Do polyethylene microplastic beads alter the intestinal uptake of Ag in rainbow trout (*Oncorhynchus mykiss*)? Analysis of the MP vector effect using *in vitro* gut sacs

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Capsule

MPs have negligible impact on the intestinal uptake of contaminants, but may serve to introduce labile forms.

Abstract

Microplastic (MP) vector effects have been well described in the literature but surprisingly little is in known about the impact of MPs on the intestinal uptake of contaminants. The present study aimed to determine whether the intestinal fate of Ag was affected by the presence of polyethylene MP beads. Ag (added as ^{110m}Ag) was introduced into the lumen of rainbow trout (*Oncorhynchus mykiss*) anterior/mid-intestine gut sac preparations as Ag only, Ag and MPs (co-exposure) and Ag-incubated MPs (where Ag was adsorbed to the MP). Results show that after 3 h exposure the distribution of accumulated Ag between the four intestinal compartments (mucus layer, mucosal epithelium, muscle layer and serosal saline) was not affected by either MP condition when compared to Ag alone ($p>0.05$, One way ANOVA). Across all treatment groups mucus layer binding dominated (54.2-72.6 %) whereas relatively little Ag was transported to the blood compartment (i.e. combined muscle layer and serosal saline compartments, 8.5-15.0 %). Accompanying adsorption/desorption studies were performed in relevant media. Over 24 h, 60.6 ± 2.9% of the available Ag in artificial freshwater adhered to the surface of the PE MPs. In pH adjusted luminal fluids (pH 2.2, 4.1, 7.4 and 9.8) that span the range of conditions encountered within the rainbow trout digestive tract, there was almost complete dissociation at acid pH within 3 h (<2% remaining on MPs at both pH 2.2 and pH 4.1). Such pHs are typical of piscine stomach. Based on our finding we suggest that following the ingestion of MPs with adsorbed pollutants, desorption would occur prior to entering the site of uptake. The MPs themselves have no impact on the trans-epithelial transport of the contaminant, but the net result of the MP vector effect is to potentially introduce labile contaminant forms into the intestine.

Keywords

Microplastic vector-effect; Adsorbed pollutants; *in vitro* gut sacs; Intestinal metal uptake

1. Introduction

Microplastics (MPs, defined as <5 mm in size (Arthur, 2008)) are a ubiquitous aquatic contaminant having been found in marine and freshwater habitats (Derraik et al., 2002; Wagner et al., 2014) and in all compartments of the aquatic environment (water, sediment and biota (e.g. Claessens et al., 2011; Lusher et al., 2013 Eriksen et al., 2013)). MPs have been sampled from within densely-populated tourist-friendly locales (Cozar et al., 2016) as well as sparsely populated remote locations (Free et al., 2014). Considerable research has been devoted to the sorption of chemical pollutants to plastics (see reviews by Koelmans et al., 2013; 2016) and environmentally sampled MPs have been found with hydrophobic organic contaminants (HOCs), such as PCBs (polychlorinated biphenyls) and PAHs (polycyclic aromatic hydrocarbons) adhered to their surface (Ogata et al., 2009; Rios et al., 2010, Bakir et al., 2016).The association of trace metals with MPs has been less studied, but despite the initial consideration that plastic surfaces were relatively inert to aqueous metal cations, metals have been found adhered to various plastic polymers (Ashton et al., 2010; Holmes, 2012: Rochman et al., 2014). The sorption of HOCs and metals to MPs raises the potential for chemicals to be transferred to biota that mistakenly ingest MPs. This has been termed as a "vector-effect" (Syberg et al., 2015).

Numerous studies have investigated this vector effect, but results often differ. For instance, the ingestion of low density polyethylene (PE) MPs by Japanese medaka (*Oryzias latipes*) where the MPs had sorbed various HOCs following a three month deployment in the marine waters of San Diego Bay, led to HOC bioaccumulation and hepatic toxicity (Rochman et al., 2013). In contrast, Besseling et al. (2013) showed MPs had a minimal impact on PCB

accumulation in the sediment dwelling lugworm, *Arenicola marina*, and in the case of *Allorchetes compressa* (marine amphipod) the presence of MPs decreased HOC bioavailability (Chua et al., 2013). Recognizing that there are species-specific, pollutant and polymer-specific, as well as experimental differences between the studies, these varied results demonstrate that the impact of MPs on the uptake and accumulation of pollutants is far from consistent. One of the key determinants may be whether pollutants and MPs encounter each other prior to organism exposure or whether they are introduced as a co-exposure.

