5-1997

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SEMIPERMEABLE MEMBRANE DEVICES ARE EFFECTIVE SURROGATES OF FISH IN CONCENTRATING POLYCHLORINATED BIPHENYLS

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ABSTRACT

This study examined the effectiveness of Semipermeable Membrane Devices (SPMDs) as surrogates for fish in concentrating polychlorinated biphenyls. Golden shiners (Notemigonus crysolucas) and SPMDs were exposed to three different concentrations (0.5, 1.5, and 3.0ppm) of Aroclor 1254 for 1, 3, and 5 days under laboratory conditions. Concentrations of Aroclor 1254 were measured in the SPMDs and fish tissue using extraction techniques and gas chromatography. The concentrations of PCB in SPMDs and N. crysolucas were positively correlated. This relationship compared favorably with data from other studies. The relationship between the concentration of PCB in SPMDs and tissue of fish and mollusks could be described by the equation $F=2.38 S^{0.59}$, where F and S were the concentrations of PCB in fish and SPMDs (ng/g) respectively.

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By

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B.S., University of Richmond, 1993

A Thesis

Submitted to the Graduate Faculty

of the University of Richmond

in Candidacy

for the degree of

MASTER OF SCIENCE

in

Biology

May, 1997

Richmond, Virginia

ACKNOWLEDGMENTS

I would like to thank Drs. John Bishop, Roni Kingsley, and Stuart Clough for serving on my thesis committee. I appreciate all of the input, suggestions and assistance.

I would like to thank Mr. Mike Martin of the Virginia Division of Consolidated Laboratory Services-Trace Organics Laboratory for the many hours of analytical assistance, Mr. David Grimes of the Department of Environmental Quality for assisting me in experimental design as well as his technical expertise, and James Huckins for the National Biological Survey for his advice. Thanks also to my wife, Shannon, for all of her support.

I would also like to thank CIA Laboratories for supplying the SPMDs and the University of Richmond for the funds to support this work.

Finally, thank you to all who have reviewed the many drafts that have led to this product.

TABLE OF CONTENTS

INTRODUCTION

Recent growth of environmental awareness has increased demands for assessments of water quality. The 1987 amendments to the Clean Water Act require all states to establish standards to regulate the concentrations of contaminants in their waters (U.S. Office of Federal Register, 1987). Measurements of these concentrations are needed in order to enforce laws and safeguard waters.

Of particular concern are bioconcentratable contaminants that accumulate in lipids of aquatic organisms. In this process, contaminants which have relatively low ambient concentrations, reach levels in the tissues of organisms that may be detrimental to the health of aquatic life and humans who consume the organisms (De la Torre, et al., 1995).

Two traditional approaches to estimate bioconcentrations rely on: 1) predictions based on concentrations in ambient water, and 2) measurements of tissues. For the former approach, physical-chemical properties of the contaminant, such as the partitioning of the contaminant between octanol and water (octanol partitioning coefficient) are used to relate concentrations in tissues and ambient water (Chiou, 1985). This approach relies on the applicability of the predictive model and measurements of concentrations in ambient water. Physio-chemical properties do not necessarily account for physiological and natural history features of organisms, and ambient concentrations may be below detection limits of the analytical procedures (Lebo, et al., 1996). Tissue studies involve measurements of concentrations of contaminants in indigenous organisms

collected from a sampling site, and/or indicator organisms in live boxes. Organisms collected from a site may not solely reflect conditions at that site especially if the organisms migrate (Ellis, et al., 1995).

A live box study is limited in terms of time by the lifespan and health of the organisms. Methods based on tissue analyses are costly and time consuming. The Virginia Department of Environmental Quality currently spends approximately $300 per sample for analysis of polychlorinated biphenyls (PCBs) and pesticides (Grimes, pers. comm.).

Biomonitoring, or the systematic use of living organisms as sensors of water quality is undergoing a fundamental change (Rand, 1995). A recent development in the collection of bioconcentration data is the surrogate system, which could make obsolete the use of living organisms. Like aquatic organisms, these systems concentrate pollutants by bioconcentration. They do not, however, consider dietary uptake (bioaccumulation) (Spacie, et al., 1995). The outer envelope separates the interior material, representing the lipid pool of the organism, from the water.

The semipermeable membrane device (SPMD), which was developed by Huckins et al. (1990) is a type of surrogate system. It is in the developmental stage and appears to be useful as a surrogate of aquatic organisms and sampler of contaminants. A general description of the SPMD, taken in large part from Huckins et al. (1990) and Huckins et al.

(1993), is given below. The SPMD consists of low density polyethylene tubing containing a thin film of lipid. The tubing consists of non-polar, dense polymers, and has pores with diameters of up to 10 angstroms. Triolein commonly is used as a lipid because it comprises the largest portion of neutral lipid in freshwater fish, remains in a liquid state down to a temperature of 4.9° C, and has a large molecular diameter (≥600 daltons) (Huckins, et al., 1993) compared with the molecular weight of a contaminant such as a PCB (around 200) (Lide, et al., 1994). Dialysis of triolein through the membrane (lipid carryover) is limited and varies with time, e.g., approximately 1.5 % (24 h) and 5.5% (120 h) for grass carp lipid (Meadows et al., 1993). For deployment, the SPMD is looped and attached to a float and weight in order to suspend it in the water column (Fig. 1).

Bioconcentratable contaminants, which are dissolved in water, enter the SPMDs by passive diffusion. The partitioning of contaminants between ambient water and SPMDs is relatively independent of the type of lipid in the SPMDs (Huckins et al., 1990). SPMDs also can concentrate contaminants to detectable levels that otherwise would be below detection limits for standard analytical methods (Lebo et al., 1995). The laboratory processes for identifying contaminants concentrated in these systems are simpler than those for identifying contaminants in tissue samples. In most cases, the material inside the membrane can be sent directly into a gas chromatograph with very little clean up (Meadows, et al., 1993).

The extent to which SPMDs mimic organisms must be known in order to assess the usefulness of SPMDs in estimating bioconcentrations. Previous studies have examined the kinds and concentrations of contaminants in SPMDs and organisms under field or laboratory conditions. SPMDs sequestered more kinds of contaminants than did channel catfish (Ictalurus punctatus), sauger (Stizostedion canadense), and carp (Cyprinus carpio) in the upper Mississippi River (Ellis et al., 1995), and I. punctatus in Lake Michigan (Wood, 1993) and caged I. punctatus (Gale et al., 1997). Concentrations of contaminants in SPMDs and organisms appear related, but vary according to the contaminant and organisms. Peven et al. (1996) reported that SPMDs and mussels (Mytilus edulis) concentrated polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and chlorinated pesticides at similar rates, but the individual compounds that comprise the contaminants differed in SPMDs and mussels. Herve et al. (1995) found that SPMDs and mussels (Anodonta piscinalis) concentrated organochlorine compounds at different rates, and concentrated different compounds. Prest et al. (1995b) reported similar concentrations of PCBs in SPMDs and M. edulis in Corio Bay, Australia, but greater concentrations of PCBs in clams (Corbicula fluminea) than in SPMDs in Sacramento/San Joaquin River Delta (Prest et al., 1992).

Petty et al. (1995b) suggest that dietary uptake and depuration of contaminants by organisms could result in inconsistencies between concentrations of contaminants in SPMDs and organisms. Ellis et al. (1995) suggest that high correlations might be expected for highly chlorinated compounds, which tend to be recalcitrant.

Polychlorinated biphenyls (PCBs) are highly chlorinated compounds, which can remain in the environment for decades (Mathewson, 1985). In the United States of America, mixtures of PCBs such as Aroclor were commonly used in the insulating fluid of electric transformers throughout the 1960s (Science News, 1984 and Rhee, et. al., 1993). Discovery of the health risks posed by PCBs resulted in a Congressional ban on their manufacture in 1976 (Stone, 1992) and their inclusion on the list of priority pollutants in the amendments to the Clean Water Act (U.S. Office of Federal Register, 1987). Accordingly, states are required to develop standards to regulate concentrations of PCBs in surface waters. Effects of PCB on aquatic organisms include genotoxicological (Shugart, 1995) and immunotoxicological effects (Anderson et al., 1995) as well as carcinogenic responses (Hawkins et al., 1995).

