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Selective Retention of Polychlorinated Biphenyl Components in the Mussel, *Mytilus edulis*

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Abstract. The retention properties of PCBs (polychlorinated biphenyls) were examined in the mussel *(Mytilus edulis)* over a period of 89 days. Mussels were transferred from a site of relatively high PCB contamination to a site of relatively low PCB concentration in Puget Sound, Washington. Retention of PCB components increased with increasing chlorine content. Concentrations of PCB decayed logarithmically with calculated half-lives ranging from 3 days for a PCB component with two and three chlorines to 50 days for a PCB component with six and seven chlorines. Components chosen to best represent the commercial PCB mixtures, Aroclor® 1242, 1254, and 1260, were estimated to have half-lives of 8, 23, and 39 days, respectively. The selective retention of the higher chlorinated biphenyls appear to be the result of lower water solubility of these components.

Widespread environmental contamination by polychlorinated biphenyls (PCBs) has been documented (Risebrough et al. 1968, Hammond 1972, Peakall and Lincer 1970). The determination of the environmental impact of PCBs has been complicated by the varied chemical structure and properties of the individual components. Environmental samples from different levels on the food chain have been reported to contain PCBs varying in their component make-up (Koeman et al. 1969, Jensen et al. 1969). Organisms which were fed commercial PCB mixtures later contained PCBs with the ratios of the individual components significantly altered (Grant et al. 1971, Curley et al. 1971, Matthews and Anderson 1976). To better understand one of the processes that might explain these shifts, we studied the selective retention of PCBs by the mussel *(Mytilus edulis)* in a controlled field experiment.

Methods

Puget Sound, in the northwest sector of Washington State, is a complex series of salt-water channels and inlets where the distribution of PCBs has been described (Pavlou et al. 1973, Mowrer et al. 1977). Mussels (150) were collected from a site on the Duwamish River in Seattle, Washington (known to

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have high PCB levels), and taken to a site on Eld Inlet near Olympia, Washington, where PCB levels were greater than one order of magnitude lower. The mussels were placed in a live car constructed of wood and wire mesh and positioned on the portion of the beach inhabited by the majority of the local mussels (2 m above the mean lower low-tide level). An identical control live car was placed next to the experimental cage and filled with 150 mussels from the immediate vicinity.

Mussels from the experimental cage were sampled at 0,2,4,6,9,12,18,23,28,34,40,48,59,72, and 89 days. Mussels from the control cage were sampled at the same times as the experimental mussels, with the exception of 0 and 4 days. Five to ten mussels were taken from the live cars at each sampling time, with the exception of the first three sampling times, when approximately twenty mussels were obtained, to allow for duplicate analyses.

The samples were wrapped in aluminum foil and frozen until the time for analysis. Four to six mussels, with mean lengths of 4 to 5 cm (total of 10–20 g, wet weight), were shucked and digested with BFM (perchloric and glacial acetic acids) and extracted with hexane (Stanley and Le Favoure 1965). The extract was concentrated to 5 to 10 ml, cleaned up with sulfuric acid (Murphy 1972), and treated with copper to remove sulfur compounds.

A Hewlett-Packard 5700A electron-capture gas chromatograph (^{63}Ni detector) coupled to an H.P. 3380A integrator was used to quantitatively determine the amount of PCB in each sample. Ten to 40 μl of the final hexane extract was injected onto a coiled 6′ glass column packed with 1″ 33% NaOH/KOH on Gas Chrom Q, 80/100 mesh, (Miller and Wells 1969) followed by 10% DC-200 on Gas Chrom Q, 80/100 mesh, maintained at an oven temperature of 230°C, detector temperature 300°C, and a carrier gas flow rate of 60 ml/min, 95% argon-methane.

All PCB levels in the samples were quantified by individual homolog analysis. The mean weight percent of each homolog in a PCB standard (equal quantities of Monsanto Aroclor 1242, 1254, and 1260) was determined from figures published by Webb and McCall (1973). Peaks were identified by comparing peak profile and relative retention times in the PCB standard to those in the environmental samples.