Our previous study (Khan et al., 2015) investigated how MPs can affect the uptake and distribution of a metal in fish. Zebrafish (*Danio rerio*) were exposed to aqueous Ag alone or in combination with PE MPs (microplastic beads, 10-106 µm, added at 10, 100 or 1000 MPs mL^{-1}). When Ag and MPs were individually dosed into exposure aquaria (i.e. co-exposure) there was no difference in the total tissue concentration or distribution of Ag between intestine and gills after 48 h in comparison to Ag only treatments. Therefore, MP concentration did not appear to influence Ag bioavailability or route of uptake. However, when Ag and MPs were incubated together for 96 h prior to the fish exposure (a process resulting in 75% adsorption of Ag to the MP), the total Ag concentrations in fish decreased and accumulated Ag was proportionally more associated with the intestine. This indicated that the incidental ingestion of MPs changed the uptake route and tissue localization of Ag. Based on this we suggested that there are four potential outcomes for Ag entering the fish intestine adhered to a MP: (i) Ag is released from the MPs in the digestive track and undergoes the same fate as the ingested contaminant in labile form; (ii) Ag remains adsorbed to the MP passes through the organism without effect; (iii) MPs remain in the digestive system, potentially causing blockages and a false sense of satiation, and release adhered contaminants over time; and (iv) a combination of these possible eventualities.

Studies into the internal fate of MP-adhered pollutants are scarce (Bakir et al., 2014). A so far unutilized methodology for determining intestinal fate of the MP vector is the *in vitro* isolated gut sac technique. This approach has been widely used for studying transport processes of solutes and in particular trace metals in fish gastro-intestinal tracts (e.g., Grosell et al., 1999, 2001; Bury et al., 2001; Ojo and Wood, 2007, 2008). The basis of the technique is the introduction of physiological saline containing compounds of interest (Ag and MPs in our case) directly into the lumen of gut tissues excised from fish and incubation of the sealed gut sac under controlled conditions. The technique then allows the transport of the ions to be measured between the following five compartments; gut lumen, mucus layer, mucosal epithelium ('enteroctytes'), muscle layer and serosal saline (the latter compartments representing transport to the blood side and uptake into the tissue), thereby studying the three steps in the intestinal transport process (mucus binding, accumulation in the mucosal epithelium and transport to the blood side, Ojo and Wood, 2007; 2008).

The aim of the present study was to introduce Ag, Ag and MPs (co-exposure) and Ag-incubated MPs into the lumen of rainbow trout (*Oncorhynchus mykiss*) gut sac preparations, to further investigate the fate of Ag adhered to MPs within the piscine gastro-intestinal tract. In addition, desorption studies were conducted at various pHs encountered along the fish gastro-intestinal tract to better understand the fate of Ag bound to MP once ingested by fish.

2. Methods

2.1 Rainbow trout

Rainbow trout were purchased from a trout farm (Berkshire, UK) and transported to King's College London. Fish were acclimatised in 1000 L fiberglass aquaria and maintained for 2 months at 13-14 °C in dechlorinated recirculating aerated city of London tap water ($[Na^+]$ = 0.53 mM, $[Ca^{2+}]$ = 0.92 mM, $[Mg^{2+}]$ = 0.14 mM, $[K^+]$ = 0.066 mM and $[NH_4^+]$ = 0.027 mM), which was passed through carbon, mechanical and biological filters, before use. Photoperiod was maintained at a constant 14 hour light: 10 hour dark cycle and fish were fed a daily 1 % (*w*/*w*) ration of fish chow.

2.2 Radioactivity

The gamma emitting radioisotope ^{110m}Ag (specific activity of 209 MBq mg^{-1} Ag, in 0.1 M HCl carrier, Institute of Atomic Energy POLATOM Radioisotope Centre, Poland) was used throughout this study. All radioactivity measurements were made with a Scaler Ratemeter portable gamma counter connected to a Type 43 well probe (Mini Instruments, Wiltshire, UK). A blank and standard (1 µL aliquot) were assayed within sample runs to determine background radiation levels and to monitor radioactive decay between experimental days.

2.3 Microplastic

The MPs used in the present study were from the same batch previously described by Khan et al. (2015). Briefly, clear PE MP beads were purchased dry from Cospheric LLC (Lot #: 100929-3-B, Santa Barbara, CA, USA). The composition was stated to be 100% PE with a size range of 10-106 µm. We independently confirmed both these by Attenuated Total Reflectance Fourier Transform Infrared (ATR-FT-IR) spectroscopy and measurement of particle sizes from light microscope images (Khan et al., 2015). MPs in their pristine state were hydrophobic and required treatment with a surfactant (polyoxyethylenesorbitan monooleate, purchased as 'TWEEN®80 Biocompatible Surfactant', Cospheric LLC) in order

to disperse in media. Stable dispersions were achieved following 24 h in 0.01% TWEEN®80 after which MPs were filtered from the surfactant with 1 µm nylon mesh. The previously determined weight to particle number ratio (1.0×10^6 MPs weighing 0.15 g) was again used.

