Assessing the chemical interactions and biological effects of a petrochemical and bio-based plastic with a common plastic flame retardant and solvent

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HIGHLIGHTS

- Microplastics and additives were tested on a freshwater host-pathogen system.
- Pristine bio-based plastic showed no toxic effects.
- Bio-based plastic with additives reduced host feeding and survival.
- Petrochemical microplastic had harmful impacts on fish with and without the additives.
- No significant chemical adsorption/ desorption onto either polymer type.

GRAPHICAL ABSTRACT
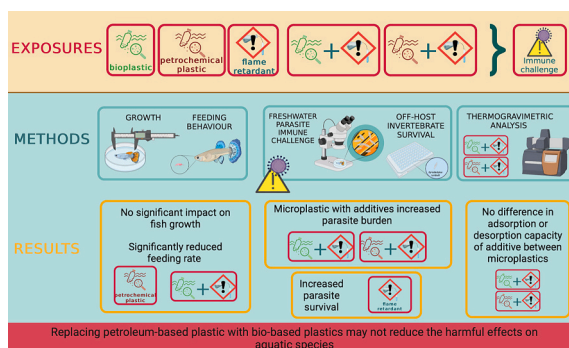
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ABSTRACT

Microplastic pollution remains a persistent environmental challenge for aquatic environments. Yet, health impact assessments of microplastics focus largely on the polymers themselves. It is important to understand the chemical behaviour and biological effects of both plastics and chemicals associated with their production, such as additives and solvents. Here, the individual and interactive chemical behaviour and biological impacts of two microplastics and two associated chemicals are assessed: polyvinyl chloride (PVC), a traditional petroleum-based plastic; polyhydroxyalkanoate (PHA) a novel bio-based plastic; triphenyl phosphate (TPhP), a common plastic flame retardant; and a widely use solvent dimethyl sulfoxide (DMSO). Thermogravimetric analysis and Nuclear Magnetic Resonance revealed no significant polymer chemical adsorption and desorption of TPhP or DMSO nor any evidence of reaction products between TPhP and DMSO. Biological assays on a freshwater fish host-parasite system, assessed fish growth, feeding, disease resistance and parasite survival. Both microplastics, the TPhP and solvent DMSO individually and interactively had no significant impact on fish growth. However, PVC alone and PHA + TPhP + DMSO significantly inhibited feeding behaviour of fish and increased mortality. Fish exposed to the solvent DMSO alone experienced the highest disease burdens. Interestingly, off-host survival of parasitic worms exposed to DMSO or TPhP + DMSO was higher than unexposed control worms. This study highlights the

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complex effects of microplastics and plastic associated chemicals on biological systems, and that novel bio-based plastics are not necessarily 'better' especially when associated with the same chemicals. Industry must be required to declare which chemicals are used in the manufacture of plastic products.

1. Introduction

The unprecedented UNEP Treaty on Plastic Pollution (2022), constituting 175 nations agreeing to tackle plastic waste, highlights the severity of this ecological crisis. International policies to restrict the use of single use plastics began with microbeads and plastic bags, and have since developed to include items such as straws and cutlery (Xanthos and Walker, 2017; Adam et al., 2020; Elliott et al., 2020). These restrictions, however, are very limited in scope with many single use plastics, such as bottles, food packaging and medical supplies, still widely in use (Chen et al., 2021). While macroplastics (>5 mm) dominate the extant mass and volume of plastic pollution, microplastics (<5 mm −1 µm) present the greatest environmental and health challenge as they are found in all food webs and even internal organs (Horton et al., 2017; Barboza et al., 2018; Triebskorn et al., 2019; Rakib et al., 2023).

The development of bio-degradable and/or chemically recyclable plastics has been driven by the need for suitable and eco-friendly alternatives to traditional petrochemical plastics (Acharjee et al., 2023). Although bio-based plastic production is a young industry, global production efforts from 2022 are expected to nearly triple by 2027 to 6.3Mt (EuropeanBioplastic, 2022). One of the first bio-based plastics brought to market in the 1980s was polyhydroxyalkanoate (PHA), a group of naturally occurring polyesters (Storz and Vorlop, 2013). Although PHAs are in principle completely biodegradable, their degradation depends on chemical composition, environmental conditions and the local microbial community (Volova et al., 2017; Dhaini et al., 2024). Like other bio-based plastics regarded as more environmentally friendly, their fate is poorly understood (Mendes and Pedersen, 2021). Many bio-based plastics are marketed as biodegradable, yet studies show they resist degradation under environmentally realistic scenarios (Bátori et al., 2018; Kalita et al., 2021).

Over 1000 assessed aquatic species have confirmed reports of microplastic ingestion (Gall and Thompson, 2015; Azevedo-Santos et al., 2021; Khoshmanesh et al., 2023). Fish as key links in food chains readily consume microplastics (Wang et al., 2020) through direct ingestion or consumption of contaminated prey (Galafassi et al., 2021). Microplastic exposure causes physiological harm to fish, including gut blockages and lesions (Ahrendt et al., 2020), impaired development (Qiang and Cheng, 2021), reduced growth and survival (Naidoo and Glassom, 2019). Common cellular level changes include cellular stress (Abarghouei et al., 2021) and immune dysfunction (Espinosa et al., 2017; Limonta et al., 2019; Masud and Cable, 2023). Moreover, bioplastic polymers appear to have analogous effects to conventional petroleum-based plastic pollution (Nik Mut et al., 2024).

