Thin-Film Solid-Phase Extraction To Measure Fugacities of Organic Chemicals with Low Volatility in Biological Samples

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Thin-Film Solid-Phase Extraction To Measure Fugacities of Organic Chemicals with Low Volatility in Biological Samples

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To investigate the environmental fate, food chain bioaccumulation, and toxicity of organic chemicals, it is often preferable to measure the chemical's fugacity rather than its concentration. However, simple methods to do this are rare. This paper presents a novel yet simple method to measure fugacities of a range of poorly volatile hydrophobic organic chemicals ranging in octanol−air partition coefficients from $10^{5.6}$ to $10^{9.2}$. Thin films of ethylene vinyl acetate coated on glass surfaces are used as solid-phase samplers of contaminated biological tissues. The technique is applied to fish tissue samples and spiked fish diets to determine method feasibility, equilibration times, reproducibility, and property characteristics of the thin films. It is concluded that the method provides an attractive technique to measure chemical fugacities in biological tissues without requiring solvent extractions and cleanup. The method is further expected to be applicable to investigate the fugacity of semivolatile and poorly volatile organic chemicals in air, water, sediments, and soil.

Introduction

Mackay (*1*) and others have argued that when studying the environmental fate and food chain bioaccumulation (*2*) of contaminants, it is more insightful to express the presence of a chemical substance in environmental media in terms of fugacities than concentrations. [Fugacity is a thermodynamic quantity equivalent to chemical potential. It can be viewed as the pressure that the chemical exerts when present in a medium and can be measured as a partial pressure in units of Pascal (Pa)]. This is because, in multimedia environments, net passive transport of chemicals between different media occurs as a result of a difference in fugacity, not concentration. However, fugacity measurements are rarely made because of a lack of appropriate methods. Several techniques do exist for direct measurements of fugacities. They include (i) sparging water (*3*, *4*), (ii) the fugacity meter for tree leaves (*5*), and (iii) static headspace analysis (*6*, *7*). Techniques for making fugacity measurements in animal tissues do not exist to date.

Recently, solid-phase extraction has proven to be a preferred method for making measurements of the presence of trace contaminants in various media including water and sediment. Several methods exist including solvent-filled dialysis membrane devices (SPMD) (*8*−*12*), C-18 Empore disks (*13*), and solid-phase micro-extraction (SPME) (*14*−*18*). In these techniques, a solid or liquid extraction medium is brought in contact with an environmental medium such as air, water, or sediments for a certain duration, after which the concentration in the extracting medium is measured. This methodology is used to make both qualitative and quantitative measurements of the presence of chemical substances. The application of the method in a quantitative fashion is in some cases difficult (especially for extremely hydrophobic organic substances) as simple chemical equilibria are not quickly achieved. In the latter case, extensive calibration is required because the increase in concentration on the solid phase over time typically depends on the chemical absorption kinetics, which varies with the type of chemical substance, environmental conditions, and method of application. To improve and simplify the quantitative application of solid-phase extraction, it would be beneficial to use solid phases that exhibit quick absorption kinetics, resulting in chemical equilibria between the medium and the solid phase after short application times.

Solid-phase extraction provides an opportunity to measure fugacities in environmental media in an indirect fashion. If the chemical substance in the environmental medium of interest reaches an equilibrium with the solid phase, then the fugacities in the medium of interest (f_M) and the solid phase (f_S) will be equal. The fugacity of the substance in the medium of interest can then be found from the concentration in the solid phase (C_S) if the fugacity capacity (Z_S) is known through calibration, since $f_M = f_S = C_S/Z_S$. Alternatively, if the goal of the study is to determine a fugacity gradient (e.g., when measuring air-to-water or sediment-to-water fluxes of contaminants), then the ratio of the concentrations on the solid phases exposed to the two media is equivalent to the ratio of the fugacities in the two media because the fugacity capacity of the solid-phase material is identical when applied to both media.

To apply solid-phase extraction techniques to measure fugacities or differences in fugacities, some important conditions need to be met: First, the extracting medium has to reach an equilibrium with the environmental medium of interest to ensure that the fugacity in the solid phase is equal to that of the environmental medium. Second, the solid phase should not extract a significant proportion of the chemical from the environmental sample being tested. Third, if the method is applied to biological samples, the extraction time should be sufficiently short to minimize tissue decay, which could affect an accurate measurement of the fugacity. Also, for the practical application of the technique, reasonably short equilibration times are required. Fourth, it is important that the extracting medium is not being contaminated during application, e.g., by absorption of lipids or other organic materials. Finally, if the method is used to measure fugacities, it is important to calibrate the method by measuring the fugacity capacity of the solid phase for the chemicals of interest at the appropriate temperature.

