Draft Guidelines for Equilibrium Passive Sampling of Sediments

1 Introduction

1.1 Objective

Passive sampling for sediments is primarly done to estimate the free dissolved concentration the sediment would be in equilibrium with. This is essentially the concentration in the pore-water. The method described here applies to hydrophobic compounds.

1.2 Principle and theory

A reference phase with a known reference phase-water partition coefficient (K_{RW}) is brought in contact with a large amount of sediment in the presence of sufficient water to make it fluid. The system is closed and shaken, thumbled or otherwise agitated to obtain equilibrium between sediment and reference phase. By measuring the concentration in the reference phase the free dissolve concentration in the water phase can be calculated using the K_{RW}

$$C_W = \frac{C_R}{K_{RW}}$$

The original free dissolved concentration in the waterphase is approached more close as the capacity ratio of sampler and sediment is lower. That is a maximal amount of sediment and a minimal size of the reference phase. The capacity ratio can be monitored by the addition of performance reference compounds (PRCs) to the reference phase prior to exposure. During exposure the PRCs will distribute between sediment and reference phase according that capacity ratio. If less that 10% of the added PRC has remained in the reference phase the original free dissolved concentration is also approached within 10%, which is sufficiently precise. By using compounds from different hydrophobicity (i.e.CB10, CB50, CB104, CB145 and CB204) an equal division over the both phases is een indication for equilibrium. The latter compounds will require more time for eqilibrium. It should be noted that for PAH the capacity of sediments is highly variable and no equal distribution should be expected for the different compounds.

1.3 Criteria material selection

Materials used as reference phase should have an open structure and a permeability that is higher than water. (Rusina et al 2006). With a closed structure the sorption would rely ony on surface sorption and when the affinity is about equal to biological meterial the biolayer could easily overrule the uptake by the sampler. Many hydrophobic soft plastics will apply with polyethylene (PE), polyoxymethylate (POM) and siliconerubber (like polydimethylsiloxane, PDMS) being most frequently used. However, POM does not have an openstructure and permeability is absent (Rusina 2006). To achieve equilibrium in the shortest time the thickness of the reference material should be as possible. The lowere limit is mainly determined by practical limitations in handling very thin materials. Additionally a certain volume of reference phase (0.3 –0.5 g) is required to collect sufficient contaminants for analysis. Thin sheets with a large surface would be ideal complicates practical handling and have high risk for tearing. The most stable way of using thin layers is coating a layer of siliconerubber on the wall of a bottle. Thickness can be as low as 10µm while still about 0.4 g reference phase is present (1 liter bottle). After sufficient precleaning the film can be used to eqilibrate with sediment amounts of 400g (dw).

2 Sediments Samples and sampling

Samples can be taken according to the OSPAR guidelines. The nature of the method described here is such that a disturbance of the surface layer and the consequent loss of some fine material is not critical for the outcome of the measurement.

In general at least 0.5 liter of sediment suspension is required for a single determination. Considering the large amount of sample use in one measurement the homogenisation is not very critical and a simple manual stirring will generally be sufficient. Application of very sandy sediments (OC content <1%) is not advisable. This has two reasons (1) the capacity of the sediment is very small and may be insufficient to equilibrate with the reference phase without moving away from the original concentration and (2) sand works as an abbrasive and can damage the sampler.

Samples should be stored cool (4°C). Long storage times are not recommended but so far no investigation was done to investigate a possible effect. Likewise it is not investigated whether freezing alters the sorption properties.

3 Material preparation

3.1 Sheet material

Sheet of reference material should be cut in appropriate sizes. Typically 0.3-0.8 g as sheet or coating can be used for 0.5 kg dw sediment. For coating bottles see Appendix X. New sheet material must be pre-extrated to remove oligomers (low MW polymers). This is done by soxhlett extraction of 100 hours with ethylacetate. A large size soxhlet that can cotain multiple sheets is convenient for this process.Onl 30% in with and height of the soxhlet volume may be filled with sheets to give space for swelling. Alternatively sheets can be shaken with ethylacetate (20 ml/sheet) for one week and refreshing the ethylacetate twice. Full extraction can be measures by measureing the non-evaporable mass in the last extract of the sheets (criteria will be given later). After extraction the sheets are dried in a fume hood and brought in a wide mouth botlle with methanol and shaken overnight. The methanol intends to extract all contaminants sorbed during the air drying process. Sheets can be stored under methanol for a prolonged period.

