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Method Article

An effective method to assess the sorption dynamics of PCB radiotracers onto plastic and sediment microparticles



Marine Pyl^{a,b,*}, Bruno Danis^a, François Oberhaensli^b, Angus Taylor^b, Peter Swarzenski^b, Marc Metian^b

^aLaboratoire de Biologie marine (CP 160/15), Université Libre de Bruxelles, Av. F.D. Roosevelt 50, B-1050 Brussels, Belgium

^bInternational Atomic Energy Agency, Environment Laboratories, 4a, Quai Antoine 1er, MC-98000, Monaco, Principality of Monaco

A B S T R A C T

One important aspect of marine plastic pollution is that small particles are ubiquitously present in seawater and can transport a large variety of co-contaminants. The sorption-desorption kinetics of these co-contaminants sorbed to microplastics (MPs) are not fully understood, partially due to the lack of any standardised procedures between studies. The present work aims at describing a new and efficient method to investigate the sorption of co-contaminants onto different types of particles using proven radiotracer techniques. This work provides recommendations as well as a thorough description of the materials, conditions and procedures required to optimise the adsorption of polychlorinated biphenyl (PCB) onto particles. Details of the controlled experimental conditions, such as the volume of the container, the concentration of particles, and specifics of the radiotracer are provided. In addition, a thorough description of the novel filtration methodology specific to these radiotracer techniques is presented, for the first time in the literature. To validate the efficiency of the method, we examined the partition coefficients (K_d) of ¹⁴C-PCB#153 onto virgin MP (10–29 μm polyethylene beads) and onto natural sediment particles that are similarly sized (1–17.8 μm) in seawater. After 40 h, plastic particles adsorbed 25.7% of ¹⁴C-PCB#153 while sediment particles adsorbed 89.3% of the same compound. Results suggest that in this scenario, polyethylene MP particles may be less effective transport vectors of ¹⁴C-PCB#153 than natural sediment particles.

- Details of experimental conditions, such as the volume of the container, and the concentration of particles and of radiotracer, were defined
- A thorough description of the filtration methodology specific to radiotracer techniques is presented
- Results highlight that virgin polyethylene MPs may be less effective transport vectors of ¹⁴C-PCB#153 than natural sediment particles.

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* Corresponding author.

E-mail address: [pymarine@gmail.com](mailto:pylmarine@gmail.com) (M. Pyl).

ARTICLE INFO

Method name: Method for studying the sorption dynamics of PCB radiotracers onto particles

Keywords: Polychlorinated biphenyls, Co-contaminant, Microplastics, Partition coefficient, K_d, Adsorption, Standardisation

Article history: Received 9 April 2021; Accepted 24 May 2021; Available online 25 May 2021

Specifications Table

Subject Area:	Environmental Science
More specific subject area:	Adsorption onto microplastic and sediments
Method name:	Method for studying the sorption dynamics of PCB radiotracers onto particles
Name and reference of original method:	None. This is an original work, not based on a pre-existing method.
Resource availability:	<p>Material:</p> <ul style="list-style-type: none"> · Micro-particles; · Radiotracer; · Pipettes and tips; · 1 ml cell counting chamber; · Metal tweezers; · 20 ml glass scintillation vials; · Filtration pump; · Polycarbonate filters; · Lab coat; · Sterile gloves. <p>Solutions:</p> <ul style="list-style-type: none"> · Ultrapure or osmotic water; · Ethanol; · Dispersant Tween-20®; · Scintillation liquid. <p>Equipment:</p> <ul style="list-style-type: none"> · Fume hood; · Vortex; · Scale; · Oven; · Orbital shaker; · Glove box · Filtration unit; · Vacuum pump; · Temperature probe and a glass-body combination pH electrode, coupled to a microprocessor-based pH and temperature meter; · Liquid scintillation counter; · Stereomicroscope (Leica DMS300).

Methodological protocol

Method details

The objective of this work was to provide a standardised methodology to study the sorption of co-contaminants onto different types of particles using novel radiotracer techniques. Further detailed information on the general interest and importance of this topic and method is reported in the background information section.

