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Establishing and Controlling Dissolved Concentrations of Hydrophobic Organics by Partitioning from a Solid Phase

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Bioavailable concentrations of hydrophobic organic substances in aqueous tests often decrease as a result of (bio)sorption, volatilization, or degradation. In the present study, partitioning driven administering (PDA) is introduced as an approach to maintain constant exposure levels of hydrophobic organics in aqueous tests. The working principle of PDA is to control dissolved concentrations by partitioning from a dominating solid phase. For this purpose, test compounds are loaded on an octadecyl Empore disk, which subsequently is placed in an aqueous test system. Stable aqueous concentrations for a range of halogenated aromatic substances (log K_{OW} 3.9−7.1) were established within minutes to hours, and the resulting concentrations indicate that equilibrium partitioning governs the release from the disk. Furthermore, PDA's capability to maintain stable concentrations against a depletion process was demonstrated, and PDA was successfully applied to dose 1,3,5-tribromobenzene and hexachlorobenzene in algal toxicity tests. Finally, the feasibility to administer concentrations up to aqueous solubility, to administer mixtures, and to dominate the fugacity of multicompartment systems is discussed.

Introduction

Current guidelines for standard toxicity, biodegradation, and bioconcentration tests are typically not suited for the large group of sparingly water-soluble compounds (*1, 2*). Most of such standard tests are carried out in static systems, in which dissolved concentrations of these hydrophobic chemicals usually drop during the experiment. Batch experiments are frequently employed in research laboratories because they are less laborious than continuous techniques and since they facilitate high numbers of experimental units, which is crucial for the precision and statistical power of results.

The first challenge of batch experiments with poorly soluble compounds is to obtain completely dissolved exposure concentrations. The most widely applied and very easy method for facilitating dissolution of poorly soluble organics is the use of cosolvents. Alternatively, aqueous stock solutions are prepared using a generator column, or dissolution of the test compounds is facilitated by heating or sonification. Each of these methods has its own merits and disadvantages as reviewed in ref *3*.

The second challenge is to maintain the initial exposure level throughout the experiment. Sorption to the test vessel, uptake by the test organism, volatilization, and biotic or abiotic degradation reactions can cause the amount of test compounds in the aqueous phase to decline. Association to colloids, suspended particles, and dissolved organic substances likewise ties up a certain amount of the test compound, thereby reducing the bioavailable fraction (*4*−*8*). If the concentration drops significantly or if the test substance is present in a biologically unavailable form, the initial concentration is no longer the true exposure concentration, and estimation of that true exposure concentration is difficult. Without knowledge of the true exposure, the interpretation of the observed effects and their extrapolation can be compromised (*1, 9*).

Urresterazu Ramos and co-workers (*10*) presented the "generator disk in the headspace" approach to obtain completely dissolved concentrations (first challenge). They prepared aqueous concentrations of halogenated aromatic compounds by placing a contaminated solid phase in the headspace of a bottle containing water. This spiking method yielded aqueous steady-state concentrations within 1 day for most of their test compounds.

The aim of the present study is to introduce "partitioning driven administering" (PDA) as an approach to establish dissolved concentrations (first challenge) and to keep them constant during a batch experiment (second challenge). This method is like the aforementioned spiking method based on the partitioning of hydrophobic substance from a dominating solid phase (see Figure 1). The solid phase is placed directly in the aqueous phase to accelerate release kinetics, and it remains there during the experiment to stabilize the exposure concentration. PDA features thus a refill function that compensates for loss processes that otherwise would lead to a decreasing exposure concentration.

We subsequently describe the working principle of PDA in combination with some model considerations that can lead to an accelerated administering. Validation experiments and the application of PDA are presented and discussed thereafter.

Working Principle and Theoretical Considerations

We choose to use a Teflon matrix holding porous octadecyl silica particles (C18 Empore disk) as the generating solid phase. Octadecyl silica is a "general" high-sorption capacity sorbent to which most molecules bind through van der Waals forces (*11*); porous silica particles were reported to facilitate fast release of polycyclic aromatic hydrocarbons due to their large surface area (*12*).

