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Accumulation and Depuration of Aqueous and Dietary PCB (Aroclor 1254) by Striped Bass (*Morone saxatilis*)

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The uptake of organochlorine pollutants such as polychlorinated biphenyls (PCB) by aquatic animals can occur from solution via surface in contact with the ambient water or can be taken up from food. Due to their hydrophobicity and low degradability, chlorinated hydrocarbons are able to accumulate to high concentrations in organisms relative to the concentrations present in the aquatic environment (Oliver and Niimi 1988).

However, there are several different controversies over whether chlorinated hydrocarbons are taken up by aquatic animals primarily through water or dietary intake. Partitioning plays an important process for the organochlorine pollutants bioconcentrated in aquatic biota through water to tissues (Bruggeman et al. 1981). Theoretical and experimental studies by Oliver and Niimi (1988) showed that PCB congers were directly uptaken from water by Lake Ontario salmonids. Various data have also demonstrated that dietary intake may be the primary determinant of PCB body burdens in various aquatic biota (Thomann 1981; Brown et al. 1982). O'Connor and Pizza (1987) proposed 51% to 83% of the PCBs in striped bass might be due to dietary uptake alone by using a seasonally variable pharmacokinetic model. A 99% of the PCB burden in Lake trout was accounted for by food chain intake in a model developed by Thomann and Connolly (1984).

As a very valuable fish species along the U.S. coasts, striped bass (*Morone saxatilis*) population has been drastically declined since the 1970's. Among the factors, persistent contaminants have been recognized as a major factor for the decline on this fish species (Fabrizio et al. 1991). Very little study has been described for the conditions of PCB uptake in striped bass through dietary intake or water. This study was designed to study the resulting PCB levels accumulated by the fish larvae through contaminated water and/or diets. *Artemia* nauplii were chosen as the live diets in the present study for the potential organochlorinated contaminants adsorption (Wang et al. 1997). Due to the migration habit of the species, PCB levels in striped bass were also measured after exposure to both PCB uncontaminated diets and water for the depuration study.

MATERIALS AND METHODS

Standard polychlorinated biphenyls (PCB), Aroclor 1254 (with 54% chlorines, Supelco Inc., USA), were weighed and dissolved in a series of dilutions with acetone to achieve 100 ng/mL of stock solution. In the present study, 0.2 and 2.0 mL each of 100 ng/mL prepared standard PCB were pipetted into Erlenmeyer flasks containing 200 mL of 0.45 µm filtered seawater (30‰ of salinity) to achieve 0.1 ng/mL and 1.0 ng/mL PCB concentrations. One gram of RACII *Artemia* cysts (Artemia Reference Center, Belgium) were hatched in a separatory glass funnel containing 2 L of filtered seawater (30‰ of salinity) under strong aeration for 24 h (20 ± 2°C). After 24-h hatching, *Artemia* nauplii were harvested under a 106 µm sieve and transferred to flasks containing seawater with 0.1 ng/mL and 1.0 ng/mL of prepared concentrations for another 48 h in order to accumulate PCB.

Twenty striped bass at two-wk-old (obtained from Hudson River Hatchery at Verplanck, NY, USA) were randomly collected and transferred to each of the 5 L white polyethylene tanks (35x26x13cm). The tanks were set up to contain either 0.1 and 1.0 ng/mL PCB contaminated or PCB-uncontaminated 5‰ filtered seawater under moderate aeration (20 ± 2°C). All the treatments were conducted in the Food Science & Nutrition Center at the University of Rhode Island and replicated twice.

One gram of contaminated or uncontaminated *Artemia* was fed to each tank daily. At day 10 and 20, half of the fish from each of the treatment tanks were collected at random. After day 20, the rest of the fish were fed with uncontaminated *Artemia* and changed to clean water for the depuration study. Every day before feeding, 1 L of water were siphoned to remove fecal detritus or dead *Artemia* nauplii and replaced with same volume of freshwater. At day 30, half of the fish were also collected. The depuration experiment was terminated on day 40. All collected samples were freeze-dried and stored under -20°C for PCB analysis.

Samples of freeze-dried *Artemia* nauplii and striped bass were first blended in a Polytron tissue homogenizer with hexane and acetone (1:1) to obtain upper layer solvents for 3 times. The extracts from the solvents were cleaned-up by using 110° C overnight activated Florisil® (100/120 mesh, Supelco, Inc.) and eluted with hexane. The elutes were concentrated and dissolved in hexane for gas chromatography analysis.

