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Short Communication

Passive equilibrium sampling of hydrophobic organic compounds in homogenised fish tissues of low lipid content

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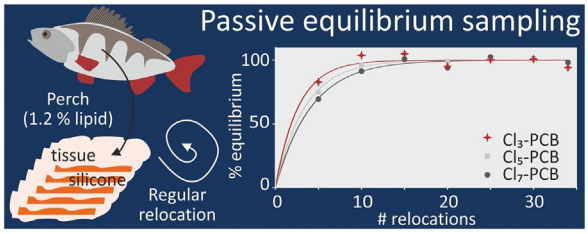
HIGHLIGHTS

- A new approach for relocation of passive samplers in homogenised tissues was successfully established.
- This procedure opens up for the use of passive samplers in fish homogenates of low lipid content.
- Organic compounds with log K_{OW} between 3.9 and 7.8 reached equilibrium in less than 3 days.

GRAPHICAL ABSTRACT



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ABSTRACT

Passive equilibrium sampling using polymer samplers in lean tissue is one of the current challenges in assessing bioaccumulation and biomagnification due to the long time needed to reach equilibrium. Despite recent progress achieved by rolling pieces of intact fish fillet with sheets of silicone, there is still a need for a passive sampling method for homogenates that achieves equilibrium before tissue decay starts. In this work, a new approach for relocation of silicone passive samplers in homogenates of lean fish was established for three homogenates with lipid contents varying from 1.2% to 6.1%. Results showed that for 20 model hydrophobic organic compounds with log K_{OW} between 3.9 and 7.8, equilibrium between the silicone and the tissue was achieved in less than 3 days at 4 °C. The concentrations in lipids obtained using passive equilibrium sampling and those from traditional total solvent extraction agreed well, within a factor of 1.3. This new procedure extends the use of passive samplers to homogenised fish tissues of low lipid content, which is highly relevant for environmental studies focused on bioaccumulation of contaminants.

1. Introduction

Hydrophobic Organic Compounds (HOCs) include a large group of legacy contaminants (e.g., polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and polybrominated diphenyl ethers (PBDEs)) and many contaminants of emerging concern. They are characterised by low water solubility, some of them being persistent and toxic, and many are bioaccumulative (Patrolecco et al., 2010; Gobas et al., 1993). The traditional approach for assessing bioaccumulation is to calculate ratios of total concentrations normalised to the mass of the main sorptive phase (e.g., storage lipids or organic carbon) in (a) biota and different abiotic media such as sediment to assess equilibrium partitioning or in (b) predator and prey to determine biomagnification. Comparability

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may be obstructed by lipid normalisation neglecting potential differences in the sorptive capacities of the different lipids and other sorptive phases of potential importance, such as proteins in lean tissues (DeBruyn and Gobas, 2007; Jahnke et al., 2014). In addition, the use of different lipid extraction protocols reduces comparability across studies.

Passive Sampling Devices (PSDs) based on polymers such as silicone, if operated in equilibrium mode, give a measure of chemical activity. The data can then be expressed on a common basis (i.e., as equilibrium partitioning concentration in silicone, in addition to chemical activity, fugacity, equilibrium partitioning concentration in lipids, etc.), circumventing the need for normalisation. PSDs have been equilibrated successfully with different media (Mayer et al., 2003; Jahnke et al., 2014; Booij et al., 2016; Gilbert et al., 2016; Allan et al., 2013; Maenpää et al., 2011; Parkerton and Maruya, 2014, Rojo-Nieto and Perales, 2015), amongst others to compare contamination of biota with high lipid content and sediments, and they offer great potential to assess contaminant transfer in aquatic food webs (Jahnke et al., 2014).

However, equilibrium sampling in lean tissues has been hampered due to the tissue in contact with the PSD surface experiencing local depletion, reducing the uptake rate. The diffusion of HOCs throughout the lean tissue, lacking "transporter agents" such as lipids, towards the PSD is then not fast enough to deliver HOCs to the tissue directly in contact with the sampler. Consequently, lean tissue in contact with the PSD surface experiences local depletion, reducing the uptake rate. Thus equilibrium may not be attained before tissue decay occurs (Jahnke et al., 2009; Rusina et al., 2017). Hence, the current challenge is to achieve equilibrium in lean tissue fast ($\ll$7 days).

