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Modeling Ingestion and Clearance Kinetics of Polydispersed Micro- and Nanoplastics in Daphnia magna Utilizing spICP-MS

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Abstract

Current models of nano- and microplastic (NMP) ingestion and clearance often lack environmental relevance because they rely on spherical, monodispersions rather than the irregularly shaped, polydispersed fragments commonly found in nature. To address this, we employed a recently published method where we metal-doped tantalum ethoxide poly(methyl methacrylate) (Ta-PMMA) polydispersion (~ 280–3350 nm) which allowed analysis of NMPs in media and organisms using single-particle inductively coupled plasma mass spectrometry (spICP-MS). The ingestion and clearance of Ta-PMMA NMPs were quantified in *Daphnia magna* following exposure to 50 µg/L Ta-PMMA (individual and group exposures) or 1.5 µg/L 200 nm AgNPs (monodispersion control) for 24 h, followed by 24 h clearance in clean media. *D. magna* were sampled four times between 3–24 h (exposure) and three times between 24–48 h (depuration). Ingestion of Ta-PMMA and AgNPs peaked at 6 and 12 h, respectively, suggesting ingestion followed by a cessation of feeding before exposures ended. Particle distributions measured in exposure media and exposed *D. magna* were consistent for individually exposed *D. magna* but differed in group exposures, where greater ingestion occurred for particles < 1 µm. Exposure set-up affected particle distribution in media, complicating direct comparisons between individual and group exposures. This research highlight the importance of incorporating environmentally relevant polydispersed NMPs into ingestion–clearance studies.

1. Introduction

Current models of nano- and microplastic (NMP) ingestion and clearance often lack environmental relevance because they rely on spherical, monodispersions rather than the irregular, polydispersed fragments commonly found in nature (Kefer et al. 2021). Determining how particles of varied sizes influence ingestion and bioaccumulation remains a central challenge in NMP research (Cunningham et al. 2023). Analysis of anthropogenic NMPs is challenging due to the presence of naturally occurring particulate and dissolved organic matter (NPM) in environmental samples (Filipe et al. 2010; Gigault et al. 2017; Nguyen et al. 2019; Pinto da Costa et al. 2019; Lusher et al. 2020; Cai et al. 2021; Gigault et al. 2021; Mitrano et al. 2021; Vogel et al. 2021; Zhou et al. 2021; Okoffo et al. 2022; Xu et al. 2023; Kozloski et al. 2024; Tran et al. 2024; Zhao et al. 2025).This is due to the inability to distinguish small carbon molecules from one another, NMPs existing at low concentrations, and aggregation with other NMPs or natural colloids affecting their overall size in analysis (Cai et al. 2021; Gigault et al. 2021; Mitrano et al. 2021; Tran et al. 2024). Thus, limiting our ability to determine environmentally relevant concentrations and size distributions of NMPs.

Researchers have employed a variety of NMP labels, sample processing techniques, and analytical approaches to overcome the challenges of naturally occurring particles (Mariano et al. 2021). For instance, fluorescence-based labeling techniques are commonly used to visualize plastic ingestion in exposure testing, yet these approaches are qualitative and prone to artifacts, including dye leaching and false positives (Ziajahromi et al. 2017; Catarino et al. 2019; Schür et al. 2019). As a result, most NMP toxicity studies rely on nominal exposure concentrations calculated from single size particle mass rather

than quantitative determination of particle number within the media or organism (Liu et al. 2021; Kokilathasan and Dittrich 2022; Li et al. 2024; Morgana et al. 2024; Cho et al. 2025 Feb 18). This analytical gap constrains interpretation of particle number-based dose–response relationships and hinders assessment of how NMP size distributions influence biological ingestion, retention, and clearance dynamics.

Emerging analytical approaches now incorporate elemental tracers into polymer matrices, enabling detection by single-particle inductively coupled plasma mass spectrometry (spICP-MS) (Kutralam-Muniasamy et al. 2024). We have recently employed poly(methyl methacrylate) (PMMA) doped with tantalum (Ta-PMMA) providing a quantifiable through single particle inductively coupled plasma mass spectrometry (spICP-MS). Cryomilling of the Ta-PMMA yielded polydispersed NMPs (280–3,350 nm) which were detectable using spICP-MS (Bevers et al. 2023; Smith et al. 2024) The inclusion of an evenly distributed, transition metal (tantalum) eliminated organic matter interferences and this approach been used to evaluate NMP transport in porous media and ingestion into zebrafish larvae and *Daphnia magna* (Tran et al. 2024; Schneiderjan et al. 2026).