SPMDs and Empore disks do not meet these criteria for very hydrophobic organic substances because their equilibration times are long, they can extract a significant fraction of chemical from the media of interest, and they are easily contaminated. SPMEs have a better potential for measuring fugacities in biological samples. They display the smallest volume of extracting medium and the largest surface area-to-volume ratio. However, the equilibration times for semivolatiles is still fairly long (*14*, *15*). The addition of methanol or hot water or the heating of a sample with a SPME fiber can shorten equilibration times, but these procedures are

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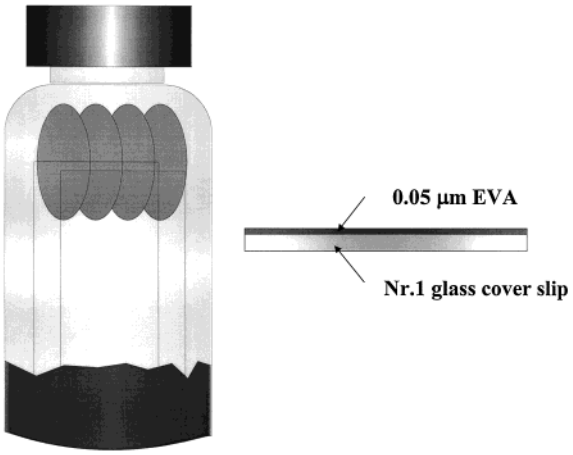

FIGURE 1. Illustrative diagram of a thin film (right) and its typical application (left) to measure the fugacity and lipid based concentration in biological matrixes.

also likely to modify the sample's matrix such that the resulting concentration measurements are a poor reflection of the chemical fugacity in the original sample. Other concerns with using SPME for fugacity measurements in biological matrixes include the potential contamination of the fibers with lipid when the SPME fibers are introduced directly to lipid-rich biological samples and incomplete desorption of the SPME fibers by the GC injector (*19*).

To measure the fugacities of semivolatile hydrophobic substances in environmental media, we have developed a new solid-phase method employing thin films of ethylene vinyl acetate. The films have a very large surface-to-volume ratio such that chemical equilibria with the films are reached quickly. The method is very simple and does not require the use of sample extraction and cleanup. In this paper, we discuss the application of the method to biological tissue samples, but the method may also be useful to measure fugacities in air, water, sediments, and soil.

Methodology

Thin-Film Preparation. Two coating solutions of ethylene vinyl acetate (Elvax 40W, Dupont, Wilmington, DE) (EVA) were prepared by dissolving 1.2 g of EVA in 200 mL of dichloromethane for the 0.05-μm films and 12 g of EVA in 83:17 (v/v) 2-octanol and dichloromethane (DCM) for the 0.33-μm films. A 22 mm diameter no. 1 glass cover-slip (Propper, Long Island, NY) was mounted on a rotating stage using a modified Fordom rotary tool (Fordom Electric Co., Bethel, CT). A total of 200 μL of coating solution was applied to the coverslip using a 1-mL Hamilton gas-tight syringe before rotating the coverslip at 5000 rpm for 10 s. Excess solution was spun off leaving thin films, which were virtually free of solvent within 1 h. To check the uniformity of the film application on the glass disks, a dye (Sudan IV) was added to the EVA solution, after which the films were visually inspected. All EVA films were of uniform thickness.

Thin Film Applications. Five methanol-rinsed thin films were placed on a rack in a 30-mL vial above 5 g of biological sample (Figure 1). The rack was designed to prevent contact between the films and the sample. A 1-mL sample of a 1% mercuric chloride solution in water was added to the samples to avoid microbial breakdown of the tissues. Air in the vials was displaced with nitrogen, and the vials were sealed airtight. Finally, the vials were rotated at 33 rpm at an incline of 20° to facilitate convection within the sample vial.

Thin-Film Analysis. Two methods for thin-film analysis were developed: solvent extraction and thermal desorption. Using the solvent extraction method, the EVA of each thin film was quantitatively transferred into a 5-mL test tube by four successive rinses with 1 mL of DCM. The DCM was then evaporated to 1 mL. A total of 200 μL of 2-octanol was added, and the DCM was further evaporated until only the octanol was left. Then 2 mL of methanol was added to precipitate the EVA. The mixture was centrifuged at 3000 rpm for 5 min, and the supernate was transferred in vials for GC analysis. The chemical extraction efficiency was tested by applying 200 μL of spiked thin-film coating solution to a small watch glass. After 5 min (to allow dichloromethane to evaporate), the spiked polymer coating was extracted using the same solvent extraction procedure as discussed above.

