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Long-Term Accumulation of Individual PCBs, Dioxins, Furans, and Trace Metals in Asiatic Clams from the Rio de la Plata Estuary, Argentina

JUAN C. COLOMBO,*,† CHARLES BROCHU,‡ CLAUDIO BILOS,† PATRICIA LANDONI,† AND SERGE MOORE‡

Química Ambiental y Biogeoquímica, Facultad de Ciencias Naturales y Museo, Universidad Nacional de la Plata, Paseo del Bosque S/N, 1900 La Plata, Buenos Aires, Argentina, and Ministère de l'Environnement du Québec, Direction des Laboratoires, 850 Boulevard Vanier, Laval, Québec, Canada H7C 2M7

The long-term accumulation of individual polychlorinated biphenyls (PCB), dioxins (CDD), furans (CDF), and selected trace metals was studied in Asiatic clams of increasing size covering a 0.5−4-year growth period. PCB bioaccumulation was congener-specific and related to compound hydrophobicity and stereochemistry as indicated the contrasted slopes obtained for the concentration vs size regressions. Tri and tetraCB with log K_{ow} < 6 showed nonsignificant slopes, indicating steady-state conditions or negative values (i.e., PCB 31 and 49) reflecting selective degradation. Penta and hexaCB with log K_{ow} 6−7 (total surface area = 240−270 Å^2) were preferentially accumulated, and the slopes increased with log K_{ow}. Except for congener 180, which continued this linear trend, hepta and octaCB showed a reduced long-term accumulation due to unfavorable stereochemistry. The selective enrichment in congeners 153, 138, 118, and 180 explained 74% of the total PCB increase observed from 10 to 35 mm clams (446−871 ng/g dw). CDD and CDF also increased with size but with much steeper gradients (136−790 and 70−400 pg/g, respectively), indicating a more rapid long-term kinetics. PCBs accounted for 83−88% of the total toxic equivalents (TEQ) calculated for the clams; pentaCBs 126 and 118 represented ∼60 and 10% TEQ, respectively. The preferential bioaccumulation of planar PCBs 77−126, mono-ortho-substituted 118 and tetra-hexa CDD/CDF accounted for 95% of the TEQ increase observed from 10 to 35 mm clams (7.1−13.1 pg/g wet weight). Among trace metals, Cu showed a consistent increasing trend with clam size (22−41 μg/g dw), similar to PCBs, suggesting continuous passive bioaccumulation. Bioregulation appeared to be effective for Zn whose concentrations increased from 10 to 22 mm clams (150−190 μg/g dw) and returned to initial values in larger animals. Ni levels were very homogeneous (3.5 ± 0.4 μg/g dw) and showed no clear pattern with clam size.

* Corresponding author fax: 54 12 75 82 66; e-mail: colombo@Isis.unlp.edu.ar.

† Universidad Nacional de la Plata.

‡ Ministère de l'Environnement du Quebéc.

Introduction

Due to their sedentarism and ability to concentrate xenobiotic compounds, bivalves have been extensively employed for biomonitoring studies in aquatic ecosystems in both marine (i.e., mussels and oysters; *1*−*5*) and freshwater (i.e., Anodonta sp., Zebra mussels, Asiatic clams; *6*−*10*) environments. In 1993, we initiated a study of the widely distributed invasive species *Corbicula fluminea* as a sentinel organism in the Río de la Plata Estuary, the second largest basin in South America. Asiatic clams showed an order of magnitude gradient in the concentration of PCBs along 150 km of the Argentinean coast of the estuary (*11*). Superposed on this clear geographical trend, size-related patterns were evident in the concentration and relative contribution of individual PCBs (i.e., larger animals showed increasing PCB levels and higher proportions of hexachlorobiphenyl 153). The analysis of trace metals in the same clams also revealed size-related variations for Cu (increasing with size) and Zn (decreasing with size; *12*). These size or age-related trends introduced a significant amount of variability that obscured the site-to-site differences.

In order to elucidate the relationship between bivalve size or age and contaminant accumulation, in this paper we report the results of a detailed study of the concentration of individual PCBs, chlorinated dibenzodioxins (CDD), furans (CDF), and trace metals in six size classes of Asiatic clams collected in the Río de la Plata Coast.

Methods

Asiatic Clams (*Corbicula fluminea*) were collected in October 1995 manually during low tide at Punta Lara, a popular beach located upstream to La Plata city (∼600 000 inhabitants) but 50 km downstream of Buenos Aires (∼6 million people), in a seriously affected sector of the estuary (*11*). Immediately after sampling, the anterior−posterior shell length was measured with a Vernier caliper, and six size classes were defined minimizing the intra-class variability and the inter-class overlapping: 10.8 ± 1.3 mm (n = 301), 14.3 ± 1.1 mm (n = 92), 18.7 ± 1.2 mm (n = 73), 22.3 ± 1.1 mm (n = 72), 31.4 ± 1.4 mm (n = 23), and 35.2 ± 1.4 (n = 11). The organisms were stored in glass containers at −20 °C until analysis.