> *It is recommended to use dedicated or disposable glassware for solvent containing oligomers of polymeric material,i.e. should never be used in the analytical processes. It is difficult to clean and residues only dissolved in "hot" ethylacetate.Consequently your soxhlet wiil not be contaminated. Residues disturb the GC process and block HPLC.*

3.2 Bottles

To keep track of the weight of the coating no labels should be used on the coated bottles. Bottles are marked by engraving a number or a letter and a number. It is advisable to develop a bottle dictionary in which the weights of borttles during use is recorded. Coated bottles used should allow air and liquid tight closure using an aluminium lined insert in the top. This is more easy achievable for bottles with a small neck diameter. Coverage off the liner in the top with foil is prerequisite as any plastic top acts as a passive sampler. Adhesive Aluminium foil tape from Tesa sticked to a semy soft (PE, Teflon will do) liner can be used. This system is sufficient toght for water but cannot withstand pressure. That means that before horizontal shaking with solvents requires acclimatisation (equilibrium between solvent and overlaying air phase) and release of pressure is required. A more secure way is to acclimatise at higher temperature than the shaking process is perfor med, i.e. a slicht vacuum is installed.

Alterantively extraction with solvents can be performed on a roller provided that the whole film is wetted sufficiently by the solvent. Before use bottles are shaken three times 24 hr with 50 ml ethylacetate. To save solvent a second extract can be used for a first extaction for the next bottle, and the third for a second extraction, etc.After extraction bottles are dried (fumehood/nitrogen) and the final film weight determined by weighing. Closed with the described lid with Alu-foil bottle can be stored in this condition.

4 Spiking, blanks and storage

4.1 Individual spiking

Sheets are taken from the methanol added after pre-extraction (3.1) and dipped with a tissue.. Spiking of sheets that easily take up solvents can be done by simply dosing the solution on the individual sheets and let the solvent evaporate. (check by weight). The PRC concentration should be such that no more than 10-20% of the sheet weight is added as spike (i.e. ±50µl). Bottles are spiked in a similar way by dosing the PRC solution on the film.

4.2 Batch spiking

For all types of sheets, including those that do not easily sorb solvents, a batch spiking procedure can be applied (Booij et all, 200x). The methanol in wich the batch of sheets (10-100) is stored (3.1) can be poured of and 80% (v/v) methanol is added, maximal 4 ml per g sheet. To this mixture the PRC spiking solution is added and the mixture shaken or thumbled overnight. Then 2 ml water per sheet is added and the mixture is shaken or thumbled for 48 hours. The sheets have taken up the PRCs according to their sorption capacity (proportional to weight). Sheets can be stored like this and be used for exposure to sediment when appropriate. During sheet analysis after exposure at least two non-exposed sheets are analysed for reference with each exposure.

5 Exposure to sediment

Coated bottles prepared and spiked (48 hr before use), or bottles containing a spiked sheet, are weighed with top. Then about 0.5 –0.7 kg (0.5 L) of homogenised wet sediment sample is transferred to the bottle. Homogenisation can be done using a spiral paint mixer. For homogenisation some water may be added to liquify the sample for convenient filling of the bottles. The sample weight is recorded and in parallel dryweight determination is performed on a subsample, to allow determination the actual dry weigjht of seiment exposed). Some water (reord the amount) may be added to liquify the sample allowing a proper shaking. Excessive dillution will decrease the uptake rate. To suspend clay material about 80% of water is required and at the other end sandy material already “fluidises” at 25-30%. Sandy material is difficult to shake as it quickly settles to a solid phase.

