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Adhesion-Binding of 2,2′,4,4′,5,5′-Hexachlorobiphenyl to Glass and Plastic: A Possible Source of Error for PCB Analysis

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Polychlorinated biphenyls (PCB) have permeated the global ecosystem through their general use. The relatively low vapor pressure and aqueous insolubility (HUTSINGER et al. 1974) of PCB have made them persistent contaminants in the environment. DEXTER (1977) found the solubilities of several PCB isomers in distilled water ranged from 0.31 ppm for dichlorobiphenyl to 0.61 ppb for heptachlorobiphenyl. Solubilities of PCB in artificial seawater were reported at about one-fifth the levels reported in distilled water. ZITKO (1970) reported the solubility of Aroclor 1254 was 2-3 mg/L in freshwater and 1.0-1.5 mg/L in seawater. HAGUE & SCHMEDDING (1975) found the solubility of PCB isomers decreased with increasing chlorination of the molecule and the solubility of 2,4-dichlorobiphenyl was 640 ppb compared to 0.95 ppb for 2,2',4,4',5,5'-hexachlorobiphenyl. PCB concentrations vary widely from trace levels in the ocean to 8 ppm near industrial areas in marine sediment (PAVILOU & DEXTER 1979). The low solubility of PCB in water and its high concentrations in specific marine sediment implies that PCB precipitates out of aqueous environments, particularly saline solutions. Therefore, due to the wide variations in the reported solubilities of PCB, it is increasingly important to study the physical and chemical behavior of the PCB molecule in aqueous solutions. This study is directed toward that end.

The relative insolubility of PCB in water has been implicated as a cause for PCB binding and/or adsorption. A purification system for raw sewage based on binding to polyvinyl chloride chips has been proposed (LAWRENCE & TOSINE 1976) and the flux of PCB between sediment and water which depends on an adsorption-desorption phenomena have been investigated. Adsorption of PCB to sediments such as sand and silt-clay was relatively irreversible, indicating that PCB was firmly bound to the sediment (WILDISH et al. 1980). The binding of PCB to microparticulates as well as their desorption and transfer to marine diatom cells reaches equilibrium within several hours after exposure (HARDING & PHILIPS 1978). The binding of PCB to inert surfaces can affect the estimation of PCB toxicity and concentration where binding to glass or plastic can occur.

In this work the effect of ^{14}C-PCB binding to borosilicate glass culture tubes and plastic centrifuge tubes was studied along with sedimentation of ^{14}C-PCB in distilled water. To elucidate the parameters of binding and sedimentation of PCB (2,2',4,4',5,5'-hexachlorobiphenyl), a concentration in excess of saturation in distilled water (HUTZINGER et al. 1974) was chosen.

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METHODS AND MATERIALS

2,2',4,4',5,5'-^{14}C hexachlorobiphenyl, specific activity 24mCi/mM (California Bionuclear Corp.) was supplied in a cyclohexanechloroform (4:1) solvent system. Approximately 500,000 DPM was evaporated to dryness under nitrogen gas and the ^{14}C-PCB residue was resuspended in 10 µL of ethanol and diluted in 3 mL of triple-distilled water. The solution was prepared in each of 7 borosilicate culture tubes measuring 15x75mm.

Approximately, $1.4x10^6$ DPM in 10 mL of distilled water was prepared as above in each of seven 15 mL conical polystyrene plastic centrifuge tubes. The solutions were allowed to stand at 21°C and a 0.1 mL aliquot was removed from the top layer of each tube at 0,1,24, 48,72 and 144 h. Each 0.1 mL aliquot was counted in 3 mL of hydrofluor (National Diagnostics, N.Y.) in a liquid scintillation counter.

After 144 h, the solution containing ^{14}C-PCB and distilled water was removed by aspiration from each culture and centrifuge tube. The side and then the bottom of each tube was wiped separately with cotton applicators soaked with 95% ethanol. The tips of the applicators were then counted in 3 mL of hydrofluor.

Following the wiping procedure, the tubes were rinsed with 3 mL of 95% ethanol and vortex mixed. A 0.5 mL aliquot of the ethanol wash was counted in 3 mL of hydrofluor. The plastic caps covering each tube during the incubation procedure were wiped with a cotton applicator and 95% ethanol; the applicators were counted in 3 mL of hydrofluor.

