



In-vivo passive sampling to measure elimination kinetics in bioaccumulation tests

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ARTICLE INFO

Article history:

Received 22 November 2011

Received in revised form 15 February 2012

Accepted 16 February 2012

Available online 17 March 2012

Keywords:

Passive sampling

Fish

Bioconcentration test

OECD 305

Bioaccumulation test

ABSTRACT

The application of in-tissue passive sampling to quantify chemical kinetics in fish bioconcentration experiments was investigated. A passive sampler consisting of an acupuncture needle covered with a PDMS tube was developed together with a method for its deployment in rainbow trout. The time to steady state for chemical uptake into the passive sampler was >1 d, so it was employed as a kinetically limited sampler with a deployment time of 2 h. The passive sampler was employed in parallel with the established whole tissue extraction method to study the elimination kinetics of 10 diverse chemicals in rainbow trout. 4-n-nonylphenol and 2,4,6-tri-*tert*-butylphenol were close to or below the limit of quantification in the sampler. For chlorpyrifos, musk xylene, hexachlorobenzene, 2,5-dichlorobiphenyl and p,p'-DDT, the elimination rate constants determined with the passive sampler method and the established method agreed within 18%. Poorer agreement (35%) was observed for 2,3,4-trichloroanisole and p-diisopropylbenzene because fewer data were obtained with the passive sampling method due to its lower sensitivity. The work shows that in-tissue passive sampling can be employed to measure contaminant elimination kinetics in fish. This opens up the possibility of studying contaminant kinetics in individual fish, thereby reducing the fish requirements and analytical costs for the determination of bioconcentration factors.

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1. Introduction

Fish bioconcentration experiments play an important role in the regulatory assessment of chemicals. In these experiments the uptake and elimination kinetics of a chemical is studied by exposing a group of fish to water containing the chemical, sacrificing several fish at a number of intervals during the exposure, transferring the remaining fish to chemical free water, and again sacrificing these fish at intervals during this depuration part of the experiment. The sacrificed fish are then homogenized, the chemical residue in each fish is determined, and the results are used to assemble a picture of the chemical uptake and elimination during the experiment, which then serves to determine the bioconcentration factor (BCF). The OECD 305 guideline for bioconcentration in a flow-through fish test, a recognized standard method for this kind of experiment, requires on the order of 100 fish to study one chemical (OECD, 1996). Given that regulations foresee the testing of large numbers of chemicals, many fish are required. For instance, of the 2.6 million test animals that were estimated to be needed for the implementation of the European chemicals management program REACH, ~2.5% or ~65 000 were fish for bioconcentration experiments (Van der Jagt et al., 2004). Finding alternative methods that require fewer fish is desirable.

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In-tissue passive sampling may open possibilities to reduce the number of fish required, since it would then be possible to study kinetics in a single fish, rather than sacrificing fish at 10 or more time points. Passive sampling, which has long been applied in air, water and biological fluids (Huckins et al., 1990; Cao and Hewitt, 1991; Krogh et al., 1995), was extended to biological tissue when Ossianer et al. (2008) reported that repeatable PCB signals were obtained when solid phase microextraction (SPME) fibres were inserted into lipid-rich tissue and then thermally desorbed in a GC/MS injector. When applied to harbour porpoise blubber the PCB signal from the SPME fibre correlated well with the PCB concentration in the blubber. This application of passive sampling to tissue was further explored by Jahnke and co-workers. Using polydimethylsiloxane (PDMS) sheets as their sampling material, they demonstrated the absence of fouling effects following insertion of PDMS into a wide range of lipid rich biological matrices (Jahnke and Mayer 2010); they showed that the PDMS quickly approached a partitioning equilibrium when inserted into the muscle tissue in lipid-rich fish, but that the approach to equilibrium took longer than several days when inserted into lean fish (Jahnke et al., 2009); and they showed that the concentrations in PDMS inserted into muscle tissue of fatty fish, when converted into lipid normalized concentrations using PDMS/lipid partition coefficients, were very similar to the lipid normalized concentrations determined by traditional tissue extraction (Jahnke et al., 2011).

