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Environmental Effects of Dredging Technical Notes

New Technique for Sediment/Organism Equilibrium Partitioning Studies

Purpose

This technical note reports on the results of the initial experiment testing a new procedure that employs a kinetic model and a simple short-term fish/suspended sediment exposure system to assess equilibrium partitioning (EqP) of neutral organic chemicals.

Background

Sediment Quality Criteria (SQC) based on EqP are under development by the U.S. Environmental Protection Agency (EPA). A stated purpose of SQC is the regulation of dredged material disposal. Long-term Effects of Dredging Operations (LEDO) Work Unit 32571, Relationships Between Sediment Geochemistry and Biological Effects, is researching methods to characterize the interactions between geochemical and physicochemical processes and contaminant uptake in, and effects on, aquatic organisms. This research will enable the U.S. Army Corps of Engineers (USACE) and other interested parties to best evaluate the adequacy of the EqP-based SQC as they are proposed and promulgated.

Theoretical bioaccumulation potential (TBP) calculations are based on the same thermodynamic principles as the EqP-based SQC. TBP is recommended as a screening procedure in the revised "Green Book," the national testing manual for ocean disposal of dredged material (EPA and USACE 1991). TBP is used in Tier II to indicate whether the presence of neutral organic chemicals in sediment is of little or no concern, or whether more definitive characterization by biological testing is necessary at Tier III or Tier IV levels. Revisions of the Green Book are expected to be made periodically. In the revisions, accuracy of TBP calculations may be increased by the use of empirically determined preference factors (*pf*) for specific chemicals. In sediment bioaccumulation tests, *pf* is

a constant that expresses the magnitude of the concentration difference at equilibrium, that is, the "preference" of the chemical for organism lipid versus sediment organic carbon. An alternative expression used by some workers is "accumulation factor" or AF. However, the meaning and usage are the same.

Research that further defines the geochemical, physicochemical, and physiological influences on EqP serves to improve the utility of TBP as well as to facilitate evaluation of EqP-based SQC intended to regulate dredged material disposal.

Additional Information

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Introduction

Equilibrium partitioning (EqP) studies on bioaccumulation of organic chemicals in aquatic organisms typically require long-term exposures. In the case of highly hydrophobic neutral organic chemicals, such as polychlorinated biphenyls (PCBs), dioxins, and dibenzofurans, exposures of up to 6 months have been used (Pruell and others 1990). During long-term laboratory exposures, changes can occur in the condition of both organism and sediment. Sediments can be depleted of the bioavailable fraction of chemical, thereby reducing exposure. Nutrient quality and amount may decline and sublethal toxicity may occur, affecting the health of organisms and causing loss of lipids. Metabolic degradation of bioaccumulating chemicals may occur in long-term exposures, reducing bioaccumulation. Induction of metabolizing enzymes caused by the chemical under investigation or by other chemicals in the sediment may exacerbate this effect. Growth during the exposure period can dilute tissue concentrations causing reduced apparent bioaccumulation. Spawning and other seasonal changes also affect bioaccumulation (Lee and others 1989).

Kinetic modeling using short exposures provides an alternative to long-term exposures to achieve EqP. Short exposures greatly reduce or eliminate the difficulties inherent in long-term exposures. A drawback to using kinetic models and short exposures is the necessity of frequent sampling to define the uptake curve and enable a projection of steady state. However, when short exposures are used in a research mode, it is often possible to greatly reduce the cost of analyzing a large number of samples by using radiolabeled chemicals.

The purpose of this study was to test a new approach to EqP measurements that eliminates the necessity of obtaining steady-state conditions in the exposure. The procedure determines the proportional difference at projected steady

state between neutral chemical concentration in sediment and organism using a three-compartment closed kinetic model. The exposure matrix involves suspended sediment and fish, rather than the usual deposited sediment and sediment-processing infaunal organisms. The design permits manipulation separately of each of the principal variables (the chemical, organic carbon, and organism lipid) affecting the magnitude of the difference in concentration at equilibrium between chemical in sediment and in exposed organisms. This is accomplished while eliminating the sources of error inherent in conducting long-term exposures. This new technique is intended to be used as a research tool for understanding the relative contributions to EqP of geochemical and physicochemical properties, and physiological differences among organisms; and for the empirical determination of preference factors, *pf*.