Before the start of the equilibration process the bottles are purged with nitrogen to remove oxygen as much as possible. Then the bottles with sediment are placed on a shaker at a speed that keeps the sedment in suspension. In general an orbital shaker at 125 rpm with an amplitude around 3 cm wil do the job. Other possibilities that have lower exchange (about a factor 2) are thumbling or rolling. The range of equilibrated compounds is extended to those with higher hydrophobicity by:

- longer shaking period;
- higher surface area-volume ratio of the sampler;
- highersuspension concentration (clay/water ratio);
- lower average particle size

- and higher shaking intensity

At this stage it is experienced that minimum conditions with a 5x5x0.05 cm sheet (50cm^2) will give in about 2 weeks equilibrium for upto logK_{OW}≈5-6. Sofar a maximal situation occurs in a bottle coated with a 10µm film with a muddy suspension which will equlibrate in 3 weeks for compunds upto logK_{OW}≈7-8 (all regular PCBs and PAHs).

What ever shaking conditions are avaialble at this stage a shaking time of 3 weeks is suggested, untill it is more clear what better approach is possible. Shaking should be done in the dark and preferably at 20°C.

After shaking coated bottles are emptied and shortly washed with 1-3 small portions of water(±50 ml) to remove residual sediment. By swinging the botlle the water is removed as much as possible. At this point an analytical recovery standard can be added.

Sheets can be recovered from the sediment, washed with little water, wiped with a tissue and stored in a alufoil lined vial of e.g. 40 ml. Analytical recovery standard can be spiked on the sheet.

Closed bottles or vials can be stored in the freezer untill extraction-analysis.

6 Extraction and cleanup

6.1 Solvents

To protect sheet and coatings extraction is better done with a solvent that does not cause large swelling and is not able to extract unpolymerised material from the sheets. Depending on the type of sheet used another range of solvents can be applied. Materials used for passive sampling do generally not have very strong sorption properties (Booij et al). The material-methanol partition coefficient, K_{mm} is less than 1 (log K_{mm}=0) for all compounds upto logK_{OW}<8. making methanol an appropriate extraction solvent. Less appropriate alternatives are ethanol (high boiling point) or acetone (possible radical formation that degrades PAHs). A very good alternative is acetonitril showing no degradation like acetone. The 15% azeotrope with water also gurantees a dry extract after evaporation. However Acetonitril is considered rather toxic and the boiling point is quite high. For silicone rubber based materials methanol is presently the best choice. For PE and POM a wider range of solvents, including pentane can be applied for extraction.

6.2 Extraction

The small volume of the film or sheet (<0.8 g) allows quantitative extraction with small amounts of solvent. Coated bottles can be extracted with 2 times 40 ml methanol, shaking them each time for 2 hours. Sheets upto 1 g can be extracted by 2 times 20 ml methanol and 4 hours shaking time. For sheets the extraction time is related to diffusion of compounds from the inside of the material and consequently the film/sheet thickness.

> *The alufoil lined caps are not pressuretight. Before horizontal.shaking the bottles should be equilibrated so no pressure is build during shaking. Warming the bottle in hot water before closing wil create even a vacuum during the coller extraction. In case the sheet in the vial is completely immersed in solvent the extraction vial can be shaken uprightp. Weighing bottle or flask before and after extraction may indicate losses.*

Alternatively soxhlet or even ASE extraction can perhaps be applied, however, only to extract sheets.

Combined extracts are transferred to an evaporation flask and on a waterbath evaporated using Kuderna Danish to 1-2 ml.

Extracted bottles are dried and weighed, the weight registered in the bottle dictionary, and the film weight calulated. Sheets are dried and the weight recorded.

6.3 Cleanup

All cleanup steps that are used for sediment extracts can also be applied for PS extracts. Compared to sediment extracts obtaine by solvennt extraction, an extract from passive sampling generally contains a very low matrix level, and even direct analyses can be considered. For accurate analyses at trace level a cleanup is likely required. Additionally it did occur that incomplete pre-extraction of sampling material caused the presence of small amounts of oligomers in the sample. The non-specific cleanup described below will remove this material among with all other highly hydrophobic material (is often high molecular weight, including lipids).