Perhaps more concerning is that both petroleum and bio-based polymers are typically associated with the same additives (Groh et al., 2019; Aurisano et al., 2021; Akoueson et al., 2022) such as plasticizers, flame retardants, and stabilisers required to add functionality to otherwise inert polymers (Murphy, 2001; Hahladakis et al., 2018), as well as solvents used in production processes. These additional chemicals (>13,000) may end up within the environment, either as industrial effluent or through desorption from the degrading plastics (Kwan and Takada, 2016; UNEP, 2023). Thus, assessing any potential harmful effects of the polymers and associated chemicals is challenging due to the sheer number of products, potential interactions between them and the differential affects they might have on different biological systems (He et al., 2021; Kim and Lee, 2021; Wiesinger et al., 2021; Zhang et al., 2021).

Plastic additives such as phthalates and organophosphates are considered as emerging water contaminants (UNEP, 2023). Triphenyl phosphate (TPhP), a prevalent flame retardant and plasticiser used during plastic production, is particularly common in polyvinyl chloride (PVC) and textile fibres (van der Veen and de Boer, 2012; Zhu et al., 2020). TPhP is consistently identified as one of the most concentrated Organophosphate Esters (OPE) in samples of air (Wei et al., 2015), sediment, water (Li et al., 2014) and biota (Sundkvist et al., 2010). Based on limited surveillance that has been conducted within freshwaters, TPhP levels vary widely from 165 ng/L to 7900 ng/L (Lassen et al., 1999; Kolpin et al., 2002; Bacaloni et al., 2007). Beyond traditional measures of ecotoxicity such as LD50 and NOEC, assessment on chronic impacts remain limited (WHO, 1991; Lin, 2008).

Here, we assess the chemical behaviour and biological impacts of two types of microplastics, a plastic flame retardant and solvent within a freshwater environment utilising an established fish host-parasite system: a fish (guppy-*Poecilia reticulata*) and an invertebrate parasitic worm, *Gyrodactylus turnbulli*. We tested chronic effects of one of the most prevalent petrochemical polymers in global circulation, polyvinyl chloride (PVC), as well as the bio-based polyhydroxyalkanoate (PHA), both as pristine polymers with no additives. To tease apart potential effects of these compounds, the two polymers were tested individually with and without the flame retardant additive triphenyl phosphate (TPhP) and a widely used solvent dimethyl sulfoxide (DMSO).

2. Materials and methods

2.1. Sourcing plastics and their associated chemicals

The petroleum-based plastic polyvinyl chloride (PVC, CAS: 9002-86-2) was purchased from Sigma-Aldrich (Gillingham) and the poly-hydroxyalkanoate, poly (phthalic anhydride-*alt*-cyclohexene oxide), was manufactured by the School of Chemistry, Cardiff University, using commercially available monomers (full methods for production in Young et al. (2023)). Both plastic samples supplied as pristine powders with no known additives were sieved in pre-cleaned (with acetone and distilled water) stainless-steel sieves with apertures of 150 µm and 38 µm. Only microplastics retained by the 38 µm sieve were used in this study, aiming to restrict the size range to 38–150 µm. Triphenyl Phosphate (TPhP, CAS: 115–86-6, purity >99 %), a prevalent flame retardant used widely in plastic products (Groh et al., 2019; Akoueson et al., 2022) and Dimethyl Sulfoxide (DMSO, CAS: 67-68-5, purity ≥99.9 %), a widely used solvent during plastic polymer synthesis (Groh et al., 2019), were purchased from Sigma-Aldrich.

2.2. Scanning electron microscopy

The size of the microplastic samples were determined using scanning electron microscopy (SEM). Sample solutions were created by adding 0.00155 g (scale resolution of 0.00001 g) of either PVC or PHA to 6 mL of methanol. The solutions were shaken by hand before pipetting 20 µL onto the centre of a 5 cm wide circular steel plate. Once the methanol had evaporated at room temperature, the plate was fixed to a specimen stub using carbon based adhesive tape and placed into the SEM (Hitachi TM3030Plus). Individual PVC and PHA particles ($n = 36$) were measured at their widest length in ImageJ (Schneider et al., 2012).

2.3. Thermogravimetric analysis of PVC and PHA

To characterise possible absorption or desorption of TPhP and DMSO on to the microplastics (PVC and PHA), we conducted thermogravimetric analysis (TGA). TGA measures the mass loss of a sample as the

temperature increases at a controlled rate, characterising thermal events such as the desorption of chemicals retained by the sample through sorption (Guo et al., 2018). Samples were tested in different combinations (see Supplementary Material) under nitrogen carrier gas conditions. Any sorbed TPhP and/or DMSO would be desorbed during TGA analysis and shown as mass losses prior to the onset of degradation. All samples were analysed using a PerkinElmer Pyris 1TGA thermogravimetric analyser (PerkinElmer, UK).

2.4. ^1H and ^{31}P NMR spectroscopy: Potential TPhP and DMSO reaction products

To determine if water chemistry influenced any potential reactions between TPhP and DMSO, an initial screening process by mixing three types of water (i.e., distilled water, dechlorinated water destined for fish tanks and fish tank water) with TPhP and DMSO-d_6 was performed at room temperature for 14 days and characterised by ^{31}P NMR spectroscopy. ^{31}P NMR samples were prepared by dissolving TPhP in deuterated DMSO (DMSO-d_6, CAS: 2206-27-1, purity 99.8 % D), added to water in an NMR tube and sealed. ^1H NMR samples were prepared by dissolving TPhP (12.5 mg) in dimethyl sulfoxide (DMSO, CAS: 67-68-5, purity 99.7 %, 2.75 mL), added to 2.25 mL of fish-tank water reconstituted with deuterium oxide (D$_2$O, CAS: 7789-20-0, purity 99.9 % D, Sigma-Aldrich,) and sonicated. ^1H and ^{31}P NMR spectra were recorded on a Bruker Avance III 400 spectrometer at 400 and 162 MHz, respectively. Spectra were referenced externally to H$_3$PO$_4$ (^{31}P) or internally to the residual protio-solvent signal (^1H). Chemical shifts were given in parts per million (ppm) and coupling constants (J) were measured in hertz (Hz). See Supplementary Material for full methodology.