The amounts of PCB were analyzed by a Tractor MT-220 gas chromatograph (GC) equipped with a ^{63}Ni electron capture detector (ECD). The glass column was 1.5% SP-2250/1.9% SP-2401 (100-120 mesh, 2.4 m x 3.175 mm i.d.) on Supelcoport® with 25 mL/min of 95%-argon/5%-methane flow rate. The temperatures used for injector, column and detector temperatures were 250°C 200°C and 275°C respectively. PCBs were quantitated by comparing total areas of peaks with that of standard Aroclor 1254. The recovery after extraction and clean-up procedures was 85-90%. The detection limit on GC was 1.0 ng/g in this study.

RESULTS AND DISCUSSION

Table 1 shows the PCB levels in *Artemia* nauplii as the diets used in the present study for striped bass after exposure to each of 1.0 ng/mL and 0.1 ng/mL PCB concentration for 48 h. The concentration of the PCB in the *Artemia* diets pre-contaminated under 1.0 ng/mL aqueous (40 ng/g, wet wt.) was 10 times than that pre-contaminated under 0.1 ng/mL (4 ng/g). Apparently, the PCB values in *Artemia* followed the direct partitioning process that caused a significant result on the transfer of the PCB between water and this zooplankton. In the accumulation period (0-20 d), the one g contaminated diets fed to striped bass daily may uptake 40 ng and 4 ng of PCB in the 1.0 ng/mL (A1.0) and 0.1 ng/mL (A0.1) pre-contaminated *Artemia* groups, respectively.

Table 1. Average PCB levels (ng/g, wet wt.) in *Artemia* nauplii under 0.1 ng/mL and 1.0 ng/mL PCB concentrations for 48 h as the contaminated diets for feeding striped bass within accumulation period.

	PCB concentration	
	0.1 ng/mL	1.0 ng/mL
PCB in *Artemia*	4.0±0.1 ng/g	40 ± 0.3 ng/g

Apparent levels of PCB in striped bass under 1.0 ng/mL and 0.1 ng/mL individual or combinations of contaminated water and *Artemia* nauplii within accumulation period (0-20 d) are shown in Figure 1. Among these treatment groups, striped bass exposed to the combination of 1.0 ng/mL contaminated water and 1.0 ng/mL pre-contaminated *Artemia* diets(W1.0 A1.0) had the highest PCB levels, 270.62 ng/g and 568.72 ng/g (wet wt.) in day 10 and 20, respectively. The least concentrations in striped bass were determined to be 59.51 ng/g and 83.88 ng/g in day 10 and 20, respectively, from the groups fed with 0.1 ng/mL pre-contaminated diets only (W0.0 A0.1).

According to Brown et al. (1985) the aqueous PCB concentrations correlate well with the PCB concentrations in yearling pumpkinseed collected at Hudson River. Defoe et al. (1978) studied the wet weight residue of Aroclor 1248 in fathead minnow tissue and found it was directly proportional to the concentration (0.1, 0.4, 1.1 & 3.0 µg/mL) of Aroclor 1248 in the water after 30 days exposure. Aroclor 1254 is the predominant PCB mixture in striped bass examined from Hudson River (Fabrizio et al. 1991). A high degree of PCB loading in this fish species can occur within 2 wk of exposure (Jones and Sloan 1989). When striped bass were exposed to PCB contaminated water alone in the present study (Figure 1), PCB levels on 1.0 ng/mL treatments (181.56 ng/g in day 10 and 413.53 ng/g in day 20) were consistently higher than that of 0.1 ng/mL treatments (123.56 ng/g in day 10 and 135.90 ng/g in day 20). Califano et al. (1980) conducted experiments by exposing striped bass larvae to PCBs in Hudson River water (1.36 ng/mL) and found fish