Having an extract in methanol a non specific cleanup can be conveniently carried out using C18 bounded. silica cleanup. A glass cartridge with 500mg C18 Bounded silica is pre-eluted with x ml methanol. Then the extract is transferred to the cartridge washed down and eluted with x ml methanol. The amount of methanol (x) is around 6-10 ml but an elution test should determine the volume required for the target contaminants. Instead of methanol acetonitril can be applied (elutes faster because of lower viscosity). This cleanu isolates all and only compounds upto $\log K_{OW} \approx 9$, i.e. all relevant contaminants. Alternatively GPC fractionation can be applied to obtain a similar cleanup.

After this nonspecific cleanup any specific cleanup can be applied. This will depend on the target compounds and instrumental method applied. This may include removal of sulphur, e.g. by addition of copper powder and ultrasonic treatment.

6.4 Concentration

The low matrix content increases the risk of losses of target compounds through evaporation as a high matrix act as a “keeper”. Extra care in concentrating sample extracts is required and evaporation to dryness should be avoided under all circiumstances. Distilling evaporation systems include some plates for separation and are prefered over rotavapor or (automated) systems that use a nitrogen flow. The latter do not have a reflux flow that can extract the the compounds back from the vapor stream. Considering the little amount of solvent a miniature Kuderna Danish with one Snyder ball is optimal for ending in 1-2 ml extract after evaporation. To evaporate methanol a waterbath at about 95°C is required. Of course any system should be checked for its perfomance with the solvent-target compound combination used.

6.5 Phase transfer

As evaporation to dryness causes losses and a solvent transfer should be performed differently. The simplest way is to take advantage of azeotropes. An extract of 1 ml methanol or acetonitril is transferred to hexane by adding 10 ml hexane and subsequently evaporate again to 1 ml using Kuderna Danish destillation. The azeotrope (metahnol/hexane 1+3) boils at 50°C and with excess hexane the an evapoartion will end in hexane. Note that hexane and methanol are not mixable. Similarly the transfer from hexane to methanol can also be performed without the necessity to evaporate to dryness.

Application of azeoptropes for phase transfer can only be used when solvent is evaporated through a distillation process. Nitrogen blow down systems will only evaporate the upper layer (i.e. hexane). In those cases a solvent extration will have to be performed. After dillution

of the methanol with water, 5 ml water for each ml methanol phase, the target compounds can be extracted with two times 20 ml pentane or hexane.

7 Analytical QA

Analytical QA focusses on the process of analysis of the target compounds in a coated bottle or sheet. This is equal to the analyses of any other environmental matrix.

1. By executing the extraction procedure without sample the blank values are determined. Some blank values of lighter PAHs seem to be inevitable, but at the same time these compounds generally give the highest signal in PS sampling. The procedural blank can be subtracte from the results.

2. Analysing reference sheet/bottles in duplicate will give a more accurate value for the start concentrations of the PRCs, as well as some information on the repeatability. Since the reference sheets/bottles do/should not contain any of the target analytes and indicate a maximal blank value. This are not to be used to subtract from results as these compounds take part in the equilibration process and only need consideraration if they would be able to seriously increase the amount in the whole system.

3. To control the recovery of the procedure for individual analyses one ore more recovery standards can be added from the start of the analyses. Comparing nominal values with measured values will indicate the recovery. Values should be over 80%.

The QA for passive sampling is still under development. Sheets or bottles with known reference concentration to use as reference material may become avaialble in future. Intercalibrations may help further improve the methods and increase robustness. Also validation by using different materials, conditions, and methods should be performed where possible.

8 Calculation

Calculation of the free dissolved concentration (C_W) in an equilibrated system is done by:

$$C_W = \frac{N_R - Bl_R}{m_R \cdot K_{RW}}$$

In which N_R is the amount (ng) of compound measured in the extract of the sheet/bottle; Bl_R the procedural (solvent) blank (ng); m_R the mass of passive sampling material (kg) **after** exposure and K_{RW} the material-water partition coefficient (l/kg). The obtaine result is in ng/l but it is often more conveniently to express the results on pg/l.

