was scored between 0 – 2. A score of 0 for a criterion indicates that the information provided from the evidence source under review was unreliable, 1 indicates that the evidence was reliable but with restrictions and the highest score of 2 indicates that the information provided by the evidence source was reliable without restrictions. Information from studies was considered “reliable” when the overall reliability score met the median score of 20. Because the CRED method does not define a threshold for which sources are considered reliable, we considered the top scoring 50% of studies (those with higher than the median reliability score) as “reliable” for our analysis. To determine whether reliability score had an influence on the results, we performed separate statistical analyses on the reliable datasets here we had sufficient sample size. Statistical analyses showed qualitatively similar results when analysing both datasets (Figs. S1 – S2). Therefore, to increase the robustness of the statistical analyses, we included all evidence sources regardless of reliability score in our final analyses. Due to

the unequal number of studies for freshwater and marine biota, and a possible influence of salinity, we performed separate analyses on the two types of aquatic organisms. We also performed separate analyses each of the ecotoxicological responses, again due to the unequal number of studies. For the freshwater data, we analysed changes in behaviour, growth, and survival. For the marine data, there were enough studies to analyse changes in growth, survival, and cellular responses. The mean size of microplastics used in experimental studies were log<sub>10</sub> transformed prior to the analysis, to improve the assumption of normality.

### *2.3 Statistical analyses*

Multinomial logistic regression modelling was used to determine the relative importance of the log K<sub>OW</sub> of chemicals and mean size of microplastic particles in predicting whether microplastics have a positive, negative, or neutral impact on the toxicity of chemicals on ecotoxicological responses of aquatic organisms. Multinomial logistic regression is an extension of binomial logistic regression that allows for a dependent variable with two or more categories (i.e. positive, negative or neutral). Multinomial logistic models are composed of  $k - 1$  equations that contrast the odds of one outcome level  $k$  compared with a reference level (Aziz et al., 2016). The reference group was first defined as the outcome level that had the most observations and was compared with each of the other two outcome levels (hereafter, comparison group). Due to the number of statistical tests that were carried out, the value at which we accepted significance was Bonferroni adjusted. Multinomial logistic regression models were built with the function “multinom” in the “nnet” package in R Statistical Software (Venables and Ripley, 2002).

One of the assumptions of logistic regression is an absence of multicollinearity amongst independent variables, as correlated variables present within the same model could result in weakened statistical power of the model. Linear relationships between the two

continuous variables, mean size of microplastic particles and log  $K_{OW}$  of chemicals were assessed using Pearson's product moment correlation in R statistical software (R Core Team., 2020). There was a weak, positive correlation between the log  $K_{OW}$  of the chemical and the mean size of microplastic particles used in studies investigating how microplastics and chemical contaminants interact (Pearson's correlation,  $r = 0.31$ ,  $n = 103$ ,  $p = 0.001$ ; Fig. S2). Therefore, we constructed separate models for microplastic particle size and log  $K_{OW}$  of chemicals to avoid multicollinearity in the analysis.

For ecotoxicological responses where only binary outcomes were observed, we applied generalized linear models (GLM) to examine the relationship between log  $K_{OW}$  or mean size of microplastic particles and observed outcomes. Due to the binomial nature of the response variable, the GLM's used a binomial family distribution. Data were modelled using the function "glm" in the "lme4" package (Bates et al., 2015) for R Statistical Software (R Core team., 2020). Odds ratios, 95% confidence intervals and p values were calculated to evaluate the outcome for each multinomial and binomial logistic model. Generally, an odds ratio of 1 indicates that the predictor variable in question is not associated with the outcome being in either the comparison or reference group. An odds ratio  $> 1$  indicates that the outcome is more likely to be in the comparison group than the referent group. Whereas an odds ratio  $< 1$  indicates that the outcome is more likely to be in the referent group than the comparison group.