The method is composed of six main phases: choice of the experimental design (*phase 1*), preparation of the solutions and exposure (*phase 2*), sampling and filtration (*phase 3*), weighing of the particles (*phase 4*), measurement of the activity (*phase 5*), and partition coefficient calculation (*phase 6*).

This work provides specific recommendations as well as an exhaustive description of the materials, conditions and procedures required to investigate adsorption of radiolabelled chemicals onto MPs and other particles. To the best of our knowledge, such a methodological description of laboratory-based radiotracer techniques has not been presented previously in the literature. It is hoped that the proposed method will improve the consistency between future studies involving the sorption kinetics

of β -radiolabeled chemicals to particles, catalysing a better understanding of the importance of MPs as vectors of co-contaminants to marine organisms.

Phase 1 - choice of the experimental design

Choice of particles

The MPs considered in this study were high density polyethylene and polystyrene microbeads, which are among the most common polymers found in the environment [1-2]. Virgin high density polyethylene microbeads (HDPE MP) were purchased from Cospheric® as a solid powder (green fluorescent; size = 10-29 μm , 38-45 μm , 63-75 μm ; density = 1.1, 1.025 and 1.025 g cm^{-3} , respectively; specific surface area = 280, 141 and 85 $\text{m}^2 \text{kg}^{-1}$, respectively). Virgin polystyrene microbeads (PS MP) were purchased from Polysciences® as 2.5% aqueous suspension (yellow green fluorescent; size = 6 μm ; density = 1.05 g cm^{-3} ; specific surface area = 952 $\text{m}^2 \text{kg}^{-1}$). As the polystyrene beads in an aqueous suspension used in the preliminary tests did not significantly sorb ^{14}C -labelled PCB#153, it is recommended to choose polyethylene as powder instead, when dealing with sorption of POPs.

To compare the adsorption capacity of different types of particles, we recommend choosing particles comparable in terms of either size or specific surface area. Sediment particles used in this study were characterized using a particle size analyser: Mastersizer 2000 from Malvern Instrument® (size range = 0.2 - 17.8 μm , specific surface area: 3220 $\text{m}^2 \text{kg}^{-1}$).

Choice of radiotracer

^{14}C -labelled congener IUPAC#153 (2,2',4,4',5,5'-hexachlorobiphenyl) was selected as representative of PCBs for this study as it is the most abundant congener in marine biota [3]. This β -emitting radiotracer, with a purity higher than 95%, was purchased from American Radiolabeled Chemicals®, USA. The specific activity of this mother source was 12.6 mCi mmol^{-1} . A working dilution with an activity of 1.5 kBq ml^{-1} was prepared in ethanol from the mother source.

Health and safety handling of radioactive particles

In agreement with the procedures for safe handling of radioactive material established by the Environment Laboratories of the International Atomic Energy Agency (IAEA) in Monaco, the manipulation and spiking of the radiotracer ^{14}C -PCB#153 was performed under a fume hood in a supervised area. The handling of radioactive particles was performed entirely within a glove box. Personal protective equipment (e.g., gloves, lab coat and eye protection) was used throughout the lab work of the operator.

Weight

To allow the calculation of the dried particles activity in Bq g^{-1} , each filter and the associated scintillation vials (including lids) in which they would be collected after filtration were weighed at ambient temperature prior to the experiment.

Furthermore, to estimate the potential impact of the drying step (60°C during 48h) on the weight of filters and vials (including lids), a test was conducted only with seawater filtered through the filters. To do so, 10 filters, along with their associated scintillation vials and lids, were weighed at ambient temperature before (dry weight 1) and after (wet weight) the filtration of 20 ml of seawater, corresponding to the volume of the sample filtered during the experiment + 5ml rinsing. These vials were then put in an oven at 60°C for 48h to dry before being weighed at ambient temperature (dry weight 2). By subtracting the dry weight 1 from the dry weight 2, we found an average loss of 8 mg due to the exposure of the material at a temperature of 60°C during 48 hours.

When dealing with small quantities of particles, we strongly recommend conducting a similar preliminary experiment in order to account for the potential temperature effect on the weight of filters, vials, and their lids.