9 Process QA

9.1 Depletion

To obtain the true free dissolved concentration (C_W) the sediment gives to the water phase the amount depleted from the sediment required to obtain equilibrium should be minimal as every decrease in sediment concentration (C_S) will also result in a decrease of the aqueous phase concentration. It should be noted that C_W is not necessarly proportional to the total concentration in the sediment. A 10% decrease in total sediment concentration can coincide with a 90% decrease of C_W, depending on the proportion of the target compound that is available for exchange with the water phase. The distribution of PRCs over reference phase and sediment phase indicates the capacity ratio of those two phases, provided equilibrium was

obtained. This distribution factor (DF) is the ratio of the amount of PRCs that is remaining on the PS and that sorbed by the sediment:

$$DF = \frac{N_R}{N_0 - N_R}$$

Where N_R is the amount measured on the reference phase after exposure and N_0 the amount spiked on the reference phase prior to exposure. When DF is 0.1 or less the CW will not significantly be affected by depletion. DF can apply to this criterion for PAHs but be higher than 0.1 for PCBs. High DF values mean that there is little sorption capacity in the sediment and likely the sheet/bottle has depleted the sediment. The calculated C_W value is underestimated.

9.2 Equilibrium

The use of DF as estimation of depletion assumes that equlibrium was attained. Depending on the conditions this does not have to be the case. For PCBs the capacity ratios between reference phase and sediment are rather equal for all congeners and similar DF are expected for PRCs with large difference in hydrophobicty. That means that DF of PRCs like CB010 and CB 204 do not differe a lot. It still hase to be verified if that is the case for sediments on a large geographically range.

9.3 Environmental validation

Application of the described method in parallel with uptake tests equilibrating sediment living organisms with contaminated sediment. Concentrations in organism should be related to the C_W values obtained by passive sampling. Sofar, attempts have shown better agreement of internal concentration in organisms with PS results, than with total concentrations in sediment. More data will be required for full validation.

10 ANNEX A Coating of bottles

1. Bottles with small neck (max 4 cm) are engraved with a unique number.
2. Then washed thouroughly,dried at 100°C and weighed (0.001 g) without top.
3. Weights are recorded in the bottle dictionary.
4. In a working fume hood a roller is prepared on wich bottles can role horizontally.
5. Teflon tubing (>5mm ID) is fixed so it enters the bottle half way with one end, bended slightly downward, and the other end outside the bottle is bended upward and a small platic funnel is connected to it. A thinner teflon tube (1-1,5 mm ID) is placed in parallel in the bottle and the other end connected to a nitrogen supply (3-5 l/min). This should all be fixed in such a way that the bottle can turn free on the roller and bottles can be placed and removed without toching the tubing ends.
6. In a disposable glass jar or bottle of about 400 ml 6 gram slicone rubber pasta is weighed.
7. Then per gram of rubber pasta 50 ml of pentane is added.
8. The slicone rubber pasta is dissolved by shaking and sonification (±15 min).
9. A bottle is placed on the roller with the tubing inside.
10. While rolling N2 is purged for 1 minute and then stopped.
11. With a disposable measuring cylinder 25 ml pentane solution is added in the funnel.
12. The solution wil spread over the walls of the bottle and the pentane evaporate, this can be assisted with some N2 flow..
13. When the pentane is evaporated the bottle can be carefully taken away from the tubing, and rolled for another 5 minutes so the film will not sag out.
14. Another bottle is placed around the tubing and coated in a similar way.
15. The films have to cure for at least three days at ambient temperature. If the humdity is lower than 50% put a few drops of water in the bottle.
16. After curing (and drying) weights are.
17. Then the coated bottles are twice pre-extracted overnight with 50 ml ethyl-acetate.
18. The ethyl-acetate is removed from the bottle. The bottles are placed upside-down on tissue-paper for 15 min and further dried horizontally (eventually assisted by N2 flow) under the fume hood
19. After drying, weigh each bottle and record the weight in the bottle dictionary. Calculate the actual film weight for individual bottle number.