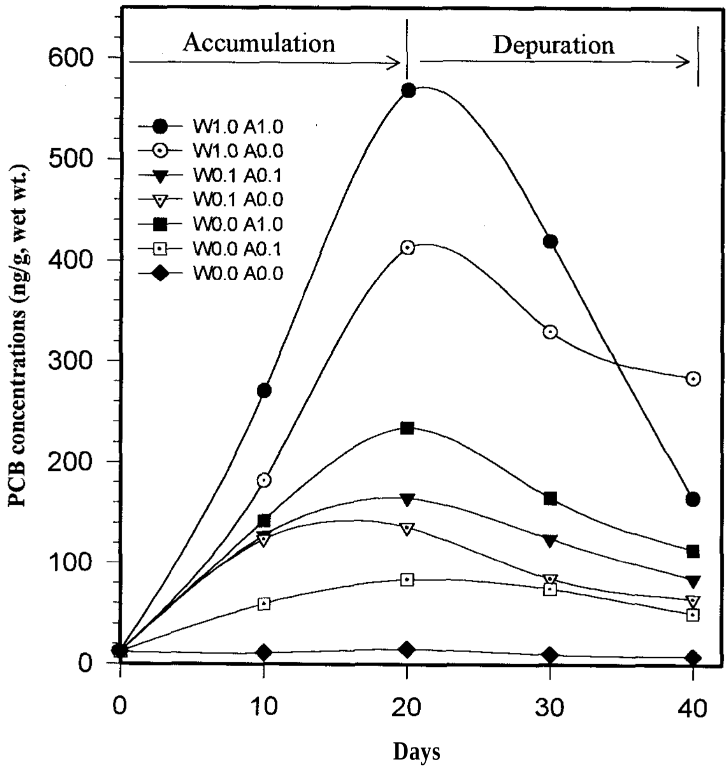

Figure 1. Concentrations of PCB in striped bass under various groups with different water and pre-contaminated *Artemia* treatments for 20 d (day 0-20) then fed uncontaminated *Artemia* diets under clean water conditions for 20 d (day 20-40). (The W1.0, W0.l and W0.0 show the PCB concentrations prepared in the water at 1.0, 0.1 and 0.0 ng/mL, respectively. The A1.0, A0.1, and A0.0 represent the *Artemia* were pre-contaminated under PCB concentrations at 1.0, 0.1 and 0.0 ng/mL, respectively.)

reached a maximum of 5.9 µg/g (dry wt.) at 48 h, at which time the fish had accumulated about 60% of the PCBs available in the water system. The value 5.9 µg/g dry wt. (or 1180 ng/g, based on 80% wet wt.) is higher than the values in this study. The differences may due to the different time of exposure, concentrations of the aqueous solutions and static state or flow through systems.

In the 20-d accumulation period (Figure l), each of 0.1 ng/mL and 1.0 ng/mL pre-contaminated *Artemia* diets applied to each of the same water concentration (W1.0 A1.0 and W0.1 A0.1) showed apparent increases of PCB levels compared to the fish exposed to contaminated water alone (W1.0 A0.0 and W0.1 A0.0). The *Artemia* with higher PCB residues could contribute to the higher increase in the

PCB body between W1.0 A1.0 (568.72 ng/g) and W1.0 A0.0 (413.53 ng/g) groups than that between W1.0 A0.1 (164.86 ng/g) and W0.1 A0.0 (135.90 ng/g) groups within 20 d. Apparently, different doses of xenobiotic chemicals in the diets with different length of exposure time may also be the factor resulting in different PCB burdens in aquatic animals. No PCB equilibrium in striped bass body seemed to be reached after 20-d accumulation exposure in all treatment groups.

The degrees of PCB accumulation in striped bass are also expressed in the linear regression equations as the function of PCB levels and exposure times (day 0-20) in Table 2. The values of linear regression coefficient (r^2) were found to relate to the PCB level and exposure time on each accumulation group. In the present experiment, the total PCB accumulated in fish may still continue with increasing higher doses of the PCB or length of exposure time. The three highest slope values, 27.84, 20.08 and 11.12 on W1.0 A1.0, W1.0 A0.0 and W0.0 A1.0 treatments, respectively, may demonstrate that 1.0 ng/mL contaminated sources have a greater degree of PCB accumulation rate than that of 0.1 ng/mL contaminated sources.

Striped bass after exposure to either contaminated water or/and *Artemia* were transferred to both PCB-free water and diets for 20-d depuration observation (Figure 1). Striped bass have the tendency to migrate into a clean water area causing the PCB concentration to decline toward a baseline concentration (Fabrizio et al. 1991). During the depuration period (day 20-40) levels of PCB in striped bass in all treatment groups were depurated to some extent. The PCB levels after 20-d (day 40) depuration were apparently lower than that after 10-d depuration (day 30) in each of the treatment group. However, the PCB level in striped bass found on WI.0 A1.0 group (165.00 ng/g) is lower than that on W1.0 A0.0 group (284.31 ng/g) after 20-d depuartion (day 40). This is consistent with the report by O'Connor et al. (1980) that PCBs accumulated from water are retained for longer

Table 2. Linear regression equations for the PCB levels in striped bass during accumulation period (day O-20) under various PCB treatments and depuration period (day 20-40) under PCB-free treatments, where y = PCB levels (ng/g, wet wt. basis); x = days.