Clam samples were thawed at room temperature and carefully washed with deionized water to remove sediment particles retained in the gills and the mantle cavity. For each size, soft tissues were removed from the shells, pooled, and homogenized using a stainless steel blender. Aliquots of ∼1 g wet weight were separated for determination of the water content (105 °C until constant weight), which varied over a narrow 81.4−83.9% range for all the samples. The remaining material was splitted for the determination of organic contaminants and trace metals. Analyses of the homogenates were effectuated in duplicate for selected sizes (10.8, 18.7, and 31.4 mm) in the case of organic contaminants or for all the samples for trace metals. Aliquots of 7−8 g wet weight of the clam homogenates were mixed with pre-extracted sodium sulfate (1.5 times in wet mass) and Soxhlet extracted with a 1:4 dichloromethane−pentane mixture (50 cycles). The extracts were concentrated at 25−30 °C under a nitrogen flow until constant weight to determine total lipids and then shipped to the Ministère de l'Environnement et de la Faune du Québec. The extracts were dissolved in hexane and spiked with three $^{13}C_{12}$-labeled non-ortho-PCBs, six ortho-PCBs, and nine $^{13}C_{12}$-labeled 2,3,7,8-CDD/F isomers. Purification was performed on multi-layer silica columns followed by fractionation of PCBs, planar PCB, CDD, and CDF on alumina columns (*13*). An additional cleanup on carbon columns was performed for the CDD/CDF fractions, which were evaporated to dryness and spiked with 50 μL of internal

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TABLE 1. Concentration of PCBs, Dioxins, Furans, and Trace Metals in Asiatic Clams from the Río de la Plata Estuary[a]

	size classes (mm)										
mean	10.8	14.3	18.7	22.3	31.4	35.1					
SD	1.3	1.1	1.2	1.1	1.4	1.4					
PCBs	**concentration (ng/g dw)**						***m***	***b***	***r***	***p***	**TEQ**
TriCB											
18	6.9	7.1	5.9	8.2	6.0	6.9	−0.01	7.12	−0.15	0.78	
31	14.7	15.6	12.7	13.7	11.1	11.7	−0.16	16.80	−0.88	0.02	
tetraCB											
52	26.0	24.7	22.4	24.6	18.6	23.1	−0.19	27.33	−0.68	0.14	
49	12.6	12.6	10.8	11.3	8.3	8.8	−0.18	14.79	−0.95	<0.01	
44	17.2	19.3	15.1	17.1	14.0	18.2	−0.06	18.09	−0.28	0.59	
40	2.4	2.5	2.4	2.5	1.5	2.4	−0.02	2.70	−0.47	0.35	
60	7.9	8.8	7.3	7.9	5.9	6.8	−0.08	9.27	−0.78	0.07	
pentaCB											
101	47.3	50.4	57.7	62.3	64.2	83.8	1.26	33.05	0.93	0.01	
86	12.1	14.5	16.0	19.3	19.0	28.1	0.53	6.41	0.91	0.01	
87	14.7	16.1	16.6	19.0	16.2	20.0	0.15	13.84	0.69	0.13	
118	31.0	37.0	44.0	47.0	55.0	71.0	1.44	15.71	0.97	<0.01	0.68−1.41
105	17.0	18.0	20.5	20.0	22.5	21.6	0.20	15.48	0.92	0.01	0.38−0.43
hexaCB											
151	17.2	18.8	23.4	25.4	24.2	35.8	0.61	10.74	0.88	0.02	
153	90.0	104.0	141.8	160.0	159.5	242.0	5.16	35.50	0.92	0.01	
141	1.4	0.2	1.9	2.4	2.0	2.6	0.07	0.30	0.70	0.12	
138	69.7	81.6	104.5	128.0	119.0	173.0	3.52	34.90	0.91	0.01	
128	9.8	11.5	14.8	15.2	13.4	18.8	0.27	8.04	0.81	0.05	
156	2.8	3.4	4.0	4.4	3.7	4.8	0.06	2.60	0.75	0.09	0.31−0.48
heptaCB											
182	14.6	16.6	22.9	24.8	24.2	32.6	0.62	8.92	0.91	0.01	
183	6.7	7.5	10.1	10.8	10.0	10.3	0.13	6.32	0.75	0.09	
171	3.1	3.7	4.6	4.6	4.3	5.4	0.07	2.80	0.82	0.05	
180	9.4	11.3	15.6	17.4	19.7	28.4	0.68	2.05	0.95	<0.01	0.02−0.06
170	5.7	6.7	8.3	9.3	7.3	7.0	0.03	6.62	0.26	0.62	0.13−0.14
octaCB											
201	0.2	0.4	0.3	0.2	0.2	0.2	−0.01	0.43	−0.69	0.13	
199	0.7	0.7	0.9	0.2	0.2	0.2	−0.03	1.09	−0.80	0.06	
196	1.4	1.6	2.3	2.3	2.3	3.4	0.07	0.74	0.90	0.02	
195	0.6	0.2	0.8	0.9	0.0	0.0	−0.02	0.88	−0.54	0.26	
planar											
77	2.9	2.9	3.1	2.8	3.3	4.1	0.04	2.24	0.81	0.05	0.32−0.41
126	0.2	0.2	0.3	0.3	0.4	0.4	0.01	0.07	0.98	<0.01	4.42−7.95
total PCBs	445.9	497.9	590.5	661.8	635.3	871.3	14.13	304.82	0.90	0.01	6.26−10.9
SD	90.3		4.8		36.7						
CDDs	**concentration (pg/g dw)**						***m***	***b***	***r***	***p***	**TEQ**
2,3,7,8-TCDD	nd	nd			nd	nd					
TCDDs	21.5	120.0			163.0	290.0	8.23	−39.74	0.90	0.10	
1,2,3,7,8-PCDD	nd	nd			nd	nd					
PCDDs	43.0	100.0			185.0	250.0	7.34	−23.68	0.97	0.03	
1,2,3,4,7,8-hexaCDD	nd	nd			nd	33.0					0.00−0.66
1,2,3,6,7,8-hexaCDD	9.5	nd			14.0	30.0					0.21−0.60
1,2,3,7,8,9-hexaCDD	nd	nd			nd	nd					
hexaCDDs	24.0	37.0			72.0	170.0	4.71	−32.01	0.86	0.14	
1,2,3,4,6,7,8-heptaCDD	21.5	nd			27.0	40.0					0.05−0.08
heptaCDDs	27.5	16.0			40.0	40.0	0.81	12.32	0.85	0.15	
OCDD	20.0	24.0			30.5	40.0	0.68	13.12	0.94	0.06	0.004−0.008
total CDDs	136.0	297.0			490.0	790.0	21.75	−69.90	0.94	0.06	0.26−1.35
SD	26.9				63.6						
CDFs	**concentration (pg/g dw)**						***m***	***b***	***r***	***p***	**TEQ**
2,3,7,8-TCDF	25.5	24.0			41.5	42.0					0.56−0.83
TCDFs	42.5	49.0			110.0	170.0	4.70	−14.68	0.95	0.05	
1,2,3,7,8-PCDF	nd	nd			nd	nd					
2,3,4,7,8-PCDF	nd	nd			nd	nd					
PCDFs	nd	37.0			30.0	79.0	1.27	14.57	0.53	0.64	
1,2,3,4,7,8-hexaCDF	nd	nd			nd	nd					
1,2,3,6,7,8-hexaCDF	nd	nd			nd	nd					
2,3,4,6,7,8-hexaCDF	nd	nd			nd	nd					
1,2,3,7,8,9-hexaCDF	nd	nd			nd	nd					
hexaCDFs	6.0	13.0			21.0	100.0	2.75	−27.98	0.76	0.24	
1,2,3,4,6,7,8-heptaCDF	14.0	16.0			nd	30.0					0.03−0.06
1,2,3,4,7,8,9-heptaCDF	nd	nd			nd	nd					
heptaCDFs	21.5	26.0			nd	48.0	1.08	10.18	0.99	0.02	
octaCDF	nd	nd			nd	nd					
total CDFs	70.0	125.0			161.0	400.0	9.80	−35.43	0.82	0.18	0.59−0.89
SD	49.5				42.4						

