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Toxicity of the water-soluble polymer PVP is dependent on molecular weight and feed concentration for a freshwater model species

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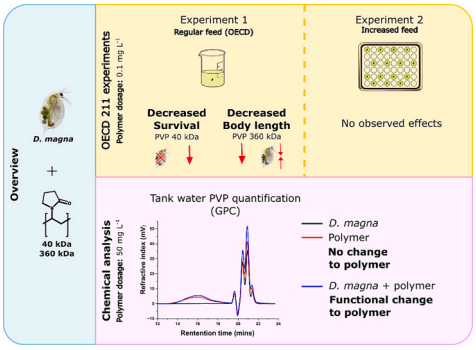
HIGHLIGHTS

- *D. magna* exposed to two different molecular weights of PVP at two feed concentrations
- PVP quantified and functional changes monitored using GPC
- Lower molecular weight PVP was more toxic to *D. magna*
- Detrimental effects of PVP not seen when feed concentration was increased
- Algal-polymer binding theory proposed to explain underlying mechanism of toxicity

GRAPHICAL ABSTRACT



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ABSTRACT

Polyvinylpyrrolidone (PVP) is a synthetic water-soluble polymer (WSP) that does not readily biodegrade and has been detected in rivers in the mg L^{-1} range. Whilst previous studies have highlighted sublethal impacts of this chemical on freshwater species, cause of this toxicity remains unclear. The current study investigates how polymer molecular weight impacts the freshwater model species, *Daphnia magna*. Following OECD 211 guidelines, *D. magna* were exposed to PVP at levels reflecting those detected in European rivers, where the effect of two different molecular weights of the polymer were compared, both representing sizes commonly used in domestic products (PVP 40 kDa and 360 kDa). Two experiments were performed which differed in algal ration; Experiment 1 followed OECD 211 recommended rationing and Experiment 2 used concentrated algal rations, both assessing *D. magna* growth, survival and reproduction. No effects of PVP on *D. magna* were seen when algae rations were high, contrary to the inhibited growth observed for *D. magna* exposed to PVP 360 kDa, and the lethal effects of PVP 40 kDa when using the OECD recommended ration. Gel-permeation chromatography (GPC) analysis of PVP tank water samples suggests a polymeric structural change occurs only in the presence of

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Pollution
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D. magna, implying that ingestion of PVP by *D. magna* causes polymer-algae binding. This polymeric change was not observed when *D. magna* were absent in an equivalent polymer-algae solution. The findings highlight the need for more research investigating the underlying toxic effects of these polymers to inform chemical legislation as well as the need to accommodate molecular weight in environmental risk assessments.

1. Introduction

Polyvinylpyrrolidone (PVP) is a water-soluble polymer (WSP) with versatile, biocompatible, film-forming and amphiphilic properties. As such, PVP is commonly used as a sustained drug delivery matrix, an emulsion stabiliser in the common disinfectant PVP-iodine, a thickener in personal care products like shampoos, and as a chemical additive in water treatment (Abdelrazek et al., 2023; AlKhatib et al., 2010; Antić et al., 2011; Kurakula and Rao, 2020; Risbud et al., 2000; Siggia, 1957). More recently, it has been used as an important component of novel bioplastics and antimicrobial films in the incentive to move away from fossil-based plastics (Aldalbahi et al., 2024; Deng et al., 2024; Menazea and Ahmed, 2020). However, its prevalent use, resistance to biodegradation and lack of regulation are a source for concern regarding PVP fate in the environment (ECHA, 2023; Huppertsberg et al., 2020; Julinova et al., 2012; Julinova et al., 2018; Robison-Smith and Cable, 2024; Trimpin et al., 2001). Furthermore, its occurrence has been quantified using pyrolysis-gas chromatography/mass spectrometry (Pyr-GC/MS) between 0.9 and 7.0 mg L^{-1} in wastewater and at 0.1 mg L^{-1} in wastewater-affected rivers, suggesting PVP is not fully removed during the wastewater treatment process (Antić et al., 2011; Antić et al., 2012).

Techniques, like Pyr-GC/MS, have relatively low detection limits and can detect PVP in wastewater influent, effluent and receiving freshwaters (Antić et al., 2011; Antić et al., 2012; Vidovic et al., 2023; Vidovic et al., 2024). However, gel-permeation chromatography (GPC) for WSP analysis has been recognised as the most readily available, cost-effective and scalable method for PVP detection, and has recently been used for PVP detection in *Daphnia magna* (Gómez-Ordóñez et al., 2012; Horváth et al., 2023; Tarring et al., 2024a; Tarring et al., 2024b). Reports of WSPs in the environment are only increasing, raising concerns of the environmental safety of WSPs including PVP (Antić et al., 2011; Antić et al., 2012; Vidovic et al., 2024). Despite current evaluations deeming PVP non-toxic, standardised environmental risk assessments are often only carried out to determine acute toxicity, and therefore the sublethal toxicity and effects of chronic exposure of this chemical have been overlooked in legislative environmental risk assessments (Johnson et al., 2024; Kurakula and Rao, 2020).

Increased reports of the presence of PVP in environmental compartments has sparked investigations to better characterise the toxicity of PVP in recent years, particularly with regards to the freshwater flea *D. magna*. When exposed to PVP for 21 days, *D. magna* demonstrated altered fecundity and reduced body length at concentrations found in wastewater, while other WSPs with lower molecular weights exerted greater toxicity, reducing offspring production after chronic exposure (Mondellini et al., 2022). Additionally, heart rate and mobility were also affected in *D. magna* exposed to WSPs at 0.001, 0.5 and 1 mg L^{-1} (Nigro et al., 2024). Notably, this study reported lethal toxicity at an intermediate dose (0.5 mg L^{-1}) of polyethylene glycol (PEG), which was the only concentration to significantly decrease survival of *D. magna*, despite the higher dose having no effect (1 mg L^{-1}). Algal feed deposits were also observed in containers but only at the intermediate concentration, hence decreased food availability was suggested as a possible cause. Given that the higher concentration did not have the same effect on *D. magna* survival, this could indicate a non-linear relationship between polymer and feed availability (Nigro et al., 2024). A comparable result was seen for *Poecilia reticulata*, where a low concentration of PVP inhibited fish weight gain over 45 days, whereas a higher concentration had no effect on the overall growth of fish (Robison-Smith et al., 2024).

In this study, we investigate the impact of molecular weight and feed concentration on the toxicity of PVP to *D. magna* over 21 days, with PVP 40 kDa and 360 kDa at an exposure dose which reflects concentrations reported in wastewater-affected freshwater environments (0.1 mg L^{-1}). Due to the chemical behaviour of PVP, GPC was used to determine whether PVP is ingested by *D. magna* and investigate interactions between the polymer and algal feed (DeMott et al., 2010).

2. Materials and methods

2.1. Chemicals

Polyvinylpyrrolidone (PVP) (number-average molecular weight (M_n) = 40,000 Da and 360,000 Da) was purchased from Sigma-Aldrich (Gillingham, UK), sodium nitrate from Honeywell (St Helens, UK), and sodium phosphate monobasic and potassium hydroxide were supplied by ThermoFisher Scientific (Loughborough, UK). Hydrochloric acid (37 %) and HPLC grade water were supplied by Fisher Scientific (Loughborough, UK). For the OECD 211 chronic exposures and the quantification tank exposures, stock polymer solutions were made at 5 mg L^{-1} and 3.33 g L^{-1} respectively by dissolving the polymers in deionised water.

2.2. Daphnia magna preparation and experimental conditions

Daphnia magna ephippia were purchased from Microbiotests toxkit (Belgium). All *D. magna* used in this experiment were maintained at 20 ± 0.5 °C with a photoperiod of 16 h light: 8 h dark in cool white light 1100 (±200) lux (testo light meter).