Phase 2 - preparation of the solutions

Addition of the particles

Solutions were prepared within glass screw-cap 20 ml scintillation vials purchased from PerkinElmer®. First, 2ml of UV-sterilized 1 µm filtered seawater was added before a mass of 9.4×10^{-2} g of HDPE MP was inoculated in each tube. Subsequently, 15 ml of seawater were poured into each tube in order to reach a total solution volume of 17 ml and a particle concentration of 5.5×10^{-3} g mL⁻¹. We found these parameters to be a reasonable compromise between the volume of the container and concentration of particles, minimizing interactions between the contaminant and the internal surfaces of the tube itself, while maximizing interactions between the contaminant and the particles. MP concentrations, in terms of number of particles, were verified by counting 3 aliquots from each sample under a microscope using a Sedgewick Rafter Counting Chamber. Interestingly, the particle mass of $5.8 \times 10^{-2} \pm 1 \times 10^{-3}$ g recovered on the filters from filtration did not compromise its handling during further analysis.

Furthermore, to compare the adsorption capacity of different types of particles, we recommend working with the same mass of particles, while reporting its equivalent quantity in terms of number of particles. Indeed, working in terms of mass has the advantage to be much more precise and faster than to work in terms of number of particles, as the counting process is highly time-consuming. We also argue that researchers should provide both particle mass and particle number, making the conversion either using the density or through an assessment of the ratio between the number and weight of particles. Notably, the conversion between particle mass and number based on the density would not be practically feasible for heterogeneous-shaped particles, such as sediments. Reporting both the mass and number of particles used should allow easier and more meaningful comparison between studies.

Homogenisation

After the addition of the particles into the solutions, vials were placed on an orbital shaker (200 rpm) for 5 min to obtain a homogeneous mixture of particles in suspension. Dry powdered MPs in their pristine state are hydrophobic and in order to be homogeneously dispersed in the water the beads required treatment with a surfactant. As recommended by the MPs manufacturer Cospheric®, we chose Tween-20® as a polysorbate-type non-ionic dispersant commonly used to maintain a homogeneous suspension of MPs in the water. Tween-20® (0.02% of the total volume) was added to the solutions containing PE microplastics to avoid aggregation and surface floating. The manufacturer guidelines suggested that a 0.1% concentration of Tween was needed to disperse the MPs in water, but homogeneous dispersion was achieved with 0.02% in solutions containing PE microplastics. The preparation of the solution of aqueous PS MPs did not require any addition of Tween®. We recommend avoiding the use of Tween® as far as possible in order to minimize its potential interference in the adsorption process. In cases where stirring or shaking did not lead to an efficient homogenisation of the solutions, we advise the use of Tween-20® at the minimum concentration needed and to first determine its effect on the adsorption. At the concentration of 0.02% used in our study, Tween-20® did not affect the adsorption of ¹⁴C-PCB#153 on sediment particles (see method validation section).

Radiotracer spiking

Once the solutions of particles in suspension were homogeneous, all vials were spiked with ¹⁴C-labelled PCB#153 to an activity of 1 Bq ml⁻¹. This activity of 1 Bq ml⁻¹ was achieved by adding 12 µl of the working dilution to the vials. 1 Bq ml⁻¹ is equivalent to a concentration of 770 ng of stable PCB#153 per litre. This concentration of ¹⁴C-labelled PCB#153 is by far higher than environmental levels, and was selected in order to both maximise the potential adsorption of the co-contaminant onto the particles, while also remaining below the solubility limit of the tracer (9.5×10^{-4} g m⁻³). The solubility limit given here was taken from Mackay *et al.*, 1992 [4] but in later experiments, we have found this to be inaccurate as the solubility limit was much greater. This spiking step was always performed under a fume hood. Furthermore, the addition of volumes in the order of microliters of the radiolabelled co-contaminant to the solutions did not affect the pH conditions.