Finally, to determine whether differences among individual studies may confound the effects observed by using log  $K_{OW}$  and mean size of microplastic particles as predictor variables in the logistic models, mixed effects logistic models were built in SAS/STAT<sup>a</sup> using the PROC GLIMMIX procedure. Here, individual studies were included as random effect, whereas log  $K_{OW}$ , mean size of microplastic particles and ecotoxicological response were included as fixed effects. There was no difference between the model with and without the

random effect, and thus below we present the results from models without the random effect.

### 3. RESULTS

#### 3.1 Evidence sources

Out of a total 3449 evidence sources identified from initial searches, a total of 156 studies were retained for meeting the inclusion criteria (Fig. 1). A total of 39 evidence sources comprising 88 observations were identified for the analysis of the effects of microplastics on chemical toxicity to aquatic organisms (Table S3). Studies that investigated the impacts of microplastics on the toxicity of chemicals affecting the feeding, reproduction and metabolism of aquatic organisms were excluded from the analysis due to insufficient observations within each category to perform statistical analysis. We also excluded studies that considered the sorption kinetics of chemicals onto microplastics and those that included heavy metals which could not be assigned a value of log  $K_{OW}$ . Chemicals investigated in the retained studies included hydrophobic organic compounds, insecticides, pesticides, fungicides, plastic additives and several PPCP's (such as roxithromycin and 17 $\beta$  – oestradiol). Log  $K_{OW}$  of the chemicals studied ranged from – 5.4 to 9.37 and size of microplastic particles used in experiments ranged from 0.05 - 3000  $\mu$ m.

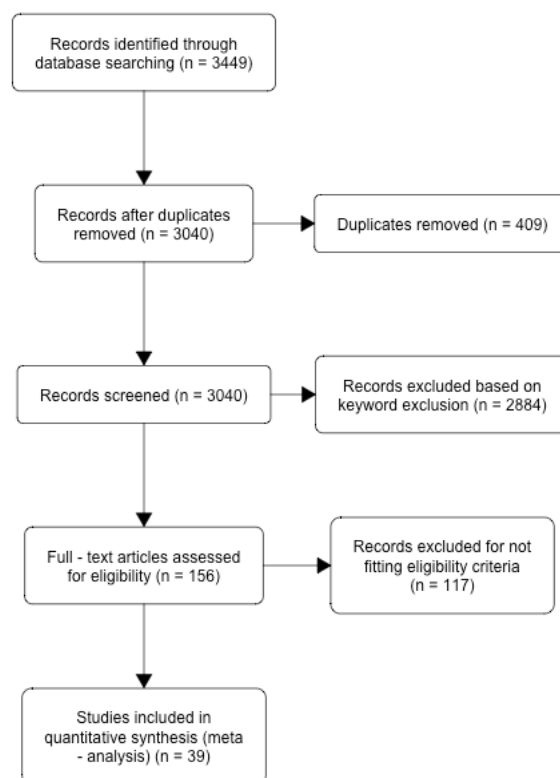


Figure 1. Evidence map of literature search and the selection process for collecting evidence relevant for the quantitative analysis of the interactive impact of microplastics and chemicals on aquatic biota.

### 3.3 The effect of $\log K_{OW}$

To test our first hypothesis, that microplastics influence the toxicity of hydrophobic chemicals, the  $\log K_{OW}$  of the chemical used in the studies was used as a predictor in each logistic model. Overall, there was no relationship between  $\log K_{OW}$  and the effect of microplastics on chemical toxicity (Fig. 2). There was a trend towards increasing probability of a neutral outcome (i.e. no influence of microplastics on the toxicity of the chemical tested) with increasing  $\log K_{OW}$  (Fig. S3) for the survival of freshwater biota exposed to microplastics and chemicals. However, the likelihood of observing a neutral outcome compared with a

positive ( $p = 0.038$ ) or negative ( $p = 0.018$ ) outcomes was not statistically significant (significance accepted at  $p < 0.004$ ).