Twenty-one peaks recorded by the gas chromatograph, representing 98% of the total PCB in the standard, were quantified. In the environmental samples, 18 of these peaks displayed quantifiable levels. The concentration of PCB (ng/g wet weight) represented by each peak was determined by quantitative comparison to standards injected daily. The concentrations of the individual peaks in each sample were summed to yield the total concentration of PCB. Subtotal concentrations of PCB, representing three commercially available preparations (Monsanto Aroclor 1242, 1254, and 1260), were determined by summing the concentrations of the appropriate individual peaks (see Figure 1). The peaks used for the subtotal concentrations represented at least 70% of the total PCB present in the Aroclor mixtures.

The concentration of PCB vs time in the mussel samples was plotted for (1) total PCB, (2) subtotal concentrations representing Aroclor 1242, 1254, and 1260, and (3) ten individual peaks. Logarithmic regression curves were calculated by the method of least squares, using the difference or multiples of differences between the experimental concentrations and the mean of the control concentrations. The half-lives of the different compounds and mixtures were calculated from the matched curves, and the Standard Error of Estimate (SEE) determined to indicate the goodness of fit of the matched curves. The formulas for these calculations are in Table 1.

Results

All PCB concentrations in the experimental mussels declined with time. The decrease in value of the individual peaks as well as the calculated total PCB and subtotal PCB concentrations appeared to be logarithmic, and the matched regression curves, described previously, provided a good fit (Figure 2). During the 89 day study period, concentrations of total PCB in the experimental mussels decreased from an initial level of 487 ng/g to a final level of 46 ng/g[2]. The mean

[2] All concentrations calculated from wet weight.

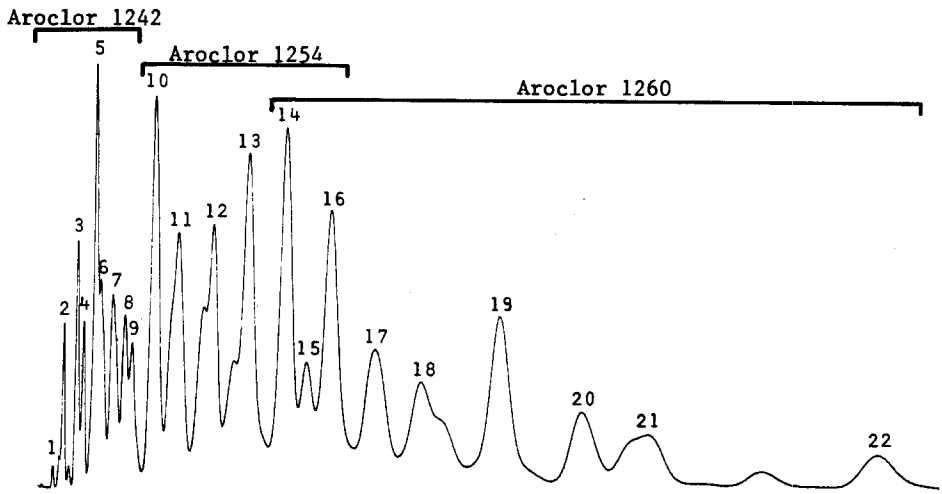

Fig. 1. Chromatogram of the PCB standard composed of equal quantities by weight of Monsanto Aroclor 1242, 1254, and 1260. Peaks 2–9 were used to calculate concentrations of Aroclor 1242, peaks 10–16 for Aroclor 1254, and peaks 14–22 for Aroclor 1260.

Table 1. Summary of total and subtotal PCB concentrations with time

Description	Peaks used[a]	Initial level[b] (ng/g)	Final level[b] (ng/g)	Mean of control[c] (ng/g)	Half life[d] (days)	SEE[d] (ng/g)
Total PCB	1–22	487	46	24 ± 7.5	23	32
Aroclor 1242	1–9	70	2	6.5 ± 4.2	8.4	5.4
Aroclor 1254	10–16	360	33	17 ± 4.9	23	24
Aroclor 1260	14–22	180	32	5.5 ± 1.5	39	13