RESULTS

There was a significant amount of ^{14}C-PCB lost from supernatant at 48 h (table 1) in the glass culture tube ($p < 0.05$), while for the plastic centrifuge tube the loss was significant after only 24 h ($p < 0.05$). After 144 h the amount of ^{14}C-PCB remaining in the supernatant of the glass culture tubes was one-third that at time zero, while in the plastic centrifuge tube there was a reduction to one-tenth the amount of ^{14}C-remaining in the supernatant compared to time zero. This indicates that more adhesion occurred in the plastic tubes than in the glass tubes. By 48 h less than half of the ^{14}C-PCB which had been initially added to the plastic tubes remained in solution; more than 72 h incubation in the glass tubes was required to reduce the concentration by half (table 1). After 144 h 35% of the initial ^{14}C-PCB remained in the supernatant of the glass tube while only 10% remained in solution in the plastic tube.

After 144 h, the amount of ^{14}C-PCB bound to the side and the bottom of the culture and centrifuge tubes was measured (table 2). In the glass culture tube there was 17 times more ^{14}C-PCB/cm^2 at the bottom than bound to the side ($p < 0.05$). In the plastic centrifuge tube there was significantly more ^{14}C-PCB bound to the bottom than to the side ($p < 0.05$). However, the amount at the bottom was only 1.5 times greater than the amount bound to the side. Therefore, more binding to the side wall was observed in the plastic culture tube than to the glass culture tubes, where sedimentation rather than binding was more predominant.

TABLE 1: Loss of 2,2',4,4',5,5'-^{14}C-Hexachlorobiphenyl (PCB) from Distilled Water in Borosilicate Galss Culture Tubes and Plastic Centrifuge Tubes[a]

	^{14}C-PCB DPM remaining in the supernatant			
	Glass Tubes		Plastic Tubes	
Incubation,h	DPM[c]	ppm[b]	DPM[c]	ppm[b]
0	470,713 ±16,347	1.18	1,546,955 ± 44,873	1.16
1	479,032 ± 4,977	1.21	1,497,193 ± 47,084	1.13
24	456,813 ± 7,103	1.16	1,067,038[c] ± 45,351	0.80
48	397,112[c] ± 5,352	0.98	650,241[c] ± 22,713	0.49
72	323,324[c] ± 3,553	0.81	412,970[c] ± 15,456	0.31
144	165,852[c] ± 9,074	0.41	155,498[c] ± 9,370	0.12

a. Each value is the mean ± the standard error of the mean (n=7). b. This value represents the mean DPM expressed in ppm. c. This is a significant difference between these values and time zero value ($p < 0.05$).

After washing the tubes in 95% ethanol, 20% of the total ^{14}C-PCB was recovered from the glass culture tubes, while 35% of ^{14}C-PCB was recovered from the plastic centrifuge tubes (table 3).

The total amount of ^{14}C-PCB recovered after all the procedures was 78% for the glass tubes and 92% for the plastic tubes. There was no ^{14}C-PCB detected by wiping the plastic caps. This indicates that there was no loss due to vaporization of ^{14}C-PCB or evaporation of the distilled water.

DISCUSSION

The binding and sedimentation of PCB in aqueous solutions can seriously effect the measurement of absolute concentrations of PCB in freshwater and marine environments, the level of PCB toxicity to cell cultures *in vitro* and the measurement of cellular uptakes and the distribution of PCB in aqueous environments.

HAGUE & SCHMEDDING (1975) speculated that adsorption of PCB onto glass container surfaces may reduce its concentration in water. They further argued that PCB's are poorly absorbed on a sand surface and therefore it is unlikely that the material absorbed on the glass surface would reduce the concentration by an order of magnitude. However, our data contradict their conclusions. After 144 h there is a significant amount of PCB binding to galss (over 20% table 3). This can result in an error in the quantitation of PCB in aqueous solutions stored in glass vials or test tubes.