Existing ingestion and clearance studies on monodispersed nano- and microplastics, or metal nanoparticles, typically have followed first-order kinetics, due to their uniform size and shape that ensures that each particle has an equal probability of ingestion and clearance (Diez-Ortiz et al. 2015; Wray and Klaine 2015; Al-Sid-Cheikh et al. 2018; E. Redondo-Hasselerharm et al. 2021; Ribeiro et al. 2022; Clark et al. 2023; Habumugisha et al. 2023; Hao et al. 2023; Dey et al. 2024; Ockenden et al. 2024; Yu et al. 2024). In natural systems though, organisms encounter heterogeneous mixtures of particle sizes, necessitating approaches that capture realistic polydispersed exposure scenarios. Thus, these studies may not capture the effect of particle size within a more environmentally relevant polydispersion on ingestion and clearance in an organism (Wang and Wang 2023a; Wang et al. 2025; Zhang et al. 2025).

The outcome of exposure studies of engineered NMPs to organisms may be influenced by the presence or absence of intraspecific interactions, which have been shown to alter ingestion and clearance of contaminants. For filter-feeding zooplankton like *D.* magna, intraspecific interactions may alter NMP hydrodynamics, feeding rates, and particle encounter probabilities. For instance, grouped daphnids generate collective swimming currents and vortex-like flow fields that keep particles in suspension longer and increase their availability (Øien 2004; Vollmer et al. 2006; Noss et al. 2013). Conversely, crowding can suppress feeding, alter filtering appendage beat frequency, and induce behavioral stress responses (Knillmann et al. 2012; Lowes et al. 2021). These interactions create exposure conditions different from isolated individuals, potentially leading to changes in particle accumulation and clearance efficiency. Current NMP researchers either expose *D. magna* individually (Wang and Wang 2023a; Wang and Wang 2023b) or in group settings (Rist et al. 2017) and not compare the two dynamics, which may lead to different conclusions. Exposure studies that rely exclusively on single-organism test chambers may not reflect the ecological complexity of environmentally relevant NMP polydispersion toxicokinetics. Thus, there is a need to compare individual and group ingestion and clearance dynamics in *D. magna* using the same NMP stock and exposure media.

Here, we apply this new methodology of Ta-PMMA NMPs and spICP-MS to evaluate the ingestion and clearance kinetics of polydispersed NMPs in *D. magna*, a model filter-feeding invertebrate representative of active particle ingestion. Exposure dose and organism dose were confirmed using spICP-MS. We hypothesized that (i) polydispersed NMPs exhibited distinct ingestion and clearance kinetics compared to monodispersed nanoparticles, (ii) kinetics differed among particle size fractions within a polydispersion, and (iii) ingestion and clearance rates were comparable between individual and grouped *D. magna* exposures. To test these hypotheses, we performed three experiments exposing *D. magna* for 24 hours to allow for ingestion of the nanoplastics, then allowed to depurate for 24 hours: 1) individual *D. magna* were exposed to 50 µg/L Ta-PMMA; 2) individual *D. magna* were exposed to 1.5 µg/L 200 nm silver (Ag) nanoparticles, which was to the particle count of the entire size range of Ta-PMMA particles, to compare monodispersions to polydispersions; and 3) grouped *D. magna* (n = 10) were exposed to 50 µg/L Ta-PMMA to compare individual versus grouped *D. magna* kinetics. Results from this work will help improve our understanding of environmentally relevant NMP ingestion and clearance kinetics by providing quantitative, particle-resolved kinetics that better reflect environmentally relevant exposure conditions.

2. Materials and Methods

2.1 Chemicals and Reagents

A description of where poly(methyl methacrylate) (PMMA), tantalum(V) ethoxide ($Ta(OC_2H_5)_4$, spherical 200 nm silver (Ag) Citrate stabilized nanospheres, as well as all other reagents were purchased is found in **SI Text 1**.

2.2 Synthesis of Tantalum Tagged PMMA Particles and Determination of Ta-PMMA Stock

Concentration by Traditional ICP-MS

A detailed description of the synthesis of the tantalum tagged PMMA (Ta-PMMA) can be found in **SI Text 2** and extensive characterization data is presented in previous work for the exact particle used in this study can be found in Smith et al., (2024).