Incubation

Once the radiotracer was spiked into vials, they were placed in the dark on the orbital shaker (200 rpm) to maintain suspension of the homogeneous mix of particles during the entire duration of the experiment. A short incubation duration (24–48h), corresponding to the time needed for the equilibrium to be reached, is commonly used in papers that studied the sorption kinetics of organic pollutants onto the external surface of polymers [5]. Throughout the exposure, the temperature was maintained at 22°C (room and seawater temperature), salinity at 38 ‰, and the pH at 8.1. Temperature and pH were measured using a temperature probe and a glass-body combination pH electrode, coupled to a microprocessor-based pH and temperature meter (Hanna® pH 211), and calibrated with standard pH buffer solutions. As the sorption processes are highly dependent on abiotic parameters, we recommend a daily verification of the stability of the laboratory conditions such as temperature, salinity and pH.

Phase 3 - sampling procedure

Sub-samples of the solution (water and particles)

To measure the initial activity in the solution and to ensure that the spike has been correctly carried out, 1 ml of each vial was sampled and measured immediately after spiking (**Water 1**). At the end of the incubation time, another 1 ml was sampled from each vial in order to assess the total activity of the solutions just prior filtration (**Water 2**).

Particles

To avoid contaminating the laboratory space with radioactive particles, we strongly recommend isolating all workspaces during the filtration process with hermetic enclosure devices such as a glove box. In this work, we used a glove box (890mm wide x 600mm deep x 700mm high) composed of a transfer chamber purchased from Sicco®. We found this device essential for the protection of operators as it allows safe handling of hazardous materials such as radiolabelled contaminants sorbed onto particles, in a controlled and static environment. The handling of samples within an enclosed device, such as a glove box, could also be beneficial to studies assessing MPs levels from environmental samples. Samples can be processed safely without exposing them to air, minimizing airborne contamination, and therefore the risk of cross-contamination. In terms of filtration equipment, a Merck Millipore™ sampling vacuum manifold coupled with a vacuum pressure pump was used. This Merck Millipore™ filtration unit has the advantage of being able to carry out twelve simultaneous filtrations as the device includes:

- a barrel, within which filtrates are collected in twelve borosilicate glass tubes (23 ml capacity, purchased from Fisher®),
- an interior lid, composed of twelve 25 mm numbered discs of filtration grids, on top of which the filters are placed,
- an exterior lid, composed of twelve chambers, in which samples can be poured,
- a central screw for sealing.

Furthermore, polycarbonate filters (diameter of 25 mm and mesh size of 1 µm) purchased from Sigma Chemicals®, USA, were used in this study. In agreement with Besson *et al.*, 2020 [6], we chose to use polycarbonate filters as their thinness minimizes the sorption of the radiotracer onto the filter itself during the filtration process.

At the end of the incubation time, the particles were separated from the solution by vacuum filtration. Each step of the protocol was designed to optimise the efficiency and accuracy of these filtrations, as detailed below:

- The entire filtration procedure was performed within a glove box. As the vacuum pump was placed outside the box, the pump was turned on before entering the glove box.
- Once in the glove box, new collection tubes were placed in the barrel of the filtration unit. Then the interior lid was set up and few drops of osmotic water were placed on each filter plate before the addition of the filters. Once the filters were placed, the exterior lid was placed on top of the interior lid.

- The two lids (interior and exterior) were then lifted together and put aside in order to access the collection tubes within the barrel. The osmotic water was removed from each collection tube.
- Once the collection tubes were empty, the two lids were placed on top of the barrel. The central screw was closed tightly to ensure the unit was properly sealed.
- Solutions of each sample were poured into their respective filtration chambers. When fewer than 12 samples were filtered at the same time, stoppers were put on unused chambers to speed up the filtration process.
- Once the twelve filtrations were completed, the central screw was unscrewed and the two lids were lifted up simultaneously in order to access the filtrates collected by the tubes within the barrel. A 2 ml sub-sample from each filtrate was collected in scintillation vials. At this step, we strongly recommend checking for drops within the barrel in order to spot any leaks, spills or other problems that could have occurred during the filtration.
- After the collection of the 2 ml, the collection tubes containing the rest of the filtrates were saved for further filtration if needed, and new collection tubes were placed in the barrel.
- The two lids were put back together on top of the barrel. The central screw was closed tightly again to ensure the unit was properly sealed.
- Then, in order to maximize the number of particles collected in the filters, the vials of each sample were rinsed thoroughly with 1 ml of seawater prior to being poured into their respective filtration chambers. Three rinses were performed per vial. Once the rinse filtrations were completed, the walls of each chamber were cleaned with 2 ml of seawater. As a result, each filter was rinsed with a total of 5 ml of seawater.
- After rinsing, each filter was collected in scintillation vials that had been previously weighed.
- Finally, every component of the filtration unit was decontaminated by thoroughly rinsing with ethanol first, then decontamination soap, and finally distilled water.