2.5. Freshwater fish host-parasite system

This study used size matched, ornamental juvenile guppies (standard length: 10–14 mm) bred from a stock originally acquired from a pet shop in Nottingham in 1997. Due to their development stage, fish could only be visually sexed at the end of the experiment, not at the start. Prior to the experiment, all fish were maintained following standardised procedures (Reynolds et al., 2018). In brief, this involved housing guppies in 70 L breeding tanks at 24 ± 0.5 °C under a 12 h light: 12 h dark light cycle (lights on 07:00–19:00). Fish were fed dry food flakes (Aquarian®) every day and freshly hatched *Artemia* nauplii every alternate day. The ectoparasite used in this study was the *Gt3* strain of *G. turnbulli,* isolated from a Nottingham aquarium in 1997 (King and Cable, 2007) and cultured using established methods in our laboratory since isolation with the addition of naïve fish to prevent extinction (Reynolds et al., 2018).

2.6. Experimental design

To investigate the individual and interactive impacts of plastics and their associated chemicals, fish were exposed to one of the following seven treatment groups: 1) PVC; 2) PHA; 3) TPhP + DMSO; 4) PVC + TPhP + DMSO; 5) PHA + TPhP + DMSO; 6) DMSO and 7) Control (dechlorinated water), $n = 86$ for each treatment. Due to the structural nature of TPhP and its extremely low solubility in water, the pure chemical was dissolved in DMSO before application, which is also standard protocol for testing the ecotoxicity of TPhP (Yu et al., 2024). Due to the large number of fish utilised in this study, the experiment was carried out in two batches with a two-week delay between the start dates of each batch. For exposure to microplastics, a concentration of 0.05 mg/L of plastic was used in each treatment; representing a relatively high concentration of microplastics found in European freshwaters (see Horton et al., 2017). For exposure to TPhP, the additive was dissolved into 1 L of DMSO to create a stock solution of TPhP, which was pipetted into the individual fish containers for a final concentration of 0.008 mg/L of TPhP and 0.01 % (v/v) DMSO. This TPhP concentration is at the

higher end of levels previously detected within freshwater systems (Lassen et al., 1999). A DMSO group was exposed to the same concentration of 0.01 % (v/v) DMSO but without the additive TPhP.

All fish were isolated in 500 mL tanks with dechlorinated water for the duration of the experiment. Separating into individual tanks was carried out two days prior to the first day of the experiment to allow the fish to acclimate. To ensure that water quality deterioration was not a confounding variable, 100 % water changes were performed every 48 h at 10 am. This standardised water changes also allowed for timing of feeding to remain consistent throughout the experiment. Fish were fed 10 % of their body weight in dry food flakes (Aquarian®) and 1 mL of freshly hatched *Artemia* nauplii on alternate days. Fish were exposed to their relevant treatment for a period of 21-days to ensure that fish had sufficient exposure time to detect any potential chronic effects of treatments (Fig. 1). The corresponding microplastic and/or TPhP + DMSO pollutant combination was dosed with a small spatula (for microplastics) and pipette (for TPhP and DMSO) onto the surface of the water after feeding. Our previous studies have confirmed that the guppies consume microplastics as detected in faecal matter (MacAulay et al., 2023).

2.6.1. Host growth

To assess any growth impact of the experimental treatments on the fish, standard length (length from the tip of the nose to the peduncle) was measured each week across the experiment. To do this, fish were anesthetized using 0.02 % MS-222 and measured using vernier callipers with a 0.1 mm resolution. An initial length was obtained two days prior to the first day of the experiment where size matching occurred to ensure no significant difference in starting lengths between treatments (GLM: $P > 0.05$). Due to restrictions in the number of fish available and the minimum number of fish required for sufficient statistical power, fish in the second batch of the experiment were on average 0.21 mm smaller than the first batch (GLM, $P = 0.005$). Two days after the end of the experiment, final growth measurements were taken, and the sex of all fish could be identified based on secondary sexual phenotypes (Magurran, 2005).

2.6.2. Host feeding

To assess the impact of plastics and their associated chemical on fish feeding metrics (latency and bite rate), measurements were taken every six days to align with the feeding of freshly hatched *Artemia* nauplii. A sterilised syringe was filled with 1 mL of freshly hatched *Artemia*, and the tip submerged in the tank before slowly releasing the food (circa. 1 s.). Latency was recorded as the time from when the food was released until the first bite was taken. Bite rate was the total number of bites taken in the first 30 s after the first bite. Where the fish did not bite within the first minute, a latency of 60 s and bite rate of 0 was recorded. To ensure consistency and reliability between the two observers, duplicate recordings of the same fish at the same time were performed to enable an inter-observer reliability test. There was no significant variation between the observers in either bite rate (Pearson's correlation, df = 89, $r = 0.90$, $P < 2.2e^{-16}$) or latency (as latency did not meet parametric assumptions, a Spearman's-rank correlation was conducted: df = 89, $r = 0.86$, $P = 7.929e^{-16}$).