Materials and Methods

Materials

PCB-52 ([^{14}C] 2,2′,5,5′-tetrachlorobiphenyl-UL) was obtained from Sigma Chemical Company. A 1:50 dilution with methanol of an 11.9 µCi/mL stock solution (409 µg/mL in toluene) provided a working solution of 0.238 µCi/mL or 8.18 µg/mL of PCB-52.

Japanese medakas, *Oryzias latipes*, were purchased from Carolina Biological Supply and were acclimated to laboratory conditions for at least 14 days at ambient temperature (≈ 23°C), in 120-L glass aquaria. Fish were maintained in filtered, aerated, dechlorinated tap water and were fed Aquarian® tablet food twice daily. A 12-hr dark/12-hr fluorescent light photoperiod was controlled by an automatic timer. Fish of both sexes were randomly used in the experiments. Air-dried and milled sediment from Barataria Bay, Louisiana, was stored at 4°C until use.

Fish and Sediment Exposure to PCBs

A 2-L Florence flask was used as the exposure vessel (Figure 1). Two liters of dechlorinated water were placed in the flask containing 400 mg of sediment. Two medakas (0.5 to 1.2 g combined fresh weight) were placed in the flask. The sediment was suspended in the water by continuous stirring using a magnetic stir bar rotated at approximately 150 rpm's, and 0.1 mL of the PCB solution was added by pipet. Fish were not fed during exposures.

After various time intervals (0 to 120 hr), fish, sediment, and water were collected and analyzed for total radioactivity. Nine time intervals were used with three replications at each interval. One experimental unit (Florence flask, fish, water, and sediment) was taken down at each sampling. Fish were netted and rinsed with distilled water and their combined weight was taken. PCBs and lipids were extracted (Lake and others 1990) from the combined sample following homogenization in 20 mL acetone (x2) using a Brinkmann PCU-2-110 Polytron homogenizer. The acetone extract was partitioned between hexane and