In-tissue passive sampling was extended from *in vitro* to *in vivo* conditions by Zhou et al. (2008). They inserted PDMS coated SPME fibres into the dorsal muscle of rainbow trout that had been ex-

posed to water containing different concentrations of several pharmaceutical chemicals. After 20 min the fibres were removed, extracted, and analysed for the pharmaceutical chemicals. A linear relationship between the concentration in water and the concentration in the passive samplers was observed, and the concentrations in the passive samplers were higher in fish that had been exposed for 14 d compared to 7 d. The method was applied to quantify the concentrations of pharmaceutical chemicals, bisphenol A and atrazine in fish exposed to wastewater treatment plant (WWTP) effluent and in wild fish from a WWTP recipient (Wang et al. 2011). This work was extended to show that the same sampler could be used to simultaneously sample different tissues by placing two distinct PDMS segments on the fibre (Zhang et al., 2010). These authors point out that the method offers the opportunity to study bioaccumulation kinetics in individual fish.

In this work we explore the possibility of applying in-tissue passive sampling to quantify chemical kinetics in fish bioconcentration experiments. A passive sampler consisting of an acupuncture needle covered with a PDMS tube is described. The chemical uptake kinetics into the passive sampler and the reproducibility of the method are first examined. Then the passive sampler is employed in parallel with the established whole tissue extraction method to study the elimination kinetics of a broad range of chemicals in rainbow trout, and the results from the two approaches are compared.

2. Methods

2.1. Chemicals

Ethyl acetate ('Pestiscan') was purchased from Labscan Ltd., Dublin, Ireland, acupuncture needles (0.35 × 25 mm) from Helio Medical Supplies Inc., San Jose, CA, USA, hypodermic needles (Ø 0.9 × 40 mm) from B. Braun, Melsungen, Germany, and silicone tubing (0.3 mm i.d., 0.16 mm wall thickness) from A-M Systems, Inc., Sequim, WA, USA. The water was of milli-Q grade from a milli-Q ultrapure water system, MilliQ PLUS 185 from Millipore Stockholm, Sweden. The chemicals to which the fish were exposed, their sources, the internal standards used for the analysis of these chemicals and their sources are listed in Table S1 in the Supporting Information.

2.2. Fish

Rainbow trout (*Oncorhynchus mykiss*) were purchased from Vilstena fiskodling, Fjärdhundra, Sweden (uptake kinetics and reproducibility experiments) and Näs Fiskodling AB, By Kyrkby, Sweden (fish elimination experiment) when they had a mass of 120–140 g (uptake kinetics experiment), 150–180 g (reproducibility experiment) and 40–80 g (fish elimination experiment). They were held in the laboratory aquarium facility for at least 2 weeks before the experiments. Before and during the experiments they were fed daily at a rate of 1% of body mass per day.

Prior to the experiments the fish were transferred to an aquarium containing elevated concentrations of the test chemicals, or injected with chemicals dissolved in corn oil. The elevated concentrations were generated using a passive dosing system as outlined in Adolfsson-Erici et al. (2012). The water exchange rate in the aquarium was six times the water volume in the aquarium per day. After exposures lasting from 3 to 24 d the fish were transferred to an aquarium containing clean water and the experiments were conducted.

2.3. Preparation, deployment and extraction of the passive samplers

Silicone tubing was cut in 2.5 cm pieces and soaked in ethyl acetate for several hours. A piece of the wet tubing was drawn over

the acupuncture needle and pushed up so that the whole needle and 1–2 mm of the handle were covered by the tube (Fig. 1a). The solvent was allowed to evaporate which caused the silicone tubing to shrink so that it sat firmly on the needle.

Prior to deployment of the passive sampler the fish was transferred from the aquarium to a bucket, and from there to a sampling box. For the first experiments the samplers were inserted while the fish were held on the lab bench, while for the fish elimination experiment a sampling box was used. The sampling box (220 mm long × 75 mm wide × 50 mm deep) was just large enough to hold the fish. The front part of the box, which contained the head of the fish, was covered to reduce the stress. The water level was high enough to cover the gills. Massage gloves were used for all handling of the fish. A small hole was punctured in the skin of the dorsal muscle using a hypodermic needle. The passive sampler (i.e. the silicone coated acupuncture needle) was inserted through the hole into the muscle until all of the silicone tube was below the skin. The lip formed by the upper end of the silicone on the handle of the needle prevented the needle from falling out of the fish. The fish was then transferred back to the bucket (reproducibility and fish elimination experiments) or to the aquarium (kinetic experiment). Typically two samplers were deployed, one on each side of the dorsal fin (see Fig. 1b), but in one experiment four were deployed (see below). With two people working together the procedure took approximately 1 min. No signs of oxygen stress were observed after deployment.