2.3. OECD 211

Following OECD 211 guidelines (Test No. 211: *Daphnia magna* Reproduction Test, 2008), (OECD, 2012) two 21 day chronic exposure tests to 0.1 mg L^{-1} PVP at two molecular weights were conducted to reflect levels detected in rivers receiving wastewater effluent (Antić et al., 2011). Three days before commencing the 21 day trial, ephippia were added to 250 mL M7 Elendt medium and monitored daily. Following appearance of the first nauplii, individual daphnids were transferred into their own container within one of the three treatments until 15 replicates per treatment was achieved: i) Control (M7 + deionised water), ii) PVP40 (M7 + 0.1 mg L^{-1} PVP 40 kDa) and iii) PVP360 (M7 + 0.1 mg L^{-1} PVP 360 kDa). Parent animal welfare and condition were monitored daily, and any offspring counted and recorded. Following the 21 day exposure trials, each individual daphnid was fixed to a small drop of Vaseline® and their carapace length was recorded using callipers (from the top of the eye to base of the carapace spine). Newly hatched nauplii carapace length was consistent, averaging 0.6 mm, so length of daphnids were compared at 21 days old (Table S1). Other than feed concentration, other variables differing between the two experiments were water change frequency and container size, details of which are outlined below (Sections 3.3.1 and 3.3.2). Primarily, these differences enabled accurate, higher feed dosing in Experiment 2, without adding high volumes of algae media.

2.3.1. Experiment 1: Normal feed

Glass beakers were used to house each daphnid containing 50 mL of exposure medium, consisting of 48 mL M7 medium, 1 mL concentrated algae, and 1 mL concentrated polymer solution or deionised water for the controls. Feed concentrations here equated to an organic carbon (C)

dose of 0.2 mg C daphnid^{-1} day^{-1}. Tank water was refreshed every 3 days.

2.3.2. Experiment 2: Increased feed

Each daphnid was housed in an individual well of a 48-well plastic plate containing 1.8 mL of exposure medium, consisting of 0.9 mL M7 medium, 0.45 mL concentrated algae, and 0.45 mL concentrated polymer solution or deionised water for the controls. Water changes occurred every day to ensure M7 medium salts concentrations were not depleted given the smaller volume. Feed mass here equated to a C dose of 2 mg C daphnid^{-1} day^{-1}; ten times that of Experiment 1.

2.4. Statistical analysis

All statistical analyses were performed using R (R version 4.4.2). For both experiments, reproduction of *D. magna* was assessed using a GLMM to analyse the relationship between offspring production and treatment using a glmmTMB model with zero inflation component. The model was performed with negative binomial family and the zero-inflation component was set to 1. Treatment was incorporated as a fixed factor along with individual daphnid ID as a random effect to account for pseudo-replication in the model. Experiments 1 and 2 were analysed independently with no statistical comparisons made between them.

A Kaplan-Meier Survival Curve was plotted to enable visualisation of survival probabilities over the 21-day exposure. GLMs were applied to compare carapace size at the end of the experiment and the number of reproductive cycles and clutch sizes. The residuals for the growth data in Experiment 1 were better fitted to a GLM (gaussian, link = identity). Experiment 2 applied a GLM with inverse gaussian family and 1/muˆ2 function to improve model fit. Poisson GLMs were applied to compare number of total offspring produced, reproductive cycles and average clutch sizes. A negative binomial GLM was applied when overdispersion was detected. Model selection was based of the model with the lowest Akaike information criterion (AIC).

2.5. Polyvinylpyrrolidone tank exposures and quantification

2.5.1. Daphnia magna preparation and experimental conditions

Live *D. magna* were purchased from Aquatic World, UK in August 2024, and thereafter maintained at Cardiff University at a constant temperature and light exposure (20 °C ± 1.0 °C, 12:12 h light:dark cycle).

2.5.2. Exposure and quantification study

To investigate the interaction of *D. magna* with PVP, duplicate exposure tanks (1.5 L) were set up under the following conditions. i) Control tank (50:50 M7/deionised water only), ii) Control_PVP40 (50:50 M7/deionised water +50 mg L^{-1} PVP 40 kDa) iii) Exposure tank_PVP40 (50:50 M7/deionised water +50 mg L^{-1} PVP 40 kDa + *D. magna*), iv) Control_PVP360 (50:50 M7/deionised water +50 mg L^{-1} PVP 360 kDa) and v) Exposure tank_PVP360 (50:50 M7/deionised water +50 mg L^{-1} PVP 360 kDa + *D. magna*). A higher exposure concentration was required for this element of the study due to the limits of detection required for this polymer using the most up-to-date methods published at the time of this study (Tarring et al., 2024b). Approximately 1 g (wet weight) of *D. magna* was added to these tanks with an initial feed of concentrated *Raphidocelis subcapitata*. Additional feeds were administered ad libitum and were consistent between the tanks with the volume recorded to account for final polymer concentration.

2.5.3. Procedural blank and sample collection

D. magna procedural blanks were collected and rinsed in a 500 μm metal sieve with deionised water (300 mL) before being stored as duplicate samples at −20 °C. *prior* to analysis, the samples were dried in a 50 °C oven. This procedure was applied to a procedural blank containing *D. magna* (~ 0.5 g) and two polymer-spiked *D. magna* samples (~ 0.5 g *D. magna*, PVP 40 kDa (1 mL, 3 mg mL^{-1})), (~ 0.5 g *D. magna*, PVP 360 kDa (1 mL, 3 mg mL^{-1})). All *D. magna* sample masses can be found in Table S2. Two further procedural blanks only contained polymer: PVP 40 kDa (1 mL, 3 mg mL^{-1}) and PVP 360 kDa (1 mL, 3 mg mL^{-1}) with no *D. magna*. These solutions were added directly to sample vials before being dried. These procedural blanks were used to assess blank *D. magna* and polymer chromatograms, as well as the extraction of the polymers from *D. magna*.

This washing and drying process was then applied to the *D. magna* samples following the 7 day exposure period. Duplicate water samples (20 mL) were also collected from each tank at this timepoint and stored at −20 °C until analysis. Prior to analysis, the samples were frozen in liquid nitrogen and the water removed by lyophilisation.

2.6. Analytical measurements

2.6.1. GPC measurements

Aqueous GPC analysis was carried out using an Agilent InfinityLab LC series 1260 Infinity Quaternary system connected to an Agilent 1260 Infinity GPC/SEC Multi Detector Suite (MDS). Instrumentation and analysis followed the protocol outlined in previous work (Tarring et al., 2024b). Briefly, calibration of the refractive index (RI) detector for both PVP 40 kDa and 360 kDa was carried out in duplicate over a range of 10 concentrations (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0 and 4.0 mg mL^{-1}), with peak areas determined using Fityk 1.3.1 line deconvolution software.

The limit of blank (LOB) and limit of detection (LOD) were calculated using the Eqs. S1 and S2 as per the methodology of Vashist and Luong (Armbruster and Pry, 2008; Tarring et al., 2024b; Vashist and Luong, 2018). The limit of quantification (LOQ) was determined to be the sample with a standard deviation <20 % and a RI value at least five times greater than the blank. Concentration was determined using the straight-line equations determined from the PVP calibration curves (Eq. S3).

Experimental samples were directly dissolved in GPC eluent and stirred for 2 h to ensure homogenisation. All samples were filtered using a glass syringe through 0.2 μm nylon filters prior to analysis.

2.6.2. UV–vis experiments

For the OECD algal feed (Experiment 1) settling velocity measurements, M7 mineral medium (2.025 mL) was added to a plastic cuvette followed with polymer (0.75 mL, 0.1 mg L^{-1}) and algal feed (0.225 mL, 0.6 mg C 3 mL^{-1}). For the increased algal feed (Experiment 2) measurements, M7 mineral medium (1.5 mL) was added to a plastic cuvette followed with polymer (0.75 mL, 0.1 mg L^{-1}) and algal feed (0.75 mL, 2 mg C 3 mL^{-1}). For the controls in both experiments, distilled water replaced the polymer solution. All UV measurements were carried out on a Shimadzu UV-1800 UV spectrophotometer. After baselining with a 2:1 mineral medium: distilled water solution, UV measurements were taken at 684 nm in triplicate over a period of 3–4 h until a plateau in absorbance was evident. The solutions were mixed directly before the measurements to replicate the conditions for when *D. magna* water changes took place in Experiments 1 and 2.