2.6.3. Experimental infections

To determine the impacts of the experimental treatments on disease resistance after 21 days exposure to the environmental stressors, half of the fish ($n = 43$) from each treatment were randomly selected for experimental infection. Each fish was anesthetized in 0.02 % MS-222 and infected with two gyrodactylid worms using established protocols (King and Cable, 2007). Briefly, this involved sacrificing a heavily infected donor fish and bringing a mildly anesthetised experimental fish near the donor fish and observing two worms transfer from the donor to the caudal fin of the experimental fish, under a dissecting microscope with fibre optic illumination. Parasite infections were then monitored
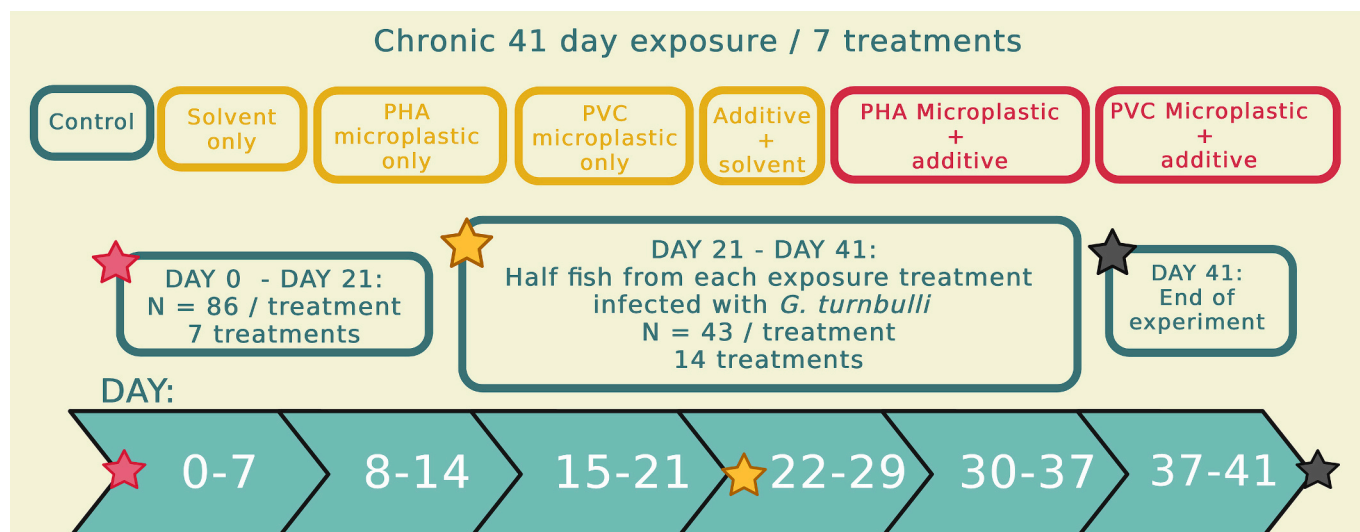
Fig. 1. Experimental design: *Poecilia reticulata* exposed to microplastics and/or plastic additive and solvent for the duration of the experiment. Half the fish from each treatment were infected with *Gyrodactylus turnbulli* on day 21 of chemical exposure with infections monitored every two days. Growth measurements were taken every 7 days for the entire duration of the experiment and feeding behaviour measured every 6 days.

every 48 h by anesthetizing the fish and using a dissecting microscope to count the number of worms on the entire surface of the fish. Infections were monitored for a maximum of 17 days, which corresponds to the duration over which sufficient data can be gathered to characterise host infection response profiles (van Oosterhout et al., 2003).

2.7. In vitro survival of parasites

As chemicals associated with the plastics (i.e., TPhP and DMSO) were likely to interact with the parasitic worms, the impact of TPhP and DMSO on parasite survival when off the host was also investigated. A heavily infected donor fish was sacrificed, and the caudal fin and parasitised scales were removed using watchmaker forceps. Individual *G. turnbulli* worms were isolated using a micro-pipette and placed into a well of a 96 well plate (Greiner Bio-One, Cellstar ®) containing 200 μL of dechlorinated tap water. Plates were allocated to one of three treatment groups: 1) Control (i.e., dechlorinated water only); 2) TPhP + DMSO; and 3) DMSO only, with $n = 38$ worms per plate. TPhP + DMSO and DMSO was then added to the corresponding wells at a concentration of 0.008 mg/L and 0.01 % v/v respectively, identical to the concentration fish hosts were exposed to during experimental infections (see above). Worms were observed every hour for survival until the last worm expired and time of worm death was recorded for every worm in each treatment. Worm death was determined if they unresponsive to a needle movement in the water column.

2.8. Ethical permission

All work was approved by the Cardiff University Animal Ethics Committee and conducted under UK Home Office licence PP8167141.

2.9. Statistical analyses

All statistical analysis was performed using RStudio version 4.2.1 (R. Core.Team, 2022). Generalised Linear Models (GLMs) were used to analyse the relationship between the experimental treatments and biological metrics: disease susceptibility, growth, feeding behaviour and host survival (full details of model selection and statistical analysis in Supplementary Material). All models included batch, sex, infection status (except models testing disease susceptibility) and standard length (except models testing growth) as explanatory factors. Final models were refined, and selection was based on the lowest AIC values and

satisfying the underlying model assumptions, i.e., normality of standardised residuals and homogeneity of variance. In addition, the reference treatment against which all other treatments were compared were control fish, not exposed to any plastics or their associated chemicals.