For each of the other ecotoxicological responses tested, behavioural, growth and cellular changes, there was no significant relationship between the  $\log K_{OW}$  of the chemical investigated and the effect of microplastics on chemical toxicity (Fig. 2). To determine whether environmental salinity influences the way that chemicals interact with microplastics, freshwater and marine studies were considered separately. The  $\log K_{OW}$  of chemicals did not significantly affect the influence of microplastics on chemical toxicity on the behaviour, growth, survival, or cellular responses of organisms in freshwater ecosystems (Fig. 2). Similarly,  $\log K_{OW}$  did not have a significant effect on the likelihood of microplastics influencing the effect of chemicals on the growth, survival, and cellular responses of organisms in marine ecosystems (Fig. 3). Neither outcome category (positive, negative, or neutral) was more likely for any given value of  $\log K_{OW}$  (Figs. S4 – 10), rejecting our first hypothesis.

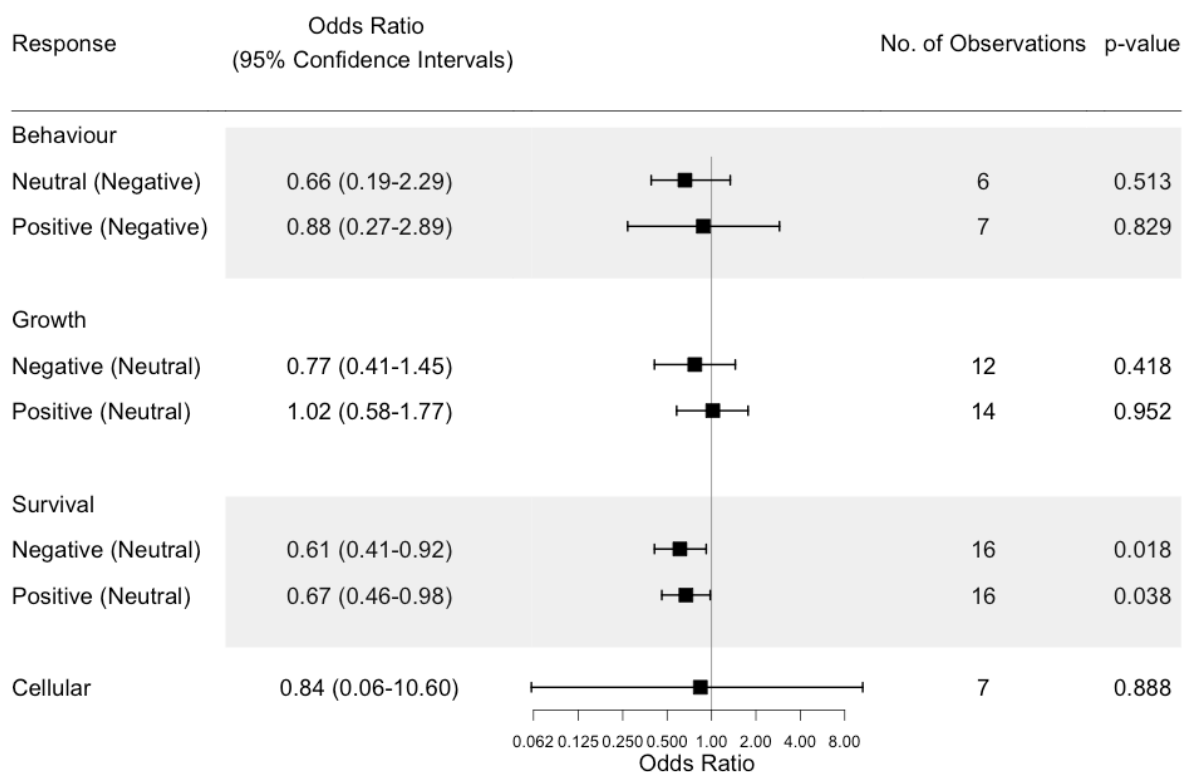


Figure 2. The effect of microplastics on chemical toxicity, determined by mean odds ratios (illustrated as filled squares) with 95% confidence intervals (horizontal bars), for the four different response categories of freshwater biota exposed to microplastics and chemicals along a gradient of log  $K_{OW}$ . Vertical line that intercepts the value of 1 indicates no difference in the response variable. An odds ratio  $> 1$  indicates that the outcome is more likely to be in the comparison group than the reference group (appears in parentheses). Whereas an odds ratio  $< 1$  indicates that the outcome is more likely to be in the reference group than the comparison group. Although the mean odds ratio and confidence intervals for survival do not overlap the vertical line, this effect was marginally non-significant after the Bonferroni correction (alpha level of  $p < 0.004$ ).