After the different periods of time in the bucket or aquarium the fish was transferred back to the sampling box and the passive samplers were pulled out. Each passive sampler was wiped clean with a lint-free tissue and inserted in a 0.4 mL glass GC-vial containing 300 µL of internal standard in ethyl acetate. After a couple of minutes the silicone tube slid off the acupuncture needle. It was cut in half and re-inserted in the vial. After 30 min of extraction, the silicone was removed, allowed to dry overnight, and weighed. The extract was concentrated to ~100 µL using a stream of nitrogen.

Some of the fish were kept for up to 4 weeks after recovery of the needles. No adverse effects of the sampling procedure were observed.

2.4. Preparation of fish samples

The whole fish was homogenized using a Kenwood multipro food processor. The homogenate was extracted by shaking it with 220 mL n-hexane/acetone (5:1) in a 1 L Erlenmeyer flask on a KS 501 digital shaker for 2 h. The homogenate was allowed to stand for sedimentation, after which the organic phase was decanted. The extraction procedure was repeated with 100 mL hexane. An aliquot corresponding to 20 g fish was taken from the combined organic phases, reduced to 40 mL, and washed with 30 mL 9% sodium chloride in 1 M phosphoric acid. The aqueous phase was re-extracted with 10 mL hexane. The combined organic phases were then reduced to 8 mL. 100 µL of the solution was transferred to a pre-weighed test tube and evaporated under a stream of nitrogen to dryness, after which the fat content was determined. The residue was redissolved in 2 mL hexane containing the internal standards and treated with 4 mL sulphuric acid monohydrate for lipid removal. The organic phase was reduced to 1 mL and analysed by GC/MS.

2.5. Instrumental analysis

Gas chromatographic separation was performed on a CarloErba GC8000 gas chromatograph using a 30 m × 0.25 mm i.d., DB-5MS J&W Scientific column with a 0.25 µm film-thickness. Injections (1 µL) were made in the splitless mode (260 °C) with the GC oven at 80 °C. This was held for 2 min, and then raised to 300 °C at a rate

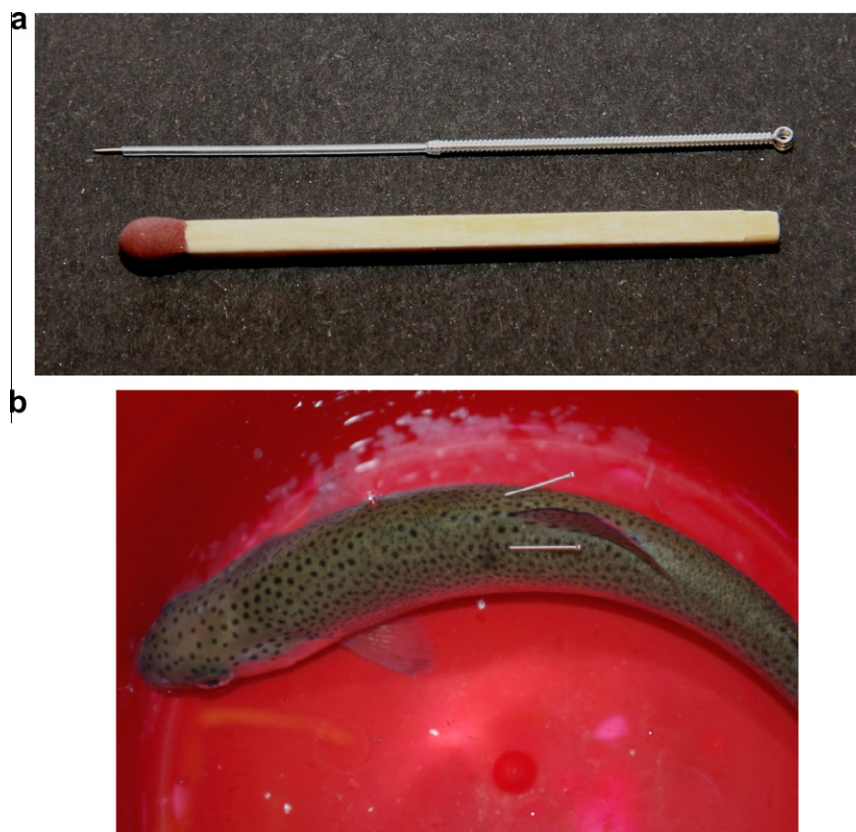


Fig. 1. (a) Passive sampler, showing the acupuncture needle coated with the silicone tube; (b) two passive samplers deployed in a rainbow trout.

of $10\text{ }^{\circ}\text{C min}^{-1}$. The carrier gas was helium and the transfer line temperature was $310\text{ }^{\circ}\text{C}$. Mass spectrometric determination was made using a Finnigan Voyager low resolution mass spectrometer in SIM mode employing electron impact ionization. The concentrations in the passive samplers were normalized to the mass of the silicone tube, whereas the concentrations in the fish were normalized to the lipid content.