The first step of PDA is the loading of test substances onto the solid phase. The loaded disk is then placed in the aqueous phase of a batch experiment. The total amount of test compound (n_{total}) will partition into all compartments of the test system; some of it might become degraded or leave the system:

$$n_{tot} = n_{C18} + n_{aqueous} + n_{biota} + n_{headspace} + n_{degraded} + n_{export} + \ldots \quad (1)$$

Constant dissolved aqueous concentrations can be established if the amount on the disk (n_{C18}) remains nearly unaffected by the mass transfer into the system ($n_{C18} \cong n_{total}$)

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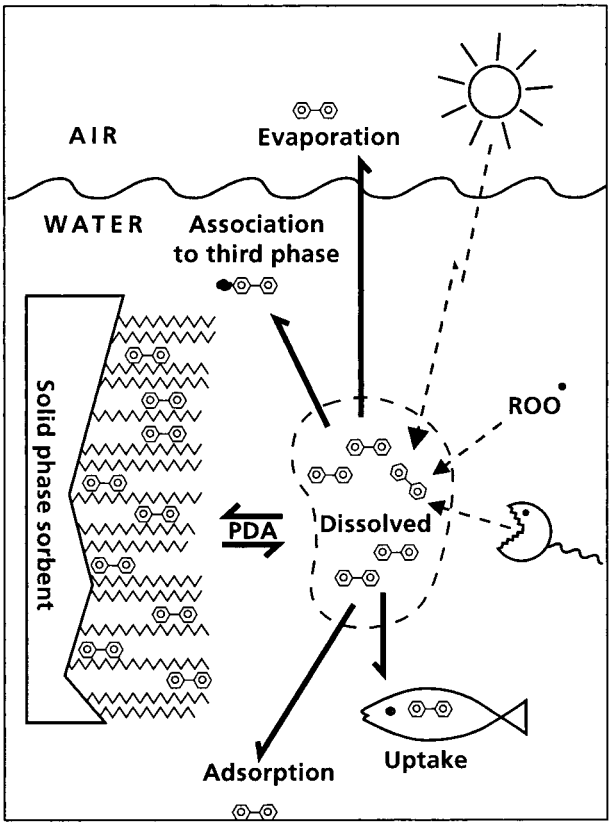

FIGURE 1. Batch experiment presented as multiphase system. Partitioning driven administering controls the dissolved concentrations of hydrophobic organics against a number of degrative (– –) and nondegrative (—) depletion processes by partitioning from a dominating solid phase.

and if the mass transfer into the water is sufficiently fast to keep up with all depletion processes.

The first requirement is generally fulfilled for substances with partition coefficients $K_{C18,aq}$ of more than 10 000, when using 1 mL of C18/L of water and in the absence of depletion processes. According to a QSAR by Verhaar et al. (*13*), log $K_{C18,aq} = 0.995 \log K_{OW} + 0.7$, such partition coefficients above 10 000 can be expected for compounds with a log K_{OW} above 3.3. The amount of sorbing phase that is required in the presence of depletion processes depends on the degree of depletion and on the partition coefficient of the test substance. Nevertheless, 1 mL of C18/L of water is expected to meet this requirement for most aqueous batch experiments.

Second, the release kinetics need to be sufficiently fast to stabilize the exposure. In an attempt to shed light on the release kinetics of the method and to identify ways to accelerate the release in case of strong depletion, a first-order one-compartment model is assumed. The time course of the aqueous concentration C_{aq} can then be described according to

$$C_{aq} = C_{C18}\frac{k_1}{k_2}(1 - e^{-k_2 t}) \quad (2)$$

in which k_1 is the delivering rate constant and k_2 is the re-uptake rate constant from the water back into the C18. The latter determines the kinetics of the system and can, for instance, be used to estimate the required time to reach 90% of the aqueous steady-state concentration: $t_{90\%} = \ln(10)/k_2$. By assuming a two-film resistance model, k_2 can be expressed in fugacity terms according to (*14*) as

$$k_2 = \frac{D_{C18/aq}}{V_{aq}Z_{aq}} \quad (3)$$

in which Z_{aq} denotes the fugacity capacity and V_{aq} is the volume of the aqueous phase. $D_{C18/aq}$ is the inverse of the total resistance for mass transfer between the two phases. This total resistance can be viewed as two resistances in series, one within the octadecyl phase and one within the aqueous phase. The water resistance for hydrophobic organics can be expected to be the limiting component of $D_{C18/aq}$, since the concentration of such compounds is very low in the aqueous phases as compared to the octadecyl phase (*14*):

$$D_{C18/aq} \cong D_{aq} = k_{aq}AZ_{aq} \quad (4)$$

where A is the surface area and k_{aq} is the mass transfer coefficient in the aqueous laminar boundary layer that can be expressed as

$$k_{aq} = \frac{d_{aq}}{l} \quad (5)$$

where d_{aq} ($m^2\ s^{-1}$) is the diffusion coefficient in water and l (m) is the average thickness of the undisturbed laminar boundary layer. Substitution of eq 2 into eq 4 yields

$$k_{aq} \cong \frac{d_{aq}}{l}\frac{A}{V_{aq}} \quad (6)$$

suggesting two ways to accelerate the administering. The first way is to reduce the thickness of the unstirred boundary layer l, for instance, by means of shaking. The second is to maximize the surface area relative to the receiving water volume (A/V_{aq}), which can be achieved by using a sufficiently sized piece of Empore disk. Additionally, kinetics depend on the diffusion coefficient d_{aq}, which can be expected to decrease with increasing molecular volume of the organic solute (*15*).