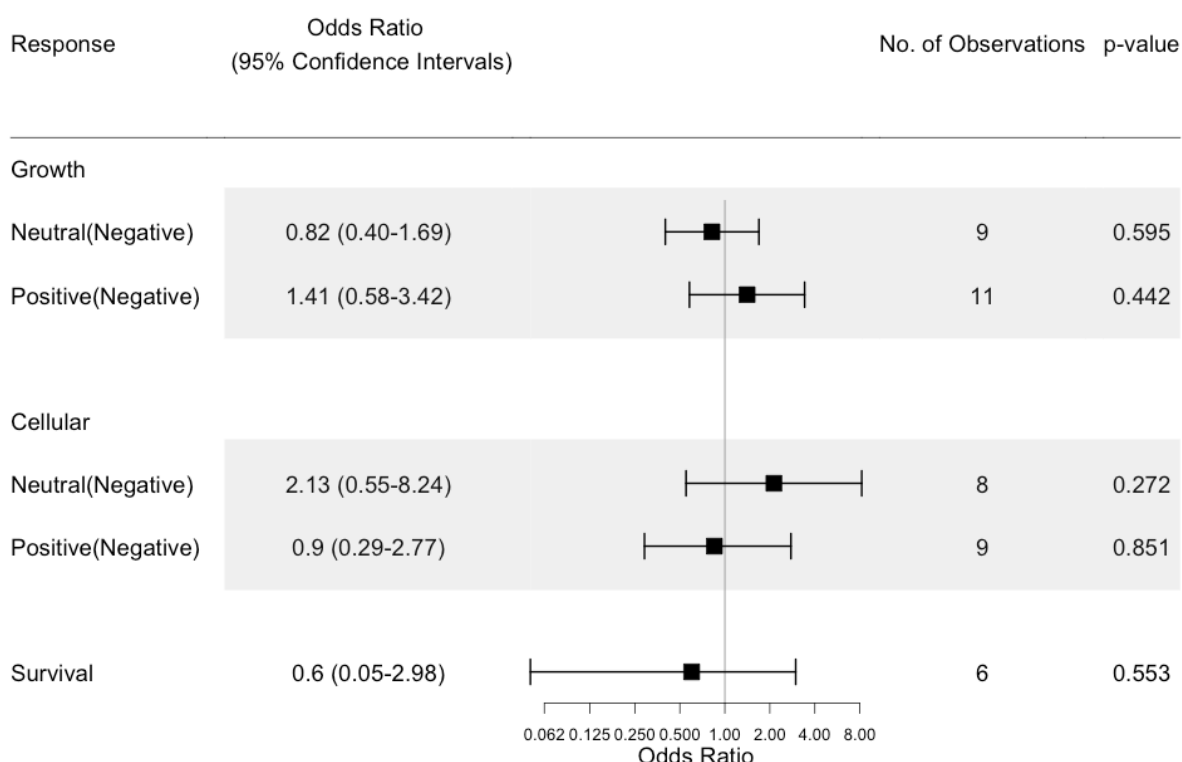


Figure 3. The effect of microplastics on chemical toxicity, determined by mean odds ratios (illustrated as filled squares) with 95% confidence intervals (horizontal bars), for the three different response categories of marine biota exposed to microplastics and chemicals along a gradient of log  $K_{OW}$ . Vertical line that intercepts the value of 1 indicates no difference in the response variable. An odds ratio  $> 1$  indicates that the outcome is more likely to be in the comparison group than the reference group (appears in parentheses). Whereas an odds ratio  $< 1$  indicates that the outcome is more likely to be in the reference group (appear in parentheses) than the comparison group. Statistical significance was accepted at  $p < 0.004$ .