Thermal desorption involved placing the thin films in a specially designed thermal desorption unit (Figure 2) that was attached directly to a 30-m HP-5 GC column installed in a HP 5890 series II gas chromatograph. Temperature of the thermal desorption unit was regulated using an Omega temperature control unit and a 500-W heating element. EVA films were desorbed at 150 °C for 60 min while maintaining the GC oven temperature at 35 °C. The advantage of thermal desorption is that all analyte is used for GC analysis, hence reducing the detection limit. The chemical extraction efficiency was determined by injecting a standard solution of the test chemicals directly on a (nonexposed) film situated in the thermal desorption unit.

Spiked Fish Food Experiments. Silver Cup trout chow (lipid content: 8.0 ± 1.2%) containing 10 ppm 1,2,4,5-tetrachlorobenzene, 10 ppm pentachlorobenzene, 10 ppm hexachlorobenzene, 50 ppm 2,2′,5,5′-tetrachlorobiphenyl, 100 ppm 2,2′,4,4′,6,6′-hexachlorobiphenyl, and 10 ppm mirex was prepared by dissolving the test chemicals in 30−60 bp reagent-grade petroleum ether and then adding this solution to the trout chow. The chow and solution were then stirred continuously for 8 h after which the petroleum ether was evaporated. In 30-mL vials, 2 g of fish food was added with 1 mL of 1% mercuric chloride and 2 mL of distilled water. Then, five films were added, and the vials were sealed. After 1, 2.5, 4.5, 5.5, 19, 22.5, 35, 43, 96, and 144 h (for 0.05-μm films) and after 2, 9, 19.5, 60, and 132 h (for 0.33-μm films), 80 μL of nitrogen was removed for gas-phase concentration analysis (i.e., required for the fugacity calibration) and immediately analyzed by GC, after which the vials were opened, films were collected, and food was removed for concentration analysis. The films were analyzed immediately using the solvent extraction method. All experiments were carried out in triplicate and conducted separately for the thick and thin films. To determine the limit of quantitation (LOQ), 2 g of uncontaminated dried fish food was added together with 1 mL of 1% mercuric chloride, 2 mL of distilled water, and 5 films and exposed for 132 h. The LOQ was determined as the mean response plus 10 times the standard deviation of the mean response.

Fish Tissue Experiments. Adult rainbow trout (*Oncorhynchus mykiss*), weight 365 (± 66) g and lipid content 6.6 ± 0.72%, were exposed in a 240 L/h flow-through system to a diet of Silver Cup trout chow containing 2,2′,4,4′,6,6′-hexachlorobiphenyl (HCBP) for a period of 73 days as described in ref *20*. The carcass, i.e., all tissues except the intestinal tract and liver, of the fish that was exposed for 73 days was ground by passing once through a hand-cranked meat grinder followed by 5 min of vigorous hand mixing. A 50-g sample of tissue was then homogenized in a blender (Oster, USA) along with 50 mL of water for 30 s. A total of 2 mL of this homogenate was introduced into 30-mL vials together with 2 mL of distilled water and 1 mL of 1% mercuric chloride. Three 0.05-μm films were then added, after which the vials were purged with nitrogen, sealed airtight, and placed on an inclined turn table for gentle mixing at 20 °C. After 0 (control), 1, 4, 24, 48, and 96 h, 80 μL of nitrogen was removed for gas-phase concentration analysis and im-