Experimental Section

Solvents, Chemicals, and Sorbent. Nonane (>99% pure) and hexane (>95% pure), which was redistilled prior to use, were obtained from J. T. Baker (Deventer, The Netherlands). 1,2,3-Trichlorobenzene (>99% pure), 1,2,3,5-tetrachlorobenzene (>99% pure), 1,4-dibromobenzene (>99% pure), 1,3,5-tribromobenzene, 1,2,4,5-tetrabromobenzene (>98% pure), 4,4′-dichlorodiphenyltrichloromethane (DDT, >98% pure), and 3,3′,4,4′-PCB (IUPAC No. 77) were from different suppliers. 2,2′-PCB (IUPAC No. 4), 2,4,5-PCB (IUPAC No. 29), 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD), and 3,3′,4,4′-2-tetrachlorobenzyltoluene (Ugilec 87 according to ref *16*) were custom synthesized for previous studies. Phenanthrene (>96% pure) was from Sigma (St. Louis, MO), and fluoranthene (>98% pure) was from Aldrich (Steinheim, Germany).

C_{18} Empore disks were in part a kind gift of 3M (Zwijndrecht, Belgium) and in part obtained from Varian (Houten, The Netherlands). According to documentation obtained from the supplier, these disks consist of 10% PTFE matrix and 90% of silica sorbent with chemically bonded octadecyl groups (18% carbon content, endcapped). The C18 content per gram of Empore disk was calculated to be about 0.19 g or 0.24 mL assuming the density of octadecane to be 0.78 g/mL. Empore disks were cleaned prior to use by three washes with methanol and three washes with hexane.

Chemical Analysis. Aqueous concentrations of halogenated aromatics were extracted with hexane using DDT as internal standard. When necessary, preconcentration was done by addition of 50 μL of nonane to 1 mL of extract followed by evaporation of the hexane. Final extracts were measured by gas chromatography on a Carlo Erba HRGC

3500 or a Varian Star 3400 CX gas chromatograph both equipped with a J&W Scientific DB 5MS column and a ^{63}Ni electron capture detector.

Loading of Empore Disk. The Empore disk was sized to obtain water to C18 volume ratios of less than 1000. The test compounds were dissolved in hexane, and up to 4 mL of this hexane stock was added to the Empore disk in a vial. The hexane was then evaporated under a laminar nitrogen flow or by placing the open vial in the fumehood. The disk turned from gray when wetted to white when dry. The dried disk was transferred to another vial to avoid a test compound that was attached to the loading vial to interfere with the administering. Before use, the loaded disks were stored in closed vials for at least 12 h and not more than 7 days. The loading of disks, their storage, and all experiments were performed in 40-mL borosilicate glass vials with Teflon-faced silicone septum screw caps (EPA vials).

The loading efficiency was investigated for 12 halogenated aromatics that were loaded as an equimolar mixture at about 1 mmol/L of C18 in triplicate. The dry disks and the loading vials were extracted with hexane. This hexane was spiked with DDT as internal standard for determining the volume of final extracts. The spatial distribution of the disk loading was investigated for the model substance fluoranthene. The loaded disks were mounted between two Teflon-faced septa, and cross sections were studied on a Zeiss Axioskop fluorescence microscope (Oberkochen, Germany) at an excitation wavelength of 365 nm (slit width 12 nm).

Establishing Dissolved Concentrations. Empore disk rings with an inner diameter of 22 mm, an outer diameter of 24 mm, and a C18 volume of 13.8 μL were loaded in triplicate with equimolar mixtures of 11 halogenated aromatics at nominal loadings of 0.5, 1.6, and 5 mmol/L of C18. The dry disks were brought into vials containing 10 mL of water that were gently shaken on a rotary shaker (100 rpm, orbit 30 mm) at 24 ± 1 °C. The 1-mL water samples were taken at different times for GC analysis. Phenanthrene was administered to 10 mL of water using 25-mm Empore disks (43 μL of C18) at room temperature. The nominal disk loading was 40 mmol of phenanthrene/L of C18. The release of phenanthrene into the aqueous phase was measured in aqueous subsamples of about 2 mL in a quartz-glass cuvette using a Perkin-Elmer LS50B fluorimeter at an excitation wavelength of 250 nm (slit width 2.5 nm) and an emission of 366 nm (slit width 5 nm). Release curves under static conditions and under two levels of agitation (250 and 400 rpm, 3 mm orbit) were recorded. Phenanthrene concentrations were confirmed on a Merck-Hitachi HPLC system with fluorescence detection.