2.6. Experiments

2.6.1. Kinetics of chemical accumulation in the passive samplers

Four rainbow trout (average mass 127 g) were injected intraperitoneally with hexachlorobenzene, PCB 53, and DDT dissolved in corn oil. The injection volume was 0.5 mL and each fish received ~ 5 , 4 and $6\text{ }\mu\text{g g}^{-1}$ body weight of the substances, respectively. After 1 d in clean water, two passive samplers were deployed in each fish. After the foreseen deployment time the samplers were recovered from all four fish and two new samplers were immediately deployed in the same fish using new holes through the skin. Different deployment times were used, namely 5, 15, 60, 180, 1080 and 3600 min. One of the fish died before completion of the experiment, perhaps due to injuries from the intraperitoneal injection. Each sampler was extracted and analysed independently.

2.6.2. Reproducibility of the passive sampling method

Four rainbow trout were exposed to pentachlorobenzene, PCB 53, chlorpyrifos, endosulfan and DDT for 3 d. After 1 d in clean water, four passive samplers were deployed in the dorsal muscle of each fish. After 2 h the samplers were recovered, extracted, and analysed twice. The mean value of the two analyses is reported.

2.6.3. Application of the method to studying chemical elimination in fish

A total of 36 rainbow trout were exposed to 2,3,4-trichloroanisole, p-diisopropylbenzene, musk xylene, chlorpyrifos, 4-n-nonylphenol, 2,4,6-tri-*tert*-butylphenol, pentachlorobenzene, PCB 9, hexachlorobenzene, and p,p'-DDT for 24 d, after which they were transferred to clean water. Four fish were sacrificed at each of nine time points: immediately, and after 10 h, 1 d, 2 d, 4 d, 10 d, 16 d, 30 d, and 57 d. Only 3 fish were sacrificed on day 30 as one of the fish had jumped out of the aquarium. The fish were homogenized and analysed as described above. Immediately prior to being sacrificed, two passive samplers were deployed in each fish, recovered after 2 h, extracted, and analysed.

Ethical approval for the experiments was obtained from Stockholms Norra Djurförsöksetiska Nämnd (permit # N 31/09).

3. Results and discussion

3.1. Quality assurance

Stable isotopes of the test chemicals were used as internal standards in the analytical procedures with a few exceptions (2,3,4-trichloroanisole, p-diisopropylbenzene and endosulfan). The recoveries of the internal standards from the fish samples were $>80\%$. The repeatability of the fish extraction was tested by analysing five replicates. The relative standard deviation of the concentrations was $<4\%$ for all of the substances except 4-n-nonylphenol (13%). Repeated extraction of the silicone tubing revealed that $>98\%$ of the extractable quantity of all chemicals was present in the first extract. Procedural blanks were determined with each round of extractions. Quantification limits were determined as five times the blanks or five times the noise and ranged

from 2–10 ng g⁻¹ silicone, depending on the chemical. At the time of the kinetic experiment, the method for needle insertion was not fully optimized and a few samplers fell out of the fish during the deployment period. These samplers were not analysed. No peak distortion or shifts in analyte retention time were observed, indicating that matrix effects were minor.

3.2. Kinetics of chemical accumulation in the passive samplers

The uptake kinetics in the passive sampler was studied by deploying samplers in the same fish for different periods of time. The fish had been exposed to hexachlorobenzene, PCB 53, and DDT. The passive samplers were deployed for periods ranging from 5 min to 3600 min. The results for one fish are shown in Fig. 2, and the results for three further fish are shown in Fig. S1 in the Supporting Information. In most cases near linear uptake kinetics was observed up to a deployment time of 1080 min, while there was little change in the concentration in the passive sampler between a deployment time of 1080 min and 3600 min. This indicates that it takes at least a day for the passive sampler to approach steady state.

The slow uptake kinetics for these passive samplers is consistent with recent work on in-tissue passive sampling of organic contaminants in fish. Jahnke et al. (2009) measured the uptake kinetics of PDMS discs inserted into fish muscle tissue in the laboratory. They found that the uptake kinetics was rapid for eel, a lipid-rich fish. However, the kinetics was slow in a number of leaner fish species including sea trout, for which a steady state was not approached during a 7 d exposure. On the other hand, the slow uptake kinetics stand in contrast to the work of Zhang et al. (2010), who reported that equilibrium was achieved within 15 h between their PDMS samplers and rainbow trout dorsal muscle for a range of pharmaceutical chemicals under *in vitro* conditions. The longer equilibration times in our study could be due to different properties of the chemicals tested or different dimensions of the sampler.