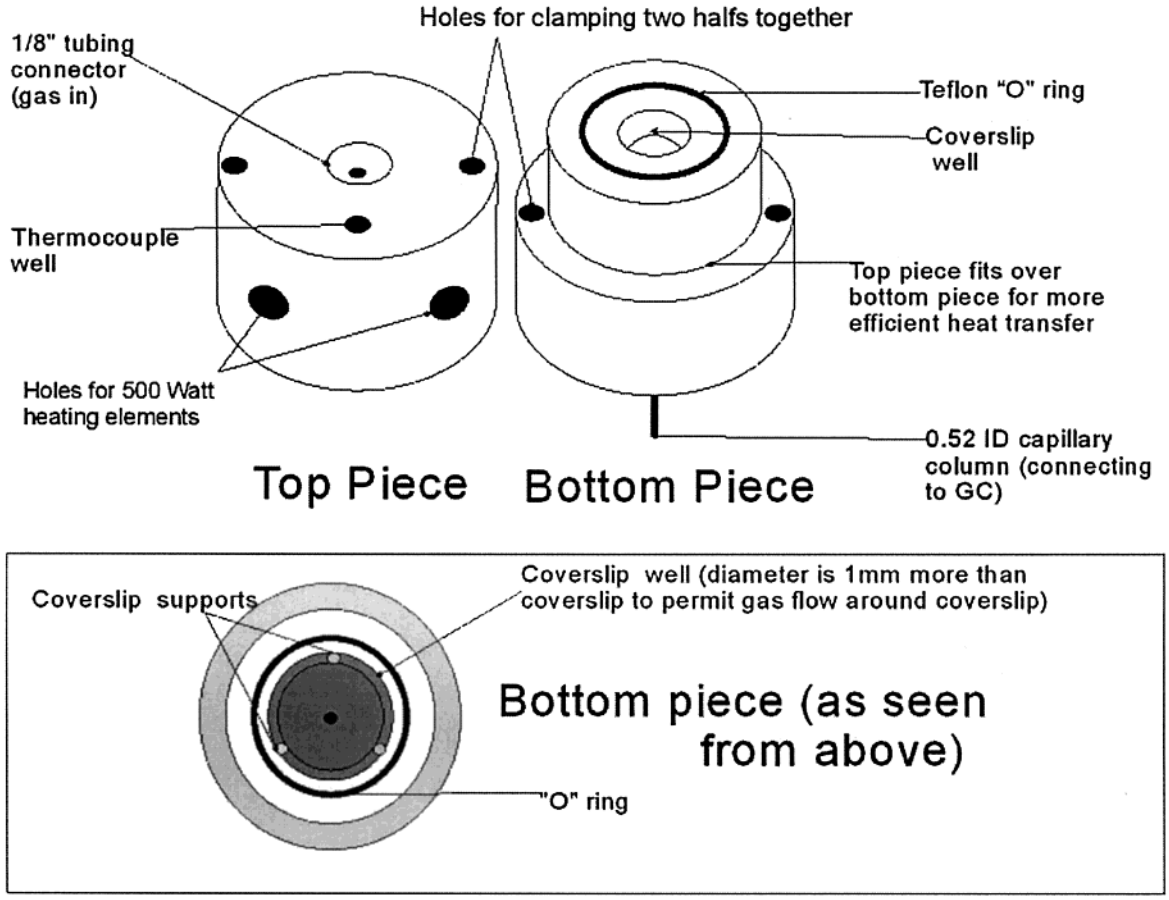

FIGURE 2. Diagram of the thermal desorption unit designed to analyze thin films.

mediately analyzed by GC, after which the vials were opened, films were collected, and the homogenate was removed for concentration analysis. The films were analyzed using the thermal desorption method. Experiments were carried out in triplicate. To determine the LOQ, exposures of films to uncontaminated rainbow trout tissues were conducted. The LOQ was determined as the mean response plus 10 times the standard deviation of the mean response in the blanks.

Concentration Analysis. Fish and spiked food samples (approximately 1 g) were each homogenized in a mortar with 5 g of granular anhydrous sodium sulfate. The homogenized sample was transferred to a column containing (from bottom to top) glass wool, 1 g of granular sodium sulfate, 12 g of acidified 60-mesh silica gel, and 18 g of sodium sulfate. The column was eluted with 250 mL of petroleum ether over a 6-h period with recoveries ranging between 89 and 99%. The eluent was then analyzed by GC.

Gas Chromatography. GC analysis was conducted on a HP 5890 series II equipped with a cool-on-column injection port, electron capture detector, and a 30 m × 0.53 mm × 2.65 μm (film thickness) HP-5 column. Carrier gas was helium at a flow rate of 20 cm/s (at 35 °C). The temperature program was 35 °C to 270 °C at 20 °C/min. External standards were used for sample quantification.

Data Analysis. To estimate equilibrium times for the analytes, nonlinear regression (Systat 8.0, SPSS Inc., 1998) was performed using the model:

$$C = A(1 - \exp(-Kt)) \quad (1)$$

where C is the concentration in the thin film (μg/g), t is time (h), and A and K are the parameters to be correlated. The time to reach 95% of equilibrium, t_{95}, was calculated as $3/K$.

TABLE 1. Properties of Thin-Film Solid-Phase Extraction Compared to C-18 SPME Fibers

	thin film (0.05 μm)	thin film (0.33 μm)	SPME fiber
coating thickness (μm)	0.049 ± 0.002	0.326 ± 0.056	100
coating vol (mm^3)	0.019 ± 0.0008	0.126 ± 0.022	0.61
surface area (mm^2)	380	380	9.4
surface area/vol (mm^{-1})	20 400	3 016	15.4

Relationship with Chemical Properties. Measured octanol−air partition coefficients (K_{OA}) for the chlorobenzenes and 2,2′,4,4′,6,6′-hexachlorobiphenyl (PCB155) at 20 °C were obtained from ref *21*. The K_{OA} for 2,2′,5,5′-tetrachlorobiphenyl (PCB52) was estimated from a measured value (*22*) for PCB53 corrected for ortho-substitution and is in agreement with the calculated value of $10^{7.95}$, derived from K_{OW} and the Henry law constant in ref *23*. The K_{OA} for mirex was calculated from data in ref *23*.