TABLE 1 (Continued)

metals	concentration (μg/g dw)						*m*	*b*	*r*	*p*	TEQ
Zn	152.8	173.5	181.4	189.2	145.2	151.6	−0.74	182.00	−0.39	0.44	
SD	3.8	6.2	8.5	0.2	4.2	5.9					
Cu	22.0	24.3	28.1	30.7	33.9	40.6	0.69	14.63	0.98	<0.01	
SD	0.7	0.9	0.4	0.6	0.1	0.6					
Ni	3.3	4.1	3.4	3.7	3.0	3.6	−0.01	3.75	−0.26	0.61	
SD	0.03	0.43	0.01	0.01	0.08	0.13					

[a] CB, chlorobiphenyls. CDD, chlorinated dibenzodioxins. CDF, chlorinated dibenzofurans. SD, standard deviation. nd, not detectable. *m* and *b* are the slope and intercept of the regressions of the pollutants versus clam size. *r* is the correlation coefficient, and *p* is the probability. TEQ, toxic equivalents in pg/g wet weight for the smallest and biggest clam.

standards containing four $^{13}C_{12}$-labeled CDDs and CDFs. Planar PCB fractions were also evaporated to dryness and spiked with 100 μL of $^{13}C_{12}$-labeled 1,2,3,4-TCDD as an internal standard. Planar PCBs and CDD/CDF were analyzed by HRGC/HRMS (Hewlett-Packard 5890-VG AutospecQ) using a 0.25 mm × 60 m DB5 capillary column. The oven temperature was programmed from 100 (1 min hold) to 200 °C at 40 °C/min, from 200 to 235 °C at 3 °C/min with a 10-min hold, and then up to 310 °C at 8 °C/min with a final plateau of 15 min. The HRMS was operated in the electron impact mode (34 eV, 260 °C source temperature) with a static resolution of 10 000 (ion monitoring mode). Non-planar PCBs were concentrated to 400 μL, then spiked with 100 μL of internal standard, and analyzed by HRGC/MS−MS (Saturn II Varian) using a 0.25 × 30 m DB5-MS capillary column. The oven temperature was programmed from 90 to 130 °C at 4 °C/min, from 130 to 170 °C at 15 °C/min, from 170 to 250 °C at 2 °C/min, and then to 310 °C at 30 °C/min (5-min hold). Relative response factors over a four-point calibration curve were calculated for each congener of CDD/CDF, planar PCBs, and non-planar PCBs. Results were corrected for the recovery yield of the $^{13}C_{12}$ surrogates. Procedural blanks and standard reference material (Carp-1; National Research Council Canada) were processed along with the samples.