The present study examined the extent to which concentrations of PCB in SPMDs and fish were related. The PCB was Aroclor 1254, and the fish was the golden shiner (N. crysolucas). The null hypothesis tested was that there is no relationship between the uptake of Aroclor 1254 in SPMDs and golden shiners.

SPMDs and fish were exposed to different concentrations of PCB, (0.5-3.0 ppm) in aquaria over 1-5 d. Concentrations of PCB in SPMDs and fish were positively correlated, thus disproving the null hypothesis.

MATERIALS AND METHODS

Experimental Design

Static systems of test chambers, which contained still solutions of Aroclor 1254, were used. Three replicates of chambers, each of which contained one of three different concentrations of PCB (0.5, 1.5, and 3.0 ppm), were prepared (Fig. 2). Samples of fish and SPMDs were exposed to the PCB in test chambers for durations of 1, 3 or 5 days. For example, a sample of fish and SPMDs was taken from concentration 0.5 ppm A, B, and C on day 1, day 3, day 5. Pilot studies indicated the absence of PCB in fish and SPMDs in test chambers to which no PCB was added, obviating the need for additional control test chambers.

The golden shiner, N. crysolucas, was the test species. This species was easy to obtain and maintain, and belongs to the same family (Cyprinidae) as Pimephales promelas, which commonly is used in toxicology (Cooney, 1995). Specimens were obtained from Perry Minnow Farm (Windsor, Virginia). The stock fish, which ranged from approximately 1.5 to 3.0 inches in length, and three to five grams in weight, were maintained in a 150 gallon tank containing moderately hard synthetic water (Appendix 1). Twice daily they were fed as much frozen brine shrimp as they could consume in several minutes. Water in the stock tank was filtered by three sponge filters, and was changed when it became visibly dirty by removing approximately 20% of the volume and refilling with fresh moderately hard synthetic water. Between batches of fish, the stock tank was drained and cleaned with a solution containing 10% bleach (5.25%).

Thirty fish were acclimated in each test chamber for two days before introduction of PCB. Each test chamber was a 10 gal aquarium, which was filled with moderately hard synthetic water kept in a temperature controlled room at 21°C. Injured fish were replaced with healthy fish during this period. Mortality rates of less than 2% occurred during the acclimation period, which were acceptable following standard procedures (Parrish, 1985).

Only assays which experienced fish mortalities of 10% or less were used in analyses in accordance with standard practices for acute toxicity test controls (Parish, 1985). After acclimation, percentage mortalities for the different PCB concentrations (in parentheses) were: 0,0,10 %(0.5 ppm), 0,3%,10%(1.5 ppm), and 3%,10%,10% (3.0 ppm). Fish mortality rates greater than 10% occurred in two test chambers at a PCB concentration of 3.0 ppm. Data from these chambers were excluded from analyses, and replaced with data from two additional test chambers.

Aroclor 1254 (98 % pure, AccuStandard, New Haven, CT), which is a mixture of congeners of PCB, was used because of its low cost, ease of manipulation in the laboratory and prevalence in the environment (De la Torre et al., 1995). Stock solutions of PCB in 95 % acetone at a ratio of 1 gm:20 ml were prepared. Appropriate volumes of stock solutions were introduced into test chambers to yield nominal concentrations of PCB of 0.5, 1.5, and 3.0 ppm. These concentrations bracket the chronic and acute water

quality standards for the Commonwealth of Virginia, which are 0.5 and 2.0 respectively (Virginia Department of Environmental Quality, 1992). Pilot studies indicated high mortality of fish above concentrations of 3.0 ppm.

Semipermeable membrane devices (SPMDs) were a patented design and materials were supplied by CIA Laboratories (St. Joseph, MO). They were assembled immediately before use in order to limit air borne contaminants (Petty et al. 1993). Each device consisted of a 45.72 cm section of 2.54 cm layflat polyethylene tubing of standardized pore size from CIA Labs (St. Joseph, MO), which contained 0.5 ml of 95% 1,2,3,-Tri[cis-9-octadecynol]-glycerol (triolein) (Sigma, St. Louis, MO). The triolein was partially frozen before being injected into the tubing to simplify handling. The devices were flattened to distribute the triolein throughout the device. The devices were looped and the open ends clipped together with a 1 inch binder clip. The bottom of each loop was weighted with a paper clip to maintain a vertical orientation in the water column. Three devices were placed into each test chamber. Each bag was clipped to a horizontal bar on top of the tanks so that all portions of the bag, but not the binder clip, were submerged.

Samples of fish and SPMDs were removed from each of the nine test chambers after 1, 3 and 5 d. Each set of samples consisted of eight fish and one SPMD. Fish were placed in glass cuvettes, corked, labeled, and frozen. The SPMDs were placed in individual beakers, sealed with Parafilm (Neenah, WI) and frozen.

Frozen fish were ground in a blender, placed in clean cuvettes, and re-frozen. Between samples, the blender was stripped with hydrochloric acid, followed by acetone, and rinsed with deionized water.

Sample Preparation and Analysis

Samples were prepared and analyzed at the Virginia Division of Consolidated Laboratory Services Trace Organics Laboratory (Richmond, VA) as follows.

PCB was extracted from the fish tissue and SPMD via acetone, and analyzed using a gas chromatograph (Hewlett Packard 5880A) (Appendix 2). Fish were thawed, weighed (wet weight) and ground with a mortar and pestal. Average weight of the fish samples was 7.52±1.0 grams (Appendix 3). Sodium sulfate was added gradually until the fish paste no longer appeared wet. The amount of sodium sulfate varied depending on the sample size and the moisture content of the sample. The dried fish paste was added to individual 250 ml beakers. SPMD samples were partially thawed, weighed (wet weight), and placed into 250 ml beakers.

The extraction procedure was repeated twice for each sample. Samples in 50 ml of 95 % acetone were sonicated for 20 minutes, and decanted into individual glass tubes. Extracts from fish were filtered through small funnels containing glass wool and sodium sulfate to remove water. The extracts were measured for volume and stored in glass vials

with Teflon lids.

Gas chromatography was conducted using 10 ng of 4-bromobiphenyl (i.e., 1 ml of 10 ng / ml solution) as an internal standard, and approximately 2 ml of extracts. Gas chromatographic readings were taken at six different retention times (12.91, 17.43, 19.25, 22.20, 24.40 and 28.85 min.) , which was a representative spectrum for Aroclor 1254. The PCB concentration in the extract was estimated using eq. 1

$$C_{ex} = (\Sigma^{6}_{t=1} Rt / Rs) \times (Xst / 1.8553) \qquad \text{(eq. 1)}$$

where C_{ex} was the PCB concentration (ng PCB/ml extract), Rt and Rs were the values for the area of each peak for the sample at the six different retention times and for the standard at a retention time of 6.09 min. respectively, Xst was the concentration of the standard (10 ng/ml), and 1.8553 was a constant used to correct for the internal standard. The PCB concentrations in the sample of fish and SPMD were estimated from eq. 2

$$C_s = (C_{ex} \times V_{ex}) / W \qquad \text{(eq. 2)}$$

where C_s was the PCB concentration in the sample (ppb; ng PCB / gm fish or SPMD), C_{ex} was as described above, V_{ex} was the extract volume, and W was the wet weight (gm) of the fish or SPMD sample.

Only those assays in which test organisms experience 10% or less mortality were used. Two sample containers (1.5B, 1.5C) were broken during analysis. Data for these

samples were not included in the analysis. All statistical analyses use a 5% confidence level.