2.4 Preparation of treatments for introduction into gut sacs

For each gut sac (n=6 per treatment, n=18 in total), a volume of 350 µL was prepared in luminal saline (Cortland's physiological saline, pH 7.4). For ease and consistency between replicates, each treatment was prepared in a volume of 2.1 mL and then divided into 6 aliquots of equal volume. For the Ag only treatments, 382 ng ^{110m}Ag was added to 2.1 mL of luminal saline. For the co-exposure treatment the radiolabel was added to luminal saline that contained 1000 dispersed MPs mL^{-1}, just prior to injection into the gut sacs. Lastly, for the treatment where gut sacs receive ^{110m}Ag adhered to the MPs 572 ng ^{110m}Ag was added to 25mL of luminal saline containing 2100 MPs, which was shaken at 150 rpm for 24 h in the dark. Quantities of isotope were used on the assumption that the adsorption of Ag to MPs would be 75% based on our previous study (Khan et al., 2015). Following incubation, Ag-incubated MPs were filtered through 1 µm nylon mesh and then resuspended in 2.1 mL of luminal saline. All three treatments were then split into their 6 respective aliquots and the radioactivity of each aliquot was measured to validate consistency between treatments.

2.5 Preparation of intestinal gut sacs

Uptake of radiolabeled Ag was measured in the anterior/mid-intestine region of rainbow trout. In this study, our definition of the anterior/mid-intestine was from immediately posterior of the final caeca to the change in colour and muscle striations that easily distinguish the transition from mid- to hind-intestine. To prepare the gut sacs, rainbow trout (107 ± 35 g, n=18, n=1 sac per fish) were unfed for 24 h prior to being sacrificed according to

171 approved local and national guidelines. The anterior/mid-intestine region of the gut was then
172 excised, and gently rinsed through with physiological saline to remove undigested feed and
173 feces and taking care to not disrupt the mucosal layer. One end of the intestine was sutured
174 closed using surgical thread and into the other end a short length (3 cm) of polyethylene
175 (PE50) tubing to permit loading and later sampling of the lumen contents was sutured tight.
176 Each gut sac was weighed and then filled via PE50 tubing with saline prepared with Ag or
177 Ag in combination with MPs (see section 2.4). The end of the PE50 tubing was then heat-
178 sealed to prevent leakage and the filled sac weighed again to enable accurate calculation of
179 the volume loaded. Filled gut sacs were rinsed with saline to displace external Ag and then
180 placed in 15 mL tubes containing a known volume (e.g. 5 ml) of serosal saline (Cortland's
181 physiological saline, pH 7.4) to completely submerge the gut sac, and kept at 15^{o}C with the
182 serosal saline gassed with 95% O_2:5% CO_2 for 3 h.
183
184 After 3 h, the gut sacs were removed from the incubation tubes, blotted dry with tissue paper
185 and weighed to enable calculation of fluid flux over the 3 h period. To measure fluid
186 movement from the mucosal to the serosal compartments, or visa versa, the difference in
187 weight before and after the treatments was assessed gravimetrically. The gravimetric method
188 has been determined to be the most reliable method of measuring fluid transport (Whittamore
189 et al., 2016). The lumen saline was then sampled via the PE50 tubing, after which the sacs
190 were cut longitudinally and immersed 10 times into two 5 mL Cortland's saline washes to
191 displace loosely bound Ag. The gut sections were then blotted with tissue paper. The
192 combined radioactive counts (counts per minute, cpm) from both washes and the tissue paper
193 were considered the "mucus-bound fraction" of Ag. The mucosal epithelium including the
194 enterocytes was then scraped off from the underlying muscle layer with a glass slide, and this
195 accounted for the epithelial Ag content. Counts present in the muscle layer and serosal saline

were considered to be the fraction of Ag transported from the luminal space across the epithelium. Data are presented normalized to the surface area of the gut tissue (Grosell and Jensen, 1999).

2.6. Adsorption and desorption studies

The pH profile of the stomach in trout has been shown to span the range pH 2 – 7 in the 72 h following a meal and in the mid- and hind-intestine the pH of the chyme may become more alkaline and may be greater than pH 9 (Bucking and Wood, 2009). To characterize Ag-MP adsorption/desorption within a realistic scenario of MPs adhering Ag in freshwater, and being ingested by and then passing through the rainbow trout digestive tract, adsorption was measured in aquarium water (composition Section 2.1) and desorption was measured in luminal saline adjusted to pH 2.2, 4.1, 7.4 and 9.8. The pH of the Cortland's saline remained stable for the 3h desorption studies.