For trace metal analyses, clam samples were completely mineralized with a concentrated sulfuric acid, nitric acid, and hydrogen peroxide mixture (1:20:5) at 60−200 °C in 250-mL borosilicate glass Erlenmeyer flasks. After centrifugation, the extracts were diluted to 25 mL with deionized water and stored in polyethylene bottles. Metal analyses were performed by flame atomic absorption spectrophotometry using a Perkin Elmer 3110 spectrophotometer, equipped with a deuterium background corrector, an air−acetylene flame, and Perkin Elmer or ISTC hollow cathode lamps. High-purity Johnson Matthey PLC original standards were used to prepare calibration mixtures. The accuracy of the analytical procedure was tested analyzing certified reference sediment BCSS-1 (National Research Council, Canada). The concentrations measured were within ±3−11% of the values reported. The precision of the data ranged from 2 to 11% (RSD).

Results and Discussion

Polychlorinated Biphenyls. Table 1 presents the concentration of organic contaminants and trace metals in the six size classes of Asiatic clams. Total concentrations of the PCBs measured ranged between 446 and 871 ng/g dw (3−5 μg/g lipids) and showed a consistent increase with clam size reflecting the progressive accumulation of these residues. The increase of total PCB from 10 to 35 mm clams (95%; Table 1) is partially related to the increase of the lipid pool in larger animals (13−14 to 17−18% dw). However, lipid-normalized PCB concentrations (Figure 1) still show a 60% PCB increase from the smallest to the largest clam analyzed, which corresponds to ∼0.5−4-year-old animals. The data also suggest stabilized PCB concentrations in intermediate (20−30 mm) animals. This might be related to physiological changes occurring during the life cycle of the clams, perhaps associated with differences in feeding, growing rates, and spawning. The growing rates are highest during the first year when clams could reach a shell length of about 20 mm, afterward the rates decrease to 3−7 mm/yr. Sexual maturity in the clams is attained at ∼10 mm with two reproductive periods, in the spring (September−October) and late summer (February−March; *14*, *15*).

Individual PCB congeners showed markedly different behaviors during the 0.5−4-year growth of the clams (Figure 1): the concentration of some lower chlorinated congeners decreased; several PCBs remained more or less constant; while some penta, hexa, hepta, and octachlorobiphenyls (CB) increased. For detailed analyses of these trends, we employed the slope of the regressions between the concentration of PCBs (dw) versus clam size (Table 1). Congeners 101, 118, 138, and 153 showed the highest slopes (1.3, 1.4, 3.5, and 5.2 ng g^{-1} mm^{-1}), which together represented 80% of the slope of total PCBs (14.1 ng g^{-1} mm^{-1}), whereas tri, tetra, and some octaCB showed non-significant or negative values. Considering the simplifying assumption of constant growth rates (24.3 mm in 3.5 yr = 6.94 mm/yr), these slopes indicate an average increase of about 0.7, 0.8, 2, 3, and 8 ng/g dw each month for congeners 101, 118, 138 and 153, and total PCBs, respectively. If instead, more realistic varying growth rates are considered, from 10 to 22.3 mm clams (11.5 mm in 0.5 yr = 23 mm/yr) the regressions (slopes = 1.3, 1.4, 5.1, 6.4, and 19.1 ng g^{-1} mm^{-1}) yield an increase of 2.5, 2.7, 9.8, 12, and 37 ng/g each month, respectively.

To compare the relationship between the long-term accumulation trend and the hydrophobicity of the different congeners, the slopes were expressed as percentage of the intercept to normalize the different scales and plotted against the log K_{ow} of the components (*16*). As observed in Figure 2, there is a weak relationship for the whole data set ($r = 0.39$, $n = 27$, $p = 0.04$). The scatter in fact presents a parabolic pattern: all tri and tetraCB with log K_{ow} below 6 show non-significant or negative slopes independent from K_{ow}, most penta and hexaCB (log K_{ow} 6−7) display increasing slopes with increasing hydrophobicity (% slope = 16.4 log K_{ow} −101.6, $r = 0.68$, $n = 12$, $p < 0.01$), and congeners with log K_{ow} higher than 7 show lower slopes, except heptaCB 180, which presents the highest relative value (33%) and is included in the linear trend. This parabolic trend is comparable to that previously found for the bioaccumulation factor−K_{ow} relationships of different aquatic organisms (*17*−*20*) and even for membrane−water PCB partition coefficients (*21*), supporting the interpretation that hydrophobicity (increasing linear trend) and spatial configuration of PCB congeners (steric hindrance of superhydrophobic PCBs) controls the bioaccumulation process. However, in the bioaccumulation factor−K_{ow} relationships the linear increasing phase extends from log K_{ow} ∼3 to log K_{ow} 6.5, whereas in the case of the slopes, it is only apparent between log K_{ow} 6 to log K_{ow} ∼7. This shift is probably related to the fact that the slopes reflect the accumulation tendency of the congeners along ∼0.5−4 yr of clam growth, almost the whole lifecycle (long-term kinetics), whereas the bioaccumulation factors represent a punctual relationship between