To create the stock solution used in exposures, a nominal 1 mg/L Ta-PMMA in 18 MΩ water solution was sonicated for 24 hours and vortexed before analysis to ensure there was no particle aggregation before analysis. The solution was analyzed using NexION 1000 ICP-MS (PerkinElmer, Waltham, MA, USA) equipped with a Type C Meinhard plus glass nebulizer (Meinhard, Golden, CO, USA) and a glass cyclonic spray chamber (PerkinElmer, Waltham, MA, USA). The stock solution was measured in triplicate and compared against a Ta+ metal standard curve ranging from concentrations of 2.3 to 9.6 µg/L. The concentration of the Ta-PMMA stock solution was 1.718 mg/L, and all exposure concentrations were based on this measurement (Analytical data shown in **SI Text 3**).

Ta-PMMA and 200 nm silver nanoparticle (AgNP) stock media were sonicated overnight and vortexed for 20 seconds prior to dilution into moderately hard water (pH = 7.4, conductivity = 305 µS) (MHW) to ensure particle dispersal in solution and to minimize aggregation of particles before exposure.

2.3 D. magna Individual and Group Exposures

Adult *D. magna* were obtained from an ongoing culture maintained in the Crago lab at TTU. Prior to exposure *D. magna* were transferred into a 1-L beaker with MHW and allowed to depurate 24h before exposure. During depuration and exposure period, all organisms were stored at 20°C. For the individual Ta-PMMA and AgNP exposures, *D. magna* were randomly placed into individual 20 mL scintillation vials (n = 1 per vial, 9 replicates per time-point) containing one of three exposures: control (MHW), 50 µg/L of Ta-PMMA, or 1.5 µg/L 200 nm AgNP. The AgNP exposure was calculated to be the equivalent to the particle count of the entire size range of Ta-PMMA particles present in the media (See in Section 3.1). For the group Ta-PMMA exposure, ten *D. magna* were placed into 100 mL beakers (N = 10 per beaker, 3 replicates per time-point) containing 80 mL of 50 µg/L Ta-PMMA or MHW (control). *D. magna* were collected at 3, 6, 12, and 24 h (ingestion) under a static exposure condition, then transferred to clean MHW and collected at 30, 36, and 48 h (clearance). A 100% replacement of clean MHW occurred at each clearance time point to assure minimal re-ingestion of nanoplastics during clearance. Collected *D. magna* were rinsed in clean water and placed into individual 15 mL conical tubes containing 2 mL of 18 MΩ water and stored at 4 ºC for later analysis on the spICP-MS analysis. Ta-PMMA and AgNP exposures media samples were collected at 0- and 24-h from each exposure replicate. A detailed description of spICP-MS analysis is found in **SI Text 3.**

2.4 Particle Rate Constant Calculations

Because particle burden began declining prior to end of exposure period, we designated this parameter an apparent clearance rate constant, rather than a true clearance or depuration constant. The apparent clearance rate constant (k_{app}) reflects the net balance of ingestion, egestion, and possible particle efflux during the experiment window. The apparent clearance rate constant (k_{app}) were calculated to fit a first-order toxicokinetic model using Eq. 1:

$$C_{D.\ magna}\left(t\right) = C_i e^{-k_{app}t}$$

1

Where $C_{D.magna}$ was the particle count in the *D. magna* at time *t,* C_i was the initial particle count in the *D. magna* at the start of clearance, and *e* was the natural log. The k_{app} was then utilized to compare the Ta-PMMA and AgNP exposures.

2.5 Data Analysis

Distribution data was tested for normality (Shapiro-Wilk Test) and failed. Differences in distributions amongst Ta-PMMA media exposure types were compared using one-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test using SAS JMP Pro 18 (SAS, Cary, NC, USA). Statistical

significance was set to $p < 0.05$ for all tests performed. Error bars presented in tables and graphs were expressed as mean ± standard error of the mean.

3. Results and Discussion

3.1 Ta-PMMA and AgNP Exposure Media Evaluations

The initial size distributions and total particle concentration differed between the individual and group Ta-PMMA media on a per L basis (Fig. 1A,B). At the start of the 48-h experiment, the individual Ta-PMMA exposure contained 432,301 ± 3,165 particles mL^{-1} (285–2,895 nm), whereas the group exposure contained 1,356,606 ± 250,051 particles mL^{-1} (281–3,348 nm; Table S1). These differences were present despite using Ta-PMMA originating from the same stock suspension, which were expected to be similar. These differences likely reflect variable resuspension of the Ta-PMMA stock between different sampling events. Although both media were prepared using the same preparation protocol immediately prior to sampling, different aliquots from a polydisperse suspension are susceptible to settling-related particle number drifts between events, particularly in the larger size fraction. This directly affects the group comparison.