Adsorption/desorption studies were conducted to be comparable to the treatments prepared for the gut sac exposures (Section 2.4). Adsorption onto the MPs was determined over 24 h (reflecting Ag-incubated MP treatment) and desorption was measured over 3 h to reflect the duration of the gut sac exposures. Three independent replicates were prepared with a total volume of 2.1 mL and an MP concentration of 1000 MPs mL^{-1}. Following the introduction of radiolabeled Ag (t=0 h), each replicate was sub-sampled by the removal of 100 µL at t=3, 6, 9, 18 and 24 h. The sub-sample was filtered through 1 µm mesh to capture the MPs, which were then rinsed with aquarium water to remove loosely bound Ag and the filtered MPs and the filtrate were assayed for radioactivity. After 24 h, the remaining MP suspension in each replicate was divided into four aliquots. Each aliquot was filtered and the Ag-bound MPs were resuspended into one of the 4 pH adjusted luminal saline solutions. Thus from the initial

3 replicates, there were three independent samples at each pH for the assessment of desorption. The radioactivity in each replicate was measured at t=0 h. At 1 and 3 h, 200 µL of the sample was filtered and both MPs and filtrate were measured for radioactivity as previously described.

At the end of both adsorption and desorption studies the glass vials used were assayed and showed that <5% of the Ag was bound to the containers. Both adsorption and desorption are expressed as percentages; the former is the percentage of Ag on the MPs, and the latter is the proportion of Ag measured in the pH-adjusted luminal salines compared to the total cpm at t=0 h (i.e. after introduction into luminal salines).

2.7. Statistical analysis

All data are presented as mean values ± standard deviation. Rainbow trout, gut sac and exposure parameters (presented in Table 1) were compared between the 3 treatment groups by one way analysis of variance (ANOVA) with post-hoc Tukey's HSD. The distribution of Ag between the four compartments of the gut sac that represent 'accumulated Ag' (i.e. not remaining in the lumen post-exposure, namely the mucus, mucosal epithelium, muscle tissue and serosal saline) were compared by analysis of variance (ANOVA) with post-hoc Tukey's. The difference in Ag desorption from MPs at four different pHs was also tested by analysis of variance (ANOVA) with post-hoc Tukey's HSD at the 1h and 3 h time points. Percentage data were arcsin transformed prior to analysis.

3. Results and Discussion

3.1. *In vitro* gut sac study

246

247 3.1.1. Comparability of experimental parameters amongst treatment groups

248 Experimental parameters between treatment groups were comparable suggesting consistency
249 between gut sac preparations. No significant differences were found for fish weights and gut
250 sac properties (surface area, weight and luminal saline volumes loaded and recovered into
251 and from the gut lumen) (Table 1, $p>0.05$, one-way ANOVA, n=6 per group). Calculated
252 fluid transport rates, whilst variable, were also not different between treatment groups and
253 were generally in keeping with those described in the literature (e.g. Ojo and Wood, 2007;
254 Kwong and Niyogi, 2008; Whittamore et al., 2016).

255

256 There was a significant difference in Ag concentration introduced into the gut sacs (at t=0 h)
257 between treatment groups with gut sacs injected with Ag adsorbed to the MPs after
258 incubation receiving 6.03 ± 1.80 ng Ag compared to11.82 ± 4.16 and 11.42 ± 4.89 ng Ag in
259 Ag only and co-exposure treatments, respectively (Table 1, $p<0.05$, one-way ANOVA post
260 hoc Tukey's HSD, n=6). This resulted from the adsorption of Ag to the MPs after 24 h
261 incubation being less that was predicted from our previous study (see section 3.2, Khan et al.,
262 2015). However, the concentration difference did not appear to influence the proportion of
263 Ag recovered from all fractions (including Ag remaining in the luminal fluid) at t=3 h or the
264 proportion of Ag considered to be biologically active (i.e. Ag accumulated in mucus, mucosal
265 epithelium, muscle and serosal fluid fraction, Hogstrand et al., 2002) which were similar
266 across treatments (Table 1).

267

268 3.1.2. Influence of MPs on the intestinal uptake of Ag

269 The primary aim of this study was to determine whether intestinal Ag uptake would be
270 altered by the presence of MPs in the gut lumen or the adsorption of Ag to the MPs prior to