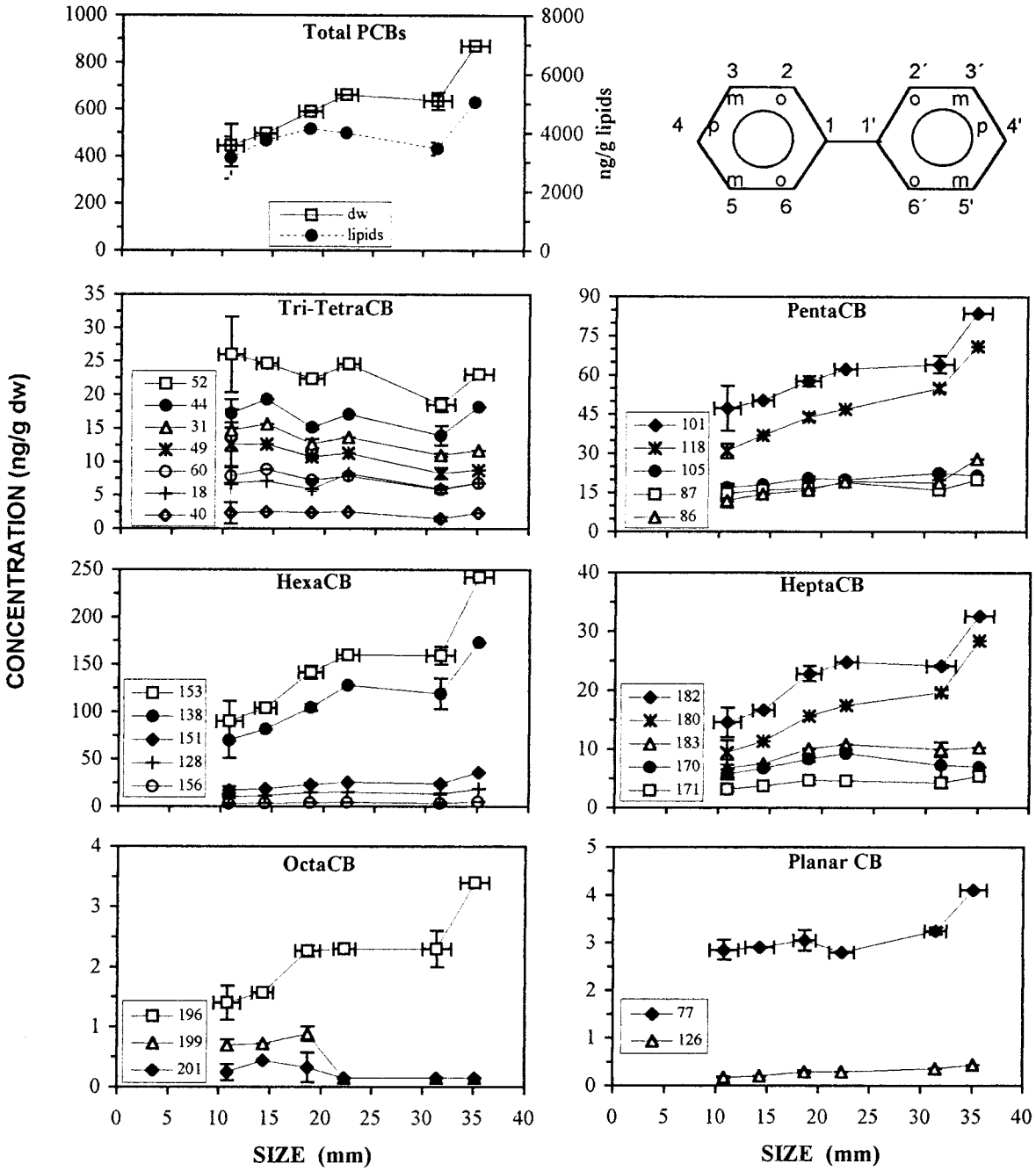

FIGURE 1. Concentration of total PCB and individual congeners in six size classes of Asiatic clams from the Río de la Plata Estuary.

the organism and the surrounding variable media and may not represent steady-state conditions, especially for super-hydrophobic (log K_{ow} > 6) compounds. Thus, it appears that although organisms can show an increasing bioaccumulation of compounds with log K_{ow} = 3−6 relative to water or sediments, only the most hydrophobic not sterically hindered compounds will show a progressive increase during the lifecycle of the clams.