Figure 1. Log size distributions of a) 0 (Red markers and line) and 24 (Blues marker and line) h media samples for the individual Ta-PMMA exposure b) 0 and 24 h media samples for the grouped Ta-PMMA exposures. Each data point represents an average of 3 for respective size range which was then fitted with a linear regression line

Log-log transformations were utilized for distribution comparisons since the NMPs have been shown to be log-normal and failed normality test (Schneiderjan et al. 2026). Log–log transformation of particle size vs particle number concentrations indicated that the individual exposure contained a greater number of smaller particles (250–550 nm), whereas the group exposure contained more particles between 650 and 1,950 nm (Fig. 1A,B; Table S1,S6). Although this relative difference persisted throughout the individual exposure, the group treatment showed a statistically significant shift in distribution between the start and end of exposure for the < 1 micron range ($p = 0.008$)(Fig. 1B; Table S1,S6), suggesting potential size-selective ingestion by *D. magna*. Feeding selectivity based on particle size is consistent with *D. magna*'s filtering morphology, as their thoracic setae retain particles ≥ 400 nm (Gophen and Geller 1984). The size-dependent efficiency, rather than the threshold alone, is consistent with the preferential depletion of the larger (> 650 nm) Ta-PMMA particles observed in the group exposure, suggesting active filtration than passive co-ingestion.

The AgNP exposure served as a monodispersed comparison to the Ta-PMMA polydispersion. Initial AgNP concentrations were 263,192 ± 45,590 particles mL^{-1} (181–346 nm), lower than the individual Ta-PMMA exposure (432,301 ± 3,165 particles mL^{-1}) (Table S2; Figure S1). The AgNP exposure concentration (1.5 µg L^{-1}) was calculated to match the total particle count of the Ta-PMMA exposure (50 µg L^{-1}), illustrating the difficulty of achieving particle-number parity in nanoparticle experiments.

Additionally, differences between replicates (Figure S3A,B; Table S1) further highlight the inherent heterogeneity of polydispersed NMPs, which complicates maintaining uniform particle concentrations and distributions even when derived from a single stock solution. Such heterogeneity underscores the challenges of modeling NMP behavior in suspension, where size and shape influence aggregation and settling dynamics. Thus, adding to the consensus that particle number concentrations remain a challenge to determine accurately, which has been seen in interlaboratory and instrumental comparison studies determining a consensus particle number concentration for nanoparticles and plastics (DeRose et al. 2022; Minelli et al. 2022; Farkas et al. 2025).

Thus, there is a need for stock characterizations and standardized protocols before for NMP exposures, given these differences in media types originating from the same stock that demonstrates mass-based concentration exposures do not define exposure. Identical nominal concentrations can correspond to substantially different particle number concentrations, which can be seen as more relevant for environmental exposures. Thus, highlighting the importance of reporting both mass and particle number concentrations with full size distributions of NMP exposure media to fully characterize what is in the exposure chamber for comparisons of ingestion relative to media.

3.2 Ingestion and Clearance of Ta-PMMA and AgNPs by *D. magna*

D. magna were observed to ingesting and excreting both Ta-PMMA NMPs (individual and grouped exposures) and AgNPs over 48 hours (Fig. 2A and B). Contrary to many monodispersed nanoparticle studies that exhibit first-order ingestion kinetics with peak body burdens at the end of exposure (Jang et al. 2014; Liu et al. 2024; Yu et al. 2024), all exposure types in this study showed rapid initial ingestion followed by clearance. Peak measured particles per *D. magna* occurred between 6 to 12 h for both polydispersion exposures and monodispersion, respectively, and subsequently decreased thereafter (Fig. 2A,B). Maximum particle loads occurred at 6 h for both individual and group Ta-PMMA exposures—26,592 ± 5,985 particles daphnid^{-1} (individual, 285–2,895 nm) and 88,338 ± 21,164 particles daphnid^{-1} (group, 281–3,240 nm)—and at 12 h for AgNPs (6,354 ± 637 particles daphnid^{-1})(Tables S3–S5; Figure S2). The early ingestion peak may reflect increased feeding activity following the 24 h clearance period prior to the start of the exposure study, as *D. magna* are known to increase particle ingestion under starvation conditions .