3. Results and discussion

3.1. Chronic toxicity tests

3.1.1. Experiment 1: Normal feed

At the end of the exposure period and at 21 days of age, control *D. magna* had an average length of 3.28 mm (n = 13), whilst PVP360 exposed animals were significantly smaller, averaging 3.16 mm (n = 13). There was no difference in the carapace length of *D. magna* exposed to PVP40 (3.2 mm), however this average was based only on the 4 surviving individuals (GLM: PVP40; t = −1.2, SE = 0.06, p > 0.05, PVP360; t = −2.65, SE = 0.04, p < 0.05). Although the current study is

limited in that only two molecular weights were investigated, these results infer an increase in toxicity with decreasing molecular weight. This is supported by a report of enhanced toxicity of PEG to *D. magna* at lower molecular weight exposure (Mondellini et al., 2022). This study implemented similar exposure conditions to the current study and also investigated PVP exposure to *D. magna*, but at a lower molecular weight over a range of concentrations (7–10 kDa, 1–10 mg L^{-1}). PVP in this instance, however, did not reduce *D. magna* final body length, but increased *D. magna* body length in an exposure to 10 mg L^{-1} of PVP 10 kDa over 21 days (Mondellini et al., 2022). These differences infer molecular weight as an important determinant of PVP toxicity and provide further evidence for a non-monotonic response slope with regard to changing molecular weight of PVP, manifested in the apparent non-linear relationship between increasing molecular weight and toxicity (Hill et al., 2018; Nigro et al., 2024).

Only two mortalities of *D. magna* occurred within the 21 day exposure period in both controls and PVP360 ($z = 0.1$, $p > 0.05$), but there was a significant reduction in survival in PVP40 (11 deaths; $z = 2.92$, $p < 0.05$). Mondellini et al. reported no acute effect on *D. magna* survival over a 48 h exposure to PVP 10 kDa at 1–50 mg L^{-1} (Mondellini et al., 2022). The same was true in the current study; 93 % survival was seen for PVP40 exposed *D. magna* over the first 48 h of the test. Survival, however, had decreased to 53 % by day 5 for this treatment, which implies that acute toxicity testing may not be appropriate for the risk assessment of PVP in freshwater environments. Significant mortality was also observed in *D. magna* exposed to an intermediate concentration of PEG (1.9–2.2 kDa) for 14 d (Nigro et al., 2024). An exposure concentration of 0.5 mg L^{-1} caused substantial mortality, despite equivalent exposures to other WSPs including polyvinyl alcohol (PVA), polyacrylic acid (PAA), and PVP having no effect on survival at the same concentration (Nigro et al., 2024). Of these polymers, PEG had the lowest molecular weight, aligning with our study. Furthermore, Mondellini et al. found PEG displayed higher toxicity at 1 kDa than 900 kDa, manifested in reduced reproductive success at 10 mg L^{-1} (Mondellini et al., 2022). Current evidence infers that the relationship between molecular weight and toxicity is polymer specific (Zicarelli et al., 2024).

Considering individuals that survived to reproduce in this experiment, an average of 41 offspring was produced per daphnid in control ($n = 15$), 38 in PVP40 ($n = 6$) and 46 in PVP360 ($n = 13$) (GLM, $p > 0.05$). PVP exposure did not significantly impact reproduction of *D. magna* (GLMM: PVP40; $z = 0.99$, SE = 0.11, $p > 0.05$, PVP360: $z = 1.24$, SE = 0.09, $p > 0.05$). Fig. 1 shows cumulative offspring production considering all $n = 15$ individuals per treatment, whilst there were no differences between reproducing individuals, the high mortality amongst juvenile *D. magna* exposed to PVP40 prevented 8 individuals reaching reproductive maturity in the current study.

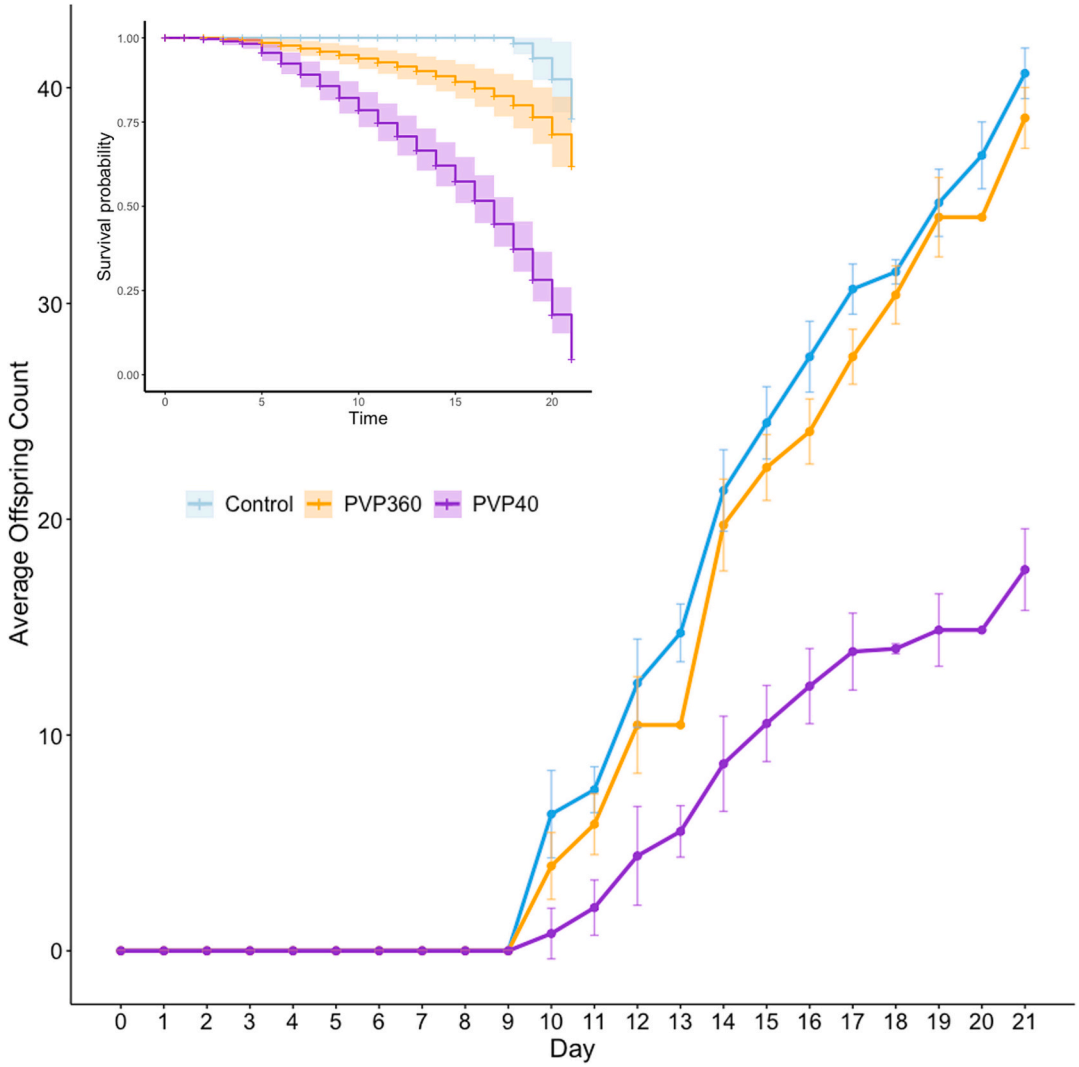

Fig. 1. The mean (±SE) cumulative *Daphnia magna* offspring counts in Experiment 1 over the 21 day exposure period to polyvinylpyrrolidone at differing molecular weights and normal feed concentration (PVP40; 40 kDa or PVP360; 360 kDa). A survival probability curve (top left insert) is also included to illustrate the significant difference in survival probabilities between treatments in Experiment 1 (Control; $n = 13$, PVP40; $n = 4$, PVP360; $n = 13$).