[a] See Figure 1. PCB levels for Aroclors 1242, 1254, and 1260 were calculated by summation of peaks listed, as described in methods.
[b] Wet weight.
[c] ±1 Standard Deviation.
[d] Half-life, SEE (Standard Error of Estimate), and matched regression curves were calculated from the following equations:

$$a = \left[\frac{(\Sigma X_i \ln(Y_i - \bar{C})) - \frac{(\Sigma X_i)(\Sigma \ln(Y_i - \bar{C}))}{n}}{(\Sigma X_i^2) - \left(\frac{\Sigma X_i}{n}\right)^2}\right]$$

$$b = \left[\frac{(\Sigma \ln(Y_i - \bar{C})) - a(\Sigma X_i)}{n}\right] \qquad \text{Half-life} = \frac{\ln(0.5)}{b}$$

$$\hat{Y}_i = \ln(b + aX_i) + \bar{C} \qquad \text{SEE} = \sqrt{\frac{\Sigma(Y_i - \hat{Y}_i)^2}{n - 2}}$$

X = days, Y = concentration of PCB in experimental mussels, $\bar{C}$ = mean concentration of PCB in control mussels, n = number of data points, $\hat{Y}$ = estimated concentration of PCB in experimental mussels (used to plot matched regression curve).

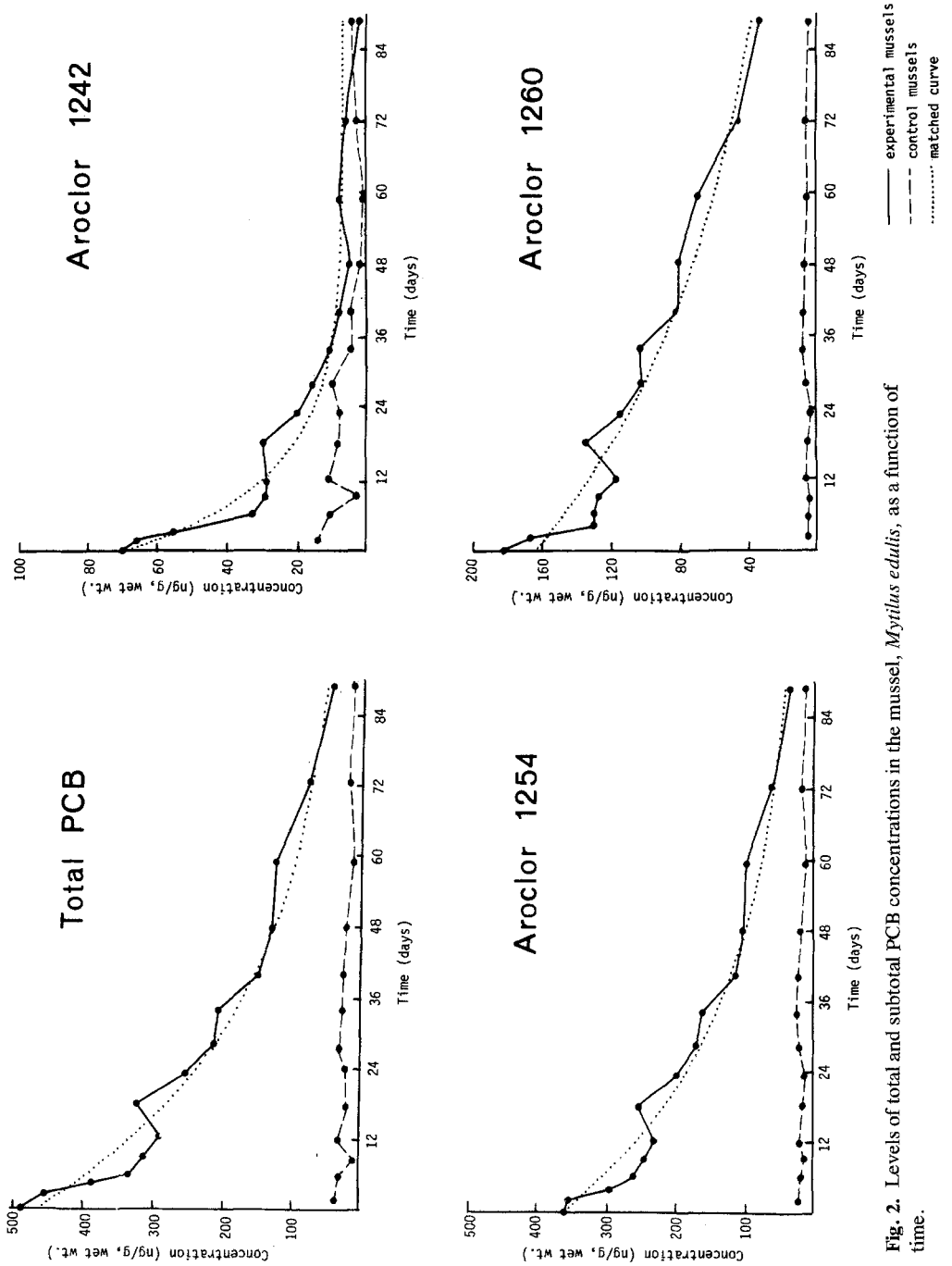

Fig. 2. Levels of total and subtotal PCB concentrations in the mussel, *Mytilus edulis*, as a function of time.

concentration in the control mussel was 24 ng/g which did not change significantly during the course of the study. The half-life of total PCB was estimated to be 23 days (Table 1).

The levels of the subtotal PCB concentrations (representing the three Aroclor mixtures) over time are shown in Figure 2 and Table 1. Peaks #14–#22, which represent Aroclor 1260, contained levels of PCB that decreased over a period of 89 days from 180 ng/g to 32 ng/g, while the mean level in the control mussels remained at 5.5 ng/g. The sum of levels under peaks #10–#16 represent Aroclor 1254, which decreased from 360 ng/g to 33 ng/g (with a mean control of 17 ng/g). Peaks #1–#9, representing Aroclor 1242, decreased from 70 ng/g to 2 ng/g (with a mean control of 6.5 ng/g). Aroclors 1242, 1254, and 1260 were found to have half-lives of 8.4, 23, and 39 days, respectively.

The level of PCB as a function of time, represented by four selected peaks, is shown in Figure 3. Data on ten peaks is summarized in Table 2. The calculated half-lives for the individual peaks range from 4.6 days for a peak representing a 2- and 3-chlorobiphenyl to 50 days for a peak representing a 6- and 7-chlorobiphenyl.