Treatments		*Linear Regression Equations*	
Water	*Artemia*	Accumulation (day 0-20)	Depuration (day 20-40)
1.0	1.0	$y = 27.84x + 5.42$ ($r^2 = 0.99$)	$y = -20.19x + 990.15$ ($r^2 = 0.98$)
1.0	0.0	$y = 20.08x + 1.59$ ($r^2 = 0.99$)	$y = -6.46x + 536.44$ ($r^2 = 0.97$)
0.1	0.1	$y = 7.64x + 24.97$ ($r^2 = 0.92$)	$y = -3.99x + 244.75$ ($r^2 = 0.99$)
0.1	0.0	$y = 6.19x + 28.50$ ($r^2 = 0.82$)	$y = -3.54x + 201.64$ ($r^2 = 0.94$)
0.0	1.0	$y = 11.12x + 18.29$ ($r^2 = 0.99$)	$y = -6.03x + 351.94$ ($r^2 = 0.99$)
0.0	0.1	$y = 3.59x + 15.86$ ($r^2 = 0.97$)	$y = -1.67x + 119.92$ ($r^2 = 0.93$)

periods, and PCB body burdens obtained from food are eliminated more rapidly than by fish that accumulated PCB from water.

Different degrees of depuration to eliminate PCB were found to be dependent on different PCB levels accumulated in the fish with different periods of time spent in PCB free environments as expressed on Table 2. All treatment groups within depuration period (day 20-40) are correlated closely with high values of r^2 between exposure time and PCB concentration in striped bass. Among the groups, the highest depuration solpe (-20.19) was found to be on the accumulation group, W1.0 A1.0. This may due to the fact that differences in PCB bioconcentrated in fish by partitioning can retain longer in the body than pollutants bioaccumulated through diets and can be depurated with a higher rate by enzymatic metabolism.

According to Khan (1977) about 50-90% of the absorbed chlorinated pesticides can be depurated within 4 wk. Larvae of chironomids retained 97.8% of total Aroclor 1242 after 7-d depuration (Meier and Rediske 1984). Cod larvae also are reported to depurate 40% of the PCB after 15-d depuration (Solbakken et al. 1984). Table 3 shows the values of percentage PCB depuration in striped bass on various treatments after transferred to PCB-free water and fed uncontaminated *Artemia* diets within 20 d. In this depuration study, all percentage depuration values are higher on 20 d than on 10 d on all experiment groups. The highest percentage depuration value was found to be at the W1.0 A1.0 group (70.99%). The PCB accumulated from dietary intake and metabolized by MFO may result in the highest percentage depuration value of 70.99% on W1.0 A1.0 treatment group compared to other groups. On the other hand, the least depuration percentage at day 20 was determined to be 31.25% from striped bass pre-contaminated under 1.0 ng/mL water only (W1.0 A0.0). Apparently, with increasing length of time for the fish living under clean diet and water environments the PCB accumulated directly from water source may significantly depurate lower PCB amounts compared to that from dietary source.

Table 3. Percentage of PCB depuration in striped bass on various treatment groups after transferred to PCB-free water and fed uncontaminated *Artemia* diets for 10 d and 20 d (day 30 and day 40 on the experiment).

Treatments		Depuration (%) in striped bass	
Water	*Artemia*	10 d	20 d
1.0	1.0	26.15%	70.99%
1.0	0.0	20.20%	31.25%
0.1	0.1	24.18%	48.44%
0.1	0.0	39.07%	52.17%
0.0	1.0	29.60%	51.45%
0.0	0.1	10.59%	39.85%

In conclusion, the present study demonstrated that PCB were significantly accumulated in the striped bass depending on different magnitudes of contaminated dietary or water sources. The individual or combination of higher PCB concentrations from the two sources have apparent effects on PCB accumulation in the fish during 20-d exposure. Following 20-d experiment under PCB-free environment, higher depuration rates and percentages were found to be from the fish previously accumulated PCB in the body through diets, compared with those accumulated PCB through water.

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