entering the intestine. Our results show that the distribution of accumulated Ag between the four intestinal compartments was not affected by either MP condition when compared to Ag alone ($p>0.05$, One way ANOVA, Figure 1); 71.6 ± 11.2 % when aqueous Ag was introduced into the lumen, 64.4 ± 14.0 % in the presence of MPs (i.e. co-exposure) and 54.2 ± 11.6 % when the Ag was presented bound to the MPs (i.e. post incubation). The enterocytes (mucosal epithelium) typically accounted for 20-30 % across treatment groups. Combining Ag in the muscle layer and serosal saline to represent transport to the blood compartment and uptake into the tissue, similar proportional distributions were again found between treatment groups with 8.5 ± 2.1 % (Ag only), 8.4 ± 3.0 % (Ag and MP co-exposure) and 15.0 ± 9.0 % (Ag-incubated MPs). This pattern of distribution is similar to that seen by Ojo and Wood (2007) with mucus binding dominating in the mid-intestine (as well as in the stomach and posterior intestine) and blood compartment uptake being approximately 10%. In the anterior intestine, blood compartment Ag was elevated to approximately one-third, but mucus binding was still significant (Ojo and Wood, 2007). Mucus binding was also shown to be significant in the intestinal uptake of Ag in the marine teleost *Platichthys flesus* (European flounder, Hogstrand et al., 2002). Thus, whilst the Ag only treatment was comparable to previously published work, the addition of MPs as either a co-contaminant or an adhered-to-vector did not alter the transport of Ag into those intestinal fractions reflective of tissue uptake.

3.2. Adsorption and desorption studies

Adsorption and desorption studies were performed to contextualize the *in vitro* gut sac experiment in terms of environmental fate and exposure of fish to MPs and co-contaminants. Over 24 h, 60.6 ± 2.9% of the available Ag in artificial freshwater adhered to the surface of the PE MPs (Figure 2A). The adsorption isotherm displayed logarithmic tendencies with 56.3 ± 4.9 % adsorption by 18 h followed by a slower rate of increase thereafter. The total

adsorption was lower than our previous study, in which approximately 75% was associated with the MPs, but the incubation period was also longer at 96 h (Khan et al., 2015). However, if the adsorption isotherm followed a similar pattern as might be expected (i.e. rise to saturation) then the increase in the last 3 days is relatively minimal compared to the rapid initial adsorption phase. These data are similar to those reported for other metals. In measuring the time to reach equilibrium between aqueous metals and virgin pellets, termed as 'system equilibrium' and defined as 63% of the new equilibrium, Holmes et al (2012) reported the majority of tested metals to reach this threshold within 24 h, an exception being Cd which reached system equilibrium after 105 h. Weathering and biofouling of MPs may increase metal binding as it was demonstrated that beached pellets generally accumulated greater metals loads than the virgin pellets (Holmes et al., 2012).

When Ag-incubated MPs were transferred into pH adjusted luminal fluids (pH 2.2, 4.1, 7.4 and 9.8) that span the range of conditions encountered within the rainbow trout digestive tract (Bucking and Wood, 2009), there was almost complete dissociation at acidic pHs within 3 h (<2% of the Ag remaining on the MPs at both pH 2.2 and pH 4.1). At pH 7.4 approximately one- third remained (31.3 ± 1.4 %) and at pH 9.8 20.1 ± 9.0 % remained (Figure 2B). The difference between Ag desorption between pH 2.2 and 4.1 compared to pH 7.4 and 9.8 were significant at both 1 and 3 h ($p<0.05$, One way- ANOVA post hoc Tukey's HSD).

For the 'organismal-level MP vector effect' (Syberg et al., 2015) to be of relevance adsorbed contaminants must be in some way bioavailable upon entering the animal. However, to date, there have been exceedingly few investigations concerning contaminant fate in physiologically relevant fluids. Utilizing simulated gastric conditions to represent the digestive processes of warm and cold blooded animals, it was determined that HOC

desorption could be 30 times greater in the gut than in seawater alone (Bakir et al., 2014). The highest desorption rates for a variety of HOCs from PE and PVC MPs were consistently found when the simulated gastric fluid (15 mM sodium taurocholate) was at pH 4 and 38ºC, conditions representative of endothermic species, but at 18ºC and at alkaline pH, desorption of HOCs from PE MPs was also observed. PBDEs (polybrominated diphenyl ethers) were shown to be leached in stomach oil of marine streaked shearwater with the likely explanation that the stomach oil acted as an organic solvent promoting the leaching of the hydrophobic PBDEs (Tanaka et al., 2015). These studies highlight the need to perform desorption experiments within physiologically relevant media to better understand the fate and potential intestinal bioavailability MP-associated chemicals. In the present study, we demonstrate that physiological conditions representative of the fish intestine and especially the acidic conditions in the stomach will also liberate much of the metal from the surfaces of PE MPs prior to the passage of the contaminants to primary site of absorption.