3.1.2. Experiment 2: Increased feed

At 21 days of age, *D. magna* in all treatments had similar average lengths of 3.35 mm in control ($n = 12$), 3.52 mm in PVP40 ($n = 12$) and 3.41 mm in PVP360 ($n = 11$) treatments (GLM: PVP40; $t = -1.59$, SE = 0.01, $p > 0.05$, PVP360; $t = -0.57$, SE = 0.01, $p > 0.05$). There was no effect of either PVP chemical on *D. magna* survival (PVP40; $z = 0.11$, SE = 0.82, $p > 0.05$, PVP360; $z = 0.4$, SE = 0.76, $p > 0.05$). In Experiment 2, only 3 individuals in PVP40 did not survive to reproduce. Mean offspring production was not statistically different (Fig. 2): 61 in control, 58 in PVP40 and 57 in PVP360 with all 15 replicates considered (GLMM: PVP40; $z = 0.64$, SE = 0.17, $p > 0.05$, PVP360; $z = -0.43$, SE = 0.16, $p > 0.05$).

From the lack of significant effects seen in Experiment 2, we infer that algae content is an important predictor of PVP toxicity. In the knowledge that the few surviving *D. magna* in the PVP40 treatment from Experiment 1 had no size nor fecundity differences from the control, this could imply that PVP is not directly toxic to *D. magna.* From this, it could be inferred that PVP affects nutrient availability by interacting with the algae, *Raphidocelis subcapitata*. Previous findings show that 1 mg L^{-1} PVP modulated 5 % of the 1123 proteins detected in *D. magna*, specifically structural, catalytic and ribosomal proteins (Nigro et al., 2024). However, this may not infer direct toxicity of the polymer. Rather, this may indicate a general stress response, as some of the proteins modulated are comparable to proteomic modulations which occur when *D. magna* experience nutritional stress, including notable decreases in structural protein actin, and catalytic protein DNA polymerase (Wagner et al., 2022). It is possible that the results presented in this proteomics study are indicative of a nutritional stress response, where an interaction between feed ration and polymer concentration may reduce nutrient assimilation in *D. magna*.

Another potential mechanism for PVP toxicity on *D. magna* can be implied from the differences between high and low molecular weight observed in the current study. The low molecular weight polymer (40 kDa) was substantially more toxic to *D. magna* in the current study causing significant mortality. Low molecular weight polymers are more likely to translocate across biological membranes, evidenced in their medicinal uses as functional carriers of encapsulated drugs, where it has been shown that PVP below 84 kDa is more readily internalised by rat epithelial cells (Duncan et al., 1981). This may explain the observed differences in toxicity between polymer sizes in Experiment 1, where 40 kDa PVP is more easily absorbed through membranes, causing greater toxicity than 360 kDa PVP. However, given that the surviving individuals in the 40 kDa group, although few, had no physiological differences from control, this theory is less well supported by the results of the current study. Direct toxicity of WSPs have been inferred from previous works (Kuskov et al., 2017; Mellati et al., 2021; Robison-Smith

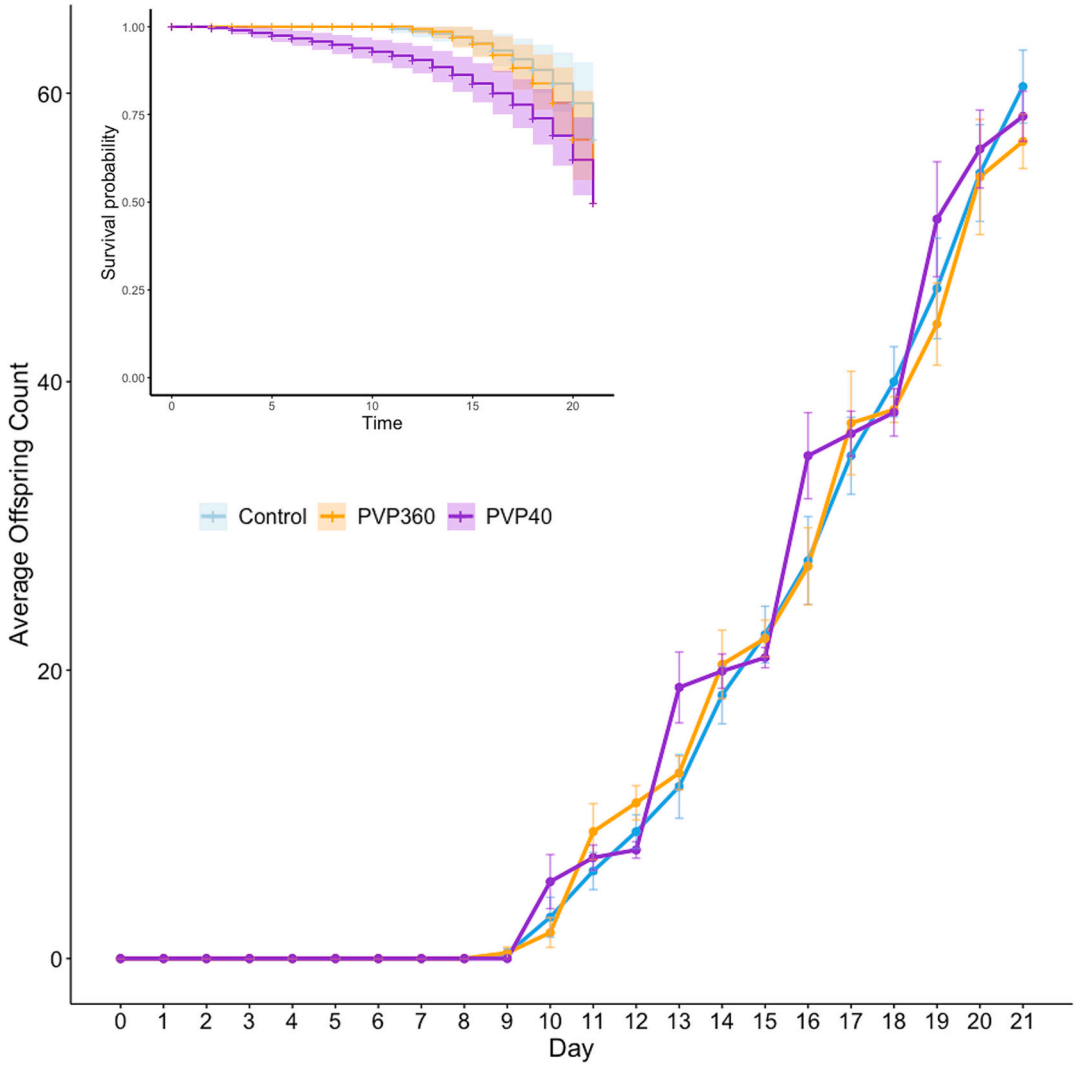

Fig. 2. The mean (±SE) cumulative *Daphnia magna* offspring counts in Experiment 2 over the 21 day exposure period to polyvinylpyrrolidone at differing molecular weights and high feed concentration (PVP40; 40 kDa or PVP360; 360 kDa). A survival probability curve (top left insert) is also included to illustrate the difference in survival probabilities between treatments in Experiment 2 (Control; $n = 12$, PVP40; $n = 12$, PVP360; $n = 11$).

et al., 2024), however future studies should focus on incrementally highlighting toxicity changes through the use of more diverse exposure models, such as cell toxicity assays, in order to distinguish between direct and indirect toxicity. Moreover, PVP cell interactions differ between cell types (Zhang et al., 2016), further elaborating the need for a more diverse suite of ecotoxicity testing of this chemical in a regulatory context.