Current *D. magna* ingestion studies have determined that larger particles get ingested quicker than smaller size particles (Rosenkranz et al. 2009; Rist et al. 2017; Yu et al. 2024). However, these studies compare two monodispersed sizes, making it difficult to compare ingestion patterns as it is not a continuous size range. This reduces the effects size has on other sized NMP ingestion rate. Ingestion rate calculations were not utilized in the Ta-PMMA exposures due to only two collection points as maximum internal concentration in *D. magna* occurred at the 6 h time-point. Since distribution data was determined to not be normally distributed, log-log size plots for the Ta-PMMA exposure medias and their respective *D. magna* exposures were utilized to determine size related ingestion patterns. Within the < 1

micron range, both exposure types had different ingestion slopes compared to their respective medias. Individually, the slopes were smaller in the *D. magna* (ranging − 0.86 to -1.77) comparative to the media (-4.09), indicating ingestion in the larger size range of the < 1 micron fraction than the expected proportion (Fig. 3A Tables S6 and S7). In the > 1 micron range, the media (-2.54) and *D. magna* (ranging − 2.56 to -4.42), were similar in distributions (Fig. 3A Tables S6 and S7). The grouped exposure had the opposite effect in the < 1 micron range where the media (-0.71) was smaller than the *D. magna* (ranging − 1.31 to -1.85), indicating that grouped *D. magna* do not up take the expected proportion at the larger end of this size fraction (Fig. 3B and Table S6 and S7). The opposite is seen in the > 1 micron range where the media (-9.03) was larger than the *D. magna* (ranging − 6.11 to -6.31 (Fig. 3B and Table S6 and S7). The use of the particle size method and 60 nm Au particles creates uncertainty for particles > 1 µm by possibly mischaracterizing them (Lomax-Vogt et al. 2025; Elinkmann et al. 2026). However, all size ingestion pattern comparisons are real as this method was applied to all samples. The uncertainty is applied to where size ingestion patterns begin. These results demonstrate different size ingestion patterns between < 1 micron and > 1 micron ranges, as well as how the individual versus group exposures altered these size-related ingestion patterns.

D. magna within the group exposure accumulated approximately four times as many particles per daphnid as the individually exposed *D. magna*, despite comparable ingestion timing (Fig. 2A). This could stem from the greater total particle abundance in the larger volume exposure (Initial Individual Media: 432,301 ± 3,165, Grouped Media: 1,356,606 ± 250,051; Table S1). Because chamber geometry, exposure volume, initial particle-number concentration, and particle-size distribution differed between the individual and group exposure systems, these experimental factors likely contributed to the observed differences in particle uptake and clearance. Larger exposure volumes and altered chamber geometry can influence particle suspension, settling dynamics, wall interactions, and organism-particle encounter frequencies independent of behavioral responses. Accordingly, while the present experimental design cannot distinguish the relative contributions of physical exposure conditions and organismal interactions, previous studies provide a useful behavioral framework for interpreting the patterns observed here.

Grouped *D. magna* often exhibit vortex-like “swarming” movements in response to environmental cues, increasing particle suspension and encounter rates (Øien 2004; Vollmer et al. 2006; Noss et al. 2013). In contrast, individuals in smaller chambers encountered fewer suspended particles due to wall effects (residing towards walls or bottom of chamber) that reduce swimming and filtering efficiency (Peters and Downing 1984; Dodson et al. 1997; Ribeiro et al. 2014). Decreased filtering behavior was also seen at increasing AgNP concentrations in a 50 mL exposure setup (Foit et al. 2012). Intraspecific competition has also been shown to increase feeding behavior and lowers reproduction in *D. magna* susceptibility to lead, copper and pesticides (Knillmann et al. 2012; Gabsi et al. 2014; Gust et al. 2016). Our results reflect these differences, as group *D. magna* had greater ingestion of particles at C_{max} relative to its media (6.5% particles per *D. magna*) as compared to both individual exposures (Ta-PMMA = 6.1% particles per *D. magna*, AgNP = 1.95% particles per *D. magna*). Although these patterns are also influenced by differences in exposure geometry and particle conditions between treatments, they are consistent with

previous observations that social interactions can modify feeding and swimming behavior in *D. magna*. Future studies that independently control chamber geometry, exposure volume, particle concentration, and size distribution while manipulating group size will be necessary to distinguish the relative contributions of physical exposure conditions and intraspecific interactions. Such studies will be important for determining when and to what extent intraspecific interaction dynamics should be incorporated into NMP ingestion and clearance studies and for improving extrapolations from individual-level responses to population-level predictions (Ananthasubramaniam et al. 2015; Zheng and Wang 2024).