To analyse the relationship between the experimental treatments and disease susceptibility, the following metrics were used: mean parasite intensity, maximum parasite burdens, peak parasite day and area under curve (AUC), clearance and the day of clearance. Here, mean parasite intensity is the arithmetic mean of gyrodactylid worms on the hosts in each treatment, excluding those that have died or cleared their infections. Maximum parasite count is the maximum numbers of worms on the host at one point in time throughout the entire experiment (defined as peak parasite day). The AUC summates total parasite burden over time, using the trapezoid rule (White, 2011). Number of fish cleared is the total number of fish that were able to completely clear their infection within the 17-day infection period. Day of clearance is the day on which fish cleared their parasites.

To determine the impact of the plastic associated chemicals on the parasitic worm survival off the host and because the time of death for each worm was known, a Kaplan-Meier estimator was created for survival analysis of *G. turnbulli* using the 'survival' package in R (Therneau and Patricia, 2023) and then analysed using a Cox's proportional hazards non-parametric model. Treatment was included in the model as an independent variable.

3. Results

3.1. Scanning electron microscopy

Despite having passed through a 150 μm sieve, PVC microplastics were on average 151.9 μm in length (min: 93.2 μm, max: 230.5 μm, Fig. S1) while PHA had a significantly smaller average length of 90.5 μm (min: 45.1 μm, max: 198.1 μm; GLM: Std. Error: 8.053, T value: 7.625, $p < 0.001$). Examination of PVC showed an undulating surface where the exterior had bumps and ridges (Fig. 2), like the surface of PHA but with a notable difference, where PHA was porous with cavities roughly 2 μm in diameter.

3.2. Thermogravimetric analysis

The TGA analysis revealed no significant changes in percentage mass loss for both the PVC and PHA that were exposed to all chemical
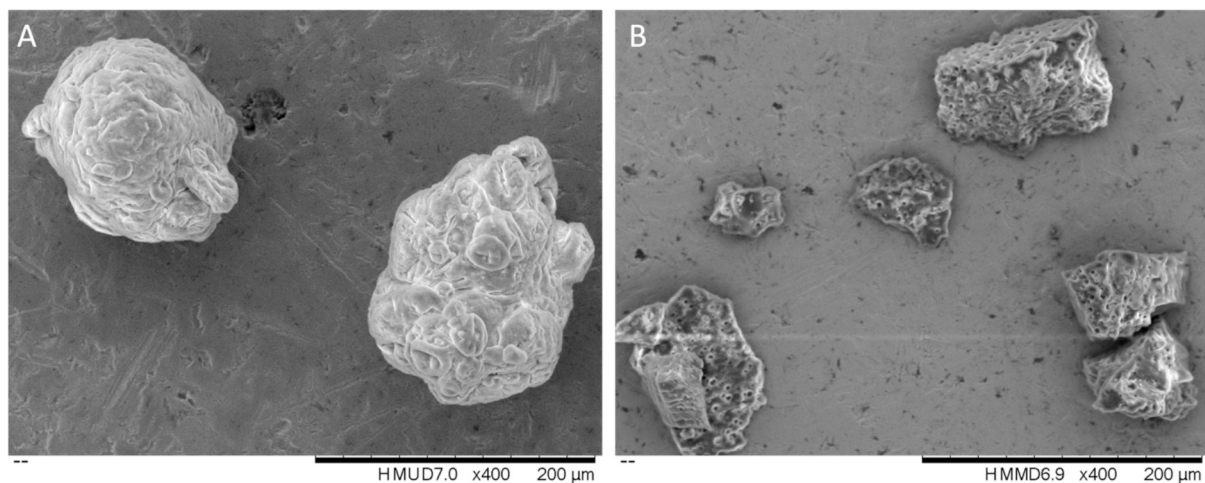
Fig. 2. Scanning electron micrographs of the two pristine polymers used for this study: A) polyvinyl chloride (PVC) and B) polyhydroxyalkanoate (PHA) microplastics with a more porous surface.

treatments (i.e. water, DMSO, TPhP and DMSO + TPhP; see Table S1 in Supplementary Material for full TGA output). Since no notable additional mass losses were observed in any of the samples, desorption of DMSO and TPhP is considered unlikely.

3.3. ^1H and ^{31}P NMR spectra of plastic associated chemicals

No reaction products were observed in the ^1H NMR spectra of the mixture containing TPhP, DMSO and deuterated fish-tank water heated at 80 °C for 14 days (see Supplementary Material, including Fig. S2, for further details of these results). Upon immediate addition of the mixture to the NMR tube (0 days), several signals were observed including signals for water and DMSO. At 7 and 14 days no additional signals were observed (Fig. S2).

3.4. Fish growth

Throughout the experiment control uninfected fish had an average growth rate of 0.17 mm/day. The most significant explanatory variables when assessing how growth rate was impacted during the experiment were infection (GLM; Std. Error = 0.08, T value = −7.96, $p < 0.001$) and host sex (GLM; Std. Error = 0.09, T value = 10.45, $p < 0.001$). No significant interactions were noted between the individual plastic and the associated chemical treatments and infections as multi-stressors.

3.5. Feeding behaviour

Control fish that were not infected had an average bite rate of 55 in the first 30 s with a latency of 2.22 s. Infections had no significant impacts on fish feeding behaviour in any treatment for either mean bite rate or mean latency (GLM; $p > 0.05$ for all outputs). However, fish exposed to PVC and PHA + TPhP + DMSO had a significantly lower mean bite rate (GLM; $p = 0.04$; $p = 0.045$ respectively). There were no interactions present between plastics and either the additive or solvent on mean bite rate and latency.