While there were slight differences in experimental design between Experiments 1 and 2, the lethal effect of PVP40 and inhibited growth caused by PVP360 dissipate entirely when algae availability increases. This aligns with observations made in a previous study, where it was hypothesised that an intermediate exposure concentration of PEG reduced food availability causing significant *D. magna* mortality, as algal deposits specific to these containers were observed (Nigro et al., 2024). Whilst this study theorised gelation caused reductions in food availability, we propose that polymer-algae binding occurs when polymer and algae are ingested and interact in the digestive tract of *D. magna*, which underscores the lethal and sublethal effects of PVP, where interactions are dependent on molecular weight. However, more research is needed to determine whether the mode of toxicity of this polymer is indirect through prevention of nutritional uptake from ingested feed or direct via polymer-gut interactions.

D. magna is a generalist zooplankton herbivore that feeds primarily on algae. Algae species resistant to digestion can reduce food assimilation efficiency in *D. magna* and ultimately inhibit growth and reduce survival (DeMott et al., 2010). These resistant algal cells remain intact throughout gut passage due to the presence of a protective 'gelatinous sheath'. The algae species used in this study, *R. subcapitata* is a green alga readily ingested by *D. magna* with high nutritional value and no gelatinous sheath (Machado and Soares, 2024). The results from the current study could suggest PVP provides a protective colloidal effect for *R. subcapitata* cells, providing synthetic digestion resistance once algae and polymer are ingested together by *D. magna*. The degree of cellular protection could be molecular weight dependent given the differences in lethality between treatments in Experiment 1 (Hubálek, 2003). The algae content of Experiment 2 may have exceeded the capacity for 0.1 mg L^{-1} of PVP to interact with a significant proportion of the free algae cells compared to Experiment 1, leaving sufficient algae without synthetic digestion resistance, enabling sufficient nutrient assimilation for *D. magna* in this experiment. Another possibility is the relative ingestion ratio of PVP per unit of food would likely be substantially reduced in Experiment 2, thereby mitigating direct toxicity on gut tissue. Although, given the severity of toxicity in Experiment 1 and other evidence of chronic PVP effects on daphnids and other freshwater organisms (Mondellini et al., 2022; Robison-Smith et al., 2024), it is unusual that no cumulative effects were observed within this timeframe. Moreover, in the knowledge that higher concentrations of WSP exposure does not necessarily lead to greater toxic effects for freshwater organisms, this could infer that more complex indirect interactions are occurring within the specific test condition of OECD 211 (Nigro et al., 2024; Zicarelli et al., 2024). Future work involving *D. magna* ecotoxicity testing could involve experiments that separate feeding and exposure phases to distinguish between these two possible mechanisms.

The digestion performance of *D. magna* increases with size and age; juvenile daphnids have a far lower food assimilation efficiency than adults (DeMott et al., 2010). This may explain the results from Experiment 1, where a high mortality rate of juveniles exposed to PVP40 and lower feed occurred, but those that did survive into adulthood were able to grow and reproduce at a similar rate to controls. Therefore, the surviving *D. magna* in this treatment exhibited better fitness in the form of assimilation efficiency, where they maintained a size where digestion performance was sufficient to overcome the reduced efficiency of nutrient assimilation caused by PVP, and gut passage time overcame the artificial defence of PVP potentially bound to algae.

3.2. Exposure and quantification study

While an excess of *D. magna* was used in each tank at the start of the 7 day exposure, by the end of the exposure a significant portion of the cultures had died. This was more noticeable in the PVP 360 kDa exposed cultures where, upon collection, it was not possible to collect a second replicate. Previously, PVP detection was only possible in a relatively large mass (~1 g), whereas in this study, it was only possible to collect samples at approximately 0.2–0.3 g (Table S2) (Tarring et al., 2024b). Due to this, it was not possible to detect PVP in any of the exposed *D. magna* samples collected (Fig. S1). Therefore, only the tank water samples were analysed further.

3.2.1. Method validation

To validate the extraction and analysis method described in section 2.5 and 2.6, a range of concentrations of both PVP 40 kDa and 360 kDa were analysed for calibration, alongside procedural blank recovery, spiked tissue recovery and spiked water recovery (Table 1). Additional information on these is given in Text S1.

The similarity in peak area, detection and quantification limits and extraction efficiencies suggest using GPC can provide robust analysis of a range of molecular weights for PVP. Freeze drying and directly dissolving the sample in eluent, and the use of broad molecular weight columns, imply that this method is not molecular weight dependent, an issue identified when pre-extraction was used (Pauelsen et al., 2023). This is particularly important for PVP, where the most used molecular weights in pharmaceutical applications range from 2.5 kDa to 3000 kDa (Kurakula and Rao, 2020). It also suggests that it may not be necessary to calibrate for a range of molecular weights when non-targeted PVP is analysed using GPC. Instead, a universal calibration could be employed, which would enable a greater range of environmental applications for future research (Cheong et al., 2015; Gómez-Ordóñez et al., 2012; Ruiz et al., 2019; Tarring et al., 2024b). However, due to the differences in the refractive index increment (*dn/dc*) between polymer types, calibration would be required for each polymer class, with the identity of the class not identifiable from GPC alone (Cheong et al., 2015; Tarring et al., 2024b).

3.2.2. Tank water GPC analysis

Water samples from tanks without *D. magna* or polymer (Control tank), without *D. magna* (Control tank_polymer) and with *D. magna* (Exposure tank_polymer) were analysed to identify any changes in polymer concentration or structure (Fig. 3). GPC analysis demonstrated the similarity in peak shape between the samples with and without *D. magna*. This was supported by the peak centre and peak maximum, which were similar, if not identical, between the samples (Table 2). This indicates that both PVP samples were not degrading over the 7 days. Therefore, it can be deduced that polymer concentration would have been consistent in Experiment 1 or 2 given the frequent water changes (Section 3.1).

However, while the extraction of the spiked sample and the control tank were below 100 % and not considerably different, the extraction of PVP 40 kDa and 360 kDa from tanks containing *D. magna* were notably above 100 % extraction. This result was unexpected, as it was hypothesised that if other matter, such as algal feed, was binding to PVP, this

Table 1
Analytical performance parameters for PVP 40 kDa and PVP 360 kDa.

Analytical performance	PVP 40 kDa	PVP 360 kDa
LOB (mg mL^{-1})	0.062 ± 0.009	0.042 ± 0.014
LOD (mg mL^{-1})	0.087 ± 0.009	0.055 ± 0.014
LOQ (mg mL^{-1})	0.223 ± 0.011	0.182 ± 0.015
Procedural blank recovery (%)	95	105
Spiked tissue recovery (%)	80	89
Spiked water recovery (%)	95	85

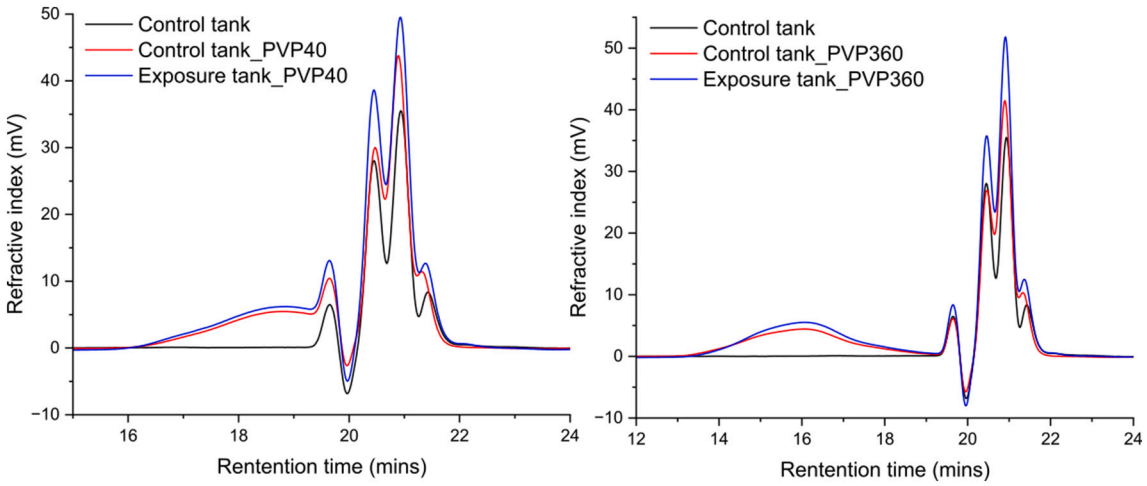

Fig. 3. GPC refractive index traces for left) PVP 40 kDa and right) PVP 360 kDa control tanks and exposure tanks.