3.3. Physiological and environmental relevance of the MP vector effect

Although MPs have been found within the gastro-intestinal tracts of numerous fish species (Lusher et al., 2013; Sanchez et al., 2014; Neves et al., 2015; Biginagwa et al., 2016), the impact of their presence has received comparatively little attention. Alterations of intestinal tissue of European sea bass including, disepithelization of villi and loss of structure in the serosa and mucosa layers, were observed during following 90 day dietary exposures to polluted PVC MPs (Pedà et al., 2016). As chemical loads were not measured the contributions of the adhered pollutants are difficult to distinguish from those of the MPs. If, as proposed by the vector effect, MPs have the potential to affect the bioavailability adhered pollutants, it is vital to understand the fate of adhered pollutants within the gut environment. Previously we reported that the prior adsorption of Ag to MPs resulted in an overall decrease

346 in whole body Ag accumulation in zebrafish, but a greater proportional localization to the
347 intestine suggesting the comparative importance of dietary uptake in this scenario (Khan et
348 al., 2015). Combining the results of the previous study with those presented here the
349 following scenario for MP vector impacts is proposed. MPs released into the environment
350 adsorb exogenous chemicals and MPs are then ingested by fish species providing a pathway
351 for chemicals to enter via the gastrointestinal tract where desorption occurs. The MPs and
352 chemical separately pass into the intestine as a co-exposure. The MPs themselves have no
353 impact on the trans-epithelial transport of the contaminant, but the net result of the MP vector
354 effect is to potentially introduce labile contaminant forms into the intestine.
355
356 The significance of this scenario is not immediately apparent, but the MP vector effect may
357 be subtle; a change in uptake route causing a localized increase in intestinal contaminant
358 concentrations which has minimal impact on whole body concentrations. Desorption into
359 labile contaminants will result in dietary impacts that have previously been reported in fish,
360 for instance ingested metals can cause cytotoxic damage localized to the intestine (Khan et
361 al., 2010a, 2010b), physiological changes to the gut environment (Glover and Hogstrand,
362 2002; Khan and McGeer, 2013) and reproductive perturbation (Boyle et al., 2008). Although
363 in the case of Ag specifically no significant deleterious effects have been attributed to dietary
364 intake despite an increase in hepatic Ag concentrations (Galvez and Wood, 1999). Vector
365 studies have typically focused on whole body accumulation and/or cytotoxic responses, and
366 results can vary with experimental parameters such as MP dose and toxicant concentration,
367 methods of preparation, species and set-ups (Phuong et al., 2016; Ziccardi et al 2016).
368 Perhaps a more informative approach may be to investigate differences in the distribution of
369 chemicals, and localized accumulation and cytotoxicity, with particular focus directed to

intestinal tissues. Such concentration increases and effects may be masked when focused on whole body impacts.

The present study is the first to utilize piscine intestinal gut sac preparations to investigate the MP vector effect and therefore the scope for comparison is limited. *In vitro* intestinal epithelium and *in vivo* intestinal loop models were employed to determine the effect of polystyrene particles (50 nm) on iron absorption in chickens (Mahler et al., 2012). Results obtained from both models showed acute exposure could disrupt intestinal iron absorption, but chronic exposure caused a remodeling of the intestinal villi causing an increase in the available surface area for absorption. This remodeling compensated for the lower iron uptake. Nanoplastics (plastic particles <100 nm) are now being recognized as an emerging concern (Koelmans et al., 2015; Lambert and Wagner 2016; Nolte et al., 2017), but to date there is limited information on their impact as a vector for adhered pollutants. Whilst it is likely that they would absorb pollutants in the environment, which in turn would desorbed at low pHs, nanoplastics would potentially be more capable of carrying contaminants across the epithelia and acting as a cellular vector (Syberg et al., 2015).

In discussing the MP vector effect it should be remembered that plastic debris is not the only waterborne particulate that sorbs chemicals and is ingested by aquatic organisms. Modeled analyses show that in comparison to other environmental vectors, such as natural prey and sediments, the flux of contaminants accumulated from ingested plastics is negligible (Gouin et al., 2011: Koelmans et al., 2016). Thus, whilst the notion that plastics sorb contaminants from the environment, including metals, is supported by laboratory and field observations, resultant adverse outcomes to individuals are not consistently found, and the overall relevance to aquatic biota remains debatable.

4. Conclusions

The present study is the first to utilize piscine intestinal gut sac preparations to investigate the MP vector effect. Results indicated that uptake of Ag in the anterior/mid intestine of rainbow trout is unaffected by the presence of MPs or prior incubation of the two contaminants. We suggest that the MPs may affect a change in the uptake route of adhered pollutants by promoting intake via ingestion, but following desorption MPs and contaminants enter the intestine unassociated. Thus role of MPs is more likely as an agent that introduces labile contaminants into the intestine. Research into the MP vector effect remains ongoing with divergent opinions on its validity. Further research is required and future foci should include (1) conducting desorption studies in physiologically relevant media, (2) a greater focus on tissue specific, namely intestinal, accumulation or cytotoxic responses which may be masked when solely investigating whole body endpoints, and (3) research into the emerging concern of nanoplastics, which are potentially more capable of cellular internalisation via endocytosis, and their potential to carry adhered pollutants across the intestinal epithelia.

Acknowledgments

The authors thank D. Asker (King's College London) for technical assistance with ^{110m}Ag.