RESULTS

Mean concentrations of PCB in fish ranged from 40 to 191 ppb (Table 1). They were positively related to ambient concentrations and increased for the first 3 days at an ambient concentration of 0.5 ppm, and over 5 days at ambient concentrations of 1.5 and 3.0 ppm (Fig. 3). The effects of ambient concentration and duration were statistically significant and interaction between the two was not, according to an ANOVA (Table 2).

Mean concentrations of PCB in SPMDs ranged from 177 to 995 ppb (Table 1). They were positively related to ambient concentrations and increased over 5 d at an ambient concentration of 3.0 ppm (Fig. 4). The effects of ambient concentration and duration were statistically significant according to an ANOVA (Table 3).

PCB concentrations in fish and SPMDs were positively related (Fig. 5). The relationship between PCB concentrations in fish and SPMDs was statistically significant according to ANOVA (Tables 4a & b). The coefficient of correlation for non-transformed and $\log_{10}$ transformed data were about the same ($r=0.78$ vs. $r=0.74$). The relationship can be described by eq. 3a and b.

$$F = 33.48 + 0.149\ S \qquad \text{(eq. 3a)}$$

$$F = 2.06 S^{0.63} \qquad \text{(eq. 3b)}$$

where F and S are the PCB concentration in fish and SPMDs (ng / gm), and the values, are the least squares regression estimates.

DISCUSSION

Risks to human health posed by bioconcentratable contaminants usually are estimated from concentrations of the contaminants in water and relationships between concentrations of the contaminants in water and aquatic organisms (U.S. EPA, 1991). Previous studies of SPMDs have focused on the use of SPMDs to estimate concentrations of pollutants in water. They emphasized the absorption and retention of pollutants by SPMDs (Huckins et al., 1990, Huckins et al., 1993 and De LaTorre et al., 1995), and relationships between concentrations of pollutants in SPMDs and water (Petty, et al., 1995a and Prest et al., 1995a). Little attention has been given to relationships between concentration of pollutants in organisms and SPMDs under controlled laboratory conditions.

The present study examined relationships between concentrations of PCB in SPMDs and N. crysolucas under laboratory conditions. Ambient concentrations of PCB in the test chambers were not measured. Nominal concentrations most likely exceeded actual concentrations, due to adsorption of PCB to the sides of the test chambers and incomplete dissolution of PCB in water (Huckins, pers. comm.). Nominal concentrations of PCB ranged between 0.5-3.0 ppm which exceeded the solubility of PCB in water. Solubility, however, depends upon the actual composition of the Aroclor but is always almost 0 (AccuStandard, technical assistance).

Concentrations of PCB in SPMDs and fish continued to increase over the duration of 5 d at nominal PCB concentrations of 1.5 and 3.0 ppm for fish and at 3.0 ppm for SPMDs. The trend suggests that at higher concentrations PCB desorbed from the walls of the test chambers as it was incorporated by the SPMDs and fish. The conditions in these test chambers, therefore, may have resembled those of steady state as PCB was released from the tank surfaces and became available for uptake by the SPMDs and fish. The lack of such a consistent trend in test chambers at a nominal concentration of 0.5 ppm suggests that PCB was depleted from the water after 3 d in these chambers.

A limited number of previous studies examined relationships between concentrations of pollutants in SPMDs and fish. Most of these studies were based on field observations, and none examined relationships between concentrations of the same compound over the wide range of ambient concentrations in the laboratory as in the present study. Gale et al. (1997) examined PCB concentrations in SPMDs and the channel catfish, *I. punctatus*, in the Saginaw River, Michigan over a period of 28 d, and the present study examined *N. crysolucas* under controlled laboratory conditions over a period of 5 d . Prest (1995b) examined PCB concentrations in SPMDs and M. edulis, in Corio Bay, Victoria, Australia over a period of 60 d. Herve (1995) examined the concentrations of PCBs in A. piscinalis in lakes in Central Finland over a four week period. Data from these studies suggest a relationship between the concentration of PCB in tissue and SPMDs. Relationships between concentrations of PCB in SPMDs and fish in the present study and those reported by Gale et al. (1997), as well as the concentrations in

clams reported by Prest et al. (1995b) and Herve et al. (1995) are remarkably similar to each other (Fig. 6a & b).

The relationship between concentrations of PCB in the SPMDs and animal tissue from the previous and present studies was statistically significant for non transformed and $\log_{10}$ transformed data according to ANOVA (Table 5a & b). The coefficient of correlation for $\log_{10}$ transformed data was higher than the coefficient of correlation for non-transformed data (r=0.95 vs. r=0.89). The relationships can be described by eq. 4a & 4b where meanings of the symbols and numerical values are the same as those in eq. 3.

$$F=10.76+0.169S \qquad \text{(eq. 4a)}$$

$$F = 2.38\,S^{0.59} \qquad \text{(eq. 4b)}$$

SPMDs appear to be valid surrogates for aquatic organisms in concentrating PCB. There was remarkable similarity for data collected on vertebrates and invertebrates and laboratory and field studies. Further studies are needed to ascertain whether similar relationships hold for other pollutants, organisms, and environmental conditions.

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Table 1. Concentrations of PCB in fish and SPMD for different ambient concentrations and durations of exposure. Values: mean ± standard deviation with number of observations in parentheses.

Ambient PCB Concentration (ppm)	Duration of Exposure (d)	PCB Concentration Fish (ppb)	SPMD (ppb)
0.5	1	40 ± 5(3)	177 ± 17(3)
0.5	3	83 ± 8(3)	226 ± 54(3)
0.5	5	82 ± 16(3)	257 ± 31(3)
1.5	1	41 ± 5(3)	308 (1)
1.5	3	101 ± 13(3)	609 ±116(3)
1.5	5	170 ± 18(3)	618 ±141(3)
3.0	1	62 ± 10(3)	253 ±117(3)
3.0	3	115 ± 56(3)	660 ±254(3)
3.0	5	191 ± 55(3)	995 ±187(3)

Table 2. Analysis of variance (two factor with replication) of effects of ambient concentration and duration of exposure on concentrations of PCB in fish tissue.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Ambient Concentration	13623	2	6812	8.72	<0.05
Duration	45425	2	22712	29.08	<0.05
Interaction	8865	4	2216	2.84	0.06
Within	14057	18	781		
Total	81970	26			

Table 3. Analysis of variance (two factor with replication) of effects of ambient concentration and duration of exposure on concentrations of PCB in SPMD.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Ambient Conentration	779353	1	779353	39.95	<0.05
Duration	508008	2	254004	13.02	<0.05
Interaction	329951	2	164976	8.46	<0.05
Within	234088	12	19507		
Total	1851400	17			

Table 4a. Analysis of variance of effects of time and ambient water concentration of PCB in fish tissue and SPMDs. Based on non-transformed values of concentrations.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	46089	46089	36.20	<0.05
Residual	23	29287	1273		
Total	24	75376			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	33.48	13.55	2.47	<0.05	5.45	61.50	5.45	61.50
Slope	0.15	0.02	6.02	<0.05	0.10	0.20	0.10	0.20

Table 4b. Analysis of variation of effects of time and ambient water concentration of PCB in fish tissue and SPMDs. Based on log10 transformed values of concentrations.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.76	0.76	28.14	<0.05
Residual	23	0.62	0.03		
Total	24	1.37			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.31	0.31	1.01	0.3239	-0.33	0.95	-0.33	0.95
Slope	0.63	0.12	5.30	<0.05	0.39	0.88	0.39	0.88

Table 5a. Analysis of variance of concentration of PCB in organisms and SPMDs. Based on non-transformed values.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	165202	165202	220.1286	<0.05
Residual	59	44230	750		
Total	60	209250			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	10.76	4.35	2.47	<0.05	2.05	19.47	2.05	19.47
Slope	0.17	0.01	14.84	<0.05	0.15	0.19	0.15	0.19

Table 5b. Analysis of variance of concentration of PCB in organisms and SPMDs. Based on log10 transformed data.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	23	23	579	<0.05
Residual	59	2	0		
Total	60	25			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.38	0.05	8.17	<0.05	0.28	0.47	0.28	0.47
Slope	0.59	0.02	24.06	<0.05	0.54	0.63	0.54	0.63