3.6. Disease susceptibility

When comparing all treatments against controls, no significance was seen in the infection burdens when analysed as a summation across time (i.e., AUC) in any of the plastic, chemical additives, and combined plastic + chemical additive treatments (Table S2 for more details).

Control fish had a mean parasite intensity of 20, this was significantly greater for fish that were exposed to DMSO with a mean parasite

intensity of 30 (GLM; Std. Error = 0.18, Z value = 2.43, $p = 0.02$, Fig. 3). While strictly speaking not significant, it is worth noting that fish exposed to PVC + TPhP + DMSO had a p value of 0.07, and these fish did have higher mean parasite burdens compared with control fish. When assessing maximum parasite burdens, fish exposed to DMSO and PVC + TPhP + DMSO had significantly higher maximum burdens (86 and 99 maximum worm burden respectively) compared to control fish with an average maximum burden of 56 worms ($p = 0.02$ and 0.04 respectively). The maximum parasite burdens were achieved on days 11, 11 and 10 respectively for those exposed to DMSO, TPhP + DMSO and PHA + TPhP + DMSO which was significantly later than the control fish on day 9 (Fig. 3).

Every treatment in the experiment had fish that were able to completely clear their infections with 19 out of 43 fish independently clearing in the control group. However, there were significantly fewer fish that were able to clear their infections in the TPhP + DMSO treatment (GLM; Std. Error = 0.30, T value = −2.60, $p = 0.009$) and the combined treatment PHA + TPhP + DMSO (GLM; 0.31, T value = −2.81, $p = 0.005$) with only 9 and 7 fish respectively clearing their infections. The day on which fish cleared their infections was not significantly different for any of the experimental treatments (GLM; $p > 0.05$) which was, on average, day 15 of the infection trial.

Throughout the experiment, there were no mortalities recorded for fish within uninfected control, DMSO, TPhP + DMSO and PHA treatments. Infection status was a significant variable in the number of mortalities recorded (GLM: Std. Error = 0.27, Z value = −3.20, $p = 0.001$). Exposure to PVC and PHA + TPhP + DMSO resulted in five mortalities in each treatment, significantly higher when compared to control fish (GLM: Std. Error = 0.50, Z value = 2.76, $p = 0.006$; Std. Error = 0.50, Z value = 2.66, $p = 0.007$ respectively). On the margin of significance, exposure to PVC + TPhP + DMSO resulted in the mortality of 3 more fish (Std. Error = 0.50, Z value = 1.95, $p = 0.05$) compared to uninfected control.

3.7. In-vitro survival of parasites

Interestingly, when analysing the impact of the flame retardant, TPhP, and solvent, DMSO, on the invertebrate parasite survival; *G. turnbulli* worms that were exposed to DMSO and TPhP + DMSO had significantly higher rates of survival than control worms in dechlorinated water (Cox PH: Std. Error = 0.24, $p = 0.02$; Std. Error = 0.24, $p = 0.02$ respectively). Control worms survived for an average of 15.24 h while those exposed to DMSO and TPhP survived for an average of 17.05 and 17.42 h respectively (Fig. 4).
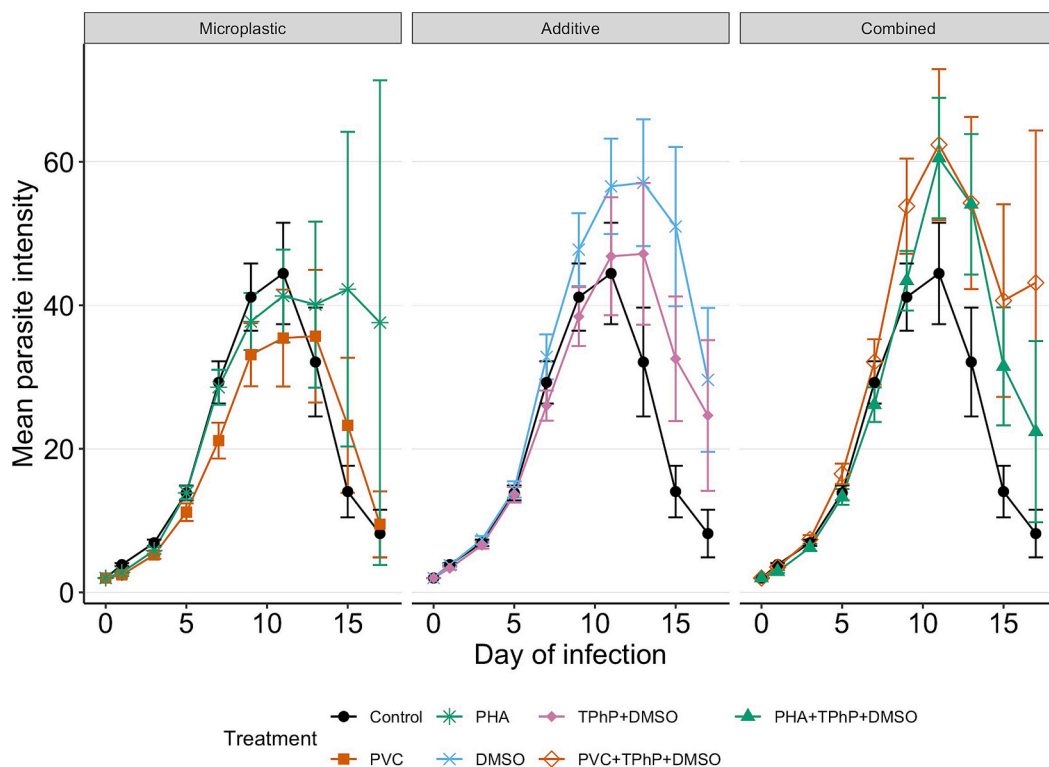
Fig. 3. Mean parasite intensities (±SE) of the host *Poecilia reticulata* after exposure to microplastics (PVC and PHA) and/or plastic associated chemicals (DMSO and TPhP + DMSO) and subsequently infected with *Gyrodactylus turnbulli*.
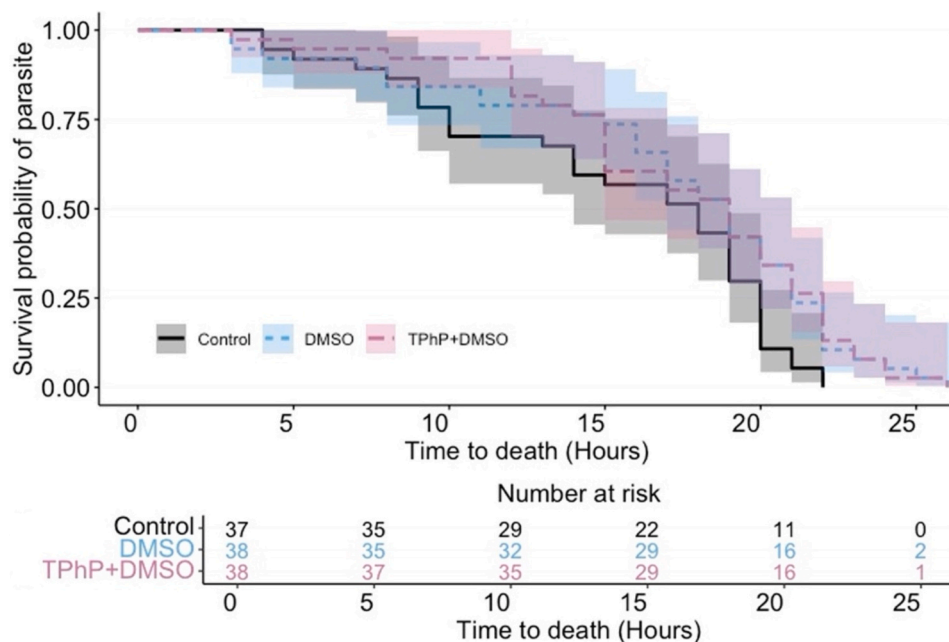