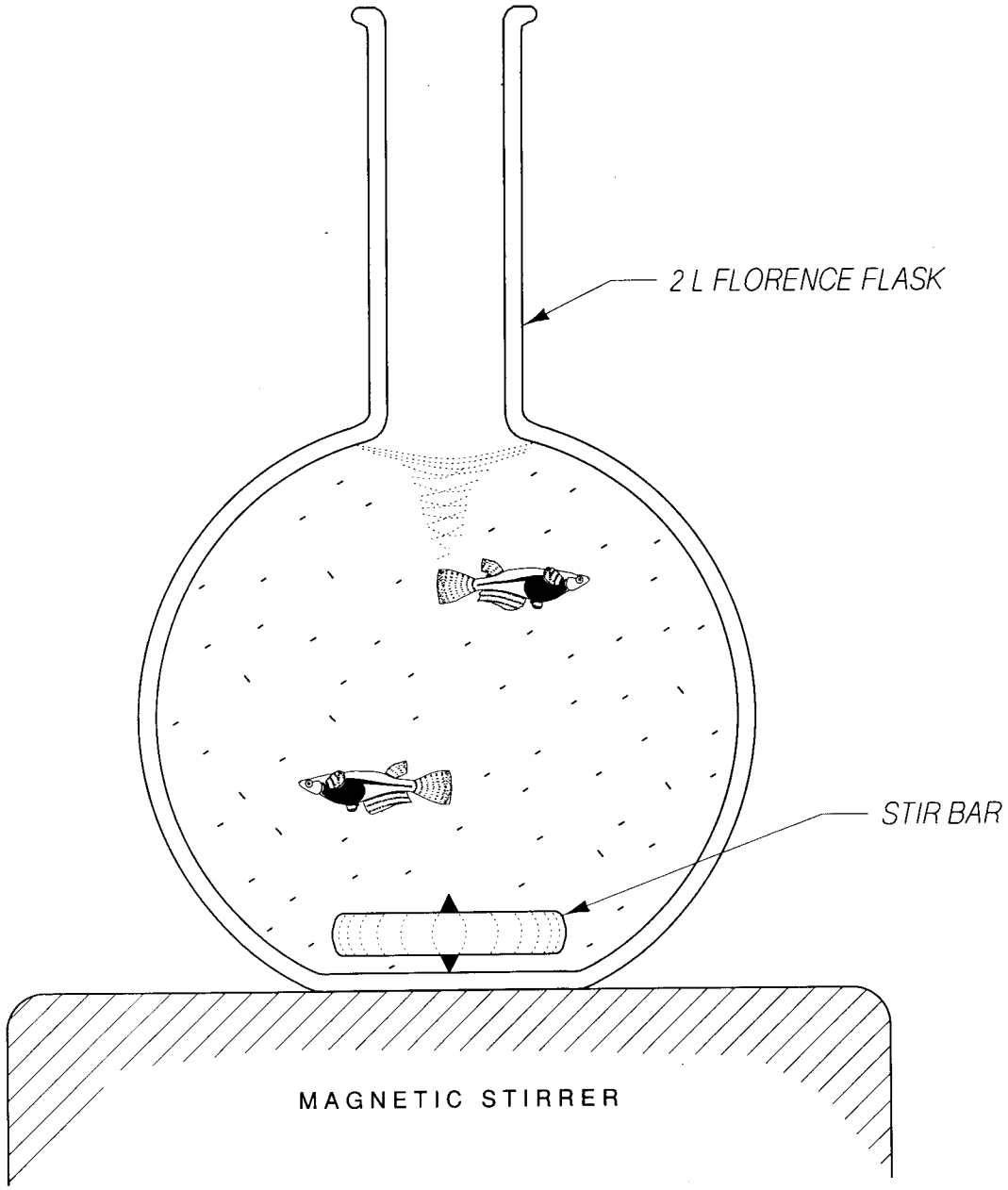

Figure 1. Exposure system, a Florence flask containing two medakas and 400 mg of sediment suspended in 2 L of water by means of a magnetic stirrer operated at 150 rpm's

water in a conical centrifuge tube. The hexane fraction was then split to provide separate aliquots for total lipids and total radioactivity measurements. The hexane extract for lipid determination was evaporated to dryness and total lipids were determined gravimetrically.

The sediment-water mixture was centrifuged and 100 mL of the supernatant were extracted with 25 mL hexane:acetone (4:1) followed by 20 mL hexane. The sediment pellet was extracted twice with 5 mL acetone and then partitioned between hexane and water. The hexane extracts of fish, sediment, and

water for radioactivity measurements were evaporated to near dryness in 20-mL scintillation vials; 15 mL of pseudocumene solvent (PCS) scintillation fluid (Amersham) was then added and the radioactivity counted on a Beckman Model 3801 Liquid Scintillation System.

Six 200-mL aliquots of the suspended sediment were centrifuged separately and the sediment pellets were analyzed by wet oxidation using the ampule method for total organic carbon (TOC) (Plumb 1981). The instrument used was an Oceanographic International Model 700 TOC Analyzer with infrared detection.

Data Analysis

Computer modeling was conducted using PCNONLIN®, version 3.0 (Metzler and Weiner 1989).

Results

The starting weight of fish in the exposure system varied from one exposure to another, while the mass of sediment put into suspension and the volume of water were constant. Therefore, the water and sediment radioactivity data for computation were adjusted based on a fish fresh weight of 1.0 g. The sediment TOC was 4.00 percent ± 0.578 (standard deviation). Lipid analyses produced highly variable results and the measurements taken were judged unusable for purposes of normalization of concentration data.

A three-compartment, closed kinetic model was used to represent PCB-52 distribution among water, fish, and sediment (Figure 2). In this model, water represents the central compartment (compartment 1) with PCB-52 being absorbed from the water by the sediment (compartment 2) and by the fish (compartment 3). Simultaneously, some of the chemical is desorbed back to the water from the sediment and eliminated to the water by the fish. These four processes are described by rate constants. Direct exchange between fish and sediment was considered insignificant and was not included in the model.