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611 **Table 1.** Rainbow trout, gut sac and exposure parameters for each treatment group (Ag only, Ag MP
612 co-exposure and Ag-incubated MPs) used in this study (n=6). All data is presented as mean values (±
613 S.D.). The different properties were compared amongst the three treatment groups by one-way
614 ANOVA (post-hoc Tukey's HSD). * denote significant differences ($p<0.05$).
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	Ag only	Ag MP co-exposure	Ag-incubated MPs
Fish			
Weight (g)	105.4 (46.7)	105.6 (26.0)	111.4 (35.7)
Gut sac			
Intestinal surface area (cm^2)	2.1 (0.5)	2.4 (0.5)	2.2 (0.4)
Weight (g)			
Pre-injection	0.20 (0.04)	0.22 (0.05)	0.19 (0.03)
Post-injection (t=0 h)	0.35 (0.06)	0.41 (0.13)	0.40 (0.08)
Post-exposure (t=3 h)	0.35 (0.06)	0.42 (0.12)	0.38 (0.08)
Volume (ml)			
Loaded (at t=0 h)	0.17 (0.05)	0.19 (0.08)	0.21 (0.06)
Recovered (at t=3 h)	0.15 (0.05)	0.19 (0.08)	0.19 (0.06)
Fluid transport rate ($\mu L\ cm^{-2}\ h^{-1}$)	0.6 (0.5)	-0.8 (1.7)	3.2 (3.4)
Ag concentration			
Introduced Ag (ng Ag at t=0 h)	11.82 (4.16)	11.42 (4.89)	6.03 (1.80)*
Recovery (post exposure, %)	73.4 (16.7)	87.3 (16.0)	71.1 (10.7)
Accumulated Ag (%)	13.1 (4.7)	20.3 (7.2)	8.9 (6.4)

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Figure legends

Figure 1. Relative Ag distribution, expressed as a proportion of total Ag accumulation, in the four compartments of the anterior/mid-intestine gut sac (mucus, mucosal epithelium (enterocytes), muscle tissue and serosal saline (these latter two comprising the blood compartment)) following exposure to Ag only (grey bars, Ag MP co-exposure (white bars) and Ag-incubated MPs (black bars). Mean values are presented with error bars showing S.D., n=6 individual guts sacs per treatment.

Figure 2. Adsorption (A) and desorption (B) kinetics of Ag with PE MPs. Adsoprtion to the MPs in artificial freshwater (presented as % adsorption (± S.D., n=3) was determined over 24 h (t=0. 3, 6, 9, 18 and 24 h). Desorption studies were conducted over 3 h in pH-adjusted luminal saline solutions (pH 2.2 (green circles, green dotted line), pH 4.1 (red circles, red short-dashed line), pH 7.2 (blue triangles, blue medium-dashed line) and pH 9.8 (grey inverted triangles, black long-dashed line)). Desorption data presented as % loss (from Ag adsorption at t=0 h) ± S.D, n=3).

634 Figure 1

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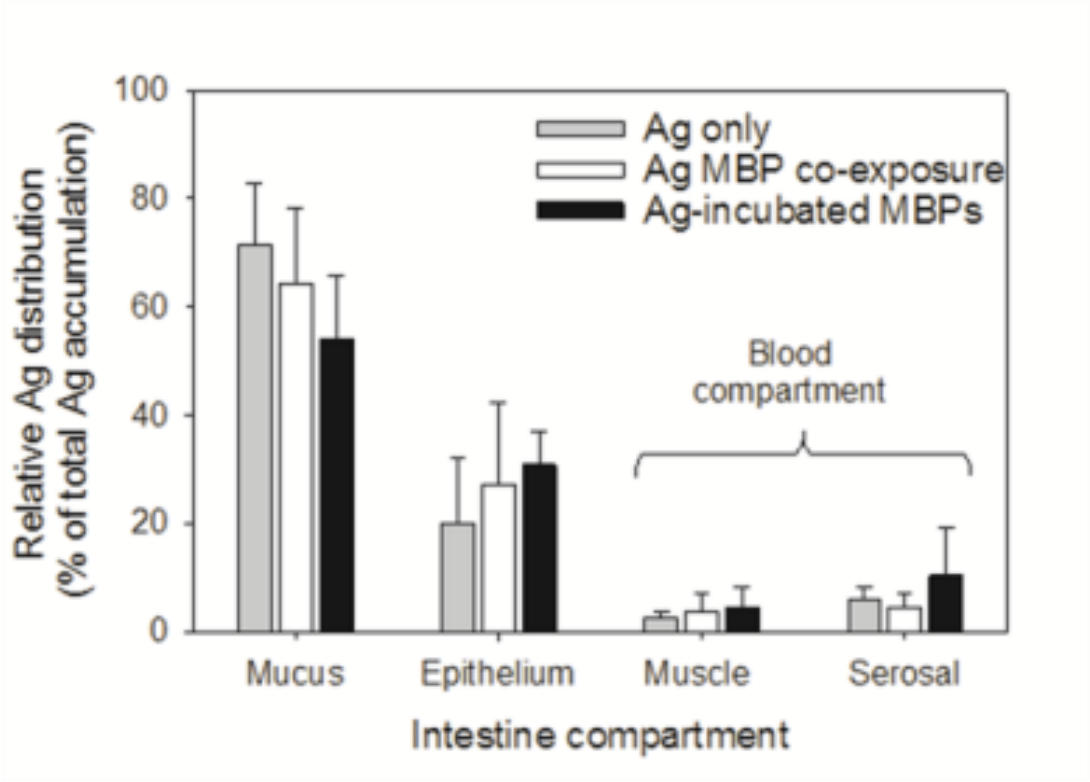

