Ultra-low dietary exposure to ^{14}C-labelled polystyrene: evidencing translocation of nanoplastics in fish

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Introduction

Plastic pollution remains a continuous threat to wildlife. However, assessing the potential tissue accumulation of nanoplastics remains challenging. Nanoplastics are beginning to be discovered in select environmental areas; for example, nanoplastics have been found in North Atlantic seawater[1] and Swedish lakes[2]. However, understanding their uptake is difficult due to the high carbon background of biological tissues. As a result, assessments of uptake have moved from the field to the lab where labelled nanoplastics can be used. Some materials incorporate a fluorescent label, but these do not provide conclusive evidence of uptake[3] and can only be used in transparent organisms such as zebrafish embryos. Metal doping of nanoplastics has been used as an alternative[4], but the exposure concentrations used are still likely to be above those in the environment. In order to use lower, more environmentally relevant exposure concentrations, sensitive techniques such as radiolabelling are required. Therefore, the aim of this study was to determine the accumulation of nanoplastics containing a radiolabel to fish at ultra-low concentrations following dietary exposure.

Methodology

Polystyrene nanoplastic synthesis

The synthesis method was adapted from Al-Sid-Cheikh et al.[5] with minor modifications. A round bottom flask was used to polymerise styrene to polystyrene nanoplastics (PS NPs) in a nitrogen atmosphere. This was initially performed on non-radiolabelled styrene to validate the synthesis, and this product was characterised using high resolution transmission electron microscopy (HRTEM), nanoparticle tracking analysis (NTA), Fourier transform infrared spectroscopy (FTIR). Then, the validated method was applied to ^{14}C-labelled styrene to produce ^{14}C-PS NPs for use in the experiment.

Fish exposure

The ^{14}C-PS NPs were incorporated into a commercially available fish diet through top dressing. Fish (n = 16/tank) were graded into 70 L tanks (n = 3/treatment) and left to settle to form hierarchies for 2 days before the start of the experiment. Fish were fed one of two diets: an unexposed control or ^{14}C-PS NPs at a concentration of 28 kBq kg^{-1} (equal to 5.9 µg kg^{-1}). In both treatment, fish were fed a ration of 2% body weight per day for 14 days. At day 3, 7 and 14, three fish were taken from each tank (n = 9/treatment) and dissected for the mid intestine, hind intestine, liver and kidney. The tissues were dried and digested into a liquid before adding liquid scintillation cocktail and subsequent analysis using liquid scintillation counting. Tissue activity was expressed as radioactivity per gram of dry weight tissue.