Our data effectively suggest that tri and tetraCB are close to a dynamic equilibrium (uptake ≅ depuration) and their residues do not build up over time. In fact, PCBs 31 and 49 showed a significant decreasing trend with size, suggesting enhanced depuration or degradation. This agrees with previous observations of very low bioaccumulation factors of less chlorinated PCBs in Río de la Plata Asiatic clams and fishes (*22*) and is consistent with the shorter biological half lives of tri−tetraCB (11−35 days for 18, 52, 49, 44) relative to pentaCB (60−67 days in fish muscle for 101−118; *23*). In addition, congeners 31 and 49 have both orto-meta and meta-para vicinal H-atoms and are easily metabolized by cytochrome P-450 1A and 2B isozymes (*24*). The increase of total PCB concentrations with clam size or age is mainly driven by congeners with log K_{ow} 6−7.4 and higher slopes, i.e., 101, 86, 151, 128, 182, 196, and especially 118, 153, 138, and 180 whose concentrations increase by 130−200% from 10 to 35 mm clams. The enhanced accumulation of these congeners is related to their favorable hydrophobicity and stereochemistry, which result in a high net uptake and low depuration. Very low or near zero depuration rate constants have been reported for PCB 153 in fish (*25*). In addition, congeners 182, 196, 153, and 180 have no vicinal H-atoms and are thus nonmetabolizable (*24*). OctaCB 199 and 201 are not detected (<200 pg/g dw) in larger animals. Their unfavorable stereochemistry (reduced planarity and large molecular volume) restricts their passage through membranes (very low uptake). The optimum log K_{ow} range (6−7) of PCBs, which display increasing slopes, corresponds to a total surface area (TSA) range of ∼240−270 Å^2 (*16*); the values for octaCB 199 and 201 are 7.20−7.62 and

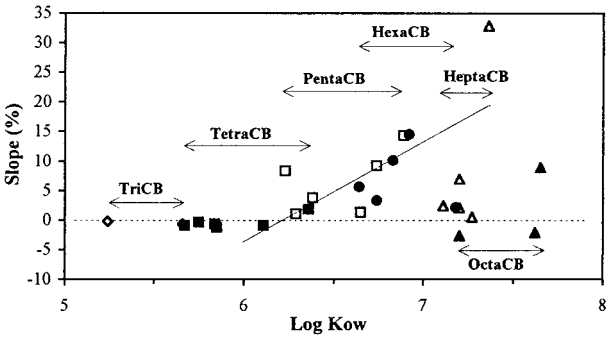

FIGURE 2. Relationship between long-term accumulation trends of PCBs in Asiatic clams and hydrophobicity (log K_{ow}) of the congeners. The slopes (as percent of the intercept) correspond to the linear regression between the concentration of the congeners and clam size. TriCB are represented by diamonds, tetraCB by filled squares, pentaCB by open squares, hexaCB by filled circles, heptaCB by open triangles, and octaCB by filled triangles. The regression line of the % slope versus log K_{ow} in the 6−7.3 range is shown in the figure.

276−288 Å^2, respectively. A loss of membrane permeation capability has been also suggested for compounds with molecular cross sectional areas greater than 9.5 Å (*26*).

The effect of the chlorine substitution pattern on long-term bioaccumulation is clearly evident in the scatter of the slopes within each congener group. To obtain a quantitative estimate of the effect of structural factors on bioaccumulation of PCBs, we calculated a steric effect coefficient (SEC; *27*) that considers that bioaccumulation is hindered by three structural characteristics: (a) the presence of two−four ortho-chlorines (2,6; 2′,6′; 2,2′,6 ; 2,2′,6,6′; Figure 1) that increase the dihedral biphenyl angle reducing the planarity; (b) the existence of three−four adjacent chlorine atoms; and (c) the substitution in the 3,5-position adjacent to chlorines ("buttressing" effect to ortho-chlorines that cannot bend back to the equilibrium position). The slopes of the concentration vs size regressions in the clams are positively correlated with SECs, indicating reduced long-term accumulation for sterically hindered PCBs: tetraCB (% slope = 14.3 SEC-13.9, r = 0.54), pentaCB excluding outlier 86 with high slope and low SEC (%slope = 82.5 SEC-72.7, r = 0.83), hexaCB (% slope = 37.2 SEC-23.9, r = 0.86), heptaCB (% slope = 194 SEC-125, r = 0.70), and octaCB (% slope = 45.5 SEC-21.3, r = 0.55). These decreasing slopes with lower SECs could be partially related to a concurrent decrease in hydrophobicity since K_{ow} are also correlated to SECs: tetraCB (log K_{ow}= 3.1 SEC+3.0, r = 0.50), pentaCB (log K_{ow}= 2.1 SEC+4.6, r = 0.73), hexaCB, excluding the outlier 156 with the highest log K_{ow} and low SEC (log K_{ow}= 0.9 SEC+6.0, r = 0.99), heptaCB (log K_{ow} = 1.5 SEC+6.2, r = 0.78) and octaCB (log K_{ow} = 3.2 SEC+5.9, r = 0.99). Nevertheless, as observed in Figure 2, this K_{ow} reduction is not sufficient to explain the decline of the slopes with lower SEC values.