Clearance of NMPs and AgNPs occurred before exposure period ended, thus we are including clearance of particles within the exposure period (6-24h NMPs, 12-24h AgNP) into apparent clearance rate calculations and comparisons. During clearance, particle counts declined from 26,592 ± 5,985 to 1,115 ± 296 particles daphnid^{-1} in the individual exposures and from 88,338 ± 21,164 to 14,617 ± 7,155 particles daphnid^{-1} in the group exposures (6–48 h and 6–36 h, respectively; Fig. 2A,B). In the group exposures, particle counts rose from 14,617 ± 7,155 particles daphnid^{-1} to 48,114 ± 4,718 particles daphnid^{-1} from 36 h to 48 h in the clean clearance period, which contradicts the monotonic clearance pattern. This increase could be due to extended time from previous water replacements where particles that were depurated were retaken up. AgNP counts decreased from 6,345 ± 637 to 140 ± 23 particles daphnid^{-1} (12–48 h; Table S6) with only ~ 2.2% of AgNPs remaining at 48 h, indicating more efficient clearance relative to the Ta-PMMA polydispersion. Apparent clearance rate constants (k_{app}) were 0.0746 h^{-1} (individual Ta-PMMA), 0.0599 h^{-1} (group Ta-PMMA), and 0.106 h^{-1} (AgNPs; Table 1), suggesting that the smaller, monodispersed AgNPs depurated more rapidly than the polydispersed Ta-PMMA particles, consistent with prior observations that clearance slows as particle size increases (Ward and Kach 2009; Rist et al. 2019; Yoo et al. 2021). Within the Ta-PMMA exposures, the individual treatment showed minimal size-specific differences (< 1 µm: 0.0744 h^{-1}; > 1 µm: 0.0711 h^{-1}), whereas the group exposure exhibited slower clearance of smaller particles (< 1 µm: 0.0567 h^{-1}) compared to larger ones (> 1 µm: 0.0737 h^{-1}). This difference may be due to exposures set-up, chamber size, or re-ingestion of particles during group exposures.

Table 1
Calculated D. magna Apparent Clearance Rate Constants for the Ta-PMMA and Ag ENP Exposures

Exposure	Apparent Clearance Rate Constant (k_{app}) (h^{-1})
Ag ENP	0.106
Individual Ta-PMMA Overall	.0746
Individual Ta-PMMA < 1 micron	.0744
Individual Ta-PMMA > 1 micron	.0711
Grouped Ta-PMMA Overall	.0599
Grouped Ta-PMMA < 1 micron	.0567
Grouped Ta-PMMA > 1 micron	.0737

4. Conclusions

This study demonstrates that metal-tagged Ta-PMMA nanoplastics analyzed by spICP-MS provide a compelling approach for quantifying ingestion and clearance kinetics of polydispersed NMPs in aquatic organisms. *D. magna* exhibited rapid initial ingestion and slower clearance of polydispersed Ta-PMMA compared to monodispersed Ag nanoparticles, highlighting the influence of particle heterogeneity on ingestion-clearance rates. Grouped exposures resulted in higher particle loads per organism and both polydispersion exposure types show size-selective accumulation, likely driven by differences in size distribution, chamber geometry, and volume. It is noted of possible interspecies interactions and hydrodynamic interactions that could enhance particle suspension. These findings emphasize the importance of considering particle polydispersity, exposure configuration, and behavioral ecology in interpreting NMP bioaccumulation studies. The work bridges the gap between monodispersed laboratory systems and environmentally realistic conditions, providing a foundation for future research into chronic exposures and the potential for transgenerational or trophic transfer of polydispersed NMPs.

Declarations

Conflicts of Interest and Competing Interests

There is no conflicts of interest to declare. The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval

This study used only *Daphnia magna*, an invertebrate. Invertebrates other than cephalopods are not regulated under institutional animal care and use guidelines, so no ethical approval was required.

Consent to Participate:

Not applicable. This study did not involve human participants.

Consent to Publish:

Not applicable. This manuscript contains no individual person's data in any form, including individual details, images, or videos.

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Author Contributions

C.H. conducted the experiments, analyzed the data, and wrote the original draft of the manuscript. E.S. helped conduct initial experiments and analyzed data for this manuscript. E.G., J.C. developed the study, designed spICP-MS methodology, analyzed data, and reviewed and edited manuscript.

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Data Availability

Supplementary information (SI) is available as a part of this manuscript. Data on the production and further information on the Ta-PMMA particles is through Smith et al. (2024).