Equations for the rate of change of PCB in water, fish, and sediment are:

$$dC_w/dt = k_{21}C_s + k_{31}C_f - k_{12}C_w - k_{13}C_w \quad \textbf{(1)}$$

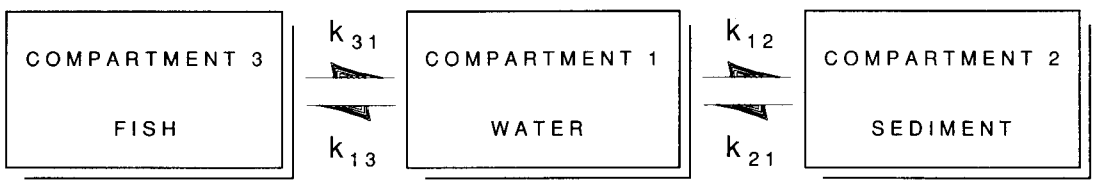

Figure 2. Three-compartment, closed kinetic model for phase-distribution of the chemical in the exposure system

$$dC_s/dt = k_{12}C_w - k_{21}C_s \quad (2)$$

$$dC_f/dt = k_{13}C_w - k_{31}C_f \quad (3)$$

where C_w is the adjusted mass of PCB-52 in the central compartment (µg/2 L), C_s is the adjusted mass of PCB-52 in the sediment (µg/400 mg), C_f is the concentration of PCB-52 in the fish (µg/g), and k_{12}, k_{21}, k_{13}, and k_{31} are the rate constants for intercompartmental transfer.

The three differential equations (Equations 1 through 3) were fitted simultaneously to the experimental data by a nonlinear least-squares technique using PCNONLIN®. Values for the least-squares estimates of the rate constants are shown in Table 1. The analysis of variance for the fitted equations is shown in Table 2.

Table 1. Least-Square Estimates of Rate Constants for Intercompartmental Transfer of PCB-52 for the Three-Compartment Model*

Rate constant	Description	Least-squares Estimate (plus/minus standard deviation)
k_{12}	Water to sediment	0.460 ± 0.035
k_{21}	Sediment to water	0.108 ± 0.016
k_{13}	Water to fish	0.066 ± 0.008
k_{31}	Fish to water	0.005 ± 0.002

* Units of rate constants are reciprocal hours (hr^{-1})

Table 2. Analysis of Variance for the Data Fitted to the Model Equations

Equation	Source	df	SS	MS	F	P	r
dC_w/dt	Model	1	2.26	2.26	—	—	—
	Error	28	0.05	0.0018	1,255.6	<0.001	0.995
dC_s/dt	Model	1	0.89	0.89	—	—	—
	Error	28	0.24	0.0086	103.8	<0.001	0.877
dC_f/dt	Model	1	1.15	1.15	—	—	—
	Error	28	0.11	0.0039	292.7	<0.001	0.959

Figure 3 shows distribution of PCB-52 among the three compartments (water, sediment, and fish) over a 120-hr period. Model-generated lines are fitted to the data. Uptake of PCB-52 by sediment was rapid as was the decline of PCB-52 concentration in the water. By the 24th hour PCB-52 water concentration stabilized. The concentration of PCB-52 in the sediment shows a