It is interesting to note that the highest slopes and SEC values of tetra−pentaCB correspond to planar and mono-ortho-substituted congeners 77, 126, and 118 (10−35 mm dw increase: 43, 144, and 129%, respectively), which account for a major portion of the total toxicity equivalents in the clams (Table 1, see below). The other mono-ortho-substituted pentaCB (105) has three adjacent chlorines with one in the "buttressing" 3-position, showing a lower slope and only a 27% dw increase. Among hexaCB, the highest slopes correspond to di-ortho (2,2′) congeners 153 (169% increase) and 138 (148% increase; SEC reduced by three adjacent chlorines with one in position 3), whereas mono-ortho-substituted 156 has a lower SEC (four adjacent chlorines with one in position 3) and slope values (70% increase from 10 to 35 mm clams). HeptaCB 170 and 180 present another interesting example of contrasted long-term bioaccumulation and only small differences in the substitution pattern. Effectively, both are di-ortho (2,2′) congeners with four adjacent chlorines and one "buttressing" 3-position in one ring (2,3,4,5). In the other ring, congener 170 has a 3′-substitution instead of a 5′ of 180, which adds three adjacent chlorines plus another "buttressing" 3′-position resulting in much lower SEC and slope values (23 vs 200% increase relative to 180). The presence of two ortho-meta vicinal H-atoms in PCB 170 also favors the metabolism by MC-type enzimes. To estimate the relative importance of hydrophobicity and stereochemistry on long-term bioaccumulation of PCBs in the clams, we performed multiple regression analysis between the slopes and log K_{ow} and SEC as independent variables for the whole data set. When SEC is added to the slope−log K_{ow} regression, the correlation coefficient increases from 0.39 to 0.59 (% slope = 9.12 log K_{ow} + 27.5 SEC-78.7, n = 27, p < 0.01). To check the possible contribution of PCB metabolism, the number of vicinal meta-para and ortho-meta H-atoms were included in the regression (higher values imply easier degradation). Meta-para vicinal H improved the correlation (r = 0.66) but changed markedly the log K_{ow} and SEC coefficients (17.3 and 43.2, respectively) and gave an unlikely positive coefficient for MP-H, implying higher accumulation (slopes) with more meta-para H-atoms. Ortho-meta H-atoms also improved the regression but gave more consistent coefficients (% slope = 8.30 log K_{ow} + 29.7 SEC-2.23 OM-H-73.2, r = 0.62, n = 27, p < 0.01), suggesting that clams have some potential to degrade PCBs through MC-type enzymes (cytochrome P-450 1A subfamily; *24*).

Dioxins and Furans. CDDs and CDFs have been measured in four size classes covering the smallest and largest clams of the series (Table 1). Total concentrations ranged from 136 to 790 and from 70 to 400 pg/g dw for CDD and CDF, respectively. The concentrations also increased with size but with steeper gradients than PCBs (Figure 3). Effectively, from 10 to 35 mm clams, the concentrations (dw) increased 471−481% as compared to 95% for total PCB (% slope = 28−31 vs 4.6%, respectively). This contrasted behavior suggests a more rapid equilibration and bioaccumulation kinetics of CDD/CDF. Biological half-lives of these compounds in fish are shorter than those of PCB congeners of comparable structure: values of 2−58 and 12−24 days in fish have been reported for CDD and CDF, respectively (*28*). However, the absorption efficiencies were lower (2−>30%) than those of PCBs, although higher values (65−90%) have been measured for 2,3,7,8-substituted congeners in rats. The marked increase of CDD/CDF levels with size in the clams suggest a faster bioaccumulation kinetics, which is consistent with the almost planar configuration of these compounds. If absorption efficiencies of CDD/CDF in clams are lower than those of PCBs, then even more reduced elimination rates must be postulated to explain the rapid rise of the concentrations with size, but this would then result in unlikely longer half-lives.

The dominance of tetra and pentaCDD/CDF observed in the clams (Figure 3) has been often reported for aquatic organisms (*29*−*31*). The dominance of tetraCDD/CDF in the clams is consistent with the higher biological half-lives reported for these homologues relative to more chlorinated congeners: 43−58 days for 1,2,3,4- and 2,3,7,8-tetraCDD versus 15 days for octaCDD (*28*). As observed for PCBs, these differences in half-lives are reflected by the percent slope of the concentration vs size regressions: tetra−pentaCDD/CDF show the highest slopes (tetraCDD = 21%, pentaCDD = 31%, tetraCDF = 32%, pentaCDF = 8.7%) whereas hepta and octo homologues show lower values (5.2−11%). This decrease in the long-term bioaccumulation of higher chlorinated CDD/CDF can be attributed to reduced uptake due to unfavorable stereochemistry; e.g., the log K_{ow} and TSA values of 1,2,3,4-tetraCDD are 6.2 and 250 Å^2, respectively, well in the optimum range determined for PCBs (6−7 and 240−270 Å^2) as