Controlling Dissolved Concentrations in the Presence of Depleting Phase. Aqueous phenanthrene solutions of the 250 rpm series were transferred to new vials, for some vials together with and for the remaining vials without the respective Empore disk. These new vials contained either 1.7 mg (0.17 g of XAD/L), 5.1 mg (0.51 g of XAD/L), or no Amberlite XAD-4 ion-exchange resin (Janssen Chimica, Geel, Belgium). This experiment was performed to investigate whether the Empore disk with an C18 phase of 33 mg (3.3 g of C18/L) was able to maintain the aqueous concentration against a depletion process, which was expected to be much greater than, for instance, the sorption to algae at typical population densities.

Algal Growth Inhibition Test. PDA was applied to dose 1,3,5-tribromobenzene and hexachlorobenzene to algal growth inhibition tests that were performed as closed tests according to ref *17*. Geometric dilution series of the test compounds in hexane were loaded on the Empore disk rings that were added to the algal medium the day before inoculation. Right before inoculation samples of 1 mL were taken for chemical analysis. The algal suspensions at the end of the 48-h test had an algal density in the range of 10^6 *Selenastrum capricornutum* cells/mL (new name *Raphidocelis subcapitata)* corresponding to 20 mg of algal dry weight/L. The algal suspensions were filtered through a binder-free dual-mode glassfiber filter (Whatman GMF 150), and final aqueous concentrations were measured in the filtrate. The end point of the test was inhibition of algal growth, which was monitored by means of electronic particle counting and in vitro fluorescence (*18*).

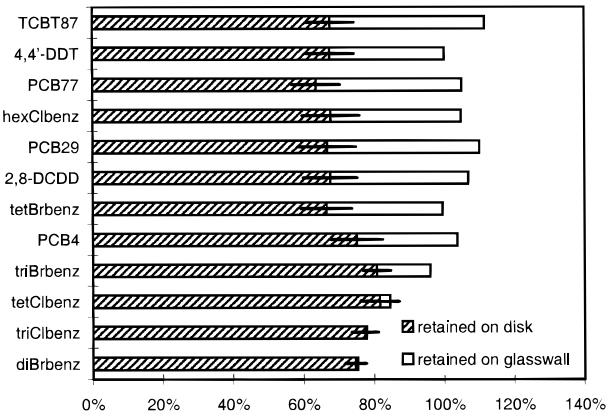

FIGURE 2. Recoveries of loading procedure (error bars = standard deviations).

Results and Discussion

Loading. A prerequisite for the predictable application of partitioning driven administering is a loading procedure that brings most of the analyte into the disk and also yields rather uniformly distributed loadings. Actual loadings of the 12 halogenated aromatics were between 63 and 82% of the nominal loading, and the standard deviations were between 3 and 8% as shown in Figure 2. The remaining fraction of the more volatile compounds was lost during the loading presumably by evaporation, whereas this fraction of the less volatile compounds was recovered in the loading vial. The loaded disks were transferred to clean vials to avoid this unbound fraction to interfere the administering. Fluorescence microscopy pictures in Figure 3 indicate a rather uniform distribution of the model compound fluoranthene between the inner part and the outer part of the disk. These experimental results indicate the loading procedure to be efficient, to be reproducible, and to produce homogeneous loadings.

Establishing Dissolved Concentrations. Aqueous steady-state concentrations for the halogenated aromatics were established under gentle shaking within a few hours. Release curves at the highest loading for compounds with steady-state aqueous concentrations of more than 1 μg/L are shown in Figure 4. The release of phenanthrene, monitored by fluorimetry, is shown in Figure 5. Without shaking (static), 90% of the steady-state fluorescence was reached after 5 h ($t_{90\%}$ = 5.0 h, k_2 = 0.43 h^{-1}). The release was accelerated under more turbulent conditions, thereby reducing $t_{90\%}$ to 30 min at moderate (k_2 = 5.2 h^{-1}) and to 8 min at firm shaking (k_2 = 17.2 h^{-1}). The shaking is expected to reduce the stagnant aqueous layer in and around the disk, and the accelerated release under shaking agrees thus with the model assumption of the stagnant aqueous layer being the main resistance for release.