Recently, Rusina et al. (2017) have proposed a new procedure to equilibrate passive samplers in lean tissue: the relocation of samplers over time, in order to avoid the local depletion of the sample. In that study, passive sampling in lean fish tissue was carried out using (i) rolling of pieces of fillet together with silicone sheets in jars and (ii) relocating the samplers in static contact with fillet or homogenised tissue. Although the rolling of pieces of fillet allows achieving equilibrium in few days, for the relocation of the samplers in static contact, equilibrium was neither attained in fillet nor in homogenate within 7 days, presumably because of insufficient frequency of relocations. Given the relevance of homogenised tissues for environmental studies (for pooling samples, for considering the whole body burden, etc.), there is a need for a passive sampling method that allows passive equilibrium sampling of lean homogenates. Therefore, we propose a new approach for relocation of silicone passive samplers in homogenates, using native samples from a polluted environment (i.e., non-spiked with pollutants) and studying the uptake kinetics for 20 HOCs with log K_{OW} ranging between 3.9 and 7.8 (Braekevelt et al., 2003; Schenker et al., 2005; Rojo-Nieto and Perales, 2012). In this study, we enhanced the relocations from once or twice a day (Rusina et al., 2017) to five times a day. We assessed the kinetics of equilibration, and we compared the results with concentrations derived from traditional exhaustive extraction.

2. Material and methods

2.1. Samples

For this study, three different homogenates (pools of eight individual fish each) were provided by the German Environmental Specimen Bank, representing different lipid contents: 1.2% for perch (*Perca fluvialis)* fillet (lowest-lipid content tissue, LLT), 3.4% for bream (*Abramis brama)* fillet (medium-lipid content tissue, MLT) and 6.1% for bream carcasses (highest-lipid content tissue, HLT). For details, see Fliedner et al. (2018).

2.2. Tissue extraction

The silicone was cleaned using Soxhlet extraction with two aliquots of ethyl acetate (Merck, LC grade) for 8 + 8 h before use and dried. Then 400 g of homogenate from each pool were arranged in alternating layers with silicone sheets (SSP-M823, Special Silicone Products), with two different thicknesses: 125 μm for LLT and 250 μm for MLT and HLT ("fish lasagna", Fig. S1). The maximum mass of silicone was calculated for each sample, based on its lipid content, in order to ensure negligible depletion (sampling less than 5% of the pollutants (Jahnke et al., 2009), for silicone mass and dimensions see Table S1).

The samples were kept at 4 °C during the experiment for up to 7 days, and the relocation of the silicone sheets was carried out manually, every three hours (5 relocations per day, static overnight). For each relocation, all the tissue was removed, thoroughly mixed, and the layers were prepared again, ensuring maximum contact of the tissue with the surface of the silicone. For all the samples, silicone sheets were collected at 7 time points to determine uptake kinetics over the course of 5 or 7 days (for details, see Table S1), with additional triplicates taken at the last sampling occasion to confirm equilibrium by comparing the concentration in sheets with different mass of silicone (Maenpää et al., 2011). The details of total extraction are given in Fliedner et al. (2018).

2.3. Silicone extraction and chemical analysis

Details about the reagents and preparation of analytical standards are given in Text S1-1. After exposure, the silicone sheets were extracted twice with ethyl acetate (1 mL of solvent per 0.1 g of silicone), spiked with 2 ng of the 7 stable isotope-labelled "indicator" PCBs (10 μL of 0.2 ng/μL in ethyl acetate) as internal standards: $^{13}C_{12}$—labelled PCBs 28, 52, 101, 118, 153, 138 and 180 (Wellington, Canada). The clean-up of the extracts was carried out using Supelclean™ EZ-POP NP cartridges (Supelco, Sigma-Aldrich, Text S1-2). The analysis and quantification of 11 PCBs, 8 PAHs and 1 PBDE (log K_{OW} ranging between 3.9 and 7.8, Table S2), were achieved with a gas chromatograph coupled to a high-resolution mass spectrometer (GC-HRMS Orbitrap Q-Exactive, Thermo Fisher, Text S1-3).