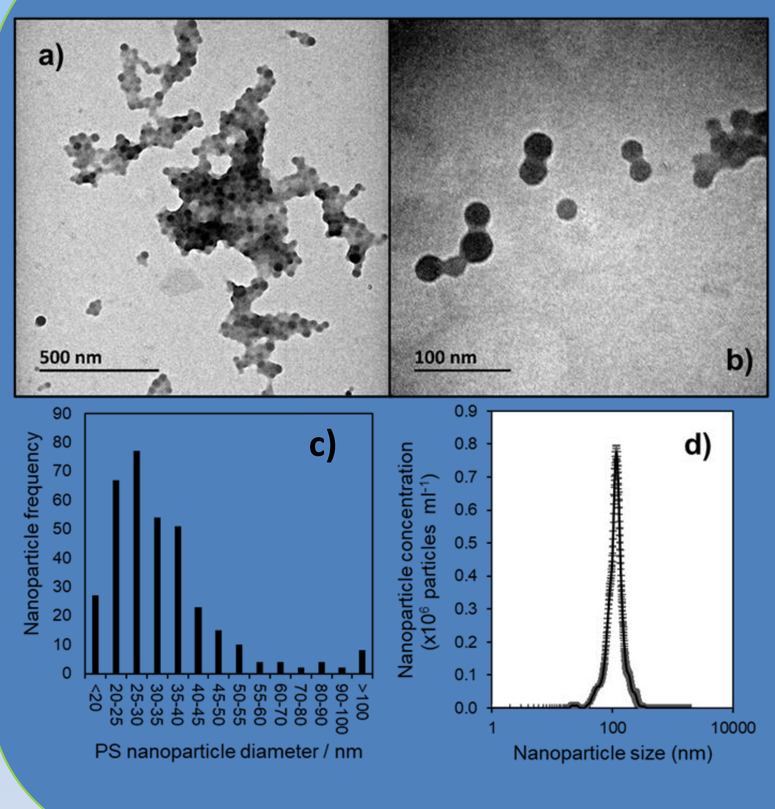
Results

Polystyrene nanoplastic characterisation

HRTEM showed the presence of particles in the final product that were spherical in shape (Fig 1a, b) and the mean particle size was measured as 35 ± 8 nm (n = 342; Fig. 1c). Using NTA, the PS NPs showed a monodisperse nature (Fig 1d), reporting a hydrodynamic diameter of 158 ± 55 nm. In comparison, the reported hydrodynamic diameter of the ^{14}C-polystyrene product was 160 nm ± 56 nm, analogous to those without the radiolabel (Fig 1), showing consistency between synthesis. FTIR analysis was conducted on the non-radiolabelled PS nanoparticles to confirm the successful polymerisation of styrene into polystyrene (Fig. 2).

Tissue radioactivity

The control samples showed some background activity in all tissue samples. For instance, the activity in the control organs ranged from around 1-31 Becquerel g^{-1} dw (Fig. 3a-d). For the ^{14}C NP treatment, the organs exhibited similar concentration, such that the mid intestine and kidney showed no significant differences from the controls at any time point. However, the liver and hind intestine showed some significant increases in tissue radioactivity (Fig. 3b-c). For instance, at day 14, the hind intestine from the ^{14}C NP treatment was over 3x higher than the control organs, reaching a tissue activity of 25 ± 6 Bq g^{-1} dw. Similar observations were made in livers whereby the ^{14}C NP treatment was significantly higher compared to the controls at day 7 and 14.



Figures 1a-b (left). HRTEM images for non-radiolabelled PS nanoparticles (a and b); and corresponding size-frequency distribution plot of PS nanoparticles (n = 342; c). Particle number concentration and particle size distribution of the non-radiolabelled PS nanoparticles, measured by supplementary nanoparticle tracking analysis (d). Data are mean ± SD (n = 3).
Figure 2 (above). FTIR spectrum of synthesised product and comparison of synthesised product in black with reference polystyrene spectrum in red.

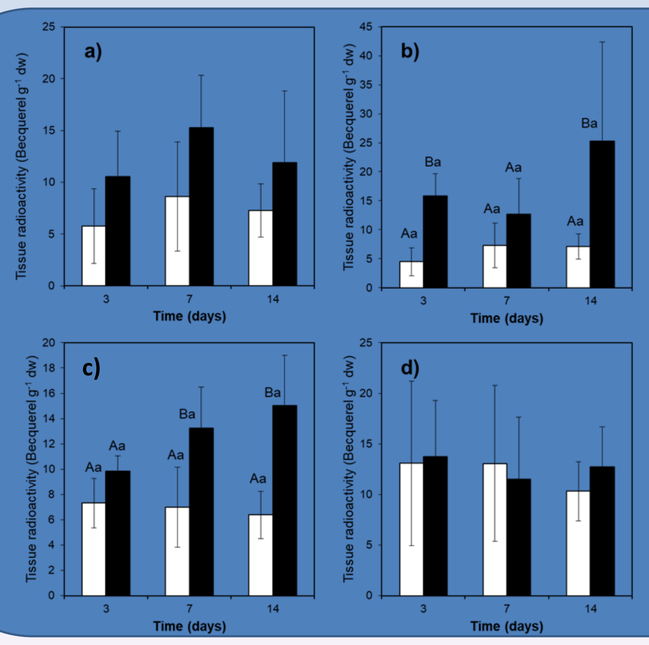


Figure 3. Tissue radioactivity in the mid intestine (a), hind intestine (b), liver (c) and kidney (d) following 14 days exposure to a control (no added ^{14}C, white bars) or ^{14}C-containing PS NPs (black bars). Different upper-case letters show significant difference between treatments within time points. Different lower-case letters show significant difference within treatments across different time points.

Conclusions

- The ^{14}C-PS NP synthesis provides a valuable system to measure the accumulation of nanoscale plastic pollution via dietary exposures in fish.
- Radiotracers are not a new tool, but its application to address plastic pollution problems has had limited use.
- Future work should focus on applying the methods used here to other biota, and long-term exposure studies should be the basis of future work to understand long term effects.

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