Discussion

Determination of the types of PCBs found in the environment is complicated by the fact that many researchers do not adequately describe the composition of the PCB mixtures encountered, other than to note their general similarity to certain standards. Both Koeman et al. (1969) and Jensen et al. (1969), however, report finding higher levels of the lower chlorinated compounds of PCB in organisms lower on the food chain. The loss of the lower chlorinated components as the PCBs are trophically concentrated has also been noted in our studies of organisms in Puget Sound, Washington (unpublished manuscript). Selective uptake, retention, and/or metabolism, three processes which are not always easily separated, could explain some of these shifts. At present the literature on these processes report different findings for different organisms, making a clear understanding of the alteration of PCBs found in the environment difficult.

Differential uptake and accumulation of higher chlorinated components has been reported in juvenile coho salmon (Gruger et al. 1975), oysters (Vreeland 1974), ring doves (Lincer and Peakall 1973), shrimp and fish tissues (Nimmo et al. 1971), and pinfish (Hansen et al. 1974). Uptake and accumulation of lower chlorinated components has been reported in juvenile Atlantic salmon (Zitko 1974), scud (Sanders and Chandler 1972), and hard clams (Courtney and Denton 1976). No selective uptake was reported in spot, *Leiostomus xanthurus,* (Hansen et al. 1971).

Studies on the metabolism of PCBs show greater consistency. More rapid metabolism of lower chlorinated components has been reported in rats (Grant et al. 1971, Hutzinger et al. 1972, Matthews and Anderson 1976), quail (Koeman et al. 1969, Bailey and Bunyan 1972), and pigeons (Bailey and Bunyan 1972, Hutzinger et al. 1972). Metabolism of PCB in pigeons (DeFreitas et al. 1972) and hens (Bush et al. 1974) were more dependent on the structural properties of PCB components than the degree of chlorination. Jensen and Sundstrom (1974) have