### 3.4 The effect of microplastic particle size

To test the second hypothesis that smaller microplastic particles enhance the negative effects of chemicals on aquatic biota, the mean size of microplastic particles used in experimental studies was used as a predictor in each logistic regression model. There was no effect of mean microplastic size on the likelihood of a positive, negative, or neutral effect of microplastics

on chemical toxicity (Figs S4 – 10) for any of the responses of freshwater (Fig. 4) or marine (Fig. 5) organisms, rejecting our second hypothesis.

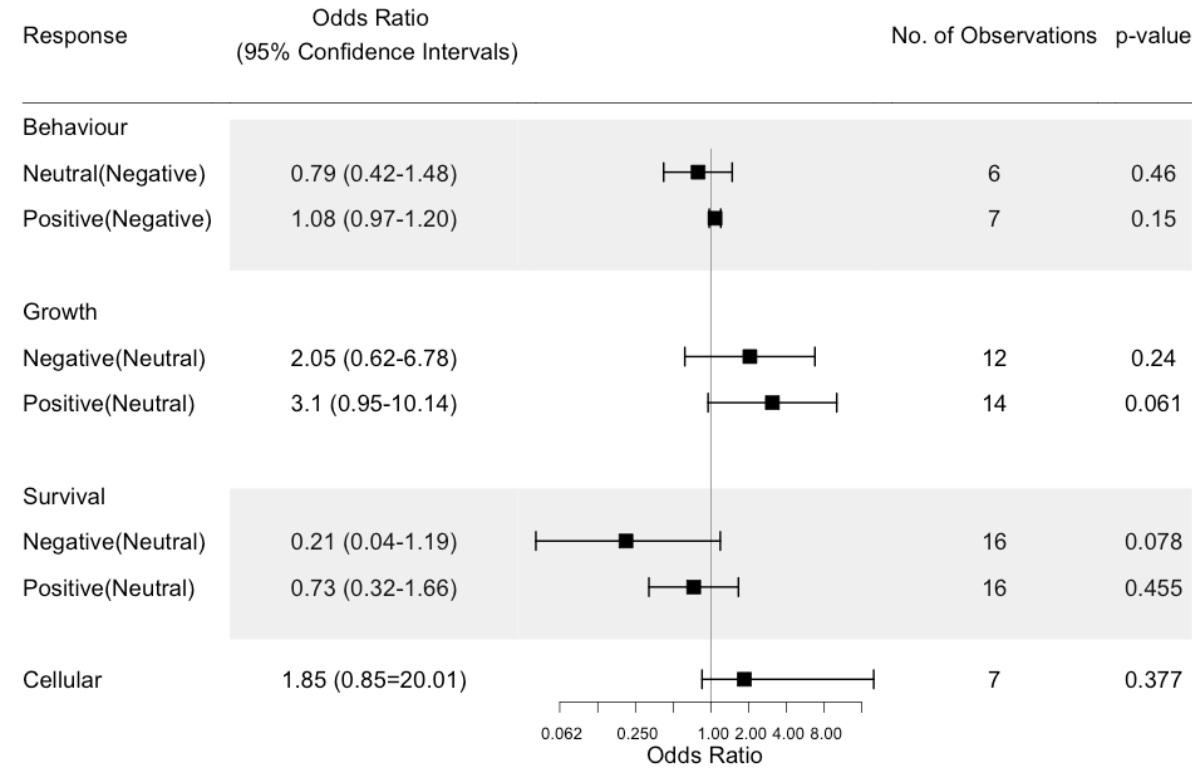


Figure 4. The effect of microplastic particle size on chemical toxicity, determined by mean odds ratios (illustrated as filled squares) with 95% confidence intervals (horizontal bars), for the four different responses of freshwater biota to chemicals along a gradient of microplastic size. Vertical line that intercepts the value of 1 indicates no difference in the response variable. An odds ratio  $> 1$  indicates that the outcome is more likely to be in the comparison group than the referent group (appears in parentheses). Whereas an odds ratio  $< 1$  indicates that the outcome is more likely to be in the reference group (appear in parentheses) than the comparison group. Statistical significance was accepted at  $p < 0.004$ .