3. Results and discussion

3.1. Time series experiments

The uptake kinetics were assessed by means of time series for all three homogenates (1.2 (± < 0.1) %, 3.4 (± < 0.1) % and 6.1 (± 0.6) % lipid), fitting the measured concentrations in silicone at different times to a one phase exponential curve, $y = y_{max}*(1-e^{-k*x})$ (GraphPad Prism 7.04, San Diego, CA), where y is the concentration at a certain time and y_{max} is the same parameter at equilibrium (in the plateau). Figs. 1a and S2 show that equilibrium was reached in less than 3 days for 20 HOCs in all the tissues. Several PAHs (Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, Benzo[ghi]perylene, Dibenz[a,h]anthracene) were not detected, and Chrysene was only detected in LLT. In addition, the plateau of the curve remained constant until the end of the experiment (7 days for LLT and MLT, 5 days for HLT), demonstrating unaltered chemical activity of the tissues throughout the experiment. In the case of LLT and MLT the kinetic uptake and the fit between the two curves were identical when considering either the time or the number of relocations, because for those tissues the silicone sheets sampled each 24 h corresponded to the silicone

sheets sampled after 5 relocations at 5 relocations per day, hence showing identical data. However, for HLT, the fit of the curves was better considering time (Fig. S2, c and d). A possible explanation could be that, in the case of tissues with higher lipid content (HLT), it is not necessary to relocate the samplers to attain equilibrium, because of the presence of sufficient lipids to function as "transporter agents" (Jahnke et al., 2009; Rusina et al., 2017), as can be observed in Table S2 (number of relocations for HLT). Contrarily, for the tissues with lower lipid contents, LLT and MLT, the number of relocations emerges as a decisive factor to achieve equilibrium. In previous studies samplers did not reach equilibrium within 7 days when there was static contact without relocation of the silicone layers (Jahnke et al., 2009) or insufficient frequency of relocations (Rusina et al., 2017) in homogenised tissue with medium or low lipid content.

Table S2 gives the parameters related to the first order association kinetics. For the 20 compounds that were determined in the tissues, the time when 95% of equilibrium was reached, t_{95}, was between 3.02 (Acenaphthylene, HLT) and 59.6 h (PCB 180, LLT). We did not observe any clear relationship between t_{95} and log K_{OW} (Fig. 1b and S3), in agreement with published results (Jahnke et al., 2009).

3.2. At equilibrium

Four silicone sheets with different mass were used for each tissue type to confirm the concentration at equilibrium at the end of the experiment (5 days for HLT, 7 days for LLT and MLT). Linear regressions using the different masses of silicone at equilibrium and the amount of analyte in the silicone (calculated from the concentration in the extract) were used to calculate the equilibrium partitioning concentrations in silicone as the slope of the linear regression (Eq. (1), Maenpää et al., 2011):

$$m_{analyte} = m_{silicone} * C_{analyte} + b \quad (1)$$

These concentrations were compared with the concentrations at equilibrium obtained from the plateau of the kinetic uptake curves, calculated as described above. For those curves where the fitting is indicated as *ambiguous* in Table S2, this affected the calculation of t_{95}, but not the concentration at equilibrium. When the curve fitting was ambiguous, the parameter k of the one phase exponential curve could not be determined, with the 95% CI being very wide, impairing the calculation of the t_{95}. However, the fitting of the curve, despite being ambiguous, allowed us to obtain the parameter y_{max} (even without 95% CI), which we used to calculate the concentration. Fig. S4 shows that both approaches agreed well, confirming the attainment of equilibrium in less than 5 (HLT) or 7 days (LLT and MLT), which is in accordance with results from the time series experiments, cross-validating these data.

For the 7 indicator PCBs, results from total solvent extraction, normalised to the lipid fraction, of the identical tissues have been published (Fliedner et al., 2018). These concentrations were normalised to the lipid fraction (1.2%, 3.4% and 6.1% lipid), given as concentrations in lipid, C_L, and compared with the concentrations in silicone translated to concentrations in lipids, $C_{L\text{-}eq}$ (Eq. (2), Jahnke et al., 2011).

$$C_{L-eq} = C_{Sil \rightleftharpoons Fish} * D_{Lip/Sil} \quad (2)$$

where $C_{L\text{-}eq}$ ($mg_{analyte}/kg_{lipid}$) was calculated using the concentration of chemical in the silicone at equilibrium with the fish tissue, $C_{Sil \rightleftharpoons Fish}$, and the compound's lipid/silicone distribution coefficients ($D_{Lip/Sil}$) from Jahnke et al., (2008) (Table S3).