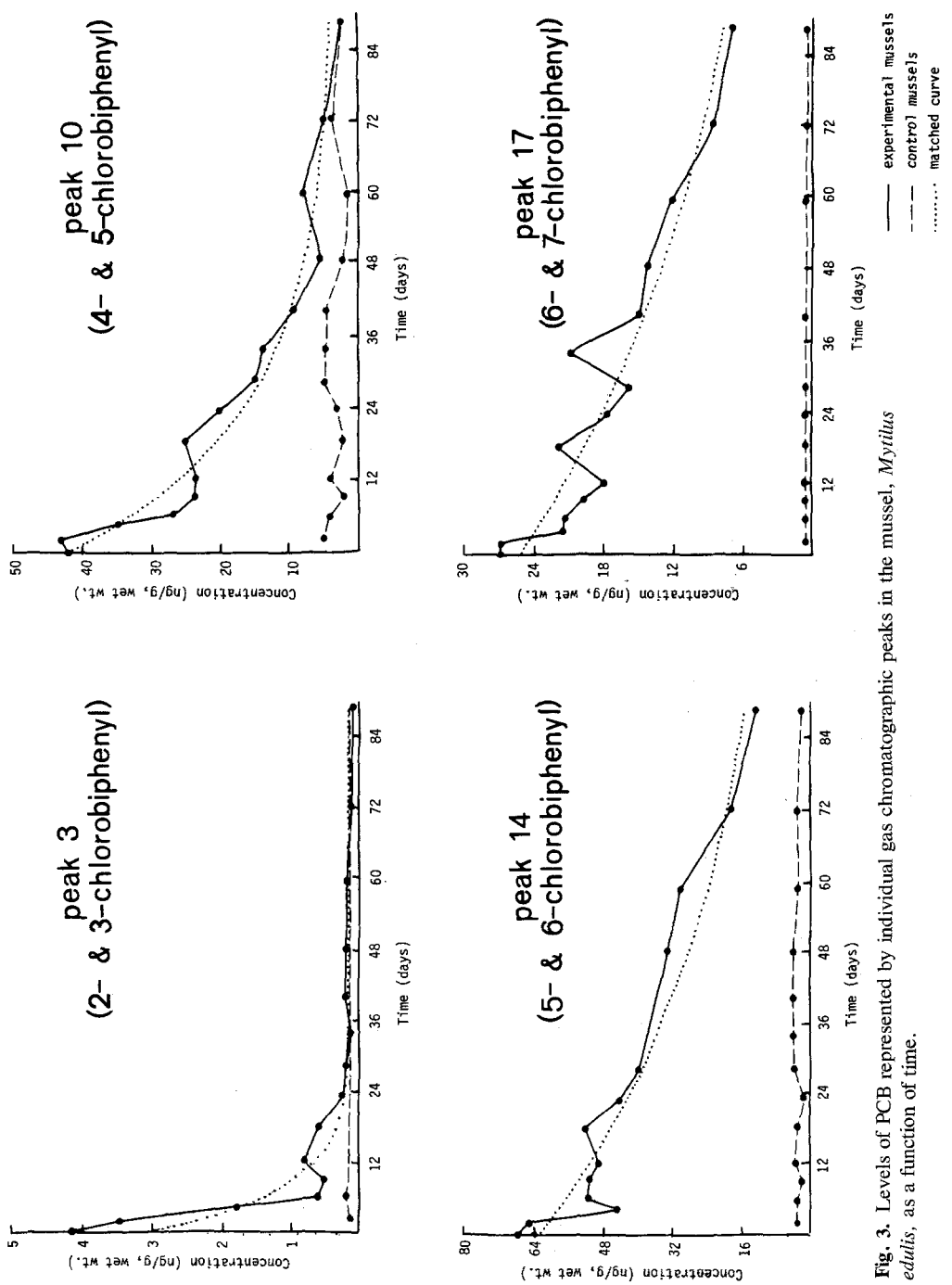

Fig. 3. Levels of PCB represented by individual gas chromatographic peaks in the mussel, *Mytilus edulis*, as a function of time.