Partition Coefficients. Disk water partition coefficients ($K_{C18,aq}$) are plotted against log K_{OW} in Figure 6 (average ± standard deviation). For 8 out of the 11 compounds, partition coefficients are based on three concentrations in triplicate (nominal 0.5, 1.6, and 5 mmol/L of C18, n = 9). Partition coefficients are based on only the highest loading for the

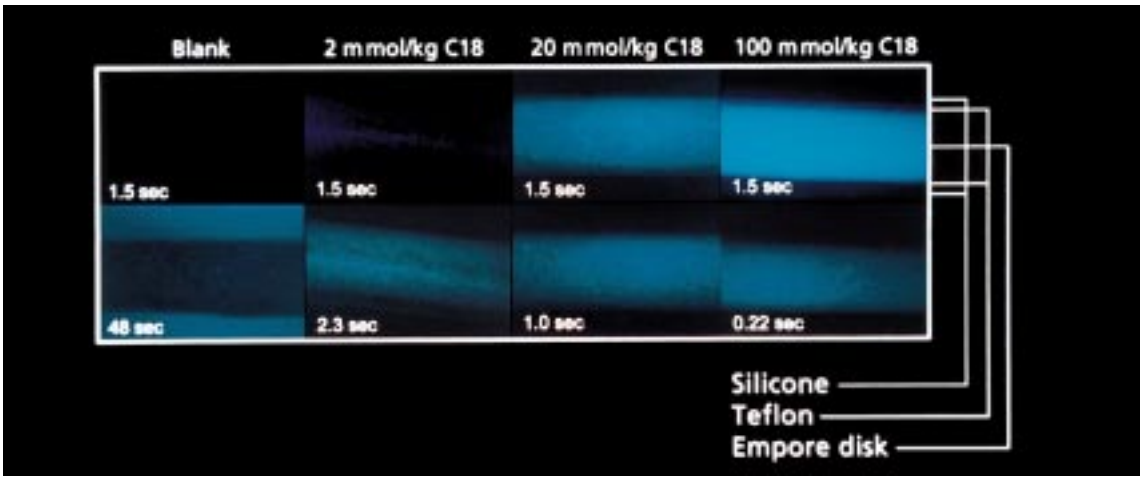

FIGURE 3. Fluorescence microscopy photos of fluoranthene-loaded Empore disks (cross sections). Upper series taken with fixed light exposure times; lower series taken with automated light exposure times.

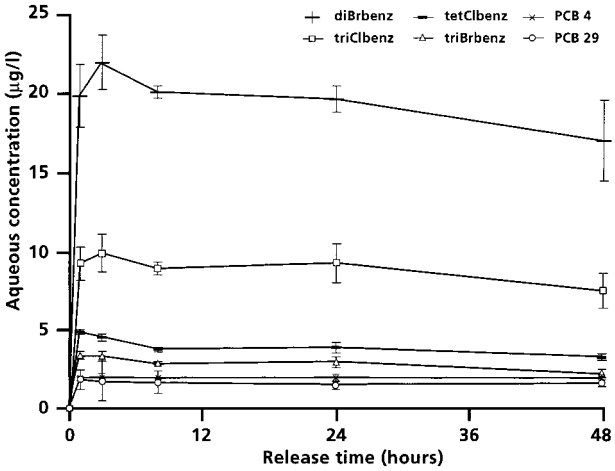

FIGURE 4. Aqueous concentrations plotted against time for all tested compounds with steady-state concentrations above 1 μg/L. Symbols represent averages ± standard deviation of three replicate vials.

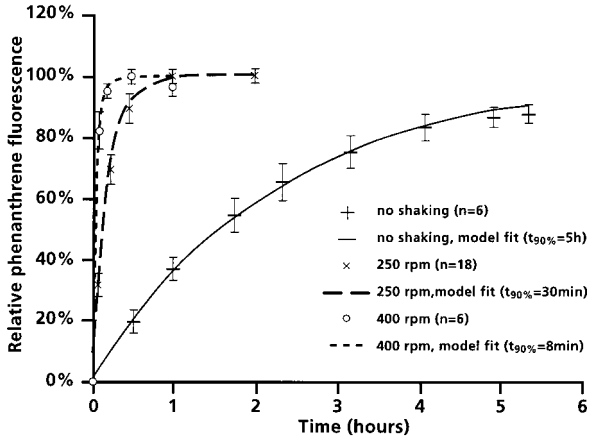

FIGURE 5. Release of phenanthrene recorded as relative fluorescence. Maximum fluorescence corresponds to about 20 μg phenanthrene/L. Symbols represent averages ± standard deviation of independent vials.

remaining three compounds (n = 3) because the lower concentrations were not quantifiable. Figure 6 also includes partition coefficients from ref *13* that were obtained by "biomimetic extraction", where the analyte partitions from the aqueous phase into the Empore disk. Comparable partition coefficients were obtained with biomimetic extraction and with the administering method, indicating that equilibrium partitioning governs the systems at steady state. The linear relation between log K_{DW} and log K_{OW} seems to bend off at a log K_{OW} between 5 and 6, which also has been reported for membrane water partitioning (*19*, *20*).