Fig. S4 shows that results obtained using both methodologies deviated by less than a factor of 1.3 ($R^2 = 0.97$ linear regression, excluding outliers, $n = 1$, Fig. 1), showing good agreement for MLT and HLT. However, two PCBs in lean tissue showed higher concentrations in the PSD-based samplers than in extracts generated by total extraction, PCB 138 and PCB 153. This deviation could be due to the potential differences in the sorptive capacities of the different lipids and/or the role that other sorptive phases besides lipids, like proteins (DeBruyn and Gobas, 2007), play in lean tissue, resulting in a less accurate estimation via lipid normalisation. Further research is necessary to unravel this issue. Despite the good agreement using both methodologies for MLT and HLT, a low bias can be observed in the figure, indicating that a slightly higher concentration was obtained using total solvent extraction than using passive sampling. This apparent bias for the medium/highest lipid content tissues may be within the uncertainties inherent to the methods and should be further studied in future work. Fig. S5 shows the correlation of the average t_{95} across compounds in each tissue with the lipid content. A negative correlation can be observed, with the time needed to achieve equilibrium being inversely correlated with the lipid content of the tissue. This

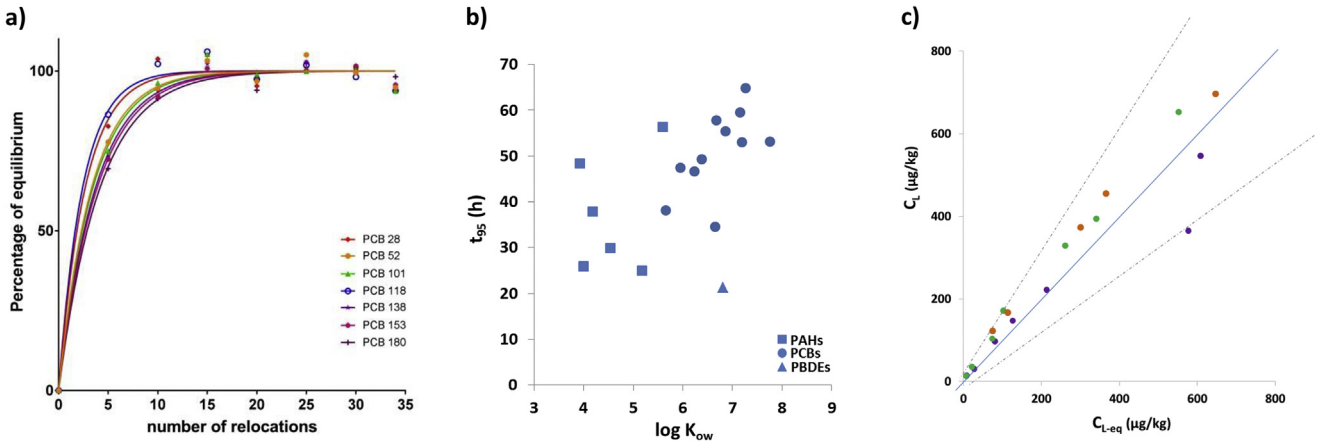

Fig. 1. Results for homogenised lean tissue: a) uptake kinetics vs. number of relocations for the 7 indicator PCBs, b) Relationship between t_{95} [hours] and log K_{ow} for all compounds and c) Lipid-normalised concentrations from total extraction (C_L) vs. concentrations in silicone transformed into equilibrium partitioning concentrations in lipids ($C_{L\text{-}eq}$), in $\mu g_{compound}/kg_{Lipid}$. Purple: lowest-lipid content tissue, LLT ((1.2% lipid), green: medium-lipid content tissue, MLT (3.4% lipid) and orange: highest-lipid content tissue, HLT (6.1% lipid). The solid line indicates 1:1 perfect fit relationship, and the dashed lines indicate 1:1.5 and 1.5:1 relationships (used to identify outliers). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

relationship can help to predict the time needed to achieve equilibrium in tissues with different lipid contents.

4. Conclusions

Using manual relocations of the PSDs, equilibrium was rapidly achieved between the silicone and non-spiked homogenised tissues with 1.2–6.1% lipid for a wide range of nonpolar compounds with log K_{OW} between 3.9 and 7.8. The passive samplers reached equilibrium with the tissues in less than 3 days, providing a new procedure which opens up for applying ESDs in homogenised tissues of low lipid content and also enables carrying out passive equilibrium sampling of small quantities of biota: for future work, time series analysis is no longer needed, making it possible to work directly at equilibrium and thus smaller amounts of tissue are required. Further optimisation is needed to automatise the procedure in order to reduce the time that laboratory staff has to invest, as well as to minimise the risk of contaminating samples.

Competing interests statement

The authors have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2018.12.134.

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