Table 2
GPC refractive index peak areas and corresponding % extraction for PVP40 kDa and PVP360 kDa tanks.

Treatment	Average peak maximum (mins)	Average extraction (%)
Spiked water recovery_PVP40	18.78	95
Control tank_PVP40	18.78 ± 0.05	96 ± 2
Exposure tank_PVP40	18.78 ± 0.03	118 ± 1
Spiked water recovery_PVP360	15.99	85
Control tank_PVP360	15.98 ± 0.01	88 ± 3
Exposure tank_PVP360	15.98 ± 0.01	117 ± 8

would cause an increase in the hydrodynamic radius of the polymer, thus decreasing its retention time (Gidley et al., 2010; Izumi et al., 2013). However, in this case, the peak height increased, without other parameters changing, which is demonstrated when the peaks were fitted (Fig. 4). While additional minor peaks were needed to fully fit the model to the PVP peak, likely due to the effects of solubilising and freeze drying the polymer, these peaks did not distinctly differ between the control and *D. magna* tanks. Again, the peaks predominantly differed in height, rather than shifting retention time.

These results indicate that the increase in peak height from PVP exposed to *D. magna* was caused by a change to polymer functionality. This could be caused by binding to ions or changes to the end groups, rather than increase in molecular weight through remaining bound to large molecules upon analysis. This change would impact the *dn/dc* of the polymer, therefore changing the magnitude of the refractive index response.

Many applications of PVP depend on its amphiphilic nature, whereby PVP can, for example, reduce metal salts and form bonds with iodine and polysaccharides through resonance forms (Jalil et al., 2018; Pourmadadi et al., 2023; Siggia, 1957; Xu and Guan, 2020). It has also been demonstrated that PVP can form attachments to mucosal membranes (Jalil et al., 2018). Therefore, it is likely that PVP is capable of complexing or forming bonds with algal components or ions present in the medium. However, in this case, it is the presence of *D. magna* that enables this binding, as binding did not occur in their absence (Table 2). This suggests two possibilities; either the digestive processes of *D. magna* allow excreted matter to bind to the PVP present in solution or, that the process of ingesting tank matter and PVP enables its binding to matter/feed, possibly including interaction with the gastrointestinal tract itself, which leaves the polymer functionally changed. This presents as the more likely theory as PVP did exert sublethal and lethal effects on *D. magna* in the current study, also aligning with previous work, which

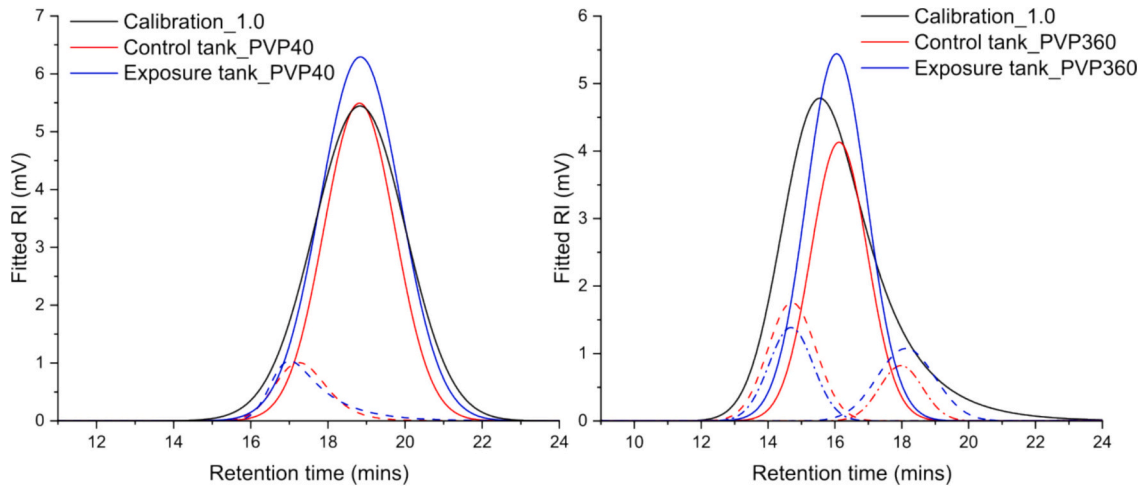

Fig. 4. Fityk-fitted GPC refractive index PVP peaks for left) PVP 40 kDa 1 mg mL-1 calibration sample, control tank and exposure tank. Right) PVP 360 kDa 1 mg mL-1 calibration sample, control tank and exposure tank. Dashed lines represent minor peaks fitted to the PVP peak to ensure good comparability of the model with the raw chromatogram peak.

indicates PVP is interacting with *D. magna* through ingestion (Nigro et al., 2024; Robison-Smith et al., 2024; Tarring et al., 2024b).

To confirm algal binding of the polymer, diffusion-ordered spectroscopy (DOSY) was investigated as a technique to probe the binding of algae to polymer. DOSY uses diffusion coefficients for individual proton nuclear magnetic resonances (^{1}H NMR), which is related to the hydrodynamic radius of the molecule (Groves, 2017). Previous work has used DOSY to investigate polymer binding to other molecules (Young et al., 2023). However, investigations here indicated the polymer concentration present to be too low for NMR and DOSY analysis. Future work should focus on optimizing a DOSY experiment at a concentration high enough to get accurate coefficients, but at a realistic concentration of polymer, which may provide further evidence for polymer-algae binding.

UV–Vis spectroscopy was used to assess any changes to the settling velocity of algae with addition of polymer (Fig. S7). As filter feeders adapted to extract suspended algae from the water column, *D. magna* food gathering efficiency decreases once algae cells benthically settle (Ebert, 2005). As the OECD 211 assays involved semi-static conditions, UV–Vis spectroscopy was applied to assess potential differences in settling rate between treatments, which could have affected food availability, which was discussed in previous work where algae deposits were observed during *D. magna* exposure to PEG (Nigro et al., 2024). At the increased algal feed concentration (Experiment 2), no differences were seen in the settling velocity of either PVP40 or PVP360 versus the control. When this was applied to the lower feed (Experiment 1), the standard deviation from the replicates increased due to the lower starting absorbance value. This made it difficult to conclude if there were differences in the settling velocity, however settling rates did appear similar. This analysis implies algal settling is not affected by PVP at 0.1 mg L^{-1}.

As a result of the above chemical analyses, the greater toxicity observed at the lower molecular weight (PVP 40 kDa) could be due to the shorter chain length; the lower number of monomers may increase the efficacy by which the polymer interacts and binds to the algae. This has previously been shown in the interaction of PVP with silver nanoparticles, where steric effects hindered the binding ability of higher molecular weight PVP (Song et al., 2014). Overall, the chemical analysis carried out in this study, alongside the OECD 211 assays, could imply PVP is not accumulating, rather being ingested, binding to other matter in the gastrointestinal tract such as algae, potentially reducing nutrient uptake before being excreted.