Results and Discussion

Thin Films. Table 1 summarizes the coating thickness, volume, surface area, and area/volume ratio of the thin films and compares these properties to those of SPMEs with a standard 100-μm polydimethylsilicone (PDMS) coating. It shows that the area-to-volume ratio of the thin films is more than 1000 times greater than that of the SPME fibers. This suggests that equilibration times in thin films have the potential to be substantially shorter than those of SPMEs. SPME fibers with a smaller PDMS coating thickness of 15 μm (*15*) and even 7 μm (*22*) have been used, but area/volume

TABLE 2. Logarithms of Octanol−Water (K_{OW}) and Octanol−Air (K_{OA}) Partition Coefficients at 20 °C, EVA Film−Air Partition Coefficient (K_{EA}) at 20 °C in Thick Films (n = 5), Fugacity Capacity (mol/m³·Pa) of EVA Films (Z_E) and Octanol (Z_O), Time To Reach Equilibrium (t_{95} (in h)), and Limit of Quantitation (LOQ in ppm in Tissue Samples) Using Solvent Extraction Technique for Some Selected Chemicals

chemical	log K_{OW} (*23*)	log K_{OA} (*21*)	log K_{EA} obsd	log Z_E obsd	log Z_O (*23*)	t_{95} (0.05 μm)	t_{95} (0.33 μm)	LOQ (0.05 μm)	LOQ (0.33 μm)
tetrachlorobenzene	4.50	5.63	5.96	2.57	2.41	<1	≤1	20	4.6
pentachlorobenzene	5.00	6.27	6.76	3.38	3.07	<1	6.2 (4.1−12)	8.0	2.0
hexachlorobenzene	5.50	6.90	7.34	3.95	3.38	3.0 (1.8−8.6)	16 (11−24)	4.1	0.75
tetrachlorobiphenyl (PCB52)	5.84	8.10[a]	8.60	5.22	4.57	32 (24−48)	81 (62−110)	10	2.4
hexachlorobiphenyl (PCB155)	6.40	8.99	8.83	5.44	5.12	38 (28−57)	103 (79−140)	4.9	1.1
mirex	7.50	9.17[b]	*c*	*c*	5.78	136 (79−500)	3000 (375−4000)	15	3
hexachlorobiphenyl (from trout tissue)	6.40	8.99	8.79	5.40	5.12	12.5 (9.4−17.6)		0.1	

[a] Estimated from a measured value (*22*) for PCB53 corrected for ortho-substitution. [b] The K_{OA} for mirex was calculated from K_{OW} and Henry law constants data in ref *23*. [c] Equilibrium was not achieved.

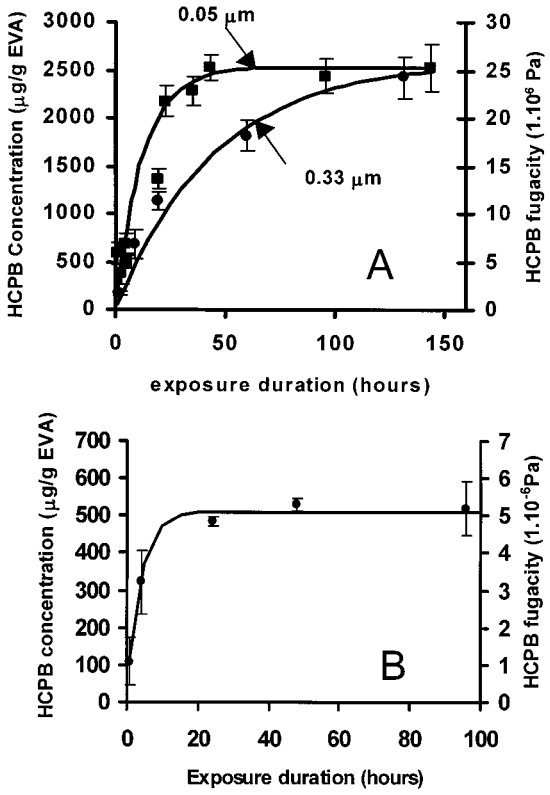

FIGURE 3. Uptake of hexachlorobiphenyl in 0.05 (square symbols) and 0.33-μm thin (round symbols) films from dried fish food samples (A) and in 0.05-μm thin films from rainbow trout wet tissue samples (B).The solid line represents the results from nonlinear regression of the concentration data with time. The error bars represent 1 SD.

ratios of these fibers are still manyfold greater than those in the thin films.