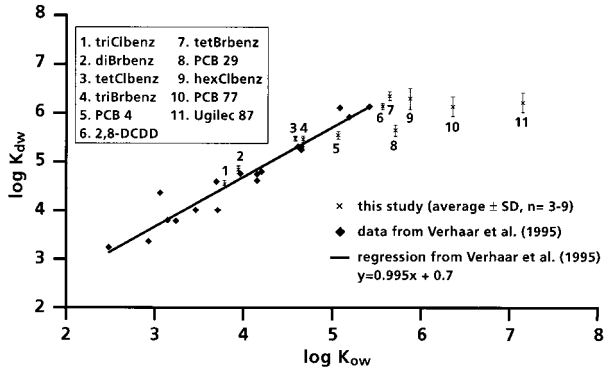

FIGURE 6. Experimental disk/water partition coefficients (K_{DW}) plotted against K_{OW} as calculated by SRC-WSKOW for Microsoft Windows (copyright William Meylan, 1994−1996). Published partition coefficients and the regression line from Verhaar et al. (*13*) are included for comparison.

Controlling Dissolved Concentrations. The aqueous phase of the release experiment with phenanthrene was transferred into new vials. Phenanthrene fluorescence decreased continuously in vials without Empore disk, and except for an initial instability, fluorescence was stable within a few percent in the vials with Empore disk (see Figure 7a). Figure 7, panels b and c, show phenanthrene fluorescence in the presence of 170 and 510 of mg XAD/L, respectively. Phenanthrene fluorescence decreased below the detection limit in vials without Empore disk, while the fluorescence in the vials with Empore disk was maintained at 78% (170 mg/L) and 72% (510 mg/L) of the fluorescence in vials with Empore disk and without XAD. Figure 7, panels b and c, demonstrate how PDA maintained aqueous concentrations against a strong depletor.

Algal Growth Inhibition Test. Algal growth inhibition tests with 1,3,5-tribromobenzene and hexachlorobenzene were included in this study in order to demonstrate an application of PDA. Expected effect concentrations for both compounds are in the range of their aqueous solubility, making both compounds difficult to test. Hexachlorobenzene was consequently in one study reported not to inhibit photosynthesis of a green alga even at aqueous solubility (*21*), and it was in another study reported to slightly inhibit photosynthesis of

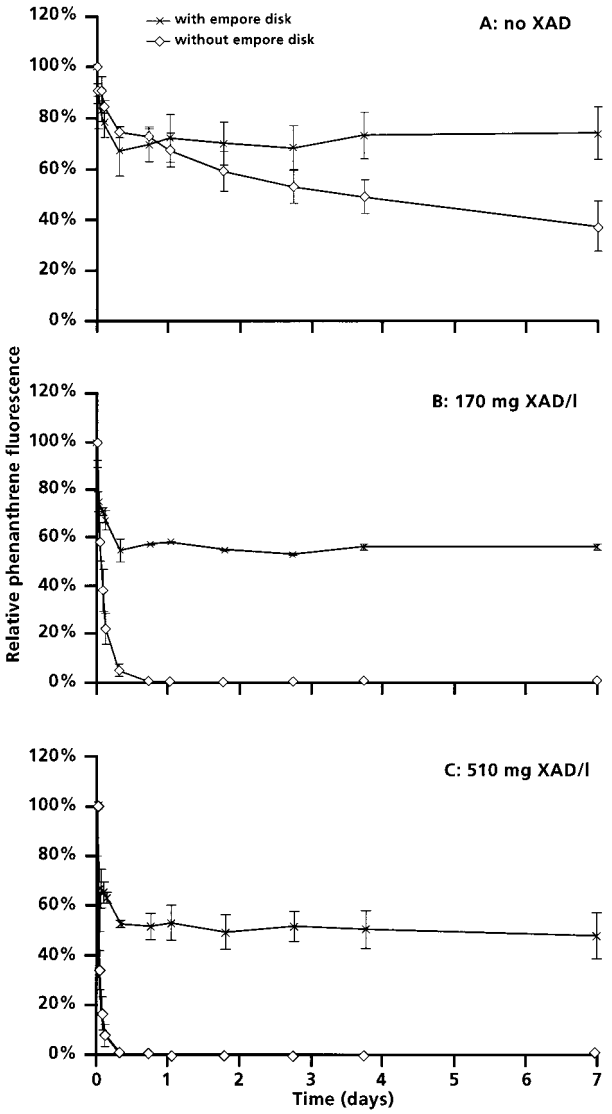

FIGURE 7. Panel a shows phenanthrene fluorescence against time in vials with and without Empore disk. The time course of phenanthrene fluorescence in vials with 170 and 510 mg of XAD/L are shown in panels b and c, respectively. Symbols represent averages ± standard deviations of three replicate vials.