The slow uptake kinetics meant that the passive sampler could only be used as a kinetic sampler in the rainbow trout. In order for it to perform as an equilibrium sampler, a deployment of 1 d or longer would be required. This is not compatible with usage of the passive sampler in fish bioaccumulation experiments, where the kinetics of chemical accumulation and elimination in the fish is measured on a time scale of several hours.

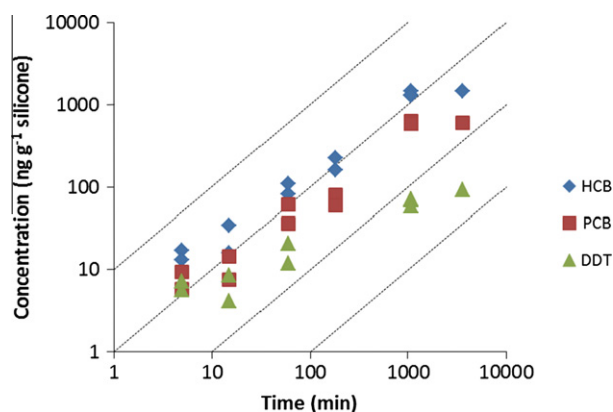


Fig. 2. Concentrations of hexachlorobenzene, PCB 53, and p,p'-DDT in passive samplers in Fish 3 plotted as a function of the time of deployment. Two passive samplers were deployed during each period with the exception of the 1080 h period when four samplers were used. The diagonal lines show the slopes of linear relationships between concentration and time on the log–log plot.

For use as a kinetic sampler a fixed deployment time of 2 h was chosen. This was sufficiently long to minimize errors caused by uncertainty in the deployment time, while being short enough to allow reasonable temporal resolution of the measurements in the fish. A longer exposure time also favours the sensitivity of the method, as more chemical accumulates in the sampler, giving a stronger signal for a given chemical activity in the fish.

3.3. Reproducibility of the passive sampling method

The reproducibility of the methodology was evaluated by deploying four samplers simultaneously in each of four fish which had been exposed to pentachlorobenzene, PCB 53, chlorpyrifos, endosulfan, and DDT. Fig. 3a shows the relative standard deviation of the four measurements made in each fish. It ranged from 13% to 56%. The variability was similar among most of the chemicals, but lower for DDT (10–22%). There was considerable variability between the fish, with fish 3 showing the best reproducibility for most chemicals.

It was hypothesized that differences in the uptake rate between samplers was a major source of the variability. To test this hypothesis, the concentrations of all of the chemicals were normalized to the concentration of pentachlorobenzene. This normalization eliminated the variability caused by differences in the uptake rate of pentachlorobenzene. The relative standard deviation of these normalized concentrations was much lower, ranging from 5–20% with the exception of DDT in fish 4 (38%) (Fig. 3b). This confirmed that differences in the uptake rate or mass transfer of chemical to the sampler were a major source of variability in the method. Furthermore, it showed that this variability could be reduced by normal-