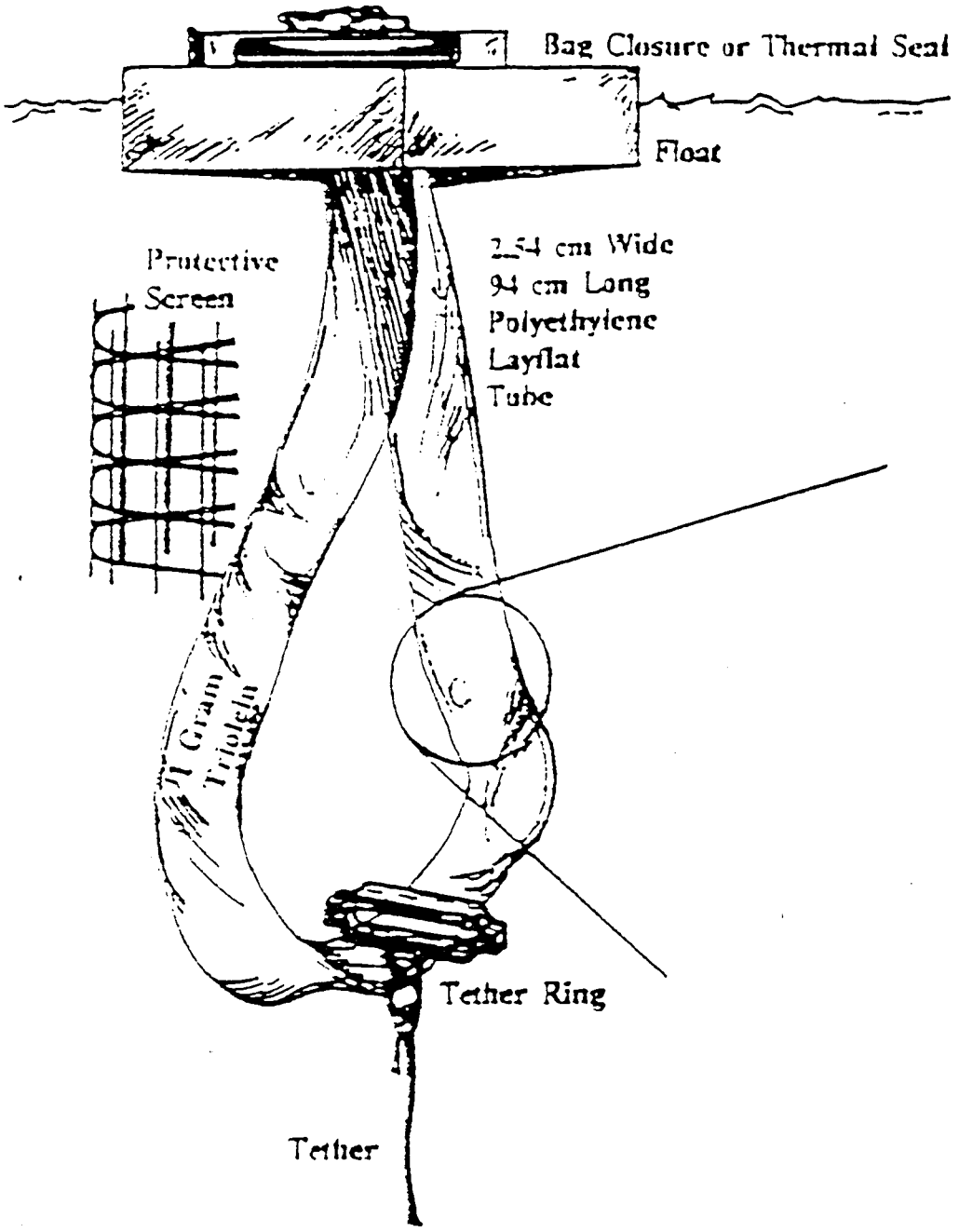

Figure 1. Possible configuration of Semipermeable membrane device. From Huckins et al., 1993.

Ambient Concentration	Exposure Time (Days)		
	1	3	5
0.5	A,B,C	A,B,C	A,B,C
1.5	A,B,C	A,B,C	A,B,C
3.0	A,B,C	A,B,C	A,B,C

Figure 2. Laboratory set up of static test chambers.

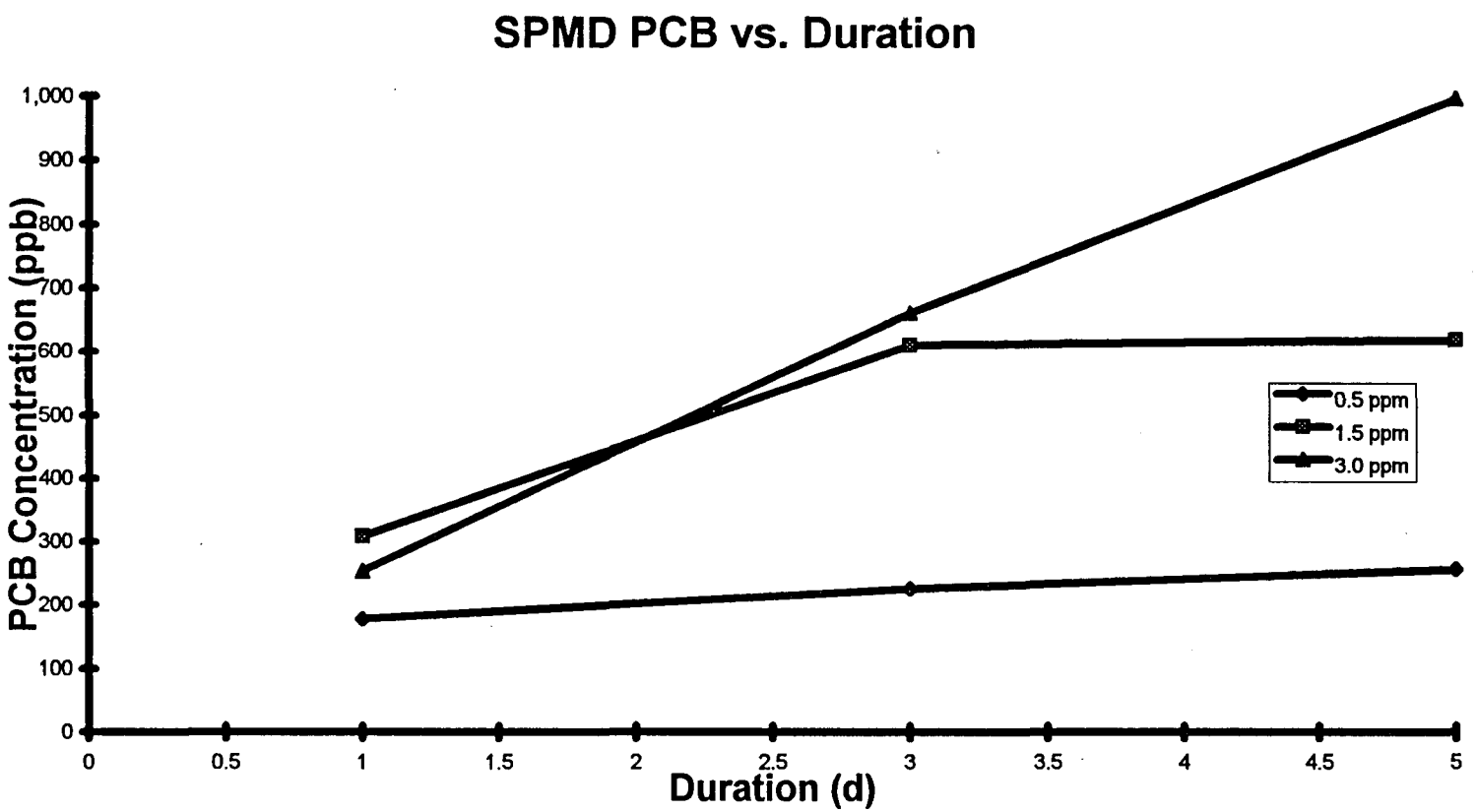

Figure 4. Mean concentration of PCB in SPMD over time at three ambient concentrations. Ambient concentrations are in ppm. Tissue concentrations are in ppb.