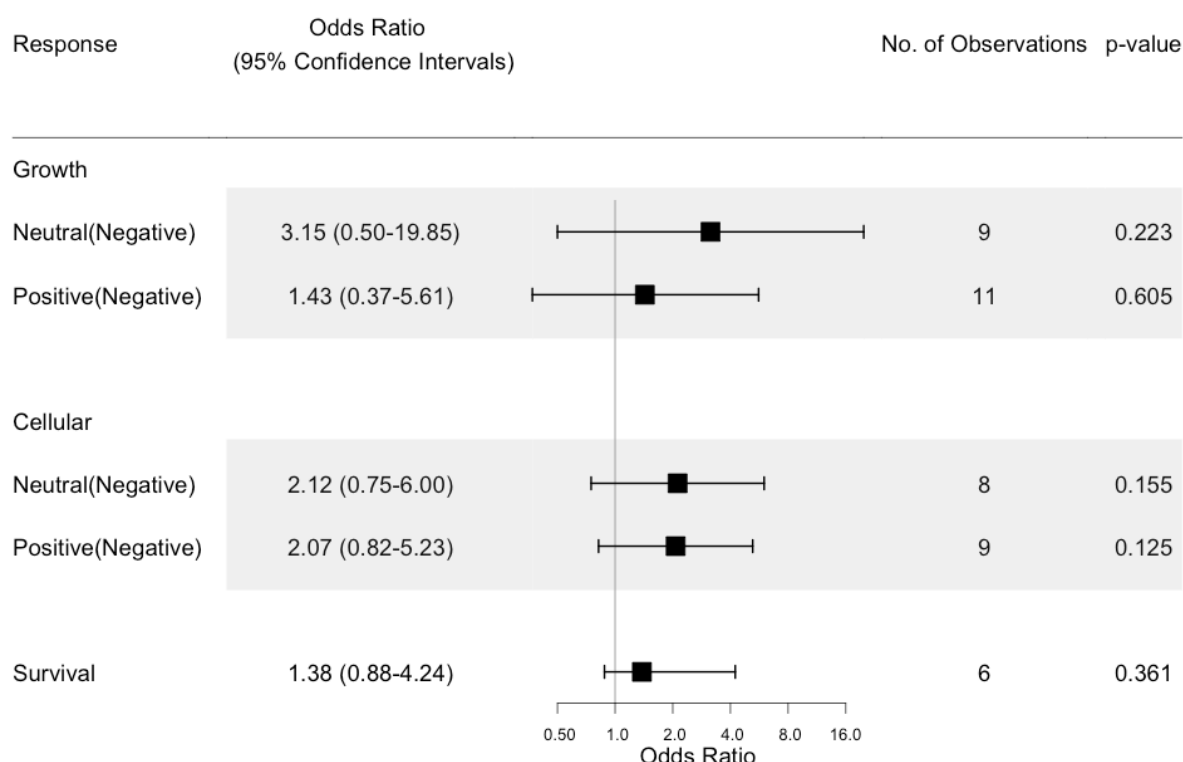


Figure 5. The influence of microplastic particle size on chemical toxicity, determined by mean odds ratios (illustrated as filled squares) with 95% confidence intervals (horizontal bars), for the three different responses of marine biota to chemicals along a gradient of microplastic size. Vertical line that intercepts the value of 1 indicates no difference in the response variable. An odds ratio  $> 1$  indicates that the outcome is more likely to be in the comparison group than the reference group (appears in parentheses). Whereas an odds ratio  $< 1$  indicates that the outcome is more likely to be in the referent group (appear in parentheses) than the comparison group. Statistical significance was accepted at  $p < 0.004$ .