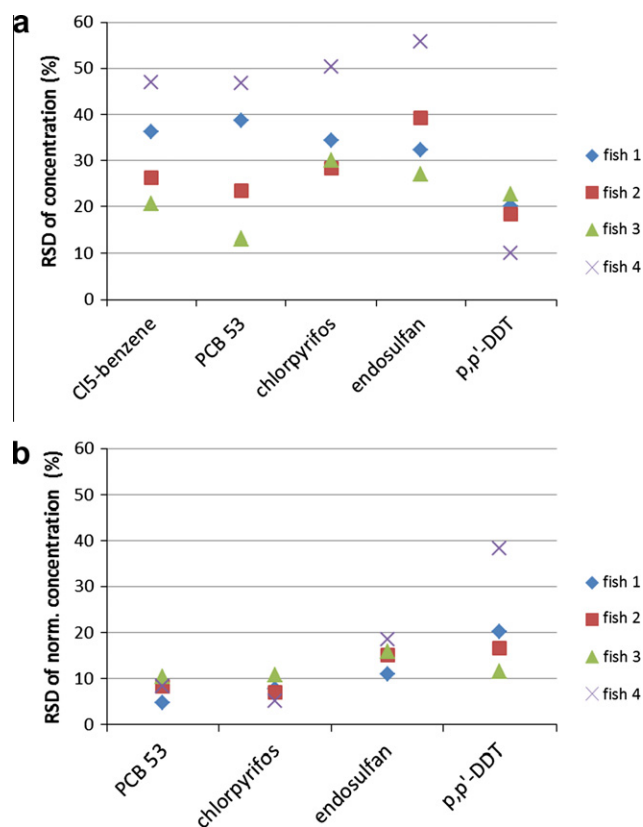


Fig. 3. Reproducibility of the passive sampling method showing the relative standard deviation of (a) concentration in the sampler; (b) concentration normalized to the concentration of pentachlorobenzene in the same sampler; for five chemicals in four fish. Four samples were collected simultaneously in each of the fish.

izing the concentrations of the analyte to the concentration of another lipophilic chemical in the fish. Differences in sampler uptake rate may have resulted from differences in the composition of the tissue that was in direct contact with or very close to the samplers. Zhang et al. (2011) proposed the use of fibres with pre-loaded standards to compensate for differences in mass transfer rates during sampling, and they applied this method in fish tissue. In the context of studying contaminant elimination kinetics it is perhaps more suitable for use a chemical in the fish with a negligible elimination rate as an internal standard, as this would correct for both variability in the sampling rate and dilution of the chemical due to fish growth. Growth dilution can increase the apparent elimination rate of hydrophobic chemicals and thus result in underestimation of the BCF (Branson et al., 1975). The use of an internal standard to correct for growth dilution in fish BCF experiments will be explored in another paper.

3.4. Application of the method to studying chemical elimination in fish

In Fig. 4 the elimination kinetics of 2,3,4-trichloroanisole, chlorpyrifos, musk xylene and PCB 9 in rainbow trout as measured with the passive sampler are compared with the kinetics measured using whole fish analysis. The results for p-diisopropylbenzene, pentachlorobenzene, hexachlorobenzene and DDT are shown in Fig. S2. 4-n-nonylphenol was not detected in the passive samplers, and the results for 2,4,6-tri-*tert*-butylphenol were highly erratic. These two phenolic compounds did not accumulate in the silicone to a sufficient extent to allow the passive samplers to be used to study their behaviour. PDMS is known to have a comparatively low sorption capacity for phenolic compounds (Sprunger et al., 2007).

The two methods gave comparable results for the eight compounds that could be quantified in the passive samplers. The whole fish analysis and the passive sampling showed first order elimination kinetics (as reflected in a linear relationship between \ln concentration and time) for most chemicals (Figs. 4 and S1). For 2,3,4-trichloroanisole, a more complex elimination behaviour was observed from the whole fish analysis, with an initial rapid de-

crease followed by nearly constant concentrations. This was mirrored by the passive sampling results. The first order elimination rate constants taken from the linear regression lines in the figures are given in Table 1. Large differences (>40%) were observed for pentachlorobenzene, which can be attributed to the poor precision of the method for this chemical in this experiment, and for 2,3,4-trichloroanisole and p-diisopropylbenzene, which was due to the shorter time period over which passive sampling data could be gathered for these two substances (see below). For PCB 9 the difference was 18%, and for the remaining four chemicals the two methods agreed within <10%. These results show that the in-tissue passive sampling method can be used to measure the elimination kinetics of some lipophilic chemicals in fish.

As a plausibility check for the passive sampling method, the lipid/silicone concentration quotients were calculated from the passive sampling and whole tissue results and compared with lipid/PDMS equilibrium distribution quotients from the literature. The median value of the concentration quotient ranged between 100 and 360 L silicone g^{-1} lipid (Fig. S3). These values are considerably higher than the lipid/PDMS equilibrium distribution quotients of 13–55 measured for several PCB congeners and selected organochlorine pesticides including p,p'-DDT and hexachlorobenzene (Jahnke et al., 2008). Since the passive samplers did not approach a steady state (see above), the concentrations in the silicone would have been below the concentrations expected at equilibrium with the fish, and hence concentration quotients in excess of the equilibrium distribution quotients were expected.