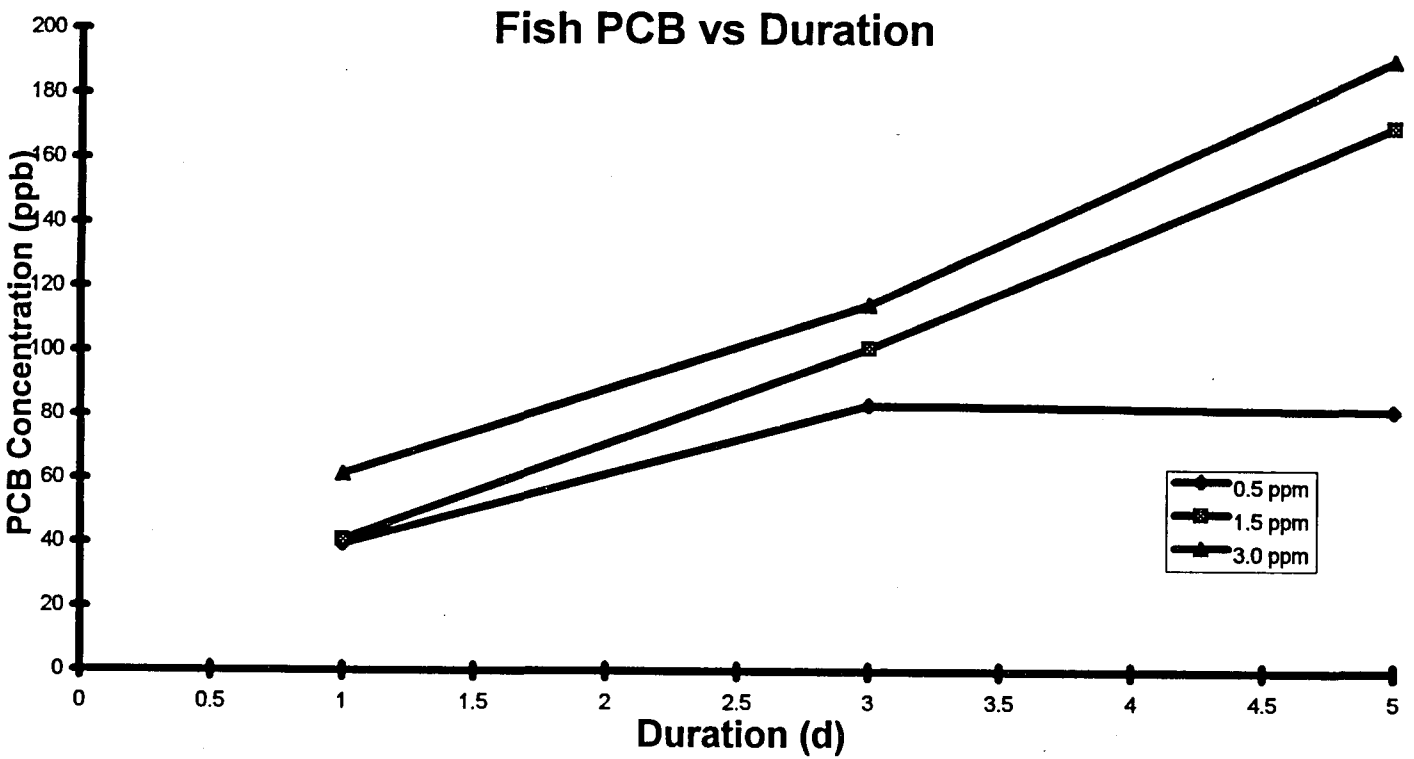

Figure 3. Mean concentration of PCB in fish tissue over time at three ambient Concentrations. Ambient concentrations are in ppm. Tissue concentrations are in ppb.