Fig. 4. Survival analysis plot with 95 % confidence intervals of *Gyrodactylus turnbulli* off the host while exposed to the solvent DMSO and the flame retardant TPhP + DMSO with a risk table showing the number of worms alive at 5-h intervals.

4. Discussion

4.1. Summary

In the current study, we assessed the chemical behaviour and biological impacts of the petrochemical polymer PVC and a bio-based PHA polymer, with two plastic associated chemicals, an additive TPhP and a solvent, DMSO. The individual and interactive impacts of the plastics and associated chemicals were tested on fish (*Poecilia reticulata*) growth, feeding rate, and disease resistance, and survival of the parasitic monogenean worm *Gyrodactylus turnbulli*. Pristine PHA was the only tested product with no apparent detrimental impact on fish tested in this study. Surprisingly, this study reveals that one of the most widely used solvents DMSO, considered biologically safe by multiple ecotoxicology

assessments, had significant detrimental impacts on disease susceptibility of *P. reticulata* to a gyrodactylid parasite.

4.2. Chemical interactions

SEM analysis indicated that PHA had a higher degree of porosity than PVC, but TGA analysis confirmed no notable desorption of DMSO or TPhP for both plastic types, at least over the 48 h tested. This lack of sorption could be explained by other factors, such as surface chemistry, functional groups, hydrophobicity, and surface charge, which also play crucial roles in adsorption and absorption processes, not tested in this study (Wang et al., 2017; Hossain et al., 2019). It is interesting to note that even though no chemical interactions were noted between PHA and the additive and solvent, when the two chemicals were combined experimentally, this did inhibit fish feeding behaviour, particularly for infected fish, and was associated with increased mortality. This effect was not seen, however, when fish were exposed to PHA microplastics on their own. This highlights the need for plastic industries to increase transparency in reporting which chemicals are being used during the manufacture process and not replacing known toxic additives with novel ones, with slight structural modifications which may retain their toxic profiles (Liang et al., 2024). Even DMSO, one of the most widely used solvents and classed by the ECHA as a solvent where '…*toxicity profile for aquatic species is of low concern*' (ECHA, 2024), is shown here to have negative impacts, specifically for disease resistance.

4.3. Fish mortality

There were no mortalities among the uninfected fish in the control, DMSO, PHA and TPhP + DMSO treatments. In contrast, all infected fish experienced increased rates of mortality. Importantly, infected fish exposed to the petrochemical microplastic PHA were significantly more likely to survive that those exposed to PVC. Petrochemical microplastics have been documented to increase levels of mortality in fish before (Naidoo and Glassom, 2019; Masud and Cable, 2023), possibly linked to ingestion and subsequent gastro-intestinal tract lesions (Dinani et al., 2021). Pathogen-induced mortality can worsen when hosts are also exposed to microplastics (Seeley et al., 2023), underscoring the risk to fish populations, particularly within aquaculture where fish face multiple stressors (Vázquez-Rowe et al., 2021).