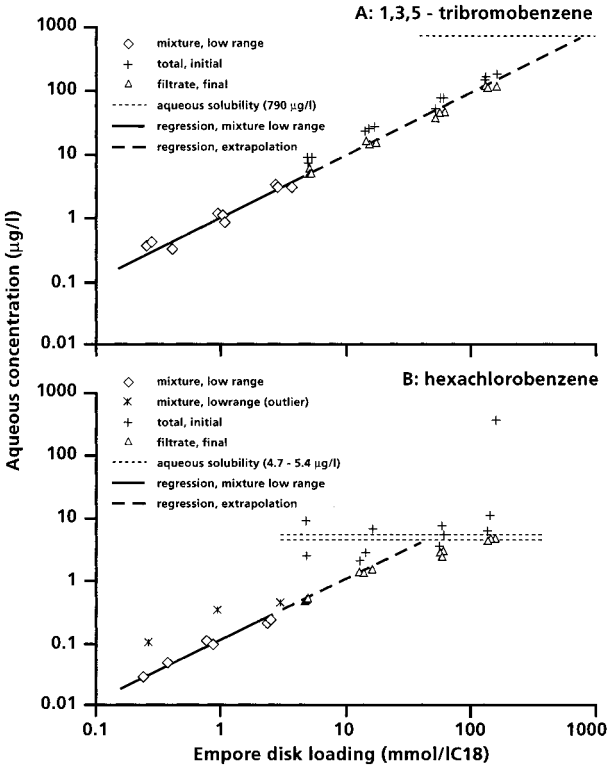

FIGURE 8. Aqueous concentrations of 1,3,5-tribromobenzene (a) and hexachlorobenzene (b) are plotted against measured disk loadings. These dosing relations are presented together with the respective regressions from the mixture experiment that was performed without algae. Three hexachlorobenzene measurements of the mixture experiment that were more than two times higher than the two respective replicates were not used for the regression shown. Aqueous solubility for hexachlorobenzene is presented as the range of three generator column values (*30*) and for 1,3,5-tribromobenzene as one experimental value (*31*).

a green alga at about aqueous solubility (*22*). The challenge of this experiment was to dose a wide range of concentrations, preferably up to aqueous solubility, and then to maintain those exposure concentrations in the presence of a growing algal population. These tests had to be run at relatively high algal densities to allow chemical analysis on the algal biomass in order to determine toxic cell concentrations, which is the analogue to critical body burdens in fish (*23*).

Aqueous concentrations of 1,3,5-tribromobenzene in the algal test extended the linear dosing relation that was derived at lower concentrations in the mixture experiment without algae (Figure 8a). On average, 68% of the initial total concentrations were actually measured on filtrates of algal cultures at the end of the test. After correction for sorption to filters, we estimate the actual exposure concentrations at the end of the test to be on average 80% of initial concentrations. Exposure concentrations could thus be kept relatively constant in the presence of the growing algal population. No toxicity was observed up to the highest concentrations, which were 20−25% of aqueous solubility.

The dosing relation of hexachlorobenzene seemed to be linear, even though the dosing was complicated by nondissolved forms of this compound (Figure 8b). Three aqueous concentrations from the mixture experiment without algae were much higher than their respective replicates, and some initial concentrations of the algal test exceeded aqueous solubility. These measurements were obtained by liquid−liquid extraction of unfiltered water samples, and nondissolved forms of the compound can contribute to such total concentration measurements. Nondissolved forms seemed to be removed in filtrates of final algal cultures since final filtrate concentrations extended the linear dosing relation derived from the mixture experiment. Nondissolved forms that are released from the disks are the trade off for the improved release kinetics that were achieved by moving the disk from the headspace into the aqueous phase. The risk that these nondissolved forms are interfering with the control of the dissolved concentration is expected to be small as long as the exchange between the solid and the aqueous phase is efficient. However, nondissolved forms that are released from the disk are complicating the evaluation of results when based on total aqueous concentrations. Problems associated with nondissolved forms of the analyte might thus be reduced when applying methods that measure freely dissolved concentrations, e.g., nondepletive solid-phase microextraction (SPME) (*24*−*26*).

Final hexachlorobenzene concentrations were up to aqueous solubility but did not exceed this level. PDA thus facilitated the toxicity testing of hexachlorobenzene at exposure concentrations up to aqueous saturation. No growth inhibition was observed, which is in accordance with ref *21* but not with ref *22*.