Phase 4 - determination of particle weight

Once the filtration was complete, scintillation vials containing particles on filters were weighed (wet weight). Then, these vials were placed in an oven at 60°C for 48h to dry, and were weighed again at ambient temperature (dry weight 2). The weight of particles retained on the filters was determined by subtracting the dry weight measured after filtration (dry weight 2, containing the particles on filter) by the dry weight of the same vials measured prior to the experiments (dry weight 1, containing the filter only). In addition, the temperature effect on the weights measured in the preliminary test was taken into account by subtracting the particle weight by the average weight loss of vials (measured to be 0.008 g) due to their exposition at 60°C for 48h.

$$\text{Particle dry weight (g)} = \text{dry weight 2 (g)} - \text{dry weight 1 (g)} - 0.008(\text{g})$$

Phase 5 - measurement of ¹⁴C-PCB activity

The activity of ¹⁴C-labelled PCB 153 was measured in **Water 1** (initial total activity), **Water 2** (total activity after exposure), **Water 3** (activity in the filtrates) and **Filter 1** (activity in the particles). Immediately after their collection, both water and filter samples were transferred to 20 ml glass scintillation vials (PerkinElmer®) and mixed with 10 ml of Ultima Gold XR® (PerkinElmer®) scintillation liquid. Radioactive samples were thoroughly mixed to homogenise the solution and kept in the dark for 24 h before counting.

¹⁴C-radioactivity was then measured using a Tri-Carb 4910 TR liquid scintillation counter (PerkinElmer®), compared to standards of known activities, and corrected for quenching, counting efficiency, background and physical radioactive decay. Counting times were adapted to obtain counting rates with relative propagated errors less than 5% (maximal counting duration was 90 min).

The activity retained by the filter itself was measured by subtracting the activities measured in Filter 1 from the activity retained on control filters. Another method consisting of measuring the activity of Filter 2 (filter of filtrate) and subtracting it from their associated Filter 1 was tested. Because the activities measured on Filter 2 (filter of filtrate) were not significantly different from the activities of the control filters, we do not consider it necessary to collect filters of filtrates.

Phase 6 - calculation of partition coefficient (Kd) and proportions of activity

The partitioning coefficients (Kds) of the PCB#153 radiotracers were determined using the standard equation corresponding to the ratio between the activity of the radiotracer in the dry particles (in Bq g⁻¹) and the activity of the radiotracers in the seawater (in Bq ml⁻¹) [7]. Both the activities measured in filtrates (Water 3) and in the water retained by the filters (controls of Filter 1) were considered to measure the quantity of PCB#153 dissolved in seawater. Furthermore, the proportions of activity were calculated by dividing the activity of the radiotracer measured in the dry particles by the sum of activities of the radiotracer in the seawater plus in the dry particles, at the time of sampling.

Method validation

To assess the efficiency of the protocol, nine solutions of 17 ml were prepared to study the adsorption of ¹⁴C-PCB#153 onto virgin high density polyethylene microbeads and sediment particles of a similar size. Three replicates were tested for all sorption experiments. Three solutions were inoculated with a mass of 9.4×10^{-2} g of MP-HDPE (concentration = $1.3 \times 10^6 \pm 1.4 \times 10^4$ MP mL⁻¹, size = 10-29 μm, density = 1.1 g cm⁻³, specific surface area = 280 m² kg⁻¹), three others with the same mass of sediment particles (concentration = $7 \times 10^7 \pm 7.4 \times 10^5$ sediment particle mL⁻¹, size range = 0.2 - 17.8 μm, specific surface area: 3220 m² kg⁻¹), and the remaining three tubes were controls, in which no particles were incubated. As discussed in the section above about the preparation of the solutions, the homogenisation of the HDPE samples required the addition of Tween-20® at the concentration of 0.02%. Three additional solutions containing sediment particles were inoculated with 0.02% Tween-20® in order to test its effect on adsorption. Tween®20 did not affect the adsorption of ¹⁴C-PCB#153 particles, as no significant difference were detectable between sediment samples with and without Tween-20®. As described in the radiotracer spiking section, all the solutions, once homogeneous, were spiked with ¹⁴C-labelled PCB#153 to an activity of 1 Bq ml⁻¹, which is equivalent to a concentration of 770 ng of stable PCB#153 per litre. The method described above was applied to all phases of the experimental procedure. The partition of ¹⁴C-labelled PCB#153 among seawater and MPs was assessed after 40 hours of exposure.