Table 2 Summary of PCB concentrations represented by selected gas chromatographic peaks with time

Peak no.[a]	RRT[b]	No. of chlorines[c]	Initial level[d] (ng/g)	Final level[d] (ng/g)	Mean of control[e] (ng/g)	Half life[f] (days)	SEE[f] (ng/g)
3	.28	2 & 3	4.1	0.1	0.15 ± 0.1	4.6	0.5
5	.37	3	8.6	<1	<1	6.7	1.0
7	.47	4	26.5	1.9	2.6 ± 1.5	9.1	2.3
10	.70	4 & 5	42	2.0	3.3 ± 1.3	15	3.3
11	.84	5	63	4.2	5.8 ± 1.6	20	4.5
13	1.25	5 & 6	69	6.6	3.0 ± 1.1	26	5.5
14	1.46	5 & 6	67	12	3.1 ± 1.1	39	6.0
16	1.74	6	57	8.2	1.4 ± 0.4	34	4.5
17	2.03	6 & 7	27	6.8	0.5 ± 0.09	50	2.4
18	2.44	6 & 7	12	3.3	0.31 ± 0.15	45	1.5
19	2.90	7	13	1.4	0.11 ± 0.08	31	1.5
20	3.32	7	3.5	0.4	<0.1	34	0.5

[a] See Figure 1. PCB levels for Aroclors 1242, 1254, and 1260 were calculated by summation of peaks listed, as described in methods.
[b] Relative retention time (*p,p'*DDE = 1.00).
[c] Webb and McCall (1973).
[d] Wet weight.
[e] ±1 Standard Deviation.
[f] Half-life, SEE (Standard Error of Estimate), and matched regression curves were determined from equations shown at end of Table 1.

suggested some structural requirements of PCB favoring more rapid hydroxylation. Isomerization of PCB has been reported in bobwhite by Bagley and Chromartie (1973).

Few studies have examined the selective retention of PCBs. No selective retention was observed in pinfish (Hansen et al. 1974) and brook trout, with the exception of one gas chromatographic peak (Hutzinger et al. 1972). Courtney and Denton (1976) found greater concentrations of the higher chlorinated biphenyls in the faeces of hard clams than in the mixture they had been fed.

The overall rates of loss of PCB have been determined for some organisms. Bailey and Bunyan (1972) reported that pigeons, after being fed PCB, lost them logarithmically with half-lives for different tissues ranging from 50 to 140 days. Hutzinger et al. (1972) reported a 61% drop in PCB concentration in brook trout during the three month period after PCB administration was terminated. Hansen et al. (1974) reported a 51% drop of PCB levels in pinfish, 8 weeks after termination of administration, and Lowe et al. (1972) found a reduction of PCB concentrations to below detectable levels in the American oyster 25 and 32 weeks after termination of administration of two different dosages of PCB. Branson et al. (1975) reported that PCB concentrations in rainbow trout decreased with a half-life of 28 days after termination of PCB administration. Courtney and Denton (1976) examined the concentration of PCB in the visceral mass and muscular foot of hard clams for a 6-month period after termination of

administration of two different dosage levels of PCB. Concentration of PCB declined significantly only in the muscular foot of the hard clams fed the higher PCB dosage.

The differential retention that we observed in mussels might result from differential solubility of the individual PCB components. Lesser chlorinated PCB components are reported to have a greater solubility in water than the more highly chlorinated biphenyls (Hutzinger et al. 1974, Wallnofer et al. 1973), although this pattern is less distinct with biphenyls with more than six chlorines. Nisbet and Sarofim (1972) have noted the potential qualitative effects this could have on the PCBs found in the environment. Vreeland (1974) suggested differential solubility as a factor potentially affecting the rate of uptake of individual chlorinated biphenyls by oysters. The fact that large volumes of water are filtered by mussels and other bivalves suggests that the solubility of the different PCB components would influence the ability of these animals to excrete the different chlorinated biphenyls. Our findings of a longer retention by the mussel of the higher chlorinated biphenyls is in agreement with the findings of decreasing solubility in water of the biphenyls with increasing chlorine content (up to six chlorines).

Conclusion

PCB concentration in the mussel decayed logarithmically with total PCB having a calculated half-life of 23 days. This rate of decrease is within the range of rates reported for other organisms. We found a longer retention of the higher chlorinated biphenyls than the lesser chlorinated biphenyls. A greater portion of the higher chlorinated biphenyls are found in environmental samples from higher trophic levels. Selective uptake, metabolism, and retention of different PCB components have been demonstrated for different organisms. Our observation of selective retention of the higher chlorinated biphenyls in the mussel appears to be caused by the lower solubility in water of these components. The selective retention we observed is one of the processes through which PCBs in the environment are altered.

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