3.3. Conclusions and environmental implications

From this study, it is proposed that *D. magna* could experience nutrient stress when too much polymer is ingested with a critical level of algal cells, leading to a reduction in digestion performance due to a protective colloidal effect of PVP once bound to algae. This binding theory is supported by chemical analysis which indicates PVP functional changes only occur in the presence of *D. magna*, which localises the interaction between PVP and algae in the digestive tract. At the studied concentration, when algal food concentration was lower, polymer binding appeared to be significant enough in relation to algae abundance to reduce assimilation efficiency of nutrients and cause inhibited growth and even mortality. Assimilation efficiency is lower in smaller *D. magna* due to reduced gut retention time, highlighting that juveniles were more vulnerable to this effect of PVP, resulting in the high mortality rate of juveniles exposed to PVP40 and lower algae concentration (47 % by 6 days old). When algae concentration was higher, it is predicted the polymer-algae interaction becomes saturated, leaving enough unbound algae cells available in the gut for sufficient nutrient assimilation, resulting in no observed effects in the high feed scenario.

Further experiments such as removing the feed variable and investigating subsequent *D. magna* survival may help elucidate if PVP is able to interact with the cell membrane. Alongside this, expanding this study to include incremental changes in molecular weight will enable a response curve to be created, and, in combination with different feed and polymer concentrations, allow a matrix testing approach to be implemented which would allow a more complete understanding of PVP toxicity. Multifaceted approaches such as this are likely to be necessary for other WSPs, where complex and non-monotonic relationships mean current singular assays are not comprehensive enough to determine ecotoxicological impacts.

The results from the current study indicate that PVP toxicity, both in a sublethal and lethal context, is dependent on molecular weight of the polymer, however the current study is limited in that only two molecular weights were tested. Despite this, the differences between the current study and comparable studies could indicate non-monotonic responses with regards to the toxicity of PVP at different molecular weights (Mondellini et al., 2022; Zicarelli et al., 2024). Current environmental risk assessment models are not appropriate safeguards for freshwater environments with regards to PVP and potentially the entire water-soluble polymer chemical class, as they do not consider soluble-polymer size dependent toxicity, or investigate biotic interactions, such as impairment of nutrient assimilation.

Polymers are currently under review for consideration under UK/EU REACH (EC, 2022; ECHA, 2023). It is important that regulatory development evolves to consider chronic effects of WSPs over their molecular size range, as the environmental risk of these polymers may currently be significantly misrepresented due to apparent non-linearity between toxicity and molecular weight. Therefore, conventional methods to assess environmental risk of these chemicals may not be reliable predictors of risk for WSPs in the environment.

CRediT authorship contribution statement

Eve C. Tarring: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Charlotte Robison-Smith:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Angel D. Ceballos-Ramirez:** Writing – review & editing, Supervision, Resources. **Isabelle Durance:** Writing – review & editing, Supervision. **Michael J. Harbottle:** Writing – review & editing, Supervision. **Jo Cable:** Writing – review & editing, Supervision. **Benjamin D. Ward:** Writing – review & editing, Supervision.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.179686.

Data availability

I have shared the link to data in the attach file step
Toxicity of the water-soluble polymer PVP is dependent on molecular

weight and feed concentration for a freshwater model species (Original data) (Figshare)

References

Abdelrazek, E.M., Elzayat, A.M., Elbana, A.A., Awad, W.M., 2023. Physical properties of copper oxide nano-composite incorporated PVP/chitosan blend matrix by casting method. Polym. Bull. 13.

Aldalbahi, A., Thamer, B.M., Abdulhameed, M.M., El-Newehy, M.H., 2024. Fabrication of biodegradable and antibacterial films of chitosan/polyvinylpyrrolidone containing *Eucalyptus citriodora* extracts. Int. J. Biol. Macromol. 266, 15.

AlKhatib, H.S., Hamed, S., Mohammad, M.K., Bustanji, Y., AlKhalidi, B., Aiedeh, K.M., Najjar, S., 2010. Effects of thermal curing conditions on drug release from polyvinyl acetate-polyvinyl pyrrolidone matrices. AAPS PharmSciTech 11 (1), 253–266. https://doi.org/10.1208/s12249-010-9378-z.

Antić, V.V., Antić, M.P., Kronimus, A., Oing, K., Schwarzbauer, J., 2011. Quantitative determination of poly(vinylpyrrolidone) by continuous-flow off-line pyrolysis-GC/MS. J. Anal. Appl. Pyrolysis 90, 93–99.

Antić, V.V., Antić, M.P., Kronimus, A., Schwarzbauer, J., 2012. Quantitative determination of poly(vinylpyrrolidone) by "on-line" pyrolysis coupled with gas chromatography. Hem. Ind. 66, 357–364.

Armbruster, D.A., Pry, T., 2008. Limit of blank, limit of detection and limit of quantitation. Clin. Biochem. Rev. 29, 49–52.

Cheong, K.L., Wu, D.T., Zhao, J., Li, S.P., 2015. A rapid and accurate method for the quantitative estimation of natural polysaccharides and their fractions using high performance size exclusion chromatography coupled with multi-angle laser light scattering and refractive index detector. J. Chromatogr. A 1400, 98–106.

DeMott, W.R., McKinney, E.N., Tessier, A.J., 2010. Ontogeny of digestion in *Daphnia*: implications for the effectiveness of algal defenses. Ecology 91 (2), 540–548. https://doi.org/10.1890/08-2103.1.

Deng, Z.A., Zhao, Z.H., Shen, C.Y., Cai, Z.H., Wu, D., Zhu, B.K., et al., 2024. Preparation of amphiphilic polyquaternium nanofiber films with antibacterial activity via environmentally friendly microfluidic-blow-spinning for green food packaging applications. Food Chem. 444, 11.

Duncan, R., Pratten, M.K., Cable, H.C., Ringsdorf, H., Lloyd, J.B., 1981. Effect of molecular-size of I-125-labeled poly(VINYLPYRROLIDONE) on its pinocytosis by rat visceral yolk sacs and rat peritoneal-macrophages. Biochem. J. 196, 49–55.

Ebert, D., 2005. Ecology, epidemiology, and evolution of parasitism in daphnia. National Center for Biotechnology Information (US), Bethesda (MD).

EC. Chemicals: Commission Seeks Views on Revision of REACH, the EU'S Chemicals Legislation. European Commission, European Commission, Energy, Climate Change, Environment 2022.

ECHA, 2023. Guidance for Monomers and Polymers. In, Agency EC, editor.

Gidley, M.J., Hanashiro, I., Hani, N.M., Hill, S.E., Huber, A., Jane, J.L., et al., 2010. Reliable measurements of the size distributions of starch molecules in solution: current dilemmas and recommendations. Carbohydr. Polym. 79, 255–261.

Gómez-Ordóñez, E., Jiménez-Escrig, A., Rupérez, P., 2012. Molecular weight distribution of polysaccharides from edible seaweeds by high-performance size-exclusion chromatography (HPSEC). Talanta 93, 153–159.

Groves, P., 2017. Diffusion ordered spectroscopy (DOSY) as applied to polymers. Polym. Chem. 8, 6700–6708.

Hill, C.E., Myers, J.P., Vandenberg, L.N., 2018. Nonmonotonic dose-response curves occur in dose ranges that are relevant to regulatory decision-making. Dose-Response 16 (4).

Horváth, Z.M., Lauberte, L., Logviss, K., Bandere, D., Mohylyuk, V., 2023. Quantification of soluplus® and copovidone polymers in dissolution media: critical systematic review. J. Drug Deliv. Sci. Technol. 89, 10.

Hubálek, Z., 2003. Protectants used in the cryopreservation of microorganisms. Cryobiology 46, 205–229.

Huppertsberg, S., Zahn, D., Pauelsen, F., Reemtsma, T., Knepper, T.P., 2020. Making waves: water-soluble polymers in the aquatic environment: an overlooked class of synthetic polymers? Water Res. 181.

Izumi, Y., Aikawa, S., Matsuda, F., Hasunuma, T., Kondo, A., 2013. Aqueous size-exclusion chromatographic method for the quantification of cyanobacterial native glycogen. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 930, 90–97.

Jalil, A., Matuszczak, B., Le, N.M.N., Mahmood, A., Laffleur, F., Bemkop-Schnürch, A., 2018. Synthesis and characterization of thiolated PVP-iodine complexes: key to highly mucoadhesive antimicrobial gels. Mol. Pharm. 15, 3527–3534.