#### 4. DISCUSSION

One of the greatest knowledge gaps in understanding the toxicity of microplastics in aquatic environments is whether or not microplastic particles mediate the toxicity of environmental contaminants (e.g. hydrophobic organic pollutants). Our findings do not support assertions

that microplastics mediate the toxicity of chemicals (Besseling et al., 2013; Brandts et al., 2018; Rochman et al., 2013b). Results across all ecotoxicological endpoints considered (behaviour, growth, survival and cellular) were consistent. Neither the octanol / water partition coefficient of chemicals ( $\log K_{OW}$ ) nor the mean size of microplastic particles used in experiments had a significant influence on observed outcome (Fig. 2 - 5). We applied odds ratios to estimate the effect sizes and to determine the association between  $\log K_{OW}$ , size of microplastics used and observed outcome in organismal responses. Odds ratios and 95% confidence intervals for all but one ecotoxicological response contained a value of 1, which indicates that the evidence available to date does not support any influence of  $\log K_{OW}$  or mean size of microplastic particles on the responses of aquatic organisms.

The strongest response to the combined effects of microplastics and chemicals was observed in the survival of freshwater organisms. There was a trend towards increasing probability of a neutral outcome for chemicals with higher values  $\log K_{OW}$ , and subsequently an increasing probability of a negative outcome for chemicals with lower values of  $\log K_{OW}$ . However, this effect was marginally non-significant. We would not expect this trend given our first hypothesis that chemicals with a higher  $\log K_{OW}$  would exert stronger negative effects on aquatic organisms than those with a lower  $\log K_{OW}$ . Although, there is some evidence suggesting that microplastics can alter the bioavailability and toxicity of chemicals with a lower  $K_{OW}$  (Zocchi and Sommaruga, 2019), possibly due to other factors influencing their interaction. The survival response of the freshwater organisms was also the response where we had the highest number of observations ( $n = 21$ ), suggesting that stronger responses may be detected for other ecotoxicological variables, as more observations accumulate.

The findings that microplastics may not alter the toxicity of chemicals agree with individual studies that observed no effects of microplastics on chemical toxicity (Batel et al., 2016; Magara et al., 2019). This is a significant result, contributing to the recent debate

suggesting that microplastics are toxic because of their chemical counterparts, rather than their physical characteristics (Oliviero et al., 2019; Rehse et al., 2018b). The results shown in the current research may be attributed to chemicals not being adsorbed onto plastic particles in sufficient concentrations to cause strong effects (Schmieg et al., 2020), as well as other mechanisms that influence the sorption and desorption of chemicals onto microplastics. A variety of interaction mechanisms, including electrostatic and pi-pi interactions, hydrogen bonding and van der Waals forces (Tourinho et al., 2019) contribute to the binding of chemicals and microplastics. Despite this, one of the main mechanisms reported in the literature is hydrophobic partitioning, where molecules remain dissolved whilst they are partitioned into a sorbing matrix (Hartmann et al., 2017). Our analysis focused on the hydrophobicity of chemicals, its influence on the binding of chemicals to microplastics and, thus, the contribution of hydrophobicity to a positive, negative, or neutral effect of microplastics on chemical toxicity. The reason that no consistent effect of microplastics on chemical toxicity to aquatic biota was observed may have been due to the simplified nature of using hydrophobic partitioning, as log  $K_{OW}$ , to describe the sorption of chemicals onto microplastics. Considering additional mechanisms that influence the binding of chemicals and microplastics, as well as other characteristics of the microplastics and the environment, remains a promising venue for future research.

The adverse effects of microplastics and associated chemicals could be reduced by rapid egestion of microplastic particles, which in turn may be influenced by the physical characteristics of microplastics such as shape and size. Spherical microplastics are likely to be egested more quickly compared with microplastic fibres (Au et al., 2015) and smaller microplastics can cross biological barriers and therefore remain within the organism for a longer period, allowing for desorption of chemical contaminants (Rist et al., 2017). The size of microplastics in this analysis did not influence observed outcome level, in contrast to our

second hypothesis that smaller microplastic particles would exert stronger negative effects due to their larger surface area. We were not able to consider microplastic morphology in this analysis due to insufficient data, although morphology was unlikely to influence the results since many sources retained for the final analysis used spherical microplastic particles.