Implications for Practical Work. So far, the working principle of PDA has been described, and the approach was experimentally verified and also applied. For both phenanthrene and the halogenated aromatics, we showed that stable aqueous concentrations were established within a practical time span, and that they were maintained against a depletion process (XAD or growing algal population). The control of aqueous concentrations was possible due to a sufficient sorption capacity of the solid phase to dominate the static system and due to sufficient release kinetics to keep up with the depletion. However, the sorption capacity of the solid phase is not unlimited, and the solid phase might need to be enlarged in situations of strong depletion. Such an increase of the solid phase relative to the water volume simultaneously accelerates the release kinetics as indicated by eq 6.

Toxicity testing of hydrophobic organics often requires exposure levels up to aqueous solubility, which in the present study was achieved for hexachlorobenzene, while aqueous solubility at similar loadings was not reached for 1,3,5-tribromobenzene (Figure 8). To generalize our findings, let us treat the loaded octadecyl phase as an ideal liquid mixture of octadecyl and the loaded analyte. The aqueous concentration (C_{aq}) of the analyte can then roughly be estimated based on its mole fraction in the liquid mixture (x_{org}) and its aqueous solubility of the subcooled liquid s_l (*27*):

$$C_{aq} \cong x_{org} s_l \tag{7}$$

At saturation, C_{aq} can be substituted with the aqueous solubility of the solid form (s_s). Upon rearranging, we arrive at eq 8:

$$x_{org,s} \cong \frac{s_s}{s_l} \tag{8}$$

Aqueous saturation should thus be reached when the mole fraction of an analyte (x_{org}) reaches or exceeds its solubility ratio (s_s/s_l), which agrees with our experimental findings. The higher loadings of hexachlorobenzene with x_{org} up to 0.046 exceeded its solubility ratio s_s/s_l of 0.009 (*15*), and aqueous saturation was indeed observed (Figure 8b). Measured tribromobenzene loadings approached but did not exceed the solubility ratio of about 0.05 (estimated according to ref *28*), and aqueous concentrations of 1,3,5-tribromobenzene were in the range of but did not reach reported solubility. The loading of the octadecyl phase has a practical upper limit, which we expect to be between 5 and 20% (mol:mol) (or 160−700 mmol/L of C18). The administering of saturated solutions seems thus only feasible for high melting compounds with s_s/s_l ratios of up to 0.05−0.20.

Many experiments are preferably performed with mixtures because most chemical products are mixtures and since chemicals in the different environmental compartments are present as complex mixtures. The disk for the release experiment was, for instance, loaded with an equimolar mixture of halogenated aromatics. A wide range of aqueous concentrations were obtained because each mixture constituent partitioned according to its own partition coefficient (Figure 4). This might be utilized to mimic aqueous mixture compositions of hydrophobic organics that are controlled by an organic phase. Hydrophobic organics that are bound to the organic fraction of sediment could, for instance, be administered to aqueous tests from an Empore disk that is loaded with a hexane extract of the sediment. The resulting aqueous mixture composition of the hydrophobic organics can then be expected to be more like the mixture composition in the porewater as compared to an aqueous solution that is spiked directly with a solvent extract.

PDA might also be applicable to control the fugacity of multiphase systems. Suspended or dissolved organic matter can sorb a substantial fraction of hydrophobic organics, and this fraction can be available for biological uptake in, for example, toxicity tests with daphnids that are fed with algae. Fliedner (*29*) suggested therefore toxicity tests of poorly water-soluble substances in the presence of different third phases such as liposomes or algal cells (food) and at high aqueous concentrations. The addition of a third phase to traditional batch experiments will cause the free concentration to decrease, which makes it hard to operate such experiments close to saturation. PDA could then be used in an attempt to dominate the fugacity of the entire system and thereby to maintain concentrations in the different compartments: water, test organism, and third phase.

The predictable distribution of test substance between the sorbed phase and the dissolved aqueous phase is crucial for the successful application of PDA. High levels of surface active substances or organic solvents in the test system that may penetrate into the Empore disk might affect the sorption behavior of the disk. This needs further investigations for the administering of substances into difficult matrixes such as biological fluids. Furthermore, the release kinetics can be affected if the effective surface area for mass transfer is reduced or if the thickness of the undisturbed aqueous boundary layer is increased. Release kinetics from the disk into a sediment sample are consequently expected to be slower than release kinetics into water. Finally, the solid phase can disintegrate due to dissolution of the silica substrate at pH values above 7.5. These interferences can limit the general applicability of the presented method to "dirty real world" conditions. However, most environmental toxicity, bioconcentration, and biodegradation data are obtained in simple batch tests, and we expect the presented method to be applicable in most of these tests. Improved control of exposure concentrations in such tests is of great value for the risk assessment of hydrophobic organics since risk assessment highly depends on the quality of the experimental data it is based on.

Acknowledgments

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