Equilibration Times. In the spiked fish food experiments, all substances, with the exception of mirex when using 0.05- or 0.33-μm films, achieved a chemical equilibrium within the 150 h of exposure when using 0.05- or 0.33-μm films. For tetrachloro- and pentachlorobenzene, equilibrium in the 0.05-μm films appeared to be achieved before the first sample was collected at 1 h, and concentrations throughout the uptake period did not vary significantly with time. A typical uptake curve is presented for HCBP in Figure 3. Nonlinear regression of the uptake data indicates that the 95% equilibration times ranged from less than 1 h for tetrachlorobenzene to approximately 136 h for mirex for 0.05-μm films (Table 2). The application of thicker film coatings resulted in generally larger equilibration times ranging from approximately 1 to 103 h for hexachlorobiphenyl. Mirex showed a nearly linear uptake curve in 0.33-μm films, indicating a high value of t_{95} with a high degree of uncertainty (Table 2). Equilibration times increased with increasing K_{OA}. These observations are consistent with a tissue−gas−film three-phase resistance model. In this model, the total resistance (R_{TOTAL}) that a chemical substance encounters when diffusing from the sample matrix into the thin film can be represented by the t_{95}. The total resistance can be viewed as the sum of the resistances for mass transport of the chemical in the biological tissue (R_T), the gas phase (R_G), and the thin film (R_F):

$$R_{TOTAL} = R_T + R_G + R_F \quad (2)$$

It can be shown (Supporting Information) that this model can be formulated as

$$t_{95} = 3 \times \{(d_F/\kappa_F) + (V_F K_{FG}/Q_G) + (V_F K_{FT}/\kappa_T A_T)\} \quad (3)$$

where d_F is the thickness (m) and V_F is the volume (m³) of the film; κ_F and κ_T are the mass transfer coefficients (m/h) for diffusion in the film and tissue, respectively; K_{FG} is the film to gas-phase partition coefficient; K_{FT} is the film to tissue partition coefficient; Q_G is the hypothetical flow rate (m³/h), representing combined diffusion and gas circulation in the vial; and A_T is the tissue-to-gas-phase area of diffusion (m²).

This model illustrates that R_F and R_T, representing diffusive processes, are likely to increase with the molecular weight of the substance (from 215.9 to 545.6 g/mol). R_G can be expected to increase with molecular weight as well, but more importantly, it increases (relative to R_T and R_F) with increasing K_{FG}, as the capacity of the gas-phase to hold chemical mass drops relative to that in the films with increasing K_{FG}. Assuming a strong correlation between K_{FG} and K_{OA}, an increase in K_{OA} from $10^{5.63}$ to $10^{9.71}$ corresponds to an approximately 10 000-fold increase in the gas-phase resistance relative to the resistance in organic (i.e., octanol-like) phases such as the biological matrix and the film. The observed proportional increase of t_{95} with increasing K_{OA} for the thin films illustrates the increased resistance for chemical mass transfer for the poorly volatile, high K_{OA} substances.

Equation 2 further illustrates that an increase in film thickness can be expected to increase t_{95} (and hence increase R_{TOTAL}).

Figure 3 shows that in the fish tissue experiments, the concentration of HCBP on the films increased quickly to achieve an equilibrium. Nonlinear regression ($n = 5$, $R^2 = 0.99$) indicates a t_{95} of 12.5 h with 95% confidence limits of 9.4−17.6 h. The observation that HCBP (log $K_{OW} = 7$) effectively achieves a chemical equilibrium within 0.05-μm films from a tissue homogenate in approximately 12.5 h, while approximately 3 days are required if the same chemical is present, in dried fish food indicates that the matrix in which the chemical resides can have a significant effect on the equilibration time. The drying of tissues appears to increase R_T, resulting in longer equilibration times.

Thin-Film Extraction. The extraction efficiency of the test chemicals from the thin films ranged from 78% to 94% using solvent extraction and from 85% to 92% applying thermal desorption. The lower efficiencies for the more volatile substances are likely due to evaporative losses during handling. Due to the very quick equilibration times of the more volatile test chemicals, losses from the films occur when transferring the films from the exposure vials to the extraction media.

Reproducibility. In the spiked fish food experiments, coefficients of variation (COV) of thin-film concentration measurements among replicates dropped with increasing K_{OW} from 20% to 5%. The use of thicker films reduced the coefficient of variation. The higher variability among replicate samples of the lower K_{OA} chemicals and the thinner films is probably due to the quick desorption of the lower K_{OA} substances during the film's handling from vial to extraction. In the fish tissue experiments, the COV in concentration among replicates was 10%.

