

TOXIC EFFECTS OF POLYCHLORINATED BIPHENYL BIOACCUMULATION IN SEA URCHINS EXPOSED TO CONTAMINATED SEDIMENTS

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Abstract—The uptake patterns and toxicity of polychlorinated biphenyl (PCB) congeners in the white sea urchin, *Lytechinus pictus*, on exposure to contaminated sediments were investigated. First-order modeling of uptake of the 10 most abundant PCB congeners or domains (containing more than one coeluting congener) by *L. pictus* indicated that a 35-d exposure was insufficient to reach steady state. Bioaccumulation of PCBs in sea urchins exhibited substantial difference between field and amended sediments, suggesting that caution must be exercised in sample preparation. Some evidence was observed of dependence of measured biota-sediment accumulation factors (BSAFs) on K_{oc} , indicating that equilibrium partitioning of PCBs may not always be achieved between biota lipid, sediment organic carbon, and water. Survival of *L. pictus* was unaffected by exposure to field and amended sediments with PCB concentrations varying more than three orders of magnitude. The growth measures (diameter, wt, and gonad wt) were significantly reduced in *L. pictus* exposed to San Diego Bay (SDB; San Diego, CA, USA) sediment, whereas they were relatively unaffected after exposure to amended sediments (with much higher PCB concentrations than SDB sediment) prepared from a New Bedford Harbor (MA, USA) sediment. The toxic effects as measured by the growth rates in *L. pictus* were likely attributable to polycyclic aromatic hydrocarbons (PAHs), which were elevated in SDB sediment (7.3 $\mu\text{g/g}$), rather than PCBs.

Keywords—Bioaccumulation Toxicity Polychlorinated biphenyl Sea urchin Equilibrium partitioning

INTRODUCTION

Polychlorinated biphenyls are widely distributed in sediments throughout the Southern California Bight (USA), yet little information is available by which to accurately assess their impacts on marine organisms. Previous studies have demonstrated reduced growth and PCB accumulation in the white sea urchin, *Lytechinus pictus*, at sites with elevated PCB concentrations [1,2; <http://www.sccwrp.org/pubs/techrpt.htm>]. These data, however, are insufficient for developing bioaccumulation models because multiple contaminants were present at the sites and too small a range of exposure concentrations was available to establish a relationship between contaminant body burden and toxicity. Regulatory and monitoring programs rely on such bioaccumulation models for purposes such as establishing sediment quality criteria for human health [3] and determining the suitability of dredged materials for ocean disposal. A critical factor in these assessments is the completeness and accuracy of the bioaccumulation data.

A popular approach is to describe bioaccumulation phenomena using the equilibrium partition theory (EPT) [4–6], in which lipid in biota and organic carbon (OC) in sediments (or soil) are relevant compartments for such partitioning. In a system consisting of sediment, biota, and water, the following partition coefficients can be defined under equilibrium conditions:

$$K_{oc} = \frac{C_{oc}}{C_w} \quad (1)$$

$$\text{BCF} = \frac{C_l}{C_w} \quad (2)$$

where C_{oc} , C_w , and C_l are compound concentrations in sediment (normalized to OC), water, and biota (normalized to lipid); K_{oc} is the sediment-water partition coefficient; and BCF is bioconcentration factor with the biota being exposed to water only. Generally, C_w is low and difficult to measure. In cases where sediment interstitial water is substantially different from the surrounding water that biota aspirate, C_w is vaguely defined. A more practical and useful parameter, BSAF, can be derived from combining Equations 1 and 2:

$$\text{BSAF} = \frac{\text{BCF}}{K_{oc}} = \frac{C_l}{C_{oc}} \quad (3)$$

Under strictly equilibrium conditions and the assumption of equal partitioning capacity for OC and lipid, BSAF equals unity. Although using the EPT to determine BSAFs provides a simple method to relate bioaccumulation to sediment contamination [7,8], its validity remains questionable [7]. Marine organisms represent a tremendous diversity of physiological (e.g., metabolic rate) and life-history (e.g., feeding mode, degree of sediment association) characteristics that may undermine the assumptions of the EPT. Bioaccumulation studies have been conducted with a variety of infaunal species [9] and indicate considerable variation in BSAFs. Few equivalent data exist for *L. pictus*, which, as an epibenthic deposit feeder, has life-history characteristics different from species (clams and polychaetes) commonly used in bioaccumulation studies.