While the flame retardant and the DMSO solvent did not cause a significant increase in host mortalities (Liu et al., 2019; Umamaheswari et al., 2021), the PVC microplastic alone and in combination with TPhP and DMSO did significantly increase fish mortality. In contrast, the bio-based plastic, PHA, which on its own was not associated with any detrimental impacts, when combined with TPhP and DMSO did lead to significantly higher mortalities, more so when compared with the PVC in combination with the same chemicals. Collectively this suggests that interactions between microplastics and their associated chemicals increases toxicity. However, PHA were smaller in size than PVC microparticles, so we cannot exclude the possibility that size and other morphological factors influence the toxic effects of the microplastics.

4.4. Growth and feeding

The growth rate of fish was only reduced when exposed to parasite infection and was not impacted by exposure to microplastics or associated chemicals individually or in combination. This supports another study that found parasite infections but not microplastic exposure reduced the growth of zebrafish (Parker et al., 2023), at least over the duration of the study. There are however reports that exposure to microplastics (both pristine and with additives) can inhibit the growth of freshwater fish (Naidoo and Glassom, 2019; Xia et al., 2020). We noted significantly lower bite rates for fish exposed to PVC and the interaction between PHA + TPhP + DMSO but feeding latency was not impacted by exposure to any combination of microplastics and TPhP or DMSO. As

there was no significant reduction in growth seen in this study in response to microplastics or TPhP and DMSO, there is likely to be no significant reduction in nutritional intake and therefore explaining reduced bite rate seen in this study is challenging. Possibly fish intentionally avoid microplastic when feeding (Ryan et al., 2019) resulting in a slower, more selective feeding pattern. In marine medaka (*Oryzias melastigma*), TPhP can impact locomotor function and eye development which is posited to impact feeding behaviour, though when the medaka were exposed to microplastics and TPhP together, locomotor behaviour returned to pre-exposure conditions (Zhang et al., 2021).

4.5. Disease susceptibility

Few studies have investigated the disease susceptibility of fish when exposed to microplastics (Masud et al., 2022; Masud and Cable, 2023; Parker et al., 2023; Seeley et al., 2023), and these have focussed on the polymers themselves as opposed to any impacts of chemical additives and their interactions with the polymers. In the current study, PVC alone and PVC with the associated chemicals increased disease burdens. In contrast, the bio-based polymer PHA had no impact on disease resistance on its own nor in combination with TPhP and DMSO. Interestingly, exposure to TPhP + DMSO significantly reduced the number of fish able to clear their infections, even though exposure to these combined chemicals did not lead to higher parasite burdens. However, when exposed to only DMSO without TPhP, the solvent had significant impacts on most of the disease metrics measured, indicating that it is detrimental to fish disease resistance. Although disease resistance was not assessed when zebrafish (*Danio rerio*) were exposed to microplastics, the observed downregulation in key genes involved in innate immunity, specifically genes involved in epithelium integrity (Limonta et al., 2019), might explain the observed impact on an ectoparasite like *G. turnbulli*.

The infection responses observed in the current study may be partially explained by the off-host survival of the parasitic worms, where parasites exposed to DMSO or TPhP + DMSO had enhanced survival rates. This suggests some degree of protection for the parasites offered by the chemicals, which may explain why fish hosted parasites for longer when exposed to TPhP and DMSO. Certainly, 0.2 % DMSO did not damage the body surface or impact survival rate of a related species, *G. kobayashii*, both on and off the host (Tu et al., 2018; Liu et al., 2022). Although these studies are not directly comparable, as previously DMSO was tested over a shorter period and at a higher concentration than in our study (3 h at 0.2 % versus our 27 h at 0.01 % v/v). For guppies, DMSO has an LD50 value of 1.9 % v/v (Rabinowitz and Myerson, 1966), almost 200 % higher than the levels used within this experiment. Typically, fish respond to gyrodactylid infection through innate and adaptive immune responses including the expression of pro-inflammatory cytokines (Konczal et al., 2020) as the ectoparasite uses hooks to penetrate and damage the epidermis of fish (Bakke et al., 2007). Interestingly, DMSO is used to treat inflammatory conditions such as arthritis, inhibiting the production of inflammatory cytokines (Elisia et al., 2016). If DMSO is acting in a similar way on this fish, then a lack of inflammatory cytokines would restrict the immune response, preventing the guppy from effectively fighting off the gyrodactylid infection.

5. Conclusion

The negative impacts of pristine microplastics can be intensified by interactions with chemicals used in their production. In this study, the biodegradable polymer PHA without additives was not associated with any adverse effects on fish health. PHA when combined with the common flame retardant and solvent TPhP and DMSO, however, caused a reduction in fish feeding and an increase in mortality. Therefore, replacing petroleum-based plastics with bio-based plastics is not an easy route for reducing toxicity, particularly if the same additives are used in

plastic production.

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Ethical approval

All animal work was approved by the Cardiff University Animal Ethics Committee and conducted under UK Home Office licence PP8167141.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

S.J. Cheung: Writing – review & editing, Writing – original draft, Project administration, Formal analysis, Data curation, Conceptualization. **N. Masud:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **C. Robison-Smith:** Writing – review & editing, Data curation, Conceptualization. **P. Hansal:** Writing – review & editing, Formal analysis, Data curation. **J. Davies-Jones:** Writing – review & editing, Software, Data curation. **B.D. Ward:** Writing – review & editing, Supervision, Formal analysis, Data curation. **J. Cable:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.scitotenv.2024.177958.

Data availability

All data pertaining for this manuscript will be made available via the data repository Mendeley upon acceptance for publication.

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