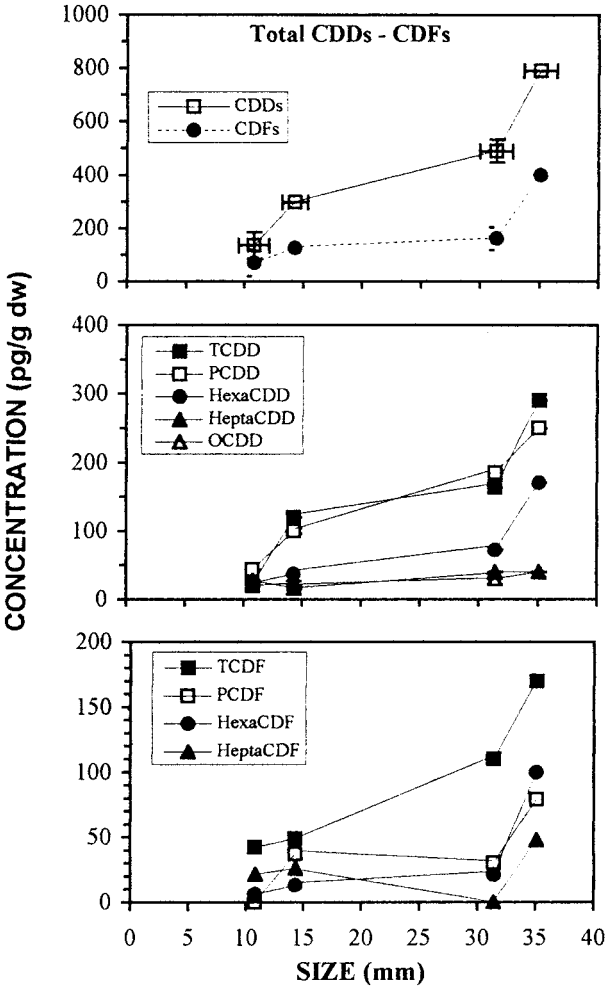

FIGURE 3. Concentration of total chlorinated dibenzodioxins (CDD), furans (CDF), and the different homologue groups in six size classes of Asiatic clams from the Río de la Plata Estuary.

compared to 7.59 and 315 Å^2 for octaCDD (*32*). A similar decreasing trend of octaCDD/F has been observed along food chains from the Northern Baltic Sea and has been attributed to reduced uptake rates of 1,4- and/or 6,9-substituted CDD/CDF related to the increased molecular size (*33*).

Toxic Equivalents. The toxic equivalents (TEQ) calculated for PCBs and CDD/CDF (*34*) are presented in Table 1. Total TEQ ranged from 7.1 to 13.1 pg/g wet weight for the smallest and largest clams of the series, respectively. These values are comparable to those reported for fish from the contaminated Saginaw River (5−13 pg/g; *35*) but are lower than the Canadian acceptable level in fish for human consumption (15 pg/g). The TEQ calculated are based on the presence of PCBs 77, 126, 118, 105, 156, 180, and 170; dioxins 1,2,3,4,7,8- and 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and octaCDD; and furans 2,3,7,8-tetraCDF and 1,2,3,4,6,7,8-heptaCDF (Table 1). PCBs account for 83−88% of the total TEQ, with pentaCB 126 as the outweighing contributor (60−62% TEQ), followed by 118 (~10%), 105 (3.1−5.7%), 156 (3.4−4.6%), and 77 (2.9−4.8%). CDD and CDF account for 3.7−10 and 6.8−8.3% of total TEQ, respectively, with hexaCDD and tetraCDF as the most important components. The increase in the total TEQ (~85%) observed from 10 to 35 mm clams is chiefly driven by the selective long-term bioaccumulation of PCBs 126, hexaCDD, PCB 118, and tetraCDF, which explains 59, 17, 12, and 4.5% respectively, of the TEQ increment.

Trace Metals. The concentration of trace metals in the Asiatic clams ranged from 145 to 189, from 22 to 41, and from 3.0 to 4.1 μg/g dw for Zn, Cu, and Ni, respectively (Table 1). These values are similar to those reported for bivalves from

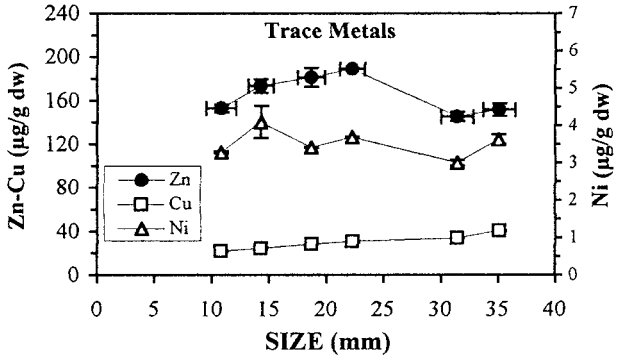

FIGURE 4. Trace metal concentration in six size classes of Asiatic clams from the Río de la Plata Estuary.

moderately polluted world rivers (*12*). No clear concentration−size relationships are observed for Zn and Ni (Figure 4); Ni shows rather homogeneous values in the six size classes (3.52 ± 0.38 μg/g) whereas Zn shows increasing values from 10 to 22 mm clams and lower levels in larger animals. The decreasing general trend with size observed previously for Zn in Asiatic clams from seven different sampling stations in the Río de la Plata also insinuated an initial increase of the concentrations up to about a 24 mm clam size (*12*). This consistent behavior and the reduction of Zn levels in larger animals suggest that this essential metal, involved in several enzymatic systems (e.g., carbonic anhydrase, hydrolases and carboxypeptidases), is being regulated by the clams.

A completely different trend is observed for Cu, which is also essential for several basic enzymes like cytochrome oxidase and monooxigenases, but shows a consistent increase with clam size ($y = 0.69$ length $+ 14.6$, $r = 0.98$, $n = 6$). This trend showing a 85% increase of Cu levels from 10 to 35 mm clams (similar to PCBs) confirms previous results suggesting a continuous passive accumulation of Cu in the clams. This would explain the relatively high Cu values found in Río de la Plata *Corbicula* as compared to other freshwater bivalves (*12*).

Acknowledgments

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