The research described in this paper was conducted to investigate three aspects of PCB bioaccumulation in *L. pictus*. The first objective was to describe the uptake kinetics of PCB congeners in *L. pictus* exposed to field sediment. The second objective was to characterize the patterns of PCB bioaccumulation in *L. pictus* exposed to various levels of sediment

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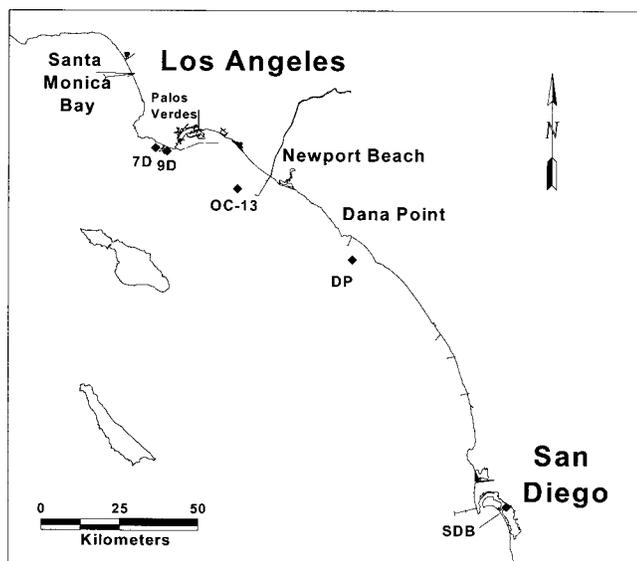


Fig. 1. Map of sampling locations in Southern California (USA). OC = Orange County (CA, USA); DP = Dana Point (Orange County); SDB = San Diego Bay (CA, USA).

contamination against EPT predictions. The third objective was to evaluate the toxicity of PCB congeners in *L. pictus* in order to better understand the effects of sediment contamination. These data will assist in the establishment of sediment quality criteria for Southern California since *L. pictus* occurs in this area. To accomplish these objectives, two sets of laboratory experiments were conducted. In the first experiment (kinetic exposure), sea urchins were exposed to field sediments from PCB-contaminated and reference areas. Sea urchins were sampled and analyzed at multiple times to study the pattern of PCB uptake and toxicity. In the second set of experiments (equilibrium exposures), sea urchins were exposed to amended sediments containing various PCB concentrations. Samples were collected at a single time to examine the relationship of bioaccumulation and toxicity to dose level.

METHODS

Kinetic exposure

Sample collection and preparation. Sediments were collected from a site in San Diego Bay near the National Steel and Shipbuilding shipyard (SDB) and also from a reference station near Dana Point (DP) in Orange County (CA, USA) (Fig. 1). Both sites were sampled from December 6 to 7, 1994, using a modified 0.1 m² Van Veen grab [10]. Sediments were placed in plastic buckets, cooled with ice, and transported to the laboratory, where they were stored at 4°C until used in experiments. White sea urchins were collected from the DP site. The animals were acclimated to laboratory conditions on DP sediments for about three weeks before the experiment was initiated.

Sea urchin exposure. Both SDB and DP sediments were homogenized and distributed into replicate polyethylene chambers (29 × 26 × 14 cm) at a depth of approximately 3 cm. Triplicate samples were also collected and frozen (−20°C) for chemical analysis. Seven DP (these samples were used for the exposure experiments to provide baseline information about the bioaccumulation behavior of sea urchins) and 21 SDB replicates were prepared in order to accommodate sampling at several times during the experiment. Fifteen sea urchins, weighed and digitally photographed to determine initial size,

were added to each tub. The ratio of sediment to animals was approximately 75:1 (g/g). An additional 45 sea urchins in three groups were used for initial gonad size and contaminant measurements.

The exposure was conducted at 15°C with seawater flowing through each chamber at 8 to 10 ml/min. Gentle aeration was also provided. The animals were fed Argent Hatchfry micro-encapsulated food (Redmond, WA, USA) at a rate of 0.36 g/tub every other day. Chemical analysis verified that the food did not contain detectable PCBs. The maximum exposure time was 66 d, with replicates exposed to SDB sediment being sampled at intermediate time points of 7, 14, 21, 28, 35, and 50 d for chemical analyses. Sea urchins exposed to DP sediment were sampled at 35 and 66 d only. At each sampling, all sea urchins from randomly selected chambers were weighed, digitally photographed, and dissected. The gonads from all individuals in each replicate were composited and frozen for chemical analysis. Five replicates were sampled at 35 d and two or three replicates processed at the other times. Replicate sediment samples were also collected at day 0 and day 35 and frozen for later chemical analysis.