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638 Figure 2

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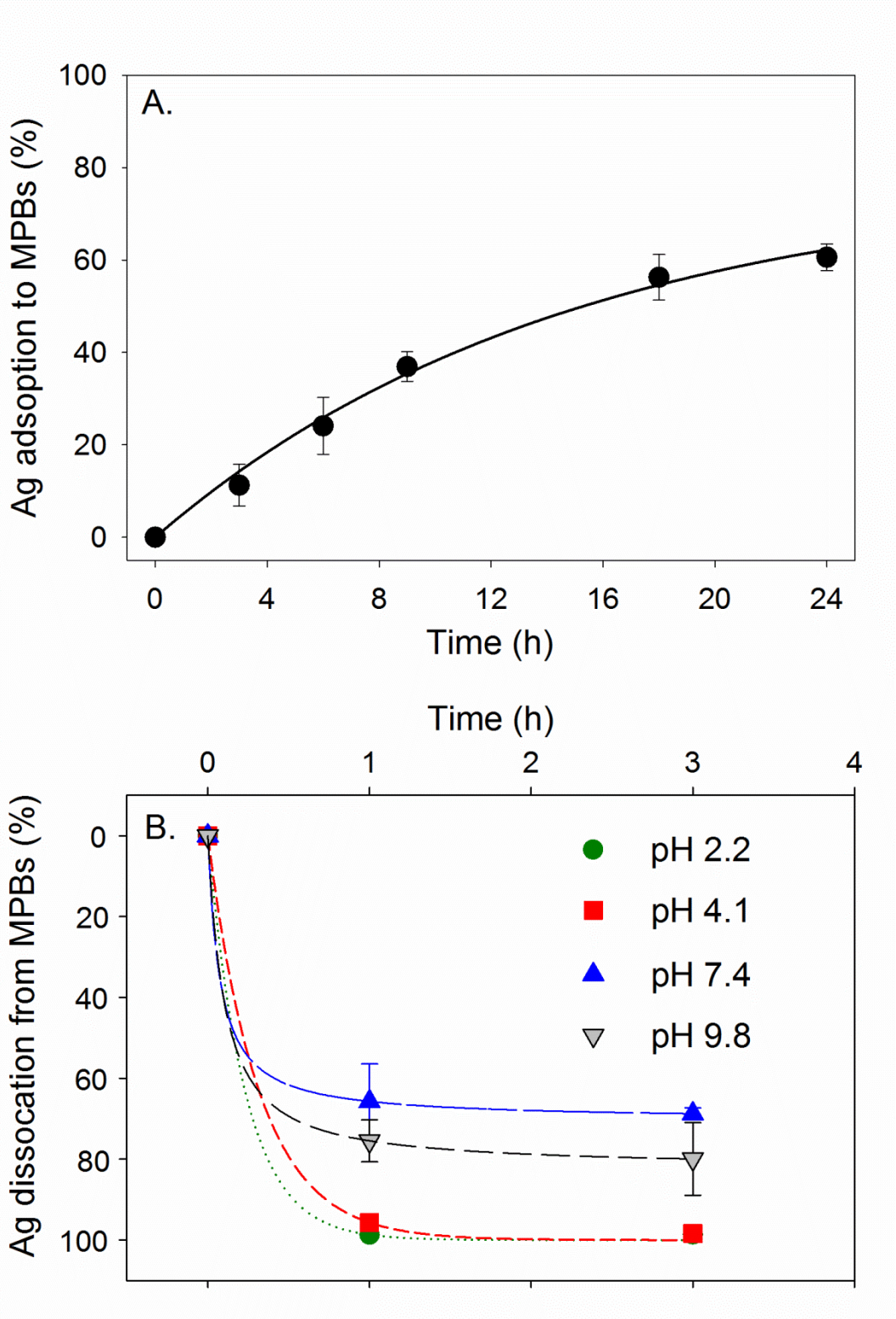

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