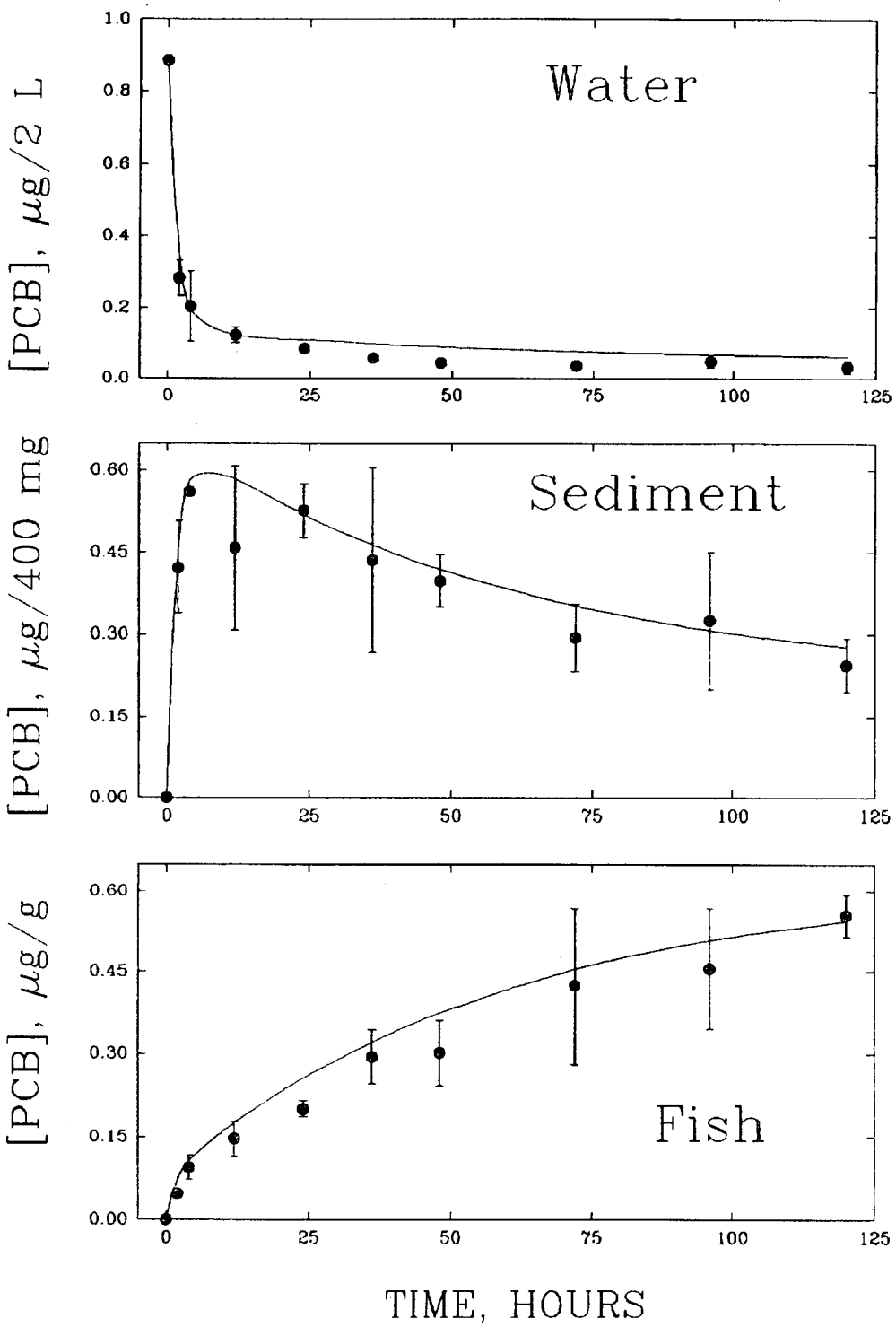

Figure 3. Distribution of PCB-52 among the three compartments — water, sediment, and fish over time; filled circles are means, vertical bars are plus/minus 1 standard deviation; lines are model-fitted estimates from the data

gradual decrease after the initial sorption phase, and this release of the chemical is reflected in the similarly gradual uptake of PCB-52 by the fish.

Discussion

The slower uptake by the fish as compared with the sediment is typical of bioaccumulation or bioconcentration curves for hydrophobic neutral organic chemicals in general and can be explained by the existence of rate-limiting processes of a physiological nature (Karara and McFarland 1992). Equation 3 can be integrated to obtain the time required to reach a proportion (P) of steady-state concentration of chemical in the fish (C_{ss}) when the exposure (water) concentration (C_w) is constant:

$$t = -1n\ (1 - P)/k_{31} \quad (4)$$

Solving Equation 4 for approximate steady state (P = 0.99) results in a time of 921 hr or about 38 days. This time requirement is the same as would be expected for PCB-52 (or chemicals of similar log K_{ow}) in a standard bioconcentration or sediment bioaccumulation study, and is the reason why the Green Book (EPA and USACE 1991) requires at least a 28-day test for demonstration of bioavailability in Tier III testing and in Tier IV bioaccumulation tests. However, it is not necessary to carry a test of bioaccumulation to actual steady state if, as was done in the present experiment, the rate constants for uptake and elimination can be obtained by time-sequenced sampling over the first hours or days of exposure. Then, since steady-state chemical concentration in tissue is