Polymer type is also an important characteristic and could influence the vector function of microplastics. Polymer type has been shown to influence the sorption of chemicals, where polyethylene and polystyrene had greater affinity for the sorption of polycyclic aromatic hydrocarbons than polypropylene (Lee et al., 2014; Rochman et al., 2013a). The concept adopted in this study was that microplastic particles are nonpolar and, therefore, attract nonpolar chemicals present in the environment. However, not all plastic polymers are nonpolar, hydrophilic microplastics such as polyamides have been shown to have higher sorption affinity for hydrophilic chemicals (Li et al., 2018; Liu et al., 2019). Many biodegradable materials, such as poly (butylene adipate co-terephthalate) (PBAT) and polyurethane (PLU) are also polar, due to the presence of oxygen-containing functional groups that facilitate their degradation (Zhao et al., 2020b). Polarity can also be altered by ions present in seawater and, thus, salinity may influence the interaction between microplastics and chemicals. There were two ecotoxicological responses (growth and survival) where we had sufficient observations to determine the effects of microplastics on chemical toxicity to both freshwater and marine organisms. Despite the possible influence of salinity, microplastics did not influence the growth or survival of either freshwater or marine organisms.

Other species - dependent factors could also influence how multiple stressors interact. A recent meta-analysis has shown that the physical effects of microplastics may differ amongst taxonomic groups (Foley et al., 2018). Whereas some species show selectivity against microplastic particles in the presence of food (Aljaibachi and Callaghan, 2018), other

species only ingests microplastic particles within a certain size range (Straub et al., 2017). Crustaceans and fish appeared to be the most frequently used organisms within evidence sources relating to the interactive effects of microplastics and chemicals on aquatic biota. This was also the case for a recent review assessing the ecological impacts of microplastics on freshwater and estuarine organisms (Jones et al., 2019). Future research should thus identify the effects of microplastics and chemical contaminants on a wider range of species within different taxonomic groups, to partition any species-specific effects.

There were not enough studies to analyse the interactive effects of microplastics and chemicals on other sublethal responses such as reproduction, feeding and metabolism. Such sublethal effects could have broader ramifications within an ecosystem, leading to altered population densities, community structure and ecosystem functioning (Ward et al., 2016). The evidence for the effects of microplastics at the ecosystem level are generally lacking regarding both physical (Green, 2016; Green et al., 2016; Seeley et al., 2020) and chemical characteristics of microplastic particles. All studies considered for this analysis only investigated the effects of microplastics on chemical toxicity impacting the health and fitness of individual species, rather than on communities and ecosystems. Given the physicochemical properties of microplastics and their movement in the natural environment, perhaps one of the biggest challenges in microplastic research is scaling up from laboratory tests on individual organisms to the entire ecosystems. Such studies are urgently needed, considering the effects observed at the individual level can influence ecosystem structure and function.

Microplastics present in the environment are one of many stressors acting on aquatic organisms. Whereas previous work has summarized the potential hazards of microplastics and associated chemicals (Koelmans et al., 2016; Wang et al., 2018), this is the first quantitative analysis of the factors (hydrophobicity of chemicals and size of microplastics) that underpin the interaction between these two major pollutants of aquatic ecosystems.

Studies assessing the interaction between microplastics and other environmental stressors (e.g. habitat degradation, climate warming, eutrophication) are becoming increasingly important in understanding the overall effect that microplastics have in the environment (Kratina et al., 2019). We did not find evidence to show that the log  $K_{OW}$  of chemicals and the mean size of microplastics influence how microplastics affect chemical toxicity. Despite assertions that these two factors influence the interaction between microplastics and chemical contaminants (Horton et al., 2018; Ma et al., 2016), the interaction between microplastics and chemicals remains complex, with numerous factors potentially having an influence on the sorption / desorption of chemicals onto microplastics. A better understanding of these mechanisms would foster a robust risk assessment and directly assist restoration and conservation of both marine and freshwater ecosystems.

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