One disadvantage of the passive sampling method was that it had a higher limit of quantification than the whole fish extraction method. This was reflected in fewer passive sampling data being collected during the elimination experiment for 2,3,4-trichloroanisole and p-diisopropylbenzene. Already after 4 h and 1 h, respectively, their concentrations in the passive samplers fell below the limit of quantification. The elimination rate constants therefore had to be evaluated based on a shorter time period, which was likely a major reason for the large differences compared to the rate constants obtained from whole fish analysis (Table 1). The higher limit of quantification can be explained by the smaller equivalent

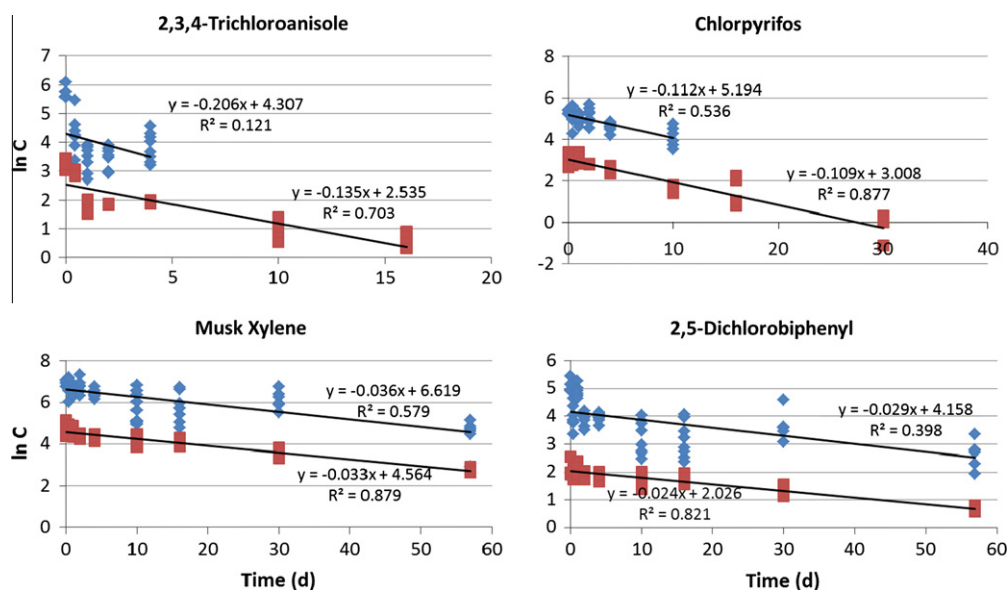


Fig. 4. Comparison of the passive sampling method (blue diamonds) and wet extraction of whole fish homogenates (red squares) for the determination of the elimination kinetics of: (a) 2,3,4-trichloroanisole; (b) chlorpyrifos; (c) musk xylene; (d) PCB 9 measured in rainbow trout. The natural logarithm of the concentration (ng g^{-1} silicone and $\mu\text{g g}^{-1}$ lipid) is plotted versus time. The whole fish homogenate data are from four fish at each time point (exception $t = 30$ d, three fish). The passive sampling data are from two samplers deployed in each of the same fish, giving 6–8 samples per time point. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Comparison of the first order elimination rate constants (in d^{-1}) in rainbow trout determined using the passive sampling method and whole fish extraction. The best estimate and its standard error are shown.

	Passive sampling	Whole fish extraction
2,3,4-Trichloroanisole	0.205 ± 0.097	0.135 ± 0.017
p-Diisopropylbenzene	0.460 ± 0.209	0.300 ± 0.023
Musk xylene	0.036 ± 0.004	0.033 ± 0.002
Chlorpyrifos	0.112 ± 0.017	0.109 ± 0.008
Pentachlorobenzene	0.009 ± 0.008	0.023 ± 0.002
PCB 9	0.029 ± 0.005	0.024 ± 0.002
Hexachlorobenzene	0.016 ± 0.004	0.017 ± 0.002
p,p'-DDT	0.015 ± 0.005	0.016 ± 0.002

amount of sample extracted by the passive sampler. With a lipid/silicone concentration quotient of the order of 200 and ~6 mg of silicon, the passive sampler extracts the amount of chemical present in ~30 µg of lipid. In the whole fish extraction several grams of lipid are extracted, and although only ~1% of this was cleaned up and analyzed, it nevertheless amounts to several orders of magnitude more lipid than was extracted by the passive sampler.

The variability in the passive sampler measurements was in general greater than the variability in the whole fish extraction measurements (see Figs. 4 and S1). As a quantitative measure of the variability, the relative standard deviation of the measurements at a given time point was calculated and then averaged across all time points for each chemical (see Table 2). For the whole fish extraction the average relative standard deviation was ~20% for most of the chemicals, while for the passive sampling method it ranged from 25–50% for most chemicals. The greater variability implies that achieving the same level of certainty in the mean concentration at a given time point will require the analysis of more samples when using the passive sampling method. Since the standard error of the mean is proportional to the square root of the number of samples, a two times higher variability for the passive sampling method would require the analysis of four times as many samples. In this study the standard error of the elimination rate constant was higher when determined via passive sampling (see Table 1). Possibilities for increasing the precision of the method were discussed in Section 3.3.