After 40 hours of exposure, control filters absorbed an average of 0.72% of ¹⁴C-PCB#153 while MP and sediment particles adsorbed 25.72% and 89.31%, respectively. As the adsorption on particles was significantly higher on sample filters than on controls, we consider that the method is efficient in the adsorption of ¹⁴C-PCB#153 onto PE MP and sediment particles. It is assumed that an extended incubation time would lead to very similar Kd values compared to ours, as the equilibrium time of organic pollutants onto the external surface of various virgin MPs have been previously proven to be reached within 48 hours [5]. Moreover, the adsorption of ¹⁴C-PCB#153 was significantly higher (two orders of magnitude) on sediment particles than on virgin HDPE MP (Fig. 1). Indeed, after 40 h, ¹⁴C-PCB#153 adsorption averaged at 217.5 ± 6.2 Bq g⁻¹ on sediment particles (Kd = 18129.1 ± 8990.8 mL g⁻¹) while it averaged at 67.8 ± 15.9 Bq g⁻¹ (Kd = 107.8 ± 32.6 mL g⁻¹) on virgin HDPE MP. The Kd value for sediment particles is 168 times higher than our result on HDPE MPs. This difference can be partly, though not entirely, attributed to the higher surface area of the sediment particles compared to the HDPE MPs that we used, as it was only 11.5 times higher.

Given the lower absorbance of ¹⁴C-PCB#153 onto virgin MP than sediment particles, they may therefore represent a less efficient uptake pathway for the considered contaminant for marine organisms. Similarly, Besson *et al.*, 2020 [6] and Johansen *et al.*, 2018 [8] also reported a higher adsorption of metal radiotracers on sediments than on virgin HDPE MP of similar size. As already reported using metal radiotracers, even weathered MPs with associated-biofilms seem to constitute smaller uptake pathways than sediment particles [8]. The fact that aged MPs generally adsorb more co-contaminants than virgin MPs needs to be tested further using a wider range of waterborne pollutants, such as organic contaminants. On the other hand, earlier studies found sorption of organic compounds to be two orders of magnitude higher in plastics than in natural sediments and soils [9-10]. This high variability of Kd measurements across studies is due to the lack of consistent approaches between experimental methodology. Therefore, to enable direct comparisons between studies, we strongly recommend the use of standard guidelines and procedures, such as the one