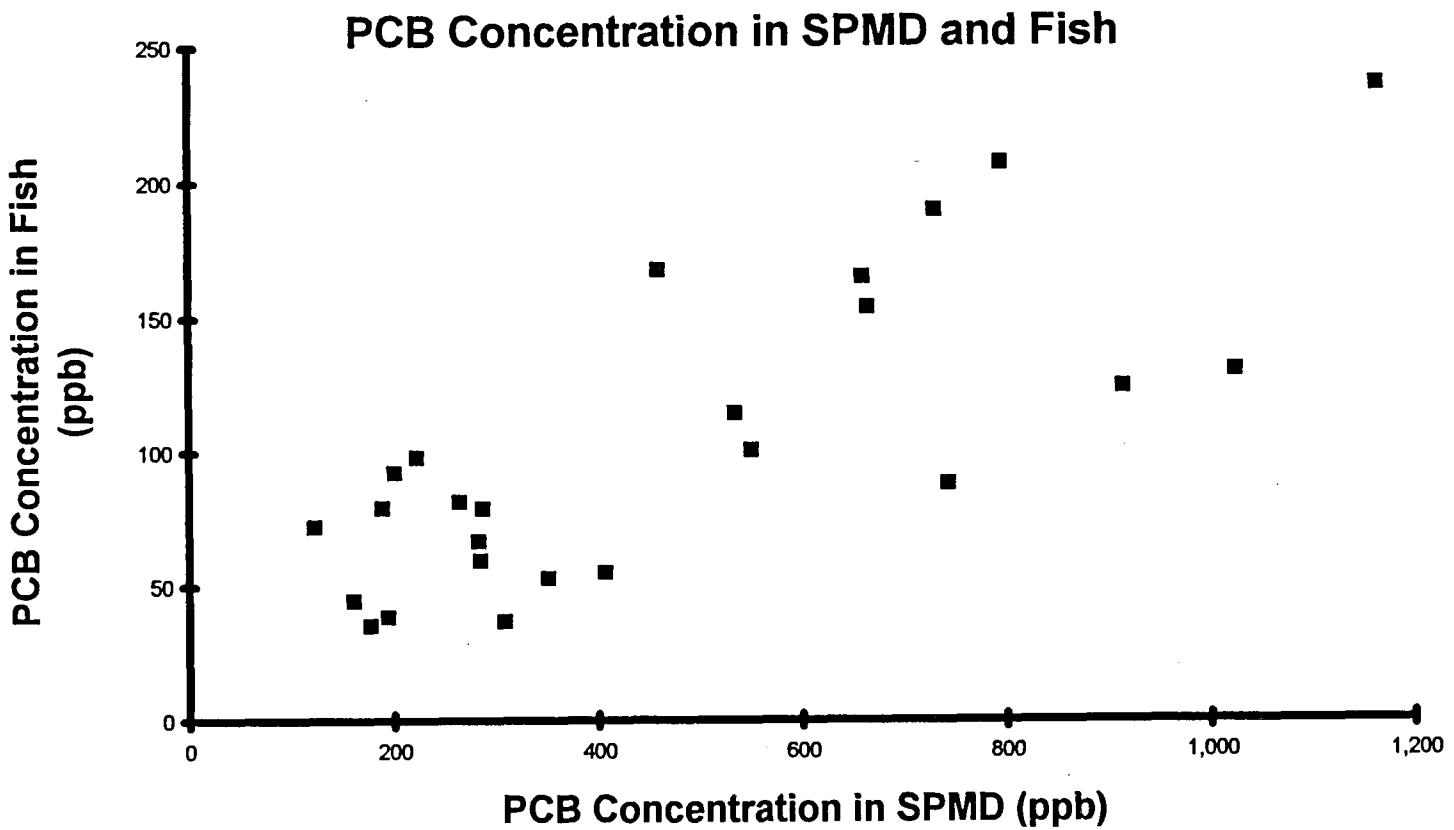

Figure 5a. Concentration of PCB (ppb) in fish tissue and SPMDs.
r-0.78

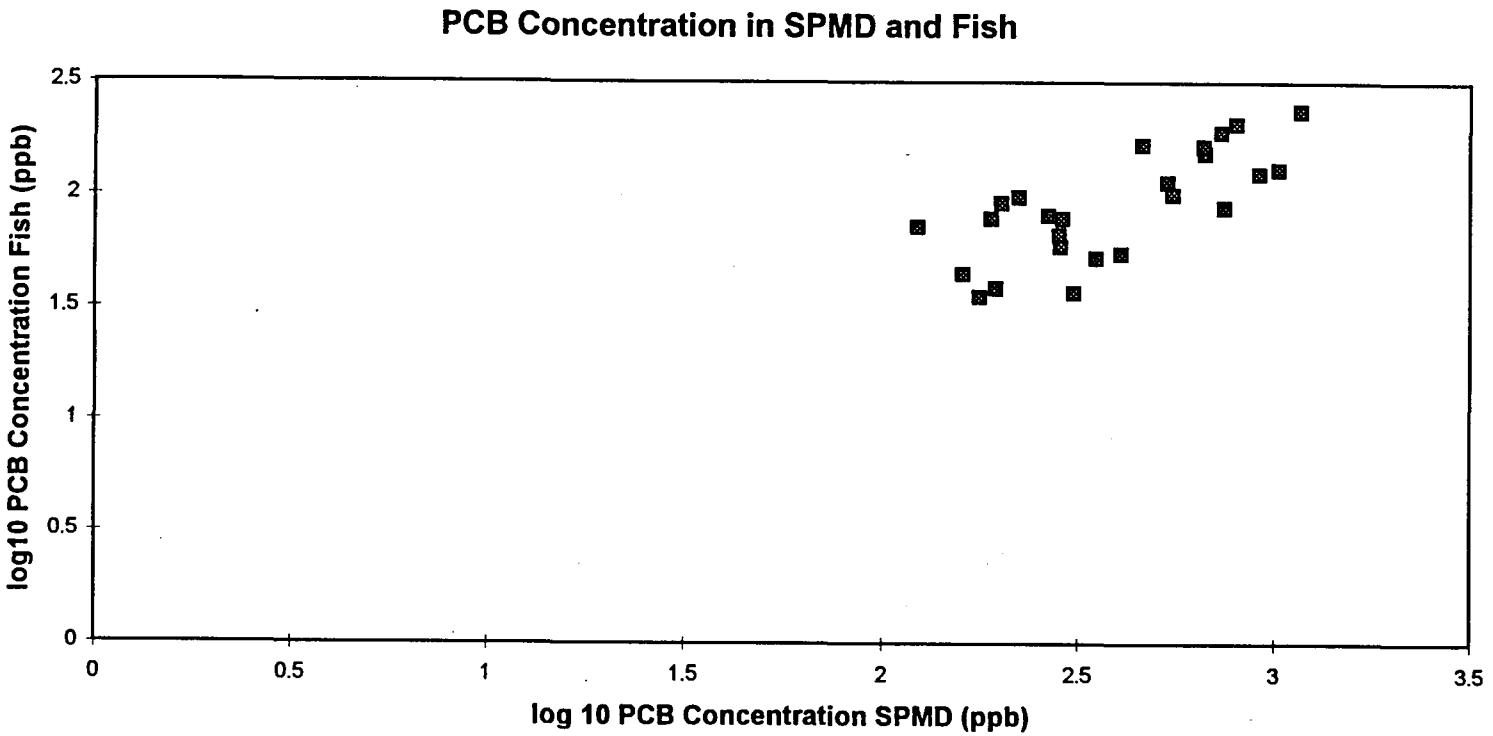

Figure 5b. Concentration of PCB in fish tissue and SPMDs in ppb. Based on log10 transformed data.

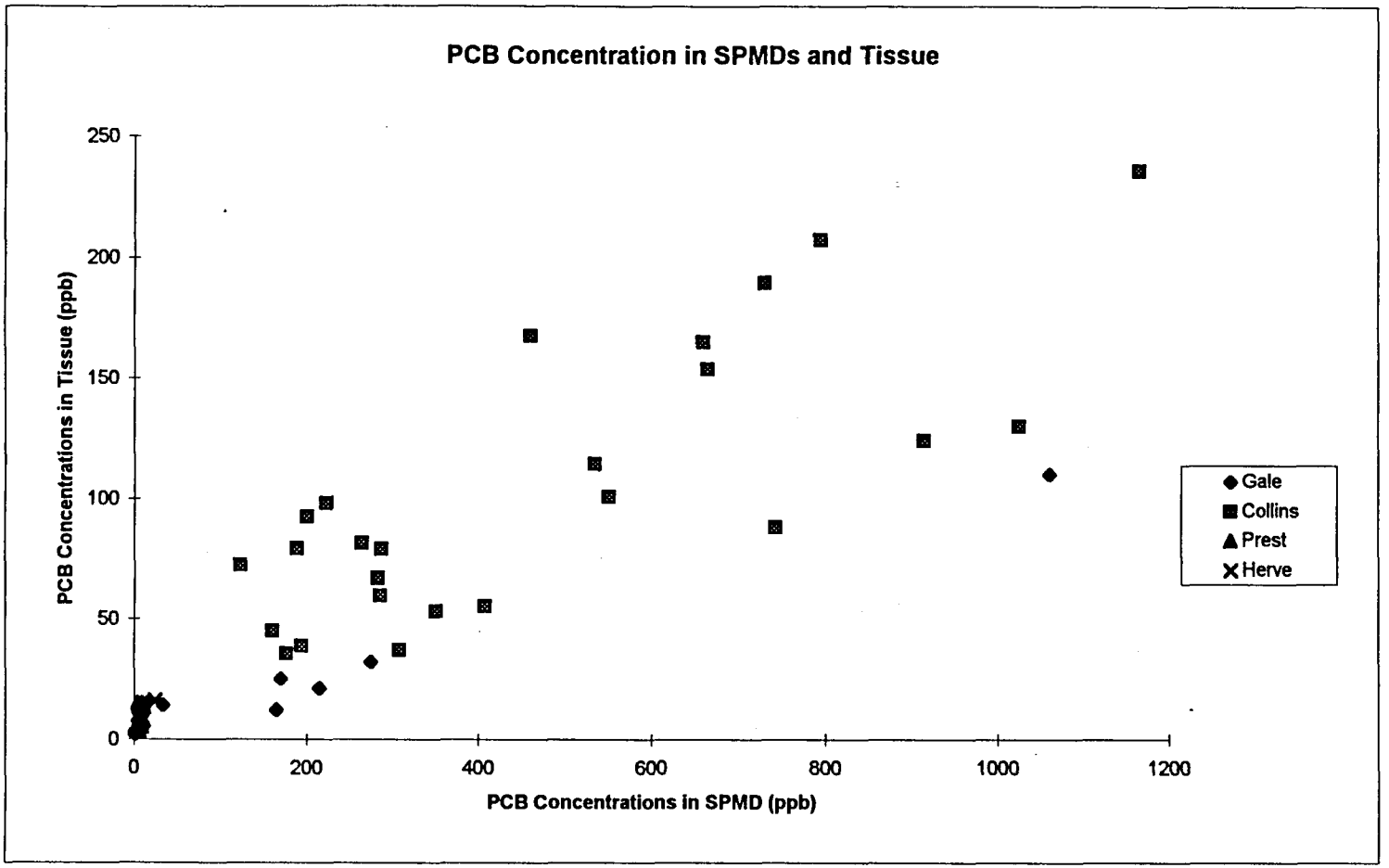

Figure 6a. Concentrations of PCB in fish, clams and SPMDs. Concentrations are ppb per unit wet weight.
For non-transformed values of concentrations.