Johnson, W., Bergfeld, W.F., Belsito, D.V., Hill, R.A., Klaassen, C.D., Liebler, D.C., Marks, J.G., Shank, R.C., Slaga, T.J., Snyder, P.W., Fiume, M., Heldreth, B., 2024. Safety assessment of vinylpyrrolidone polymers as used in cosmetics. Int. J. Toxicol. 43 (4_suppl), 5–41. https://doi.org/10.1177/10915818241267203.

Julinova, M., Kupec, J., Houser, J., Slavik, R., Marusincova, H., Cervenakova, L., et al., 2012. Removal of Polyvinylpyrrolidone from wastewater using different methods. Water Environ. Res. 84, 2123–2132.

Julinova, M., Vanharova, L., Jurca, M., 2018. Water-soluble polymeric xenobiotics - polyvinyl alcohol and polyvinylpyrrolidon - and potential solutions to environmental issues: a brief review. J. Environ. Manag. 228, 213–222.

Kurakula, M., Rao, G., 2020. Pharmaceutical assessment of polyvinylpyrrolidone (PVP): as excipient from conventional to controlled delivery systems with a spotlight on COVID-19 inhibition. Journal of Drug Delivery Science and Technology 60, 24.

Kuskov, A.N., Kulikov, P.P., Goryachaya, A.V., Tzatzarakis, M.N., Docea, A.O., Velonia, K., et al., 2017. Amphiphilic poly-N-vinylpyrrolidone nanoparticles as carriers for non-steroidal, anti-inflammatory drugs: in vitro cytotoxicity and in vivo acute toxicity study. Nanomedicine-Nanotechnology Biology and Medicine 13, 1021–1030.

Machado, M.D., Soares, E.V., 2024. Features of the microalga *Raphidocelis subcapitata*: physiology and applications. Appl. Microbiol. Biotechnol. 108, 23.

Mellati, A., Kiamahalleh, M.V., Dai, S., Bi, J.X., Jin, B., Zhang, H., 2021. Influence of polymer molecular weight on the in vitro cytotoxicity of poly (N-isopropylacrylamide) (vol 59, pg 509, 2016). Materials Science & Engineering C-Materials for Biological Applications 129, 1.

Menazea, A.A., Ahmed, M.K., 2020. Nanosecond laser ablation assisted the enhancement of antibacterial activity of copper oxide nano particles embedded though polyethylene oxide/polyvinyl pyrrolidone blend matrix. Radiat. Phys. Chem. 174, 9.

Mondellini, S., Schott, M., Loder, M.G.J., Agarwal, S., Greiner, A., Laforsch, C., 2022. Beyond microplastics: water soluble synthetic polymers exert sublethal adverse effects in the freshwater cladoceran *Daphnia magna*. Sci. Total Environ. 847, 157608. https://doi.org/10.1016/j.scitotenv.2022.157608.

Nigro, L., Magni, S., Ortenzi, M.A., Gazzotti, S., Della Torre, C., Signorini, S.G., et al., 2024. Unveiling the multilevel impact of four water-soluble polymers on *Daphnia magna*: from proteome to behaviour (a case study). J. Hazard. Mater. 469, 18.

OECD, 2012. Test No. 211: *Daphnia magna* Reproduction Test. OECD Publishing, Paris.

Pauelsen, F., Huppertsberg, S., Knepper, T.P., Zahn, D., 2023. Narrowing the analytical gap for water-soluble polymers: a novel trace-analytical method and first quantitative occurrence data for polyethylene oxide in surface and wastewater. Sci. Total Environ. 882, 163563.

Pourmadadi, M., Shamsabadipour, A., Aslani, A., Eshaghi, M.M., Rahdar, A., Pandey, S., 2023. Development of Polyvinylpyrrolidone-based nanomaterials for biosensors applications: a review. Inorg. Chem. Commun. 152, 16.

Risbud, M.V., Hardikar, A.A., Bhat, S.V., Bhonde, R.R., 2000. pH-sensitive freeze-dried chitosan-polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. J. Control. Release 68, 23–30.

Robison-Smith, C., Cable, J., 2024. Invisible plastics problem in intensive aquaculture: the case of polyvinylpyrrolidone. Rev. Aquac. 11.

Robison-Smith, C., Masud, N., Tarring, E.C., Ward, B.D., Cable, J., 2024. A class of their own? Water-soluble polymer pollution impacting a freshwater host-pathogen system. Sci. Total Environ. 907, 11.

Ruiz, M., Valette, J., Broust, F., Bonfils, F., 2019. Rapid quantification and characterization of the pyrolytic lignin fraction of bio-oils by size exclusion chromatography coupled with multi-angle laser light scattering detector (SEC-MALS). J. Anal. Appl. Pyrolysis 142, 8.

Siggia, S., 1957. THE CHEMISTRY OF POLYVINYLPYRROLIDONE-IODINE. J. Am. Pharm. Assoc. 46, 201–204.

Song, Y.J., Wang, M.L., Zhang, X.Y., Wu, J.Y., Zhang, T., 2014. Investigation on the role of the molecular weight of polyvinyl pyrrolidone in the shape control of high-yield silver nanospheres and nanowires. Nanoscale Res. Lett. 9, 8.

Tarring, E.C., Durance, I., Harbottle, M.J., Lucas, R., Read, D.S., Ward, B.D., 2024a. Water-soluble polymers: emerging contaminants detected, separated and quantified by a novel GPC/MALDI-TOF method. Environ. Pollut. 340, 10.

Tarring, E.C., Robison-Smith, C., Cable, J., Durance, I., Harbottle, M., Ward, B.D., 2024b. Detection of polyvinylpyrrolidone in Daphnia magna: development of a refractive index quantification method for water-soluble polymers in aquatic organisms. Sci. Total Environ. 935, 8.

Trimpin, S., Eichhorn, P., Rader, H.J., Mullen, K., Knepper, T.P., 2001. Recalcitrance of poly(vinylpyrrolidone): evidence through matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. J. Chromatogr. A 938, 67–77.

Vashist, S.K., Luong, J.H.T., 2018. Handbook of immunoassay technologies approaches, performances, and applications. Academic Press: Elsevier.

Vidovic, N., Antic, V., Schwarzbauer, J., 2023. Determination of the water-soluble polymer poly(ethyleneimine) (PEI) in wastewater by continuous-flow off-line pyrolysis GC/MS. J. Anal. Appl. Pyrolysis 172.

Vidovic, N., Antic, V., Schwarzbauer, J., 2024. Simultaneous identification and quantification of three water-soluble polymers (PVP, PNVCL and PEI) in wastewater samples by continuous-flow off-line pyrolysis GC/MS. Sci. Total Environ. 916, 11.

Wagner, N.D., Simmons, D.B.D., Prater, C., Frost, P.C., 2022. Proteome changes in an aquatic invertebrate consumer in response to different nutritional stressors. Oecologia 199, 329–341.

Xu, X., Guan, Y., 2020. Investigating the complexation and release behaviors of iodine in poly(vinylpyrrolidone)-iodine systems through experimental and computational approaches. Ind. Eng. Chem. Res. 59, 22667–22676.

Young, T.B., Guppy, O.G., Draper, A.J., Whitington, J.M., Kariuki, B.M., Paul, A., et al., 2023. Chemically recyclable fluorescent polyesters via the ring-opening copolymerization of epoxides and anhydrides. Polym. Chem. 14, 2478–2484.

Zhang, B.A., Ghimbeu, C.M., Laberty, C., Vix-Guterl, C., Tarascon, J.M., 2016. Correlation between microstructure and Na storage behavior in hard carbon. Adv. Energy Mater. 6 (9).

Zicarelli, G., Faggio, C., Blahova, J., Riesova, B., Hesova, R., Doubkova, V., et al., 2024. Toxicity of water-soluble polymers polyethylene glycol and polyvinyl alcohol for fish and frog embryos. Sci. Total Environ. 933, 10.