3.5. Applicability of the method to bioconcentration experiments

The good agreement between the rate constants determined with the passive sampling and whole fish extraction demonstrate that the new method can be employed for the measurement of contaminant elimination kinetics in fish. The method was tested for both persistent and metabolized chemicals with log octanol/water partition coefficients ranging from 4.1 to 6.9. *In-vivo* passive sampling has also been shown to be useful for pharmaceuticals (Wang et al., 2011). For any given chemical the applicability of

Table 2

Comparison of the variability of the passive sampling method and whole fish extraction. For each chemical the relative standard deviation (in %) of the measurements at a given time point were determined. The relative standard deviations were then averaged across all time points for each chemical.

	Passive sampling	Whole fish extraction
2,3,4-Trichloroanisole	46	17
p-Diisopropylbenzene	15	36
Musk xylene	39	22
Chlorpyrifos	29	30
Pentachlorobenzene	53	18
PCB 9	40	20
Hexachlorobenzene	37	20
p,p'-DDT	29	21

in-vivo passive sampling for the determination of elimination kinetics will depend on the chemical specific sampling rate and sensitivity of the analytical method on the one hand and the desired level of dosing to the fish on the other. For eight of the compounds studied here the sampling rate was similar (Fig. S3), while for the two phenols it was at least an order of magnitude lower.

One disadvantage of the passive sampling method is its comparatively low sensitivity, which means that the fish must be exposed to higher contaminant concentrations in order for the kinetics to be measurable. The quantification limits for the sampler of 2–10 ng g⁻¹ silicone corresponded to concentrations in the fish of 0.3–1.5 µg g⁻¹ lipid.

A second disadvantage is the lower method precision, which means that a larger number of samples must be collected. However, it may be possible to improve the precision by normalizing the contaminant concentrations to a substance that is known to have negligible elimination in the fish, as this should reduce variability caused by different sampling rates.

On the other hand, the passive sampling method has a number of significant advantages. One is the very simple sample preparation, which means lower analytical costs. A second is the potential to reduce the number of test animals required. For instance, instead of sacrificing 36 fish to study elimination kinetics as in this experiment (four fish at each of nine time points), the experiment could have been done using eight fish that were each sampled with two passive samplers at each of the nine time points. The passive sampling method also opens up the possibility of studying contaminant kinetics in individual fish, rather than having to rely on measurements from a collection of different fish to obtain a single rate constant. This could reduce the uncertainty in the rate constant by eliminating inter-individual variability. The experiment on the kinetics of chemical accumulation in the passive sampler showed that the same fish can be sampled repeatedly.

The application of passive sampling to quantify elimination kinetics may be limited for chemicals that are slowly distributed among the tissues of the fish. The chemical uptake by the sampler is presumably driven by the activity of the chemical in the sampled tissue. It is possible that this activity could decrease over time as a result of sequestration of the chemical into other tissues without the chemical actually being eliminated from the fish. This would be expected to yield a non-linear relationship between log concentration and time. No evidence of this was observed for the chemicals studied here.

A simplifying feature of employing passive sampling for measuring elimination kinetics is that no calibration of the method is required. The calculation of the first order rate constant for elimination is based only on the change in the chemical's concentration in the fish; no absolute value of the concentration is required. The study of uptake kinetics requires the measurement of an absolute concentration though, as this is required to calculate the first order rate constant for uptake. Thus, for passive samplers to be used for studying uptake kinetics the concentration in the sampler would have to be calibrated against the concentration in the fish for the chemical of interest. The measurements in the elimination experiment gave similar median calibration factors for the eight chemicals studied here (Fig. S3). Nevertheless a calibration could be necessary for new test chemicals. In this case the time and test animals required for this calibration could negate the advantages provided by the passive sampling during the experiment itself. Consequently, passive sampling is expected to be most useful for studying elimination kinetics.

Acknowledgements

We thank Kerstin Grunder for the extraction and clean-up of the fish samples and Annika Jahnke for providing Fig. 1a. We are

grateful to the European Chemical Industry Council Long-Range Research Initiative for funding.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.chemosphere.2012.02.063.

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