$$C_{ss} = k_{13}C_w/k_{31} \quad (5)$$

the ratio of absorption and elimination rate constants is the bioconcentration factor, K_b (Branson and others 1975, Clarke and McFarland 1991):

$$k_{13}/k_{31} = C_{ss}/C_w = K_b \quad (6)$$

It has also previously been demonstrated that constant exposure conditions are likewise unnecessary if the exposure concentration decreases in a predictable first-order fashion (Karara and McFarland 1992).

As with K_b, the distribution coefficient for chemical partitioning between sediment and water, K_D, can be expressed as the ratio of rate constants:

$$K_D = C_s/C_w = k_{12}/k_{21} \quad (7)$$

When C_w is the same in Equations 6 and 7, the relationship of the two can be expressed as a proportion:

$$K_b/K_D = C_{ss}/C_s = (k_{13}/k_{31})/(k_{12}/k_{21}) \quad (8)$$

Equation 8 expresses the EqP relationship between sediment and organism, commonly referred to as the bioaccumulation factor, *BAF*. For the data of this experiment (Table 1):

$$BAF = (0.66/0.005)/(0.460/0.04) = 3.10 \quad \textbf{(9)}$$

Because *BAF* for a particular chemical will change depending on the organic carbon content of the sediment and the lipid content of the organism, the data of this type of experiment are usually normalized. Normalization on organic carbon and on lipid allows calculation of the *pf*:

$$pf = [(k_{13}/k_{31})/f_{lipid}]/[(k_{12}/k_{21})/f_{oc}] \quad \textbf{(10)}$$

where f_{lipid} and f_{oc} are the decimal fractions of lipid and organic carbon in exposed organisms and in sediment, respectively.

In the present study the lipid analyses were unusable and difficulties in the analysis are being resolved for future studies. For purposes of calculation here a default value of 6.0 percent lipid was selected based on previous studies in which lipid measurements were made on medakas (McFarland, Clarke, and Gibson 1985). Substituting the rate constants measured here (Table 1) and the decimal fractions of organic carbon (0.04) and lipid (0.06) into Equation 9 gives:

$$pf = [(0.066/0.005)/(0.06]/[(0.460/0.108)/0.04] = 2.07$$

This value is in very good agreement with previously published *pf* values for PCB-52 using infaunal sediment-processing clams. Brannon and others (1989) exposed *Macoma nasuta* to sediment spiked with radiolabeled PCB-52 for up to 23 days and measured an average *pf* = 1.94. Sediment TOC was 1.06 percent. Ferraro and others (1991) exposed *M. nasuta* for 28 days to field-collected sediments having PCB-52 concentrations of 4.9 to 50 ng/g and having TOC contents ranging 0.84 to 7.37 percent. The *pf* values calculated were 2.1, 1.9, 2.1, 0.94 and 0.56.

Conclusion

A three-compartment, closed kinetic model applied to data obtained with a simple fish/suspended sediment exposure system can be used to study EqP relationships for neutral organic chemicals. The approach eliminates the difficulties inherent in long-term testing required to reach steady-state conditions and the results agree well with previous work. The simplicity of the system permits a high degree of control to be exercised over the principal variables. This new technique can be applied both to determining reliable *pf* values for TBP calculations and, in a research mode, to gain further insights into the fundamental processes of equilibrium phase distribution of neutral organic chemicals. No kinetic projections can be made without some associated error, but in the present study that error appears to have been small judging by the high

correspondence between these results and the results of similar studies using long-term exposures.

In experiments to be begun in fiscal year 1993, the procedure described in this technical note will be used to test the limits of applicability of organic carbon normalization for neutral organic chemicals in EqP. The linearity of the normalization at low organic carbon concentrations has been clearly identified as a problem in defining both the utility of EqP-based SQC and in the practical usage of TBP calculations as a screening procedure. The experimental design described in this technical note appears well suited to address this problem.

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