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Figures

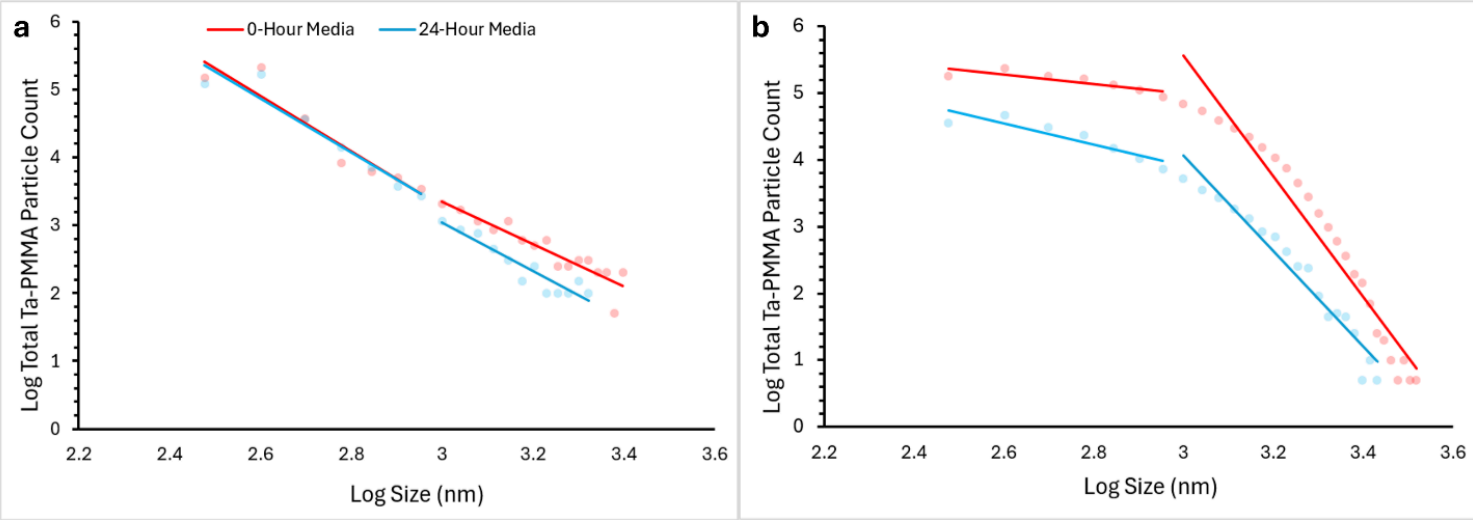


Figure 1

Log size distributions of a) 0 (Red markers and line) and 24 (Blues marker and line) h media samples for the individual Ta-PMMA exposure b) 0 and 24 h media samples for the grouped Ta-PMMA exposures. Each data point represents an average of 3 for respective size range which was then fitted with a linear regression line