11 ANNEX B Candidates for PRC compounds

Deuterated PAHs	Using GCMS nearly all deuterated PAH are applicable as PRC. A serie of Naphtalene, Fluorene, Phenanthrene, Fluoranthene, Chrysene, Benz(e)Pyrene and Coronene is suitable for PS in water as well as sediment Using HPLC all deuterated with Fluorescence the choice is more limited and and low DF values easily disturbed

12 ANNEX C Determination of Partition coefficients

12.1 Introduction

To calculate the concentration in the water phase the passive samplers are deployed or that in the sediments pore water, the partition coefficient of the sampler materials is required. The determination of this parameter is not straight forward equilibrating a sampler with water as an undisturbed determination of the free dissolved concentration is not easy possible. The slightest amount of particulate matter may significantly enhance the concentration in the waterphase. A way to circumvent that is the use of the cosolvent method. Basically the KD is determined in mixtures of methanol-water in the range of 20 to 50%. The measured LogKD values of reference phase are inversily related to the methanol content. By extrapolation to 0% methanol the KD in water only can be determined. The much lower KD values in methanol water mixtures means also lower sorption to particulate matter and bottle surfaces and can therefore be measured easier without disturbances. In general the target compounds are spiked to the passive sampler. As a check for equilibrium some of the deuterated analoges can be added to the aqueous phase

12.2 Procedure

Preparations

Any material used should first be pre-extrated as decribed in the section for the use in exposures what generally means 100 hours soxhlett extraction with ethylacetate. The sampler size can be at the same size as the used in exposures but a smaller size can be used as well.

In case the material of concern is bottle coating a sheet of his material can be obtaine by coating of a PE bottle wich, after sufficient curing is cut in required pieces and the material is pealed off.

Spiking and equilibration

The spiking with appropriate amount of test compounds (e.g. 500±50 ng for PAHs and 350±50 ng for PCBs is performed as decribed for PRC compounds. In case partition coefficients of deuterated compounds also need to be deterimend these could be subsequently spiked as well. Alternatively they can be added to the aqueous phase instead.

Bottles of 1 or 2.5 liter are filled upto 80% with methanol-water mixtures at 20, 25, 30, 35, 40, 45 and 50 % methanol prepared on weight basis. Milli-Q water (18.2 mΩ) is used. At this stage the deuterated compounds can be added to the waterphase. Introduce a spiked sheet into each bottle and an non-spiked sheet is added to a bottle containing 900 ml of 20 % methanol as procedural blank.

For partition coefficients in water only and 10% Methanol in sheets are exposed in 10 L bottles. Extraction by separation funnel is quite laborious so it is suggested to applythat

The bottles are placed upright to avoid contact with the lid that is usually made from HDPE and shaken on an orbital shaker at 150 rpm for 15 days , within which equilibrium would have been attained. (Li, 1994; 1995; communication with Smedes Foppe).

Extraction and analyses

Two seperation funnels (1 or 2 l) are rinsed with hexane and filled with 100 ml haxane each. After weighing the bottle a portion of the methanol water mixture is poured from to the first funnel. After extraction this portion is transferred to the second funnel, extracted again and subsequently wasted. Then the next portion is extrated untill the complete volume is extrated. Although sorption to the wall is considered neglegible the bottle is not further extracted as any sorbed compounds are not considered part of the aqueous phase. The bottle is weighed again. For concentrations higher than 30% methanol, smaller portions are transferred to the seperation funnel and hexane extracted water is added to the funnel to reduce the methanol concentration at or below 30%. The hexane fractions are tarnsferred to an evaporation flask by pouring it out the top while carefully preventing water to ext the funnel. Funnels are washed with two times 25 ml of hexane what is added to the extract in the same way. Measuring internal standard is added and the extract is Kurdena-Danish evaporated to the required volume for instrumental analysis as described for exposed samplers.

The equlibrated samplers are taken from the bottle and extracted sililar to exposed sheets or bottles. Provided the pre-extraction was thouroughly done no cleanup is required. For the instrumental measurement only addition of the measuring internal standaard and Kurdena-Dabish concentration is required.

The reference phase-water partition coefficients (KSW) are calculated by:

$$K_{SW} = \frac{C_R}{C_W}$$

The intercept of linear regression with the $\log(K_{SW})$ as y value and the methanol content as the x value will give the $\log(K_{SW})$ for water only