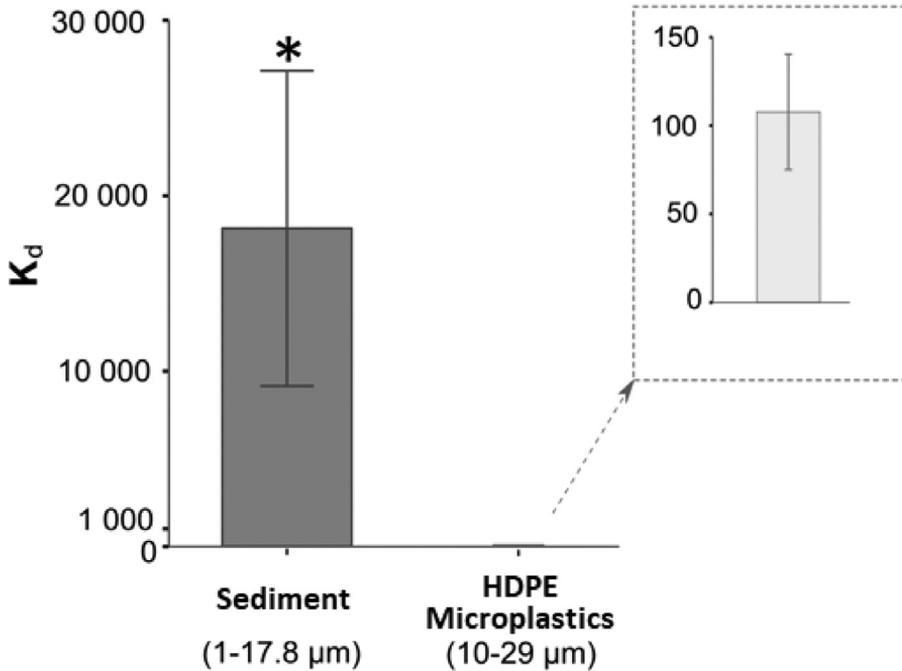


Fig. 1. ^{14}C -PCB#153 partition coefficient (K_d) and proportions of activity in high density polyethylene microplastics (HDPE ME) and sediment particles. Both graphics indicate mean \pm SE with $n=3$; * indicates p -value < 0.05 (test correspond to Wilcoxon rank sum test, with $W = 9$ for the comparisons between PE MP and sediment particles). Detailed calculations and statistical results are available in Supplementary Data.

proposed in this work. In order to enhance our understanding of the role of MP as vectors of co-contaminants to marine organisms, the further study of the sorption and desorption kinetics of several waterborne pollutants to various types, sizes and shapes of MP with different weathering and biofilm characteristics is much needed. We recommend the use of radiolabelled chemicals to address this knowledge gap, as radiotracer techniques offer strong assets in terms of rapidity, accuracy and sensitivity [6,8,11].

Conclusion

In comparison to previous studies that investigated the sorption dynamics of co-contaminants onto particles, this work offers, for the first time, a thorough methodological description of the laboratory-based procedures, while using novel radiotracer techniques. The application of standard guidelines and procedures, such as the one proposed in this work, would be a significant step towards the improvement of the consistency between different research groups. This will give rise to a better understanding of the sorption-desorption kinetics of chemicals bound to MPs and other naturally occurring particles, leading towards the clarification of the uncertainty regarding the vector effects of MPs. Knowledge of MPs as a vector for bioaccumulation of toxic chemicals would allow more rigorous risk assessments of microplastic for human health and environment.

Background information on the general interest and importance of this topic and method

The ubiquitous distribution of microplastics (MPs) in the marine environment is a global environmental concern [12]. Furthermore, their role as potential vectors of co-contaminants to marine

organisms is still a matter of scrutiny, as MPs can absorb various persistent organic pollutants (POPs; [13]). These co-contaminants may threaten marine organisms when MPs become inadvertently ingested and transferred up the food chain. However, the extent to which microplastics act as vectors of adsorbed pollutants is currently a matter of debate [13].

One of the challenges of assessing the importance of MPs as carriers for co-contaminants to marine biota is our limited understanding of the sorption and desorption kinetics of chemical compounds to MPs. To address this knowledge gap, the use of radiotracer techniques has been recently recognised as a promising approach because of their rapidity, accuracy and sensitivity, allowing the study of these kinetics within environmentally-realistic concentration ranges [6,8,11].

The sorption dynamics of POPs onto particles are driven by a myriad of factors, such as the properties of the sorbent and sorbate as well as environmental conditions, including temperature, salinity and pH [14]. As a result of these defining aspects of the chemical-physical parameters of the experimental design, and also because there is no consensus on standardised procedures, adsorption appeared to be highly variable across studies, even for similar polymer types. In order to enable direct comparisons between studies, the establishment of such a standardised method is much needed.

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.

Acknowledgements

This study was supported by the Marine Biology Laboratory of the Université Libre de Bruxelles and by the [Fonds National de la Recherche Scientifique](#), Belgium (FNRS grant n°35245388). The IAEA is grateful for the support provided to its Environment Laboratories by the Government of the Principality of Monaco. The publication was supported by the Fondation Universitaire de Belgique.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.mex.2021.101395](https://doi.org/10.1016/j.mex.2021.101395).

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