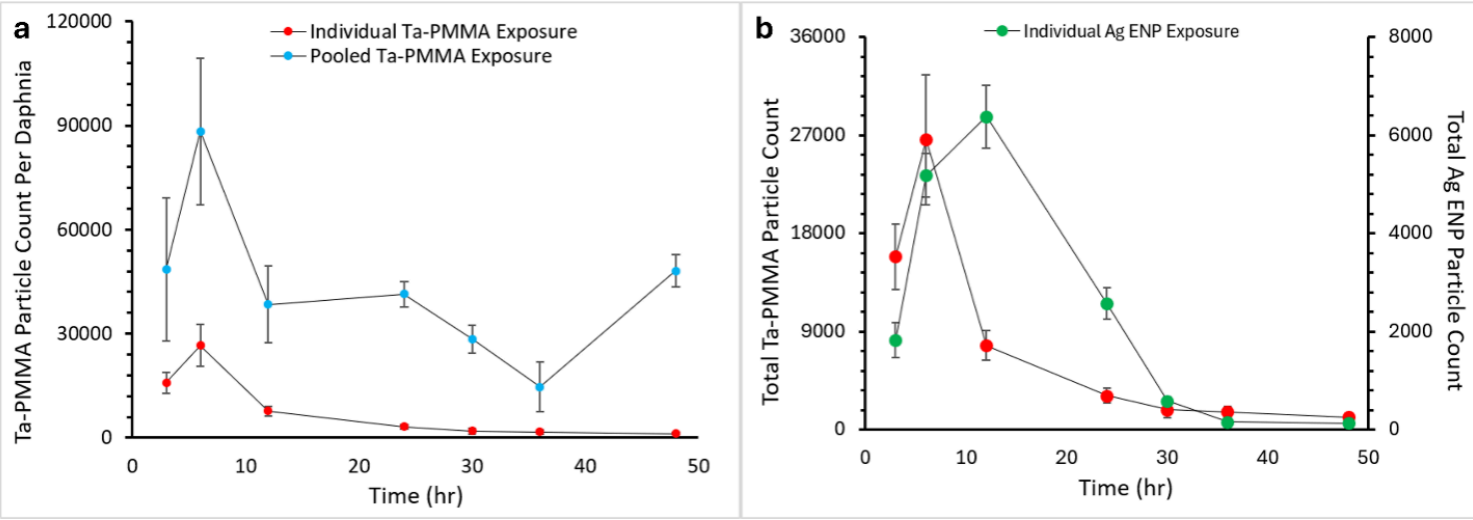


Figure 2

a) Combined ingestion and clearance models of the individual and pooled Ta-PMMA *D. magna* exposures Red points represent individual Ta-PMMA exposure and blue points represent grouped Ta-PMMA exposure Individual exposure 3-hour n=, 12-24-48-h n=8 and 6-30-36-h n=9 Grouped exposure n=3. b) Combined ingestion and clearance model of individual Ta-PMMA and 200 nm Ag ENP exposure *D. magna* exposure Green points represent the individual Ag ENP exposure with 3-24-h n=9 30-h=7 36-and 48-h n=8 Points represent mean and error bars represent standard error of mean

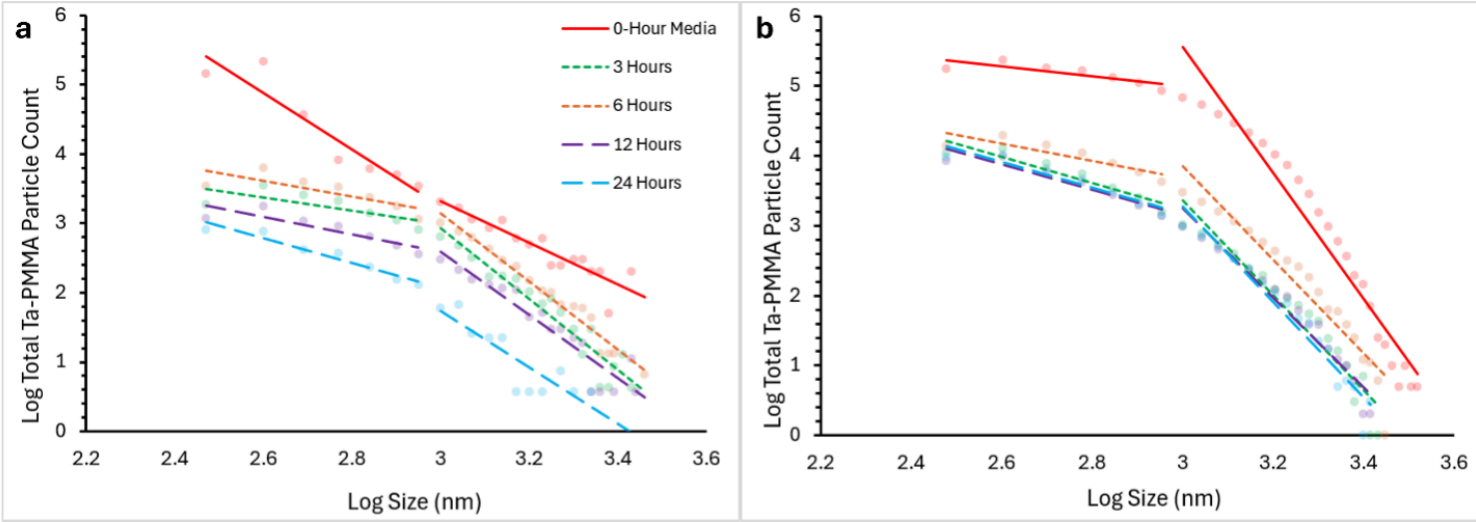


Figure 3

Log size distributions of ingestion period *D. magna* samples (3-24 h) for the a) individual and b) grouped Ta-PMMA exposures The respective 0-Hour media sample is overlayed for comparison Each distribution is divided into the <1 micron and >1 micron range Each data point represents an average of 7 (3h-green) 9 (6h-orange) and 8 (12-purple and 24h-blue) for the individual exposure 3 for the grouped exposure, and 3 for the media Each time point and media sample was fitted with a linear regression line for slope comparisons

Supplementary Files

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