Limit of Quantitation. Table 2 illustrates that in this study the method's limit of quantitation (LOQ) ranged from 20 to 4.9 ppm (in the 0.05-μm film) and from 4.6 to 0.75 ppm (in the 0.33-μm film) depending on the chemical substance when using the solvent extraction technique for film analysis. In the fish tissue experiments, using thermal desorption for analysis, the LOQ was 0.1 ppm.

This LOQ was sufficient to achieve our goal of measuring equilibration times, but it is relatively high when applying the method to very hydrophobic organic chemicals in typical biological samples taken from the field. Substantial reductions in the LOQ can be achieved by minimizing analyte dilution during the film extraction procedure and optimizing GC analysis and detection. To further reduce the LOQ, films can be rinsed with methanol prior to their use to reduce baseline interferences. In addition, reductions in LOQ are likely achieved when improving the selectivity of detection, e.g., by using GC−MS or by using radiolabeled substances.

Film−Air Partition Coefficients. Figure 4 illustrates that film−gas partition coefficients (K_{EA}) at equilibrium (calculated as the ratio of chemicals concentrations on the 0.33-μm film (C_E) and in the gas phase (C_A)) at equilibrium increased with increasing K_{OA} according to

$$\log K_{EA} = 0.89\ (\pm 0.09) \log K_{OA} + 1.19\ (\pm 0.69) \qquad r^2 = 0.97 \quad n = 5 \quad (4)$$

Figure 4 illustrates that the ethylene vinyl acetate films have a sorption potential for the test chemicals that is approximately twice that of octanol, which is a well-recognized surrogate for lipids in organisms. The correlation between K_{EA} and K_{OA} suggests that equilibrium concentrations in the films may be used as a measure of the chemical concentrations in the lipids of the sample. This was tested more directly by comparing the concentrations in the films to the lipid-normalized concentrations in the samples. This is illustrated in Figure 5, which shows that lipid-normalized concentrations

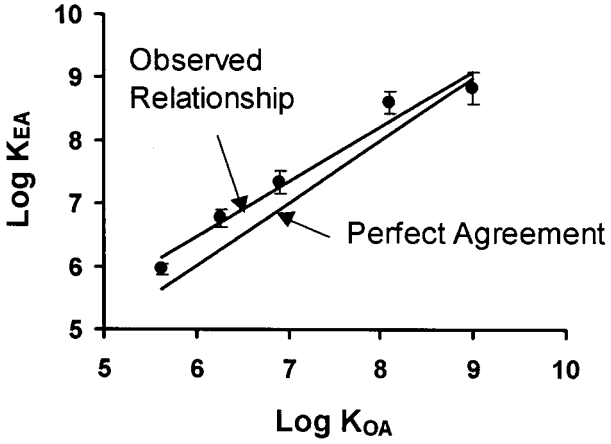

FIGURE 4. Relationship between the partition coefficients of some semivolatile and poorly volatile substances between ethylene vinyl acetate films and air (K_{EA}) and octanol and air (K_{OA}). Error bars represent 1 SD.

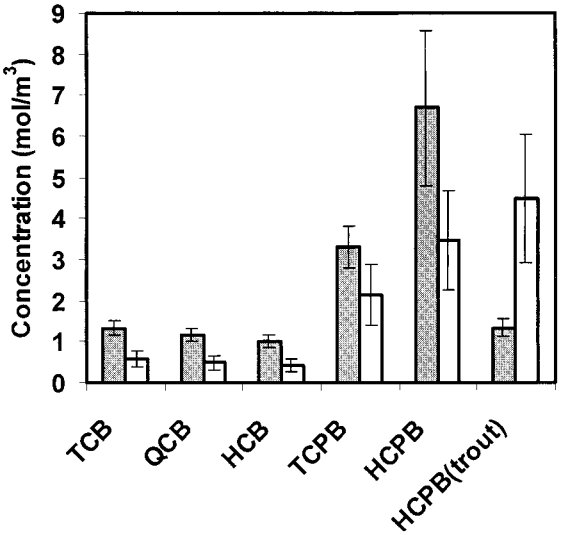

FIGURE 5. Observed concentrations of some semivolatile and poorly volatile substances in ethylene vinyl acetate films (mol/m^3 EVA), gray bars, and in the biological matrix on a lipid normalized basis (mol/m^3 lipid), white bars. Error bars represent 1 SD.

were approximately half of those measured in the films. However, the lipid-normalized concentration of HCPB in the trout tissue was greater than that in the films. These observations suggest that there may indeed be a useful relationship between lipid-normalized and EVA film concentrations that can be explored to measure lipid-normalized concentrations with EVA films but that differences in lipid solubility among various classes of lipids exist that can cause differences between lipid-normalized and thin film concentrations. The correlation between K_{EA} and K_{OA} may provide an alternative method to determine K_{OA} of very hydrophobic compounds as K_{EA}. The advantage of the films in measuring K_{OA} is that the polymers eliminates the possibility of minute quantities of octanol entering the gas phase, affecting the correct measurement of the air concentration. While differences in chemical partitioning characteristics between octanol and EVA undoubtedly exist, these differences may in some cases be small as compared to experimental error associated with K_{OA} measurements or of little overall significance when using the K_{OA} as a tool to correlate other environmentally relevant partitioning processes.