TABLE 2: Binding of 2,2',4,4',5,5'-^{14}C Hexachlorobiphenyl to Either the Side or the Bottom Borosilicate Galss Culture Tubes and Plastic Centrifuge Tubes[ab]

Tube	Side of the tube	Bottom of the tube
Glass tube		
Surface area	2.75 cm^2	0.785 cm^2
Total DPM added	470,713[b] ±18,163	470,713 ±18,163
Total DPM bound	19,258 ±1,634 (1.30x10^{-7}g)[c]	92,178 ±21,067 (6.23x10^{-7}g)[c]
Total percent bound[d]	4.1%	19.6%
Total DPM divided by surface area (DPM.cm^2)	7,003 ±535	117,424[e] ±23,105
Plastic tube		
Surface	25.9 cm^2	5.9 cm^2
Total DPM added	1,546,955 ±44,873	1,546,955 ±44,873
Total DPM bound	537,373 ±58,131 (3.63x10^{-6}g)[c]	182,093 ±8,633 (1.23x10^{-6}g)[c]
Total percent bound[d]	34.7%	11.8%
Total DPM divided by surface area (DPM/cm^2)	20,748 ±2,020	30,863[e] ±1,317

a. After 144 h incubation at 21°C. b. Each value equals the mean ± the standard error of the mean (n=7). c. The total ^{14}C-PCB bound expressed in g. d. The total percent bound equals the total DPM bound divided by the total DPM added. e. There is a significant difference between the amount of ^{14}C-PCB bound at the bottom and the side of both the glass and the plastic tubes.

Since our data show that the absolute concentration of PCB is affected by a significant loss due to binding and sedimentation, underestimation of the toxic effects of PCB is quite possible. The actual amount of PCB added to an aqueous system is decreased by binding to glass or plastic. The degree of sorption also depends on the PCB isomer since their solubilities in water are variable. Therefore, measurements involving *in vitro* uptakes and toxicity studies may be confounded by PCB binding and sedimentation to the container surfaces.

The factor of sedimentation plays an important ecological role in the understanding the distribution of PCB in rivers, lakes and oceans. The finding of high concentrations of PCB in marine sedi-

TABLE 3: Distribution of 2,2',4,4',5,5'-^{14}C Hexachlorobiphenyl in Borosilicate Glass Culture Tubes and Plastic Centrifuge Tubes[a]

	Glass Tube		Plastic Tube	
^{14}C-PCB	DPM[b]	Percent[c]	DPM[d]	Percent[c]
PCB added to each tube	470,713 ±18,163	-	1,546,955 ±49,859	-
PCB remaining in supernatant	165,852 ± 9,074	35.2	155,499 ± 9,370	10.0
Total bound to both the side and bottom	111,436 ±21,827	23.6	719,467 ±66,764	46.6
PCB in ethanol wash	91,966 ±9,699	19.5	542,874 ±32,375	35.1
PCB adhered to tube caps	0	0	0	0
Total Percent recovered		78.3		91.7

a. After 144 h incubation at 21°C. b. Percent of total ^{14}C-PCB added. c. Each value is the mean ± the standard error of the mean. d. Each tube was washed with 3 mL of 95% ethanol after wipes had been taken from the bottom and sides.

ment (PAVLOU & DEXTER 1979), the lower solubility in saline (DEXTER 1977; ZITKO 1970), along with the high concentration of PCB at the bottom of the culture tubes (table 2) indicates that PCB is sedimenting out of aqueous solutions. This process in the environment is important in understanding the ecological impact of PCB on bottom dwelling organisms, as well as understanding the flow distribution of PCB in aqueous environments.

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REFERENCES

DEXTER, R.N.: Mar. Chem 6, 41 (1977).
HAGUE, R. and D. SCHMEDDING: Bull Environm. Contam. Toxicol. 14, 13 (1975).
HARDING, L.W. and J.H. PHILIPS: Mar. Biol. 49, 103 (1978).
HUTZINGER, O., S. SAFE and V. ZITKO: The Chemistry of PCB's Cleveland Ohio, CRC Press, Inc. (1974).
LAWRENCE J. and H.M. TOSINE: Environ. Sci. Technol. 10, 381 (1976).
PAVLOU, S.P. and DEXTER, R.N.:Environ. Sci. Technol. 13, 65 (1979).
WILDISH, D.J., C.D. METCALFE, H.M. AKAGI and D.W. MCLEESE: Bull Environm. Contam. Toxicol. 24, 20 (1980).
ZITKO, V.: Bull Environm. Contam. Toxicol. 5, 279 (1970).