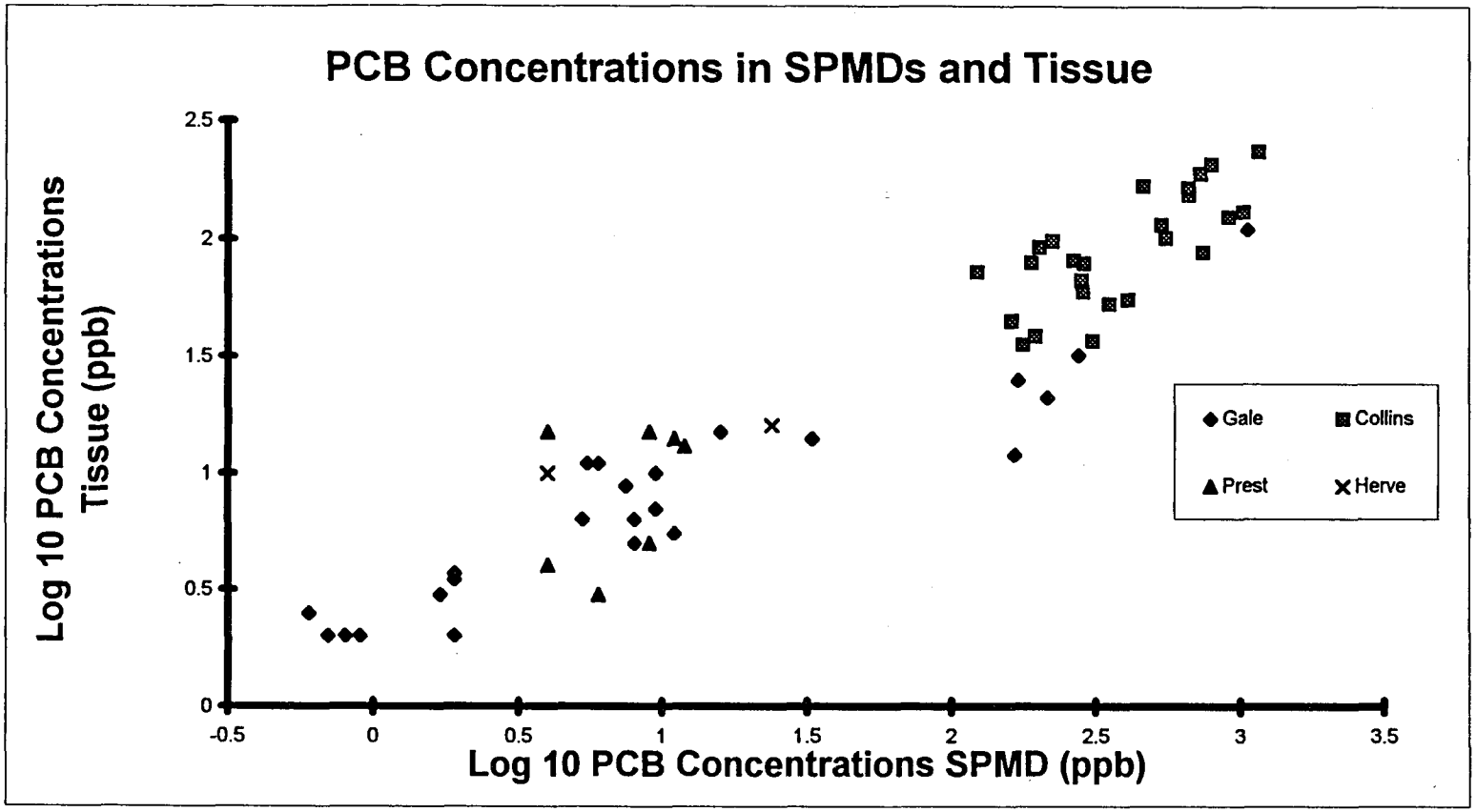

Figure 6b. Concentrations of PCB in fish, clams and SPMDs. Concentrations are ppb per unit wet weight.

r=0.95

Appendix 1.

10 Gallons of Moderately Hard Synthetic Water

41.53 ml $MgSO_4$ (120 g/L)
41.53 ml Kcl (8 g/L)
83.05 ml $NaHCO_3$ (96 g/L)
4.98 g $CaSO_4$

Appendix 2. Gas Chromatograph

Hewlett Packard 5880A

DB5 mega bore column

0.53 mm id x 30m

1 micron film thickness

Appendix 3. Raw Data

	Ret Time	Aroclor 1254 (1 ng/ml)	Fish 0.5D1A*	Bag 0.5D1A	Fish 0.5D3A	Bag 0.5D3A
	12.92	10277	6088	12816	13967	12228
	17.43	23285	11279	23993	27923	23420
	19.25	31833	15675	31318	40227	35119
	22.2	34518	14609	32888	42804	37046
	24.4	36663	10592	26098	36130	36479
	28.85	32849	6555	17701	26683	30181
Sum		169425	64798	144814	187734	174473
Extract conc. (ng PCB/ml extract)			2.79	7.45	8.80	8.81
Extract volume(ml)			89.80	53.40	90.20	52.20
Amt. of PCB in extract (ng)			250.25	397.94	793.53	460.07
Fish or SPMD weight (gm)			7.06	2.25	8.60	2.29
Sample conc. (ng PCB/gm fish or SPMD)			35.45	176.86	92.27	200.91
Int. std.	6.09	91316	125328	104743	115020	106698

* ambient conc. (0.5 ppm), day 1 (D1), replicate (A)

Fish 0.5D5A	Bag 0.5D5A	Fish 1.5D1A	Bag 1.5D1A	Fish 1.5D3A	Bag 1.5D3A	Fish 1.5D5A	Bag 1.5D5A	Fish 3.0D1A	Bag 3.0D1A	Fish 3.0D3A
7717	13393	32839	27218	51327	40405	76413	31524	71430	24849	87872
18301	26379	48026	43388	112839	77606	193068	66194	101417	37824	159050
31091	40512	54366	58104	139105	111123	253103	103275	122186	51652	199983
35771	42493	52591	56163	167303	113423	338641	110056	120474	48899	204245
38168	42361	31797	44010	117297	89174	270528	95270	81069	40823	133141
33139	35701	16115	29872	67225	59947	164498	65026	46765	27908	72929
164187	200839	235734	258755	655096	491678	1296251	471345	543341	231955	857220
7.06	8.00	9.94	11.49	37.14	18.86	55.15	18.06	23.64	10.17	36.90
96.20	72.70	31.10	59.50	26.20	65.40	23.10	58.60	16.20	28.40	20.20
679.05	581.48	309.16	683.72	973.00	1,233.67	1,274.07	1,058.16	382.91	288.95	745.43
8.34	2.20	8.40	2.22	8.50	2.31	7.60	2.30	5.30	2.35	6.00
81.42	264.31	36.81	307.98	114.47	534.06	167.64	460.07	72.25	122.96	124.24
125372	135342	127815	121370	95078	140490	126676	140692	123903	122881	125205

Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish
3.0D3A	3.0D5A	3.0D5A	0.5D1B	0.5D1B	1.5D1B		3.0D1B	3.0D1B	0.5D3B	0.5D3B	1.5D3B
66325	108462	83716	12557	10863	36797		51429	37243	13078	11030	42713
116753	222150	167588	16139	19553	54558		69168	54809	24962	20749	96343
162085	256126	245503	22829	27963	65254		48792	72933	36405	31336	116650
160184	315375	251382	23183	28360	64182		77469	68834	39278	32612	157897
133950	219516	220441	18652	24982	41682		50215	55625	35181	31203	127979
95210	127701	161373	11271	16556	22591		28831	38137	24798	22441	85085
734507	1249330	1130003	104631	128277	285064		325904	327581	173702	149371	626667
34.87	53.15	42.69	4.68	5.91	11.38		13.90	14.62	7.78	6.79	27.80
61.60	27.50	61.40	82.30	61.60	28.30		21.30	57.60	87.50	62.60	26.30
2,147.99	1,461.63	2,621.08	385.17	364.20	322.06		296.14	842.28	680.63	425.28	731.27
2.35	6.20	2.25	8.60	2.27	6.90	2.20	5.60	2.40	8.60	2.25	8.30
914.04	235.75	1,164.92	44.79	160.44	46.68	#VALUE!	52.88	350.95	79.14	189.01	88.10
113535		142677	120503	116942	135014		126344	120746	120362	118510	121479

Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish
1.5D3B	3.0D3B	3.0D3B	0.5D5B	0.5D5B	1.5D5B	1.5D5B	3.0D5B	3.0D5B	0.5D1C	0.5D1C	1.5D1C
45466	119207	64677	14511	9480	76581	42112	85049	99275	11205	14236	39178
81085	211021	106625	29183	17306	184007	94342	176403	191990	14608	26441	57825
115003	222529	139953	44472	26632	244505	149971	199950	261722	20750	37429	68289
115771	279379	133617	48889	26335	327388	165103	244602	264682	20534	37175	66622
90997	187180	97115	47222	25469	270057	146202	161709	198635	16252	28459	41188
58932	108861	61642	37314	18475	180042	102813	90291	129832	9538	16510	21142
507254	1128177	603629	221591	123697	1282580	700543	958004	1146136	92887	160250	294244
22.48	47.36	24.81	9.04	6.48	53.24	28.12	40.82	46.36	3.80	7.17	12.62
72.70	25.80	58.50	82.10	75.60	22.10	58.40	27.10	50.40	82.80	59.60	23.60
1,634.33	1,221.87	1,451.12	742.13	490.16	1,176.65	1,642.33	1,106.30	2,336.59	314.94	427.38	297.92
2.20	7.40	2.20	7.57	2.20	6.20	2.25	8.50	2.28	8.16	2.20	7.50
742.88	165.12	659.60	98.04	222.80	189.78	729.93	130.15	1,024.82	38.60	194.26	39.72
121620	128398	131162	132130	102832	129842	134268	126488	133251	131625	120452	125634

Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish	Bag	Fish
1.5D1C	3.0D1C	3.0D1C	0.5D3C	0.5D3C	1.5D3C	1.5D3C	3.0D3C	3.0D3c	0.5D5C	0.5D5C	1.5D5C
	47500	38148	13262	15934	63333	33799	32437	40838	9104	21869	55349
	64253	37359	24994	32693	141893	69816	57656	66962	20469	30049	143073
	78196	47945	36527	51477	171512	102787	71277	88564	32928	46378	192318
	76692	44678	39042	56610	217162	106687	71657	84862	36617	51189	264329
	55319	34204	35013	54778	152922	90145	45426	64891	28000	50821	213752
	36299	21913	25194	40867	88958	62453	23919	42118	30810	39071	137968
	358259	224247	174032	252359	835780	465687	302372	388235	157928	239377	1006789
	15.22	10.38	8.02	11.28	31.15	18.74	14.34	17.94	6.96	10.98	45.80
	26.20	60.40	82.20	60.60	26.20	70.50	26.10	52.40	85.20	59.20	25.20
	398.88	626.65	659.64	683.57	816.04	1,320.96	374.39	940.24	592.95	650.26	1,154.18
2.27	6.70	2.20	8.35	2.38	8.10	2.40	6.80	2.31	8.88	2.30	7.50
	59.53	284.84	79.00	287.21	100.75	550.40	55.06	407.03	66.77	282.72	153.89
	126837	116499	116890	120586	144633	133961	113617	116620	122311	117464	118482

Bag	Fish	Bag
1.5D5C	3.0D5C	3.0D5C
37691	139357	69110
87342	252502	127999
137847	319611	177796
172301	468987	175987
139333	209522	140214
101611	114286	95699
676125	1504265	786805
28.62	57.14	32.53
53.40	27.20	56.20
1,528.24	1,554.10	1,828.06
2.30	7.50	2.30
664.45	207.21	794.8109
127339	141906	130376

Appendix 4.Concentration of PCB in SPMD and fish tissue of present study.

Amb (ppm)	Day	Fish (ppb)	SPMD (ppb)
0.5	1	35	177
0.5	1	45	160
0.5	1	39	194
0.5	3	92	201
0.5	3	79	189
0.5	3	79	287
0.5	5	81	264
0.5	5	98	223
0.5	5	67	283
1.5	1	37	308
1.5	1	47	
1.5	1	40	
1.5	3	114	534
1.5	3	88	743
1.5	3	101	550
1.5	5	168	460
1.5	5	190	730
1.5	5	154	664
3	1	72	123
3	1	53	351
3	1	60	285
3	3	124	914
3	3	165	660
3	3	55	407
3	5	236	1,165
3	5	130	1,025
3	5	207	795

Appendix 5. Concentration of PCB in SPMD and tissue. Data from various studies.

Study	SPMD (ppb)	Tissue (ppb)	log 10 SPMD	log 10 Tissue
Gale et al.	8	5	0.9031	0.699
	165	12	2.2175	1.0792
	9.5	10	0.9777	1
	0.6	2.5	-0.222	0.3979
	11	5.5	1.0414	0.7404
	215	21	2.3324	1.3222
	7.5	8.8	0.8751	0.9445
	0.7	2	-0.155	0.301
	8	6.3	0.9031	0.7993
	170	25	2.2304	1.3979
	5.5	11	0.7404	1.0414
	9.5	7	0.9777	0.8451
	275	32	2.4393	1.5051
	6	11	0.7782	1.0414
	33	14	1.5185	1.1461
	1060	110	3.0253	2.0414
	16	15	1.2041	1.1761
	0.9	2	-0.046	0.301
	1.9	3.5	0.2788	0.5441
	0.8	2	-0.097	0.301
	1.7	3	0.2304	0.4771
	0.9	2	-0.046	0.301
	1.9	3.7	0.2788	0.5682
	0.8	2	-0.097	0.301
	1.9	3.7	0.2788	0.5682
	1.9	2	0.2788	0.301
	5.3	6.3	0.7243	0.7993
Present	177	35	2.2476	1.5496
	160	45	2.2053	1.6511
	194	39	2.2884	1.5865
	201	92	2.303	1.9651

Study	SPMD (ppb)	Tissue (ppb)	log 10 SPMD	log 10 Tissue
Present	189	79	2.2765	1.8984
	287	79	2.4582	1.8976
	264	81	2.4221	1.9107
	223	98	2.3479	1.9914
	283	67	2.4514	1.8246
	308	37	2.4885	1.5659
	534	114	2.7276	2.0587
	743	88	2.8709	1.945
	550	101	2.7407	2.0032
	460	168	2.6628	2.2244
	730	190	2.8633	2.2783
	664	154	2.8225	2.1872
	123	72	2.0898	1.8588
	351	53	2.5452	1.7233
	285	60	2.4546	1.7748
	914	124	2.961	2.0943
	660	165	2.8193	2.2178
	407	55	2.6096	1.7408
	1,165	236	3.0663	2.3724
	1,025	130	3.0106	2.1145
	795	207	2.9003	2.3164
Prest et al.	4	4	0.6021	0.6021
	6	3	0.7782	0.4771
	9	5	0.9542	0.699
	12	13	1.0792	1.1139
	9	15	0.9542	1.1761
	11	14	1.0414	1.1461
	4	15	0.6021	1.1761
Herve et al.	24	16	1.3802	1.2041
	4	10	0.6021	1

VITA

Christopher Gardner Collins, first son of Henry L. Collins and Pamela A. Collins, grew up in Syracuse, New York. He received his secondary education through the East Syracuse Minoa School system, graduating with honors from East Syracuse Minoa Central High School in 1989. In 1993, he received his B.S. in Biology from the University of Richmond. In 1997, he received his M.S. in Biology from the University of Richmond. He is employed by the Virginia Department of Transportation as an Environmental Program Planner.