Fugacity Capacity. The fugacity capacity (Z in mol m^{-3} Pa^{-1}) represents the increase in the chemical concentration

(in mol m^{-3}) in a medium that has to be achieved to raise the fugacity of the substance in that medium by 1 Pa (*1*). Alternatively, it can be viewed as the capacity of the medium to dissolve the substance. The fugacity capacity of the EVA (Z_E in units of mol m^{-3} Pa^{-1}) was determined as the ratio of the concentration in the thin films (C_E in units of mol m^{-3}) at equilibrium and the fugacity measured in the gas-phase (f in units of Pa), i.e., C_E/f. The fugacity f was determined as the product of the gaseous concentration (C_A), the gas constant (8.31 J/mol·K), and the temperature (293 K). The fugacity capacities are listed in Table 2 and increase from 373 to 279 000 mol m^{-3} Pa^{-1} with increasing molecular weight. A comparison of the fugacity capacities of the films and octanol shows that the fugacity capacity of the thin films for the test chemicals is comparable to that of octanol. The merit of characterizing the fugacity capacity is that fugacities in thin films can be determined from the measured concentrations in the films, i.e., as $f = C/Z$. Since the fugacities in the tissue samples and thin films are by definition equal at equilibrium, the chemical fugacities in organism tissues can be derived. Because the fugacity capacity of the films are independent of the medium to which they are applied, differences in chemical concentrations on the films are a direct measure of differences in chemical fugacities between the media or samples to which the films are applied. If applied to measure differences in fugacity, it is not necessary to measure the fugacity capacity as the fugacity capacity of the films remains identical when applied to different media and for each medium C_E is therefore proportional to the fugacity f.

Method Application. The method described in this paper is relatively simple and avoids time-consuming and expensive solvent extraction and cleanup steps typically associated with the analyses of biological samples. Its main purpose is to measure fugacities of chemical substances in biological samples. Under certain conditions, the thin film concentration is also a reasonable measure of the lipid-normalized concentration in biological samples. These measures are not identical to the whole tissue concentrations typically measured through solvent extraction techniques. The fugacities express the thermodynamic status of the chemical in the tissue or medium, which may be viewed as the "active" or "working" concentration. A high fugacity or active concentration means that more substance is available for diffusion across membranes or to bind to any receptor site via passive diffusion. Since the composition of biological tissues is typically complex and highly variable between different tissues (e.g., organs) and between different species, it is difficult to compare concentrations directly to determine differences in thermodynamic status or gradients of diffusion. For example, it is possible that two media or tissues with the same total concentration (measured through solvent extraction) display different fugacities or active concentrations (measured by the films). The medium or tissue with the highest fugacity may exhibit the greater bioavailability or potential for toxicity. The thin film method is expected to be a useful tool to explore the bioavailabilty of organic chemicals. For example, it can be used to measure the effect of food digestibility on the dietary availability of contaminants, and it may provide a better measure of the bioavailability of contaminants in sediments, soils, and water samples than the total concentration does. In cases where the total concentration of a chemical in a tissue is required, it is possible to derive the lipid-normalized sample from the concentration in the thin film. This assumes that the partitioning properties of the films and the lipids in the sample are related. It is important to verify this relationship before applying this method as the partitioning properties of lipids may vary between lipid types.

When applying the method to analyze fugacities of semivolatile and poorly volatile substances in biological tissues, it is important to choose the correct film thickness. While very poorly volatile substances (log K_{OA} greater than 7) are best measured using thin films, thicker film are recommended for the more volatile substances. The advantage of the thicker films is that equilibration times are longer (hence chemical losses during film handling are smaller, resulting in better reproducibility), and thicker films contain more chemical for detection (hence lowering the LOQ).

While this study explored the application of the method to biological samples, it is expected that it can be used in a fashion similar to air, water, sediments, and soil samples. When applied to other media such as air, water, sediments, or soils, it is important to establish in separate experiments what the appropriate equilibration times are for the analytes under study.

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Supporting Information Available

Derivation of the tissue−gas−film three-phase resistance model describing the relationship between thin-film equilibration times and K_{OA} (3 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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