Three measures of growth—changes in diameter, total body wet weight, and gonad wet weight—were determined for 11 replicates at day 0 and day 35. Sea urchin diameters were determined using computer image analysis. The initial and final diameter or body weight values were matched for each individual by assuming that the relative size of the sea urchins stayed the same throughout the experiment. Growth rates were calculated from the difference between initial and final measured values for each parameter. Since initial gonad weights could not be determined without killing the animals, the mean gonad weight of the 45 sea urchins dissected at the beginning of the experiment was compared to the final values from the individuals used in the exposure to calculate gonad growth rates.

In addition, a selected number of the individuals sampled at 35 d were induced to spawn by injection of potassium chloride. The eggs were fertilized and the developing embryos exposed to a reference toxicant (copper) using methods modified from Chapman et al. [11]. The tolerance of the embryos to copper was used as a measure of embryo health; stressed organisms would be expected to show a reduced tolerance to toxicant exposure. Embryos were exposed to copper concentrations of 10, 18, 32, 56, and 100 µg/L in addition to a control (laboratory seawater) for 72 h. The embryos were examined with an inverted compound microscope to determine the percentage of abnormal development.

Equilibrium exposure

Sample collection and preparation. Highly contaminated sediment from New Bedford Harbor was used to prepare amended sediments. A preliminary analysis indicated that this sediment had a total PCB concentration of 3,800 µg/g (dry-wt basis, same for any concentrations mentioned here). This sediment was mixed with varying amounts of relatively uncontaminated sediment from two locations (DP and OC-13, a station offshore Newport Beach, CA, USA; Fig. 1) to produce a wide range of PCB concentrations. Three sediment dose levels were prepared: A, B, and C (Table 1). The amended sediments were allowed to equilibrate at 15°C for about four weeks prior to use in exposure experiments.

Sea urchin exposure. The experiments were conducted in a similar manner to the kinetic exposure. Five replicate chambers were set up for each treatment group. Sediments collected

Table 1. Total polychlorinated biphenyl concentrations in sediment, sediment interstitial water, and sea urchin gonad samples collected before and after exposure experiments^a

Sample		Interstitial water ng/ml	Sediment		Sea urchin gonad	
			µg/g dry	µg/g TOC ^b	µg/g wet	µg/g lipid
Kinetic exposure						
DP ^c	Day 0 (<i>n</i> = 3)	ND ^d	ND	ND		
	Day 35 (<i>n</i> = 3)	ND	0.0011 (±0.0002)	0.14 (±0.02)	0.025 (±0.034) ^e	1.2 (±1.6) ^e
SDB ^f	Day 0 (<i>n</i> = 3)	ND	0.522 (±0.011)	32.2 (±2.4)	ND	ND
	Day 35 (<i>n</i> = 3)	ND	0.592 (±0.233)	42.1 (±20.4)	0.490 (±0.056) ^e	22.1 (±1.9) ^e
Equilibrium exposure 1						
R ^g	Day 0 (<i>n</i> = 3)	ND	ND	ND	ND	ND
	Day 42 (<i>n</i> = 4)	ND	ND	ND	0.050 (±0.026)	2.1 (±1.0)
A ^h	Day 0 (<i>n</i> = 3)	ND	3.87 (±0.81)	1,200 (±270)		
	Day 42 (<i>n</i> = 4)	ND	3.73 (±0.25)	1,130 (±70)	49.4 (±7.0)	1,910 (±480)
B ^h	Day 0 (<i>n</i> = 2)	5.1 (±5.3)	10.8 (±1.7)	3,260 (±530)		
	Day 42 (<i>n</i> = 5)	ND	10.3 (±1.2)	3,030 (±370)	127 (±16)	4,490 (±420)
Equilibrium exposure 2						
C ^h	Day 0 (<i>n</i> = 3)	45 (±15)	113.9 (±8.3)	16,500 (±2,500)		
	Day 43 (<i>n</i> = 5)	22 (±10)	107.2 (±5.3)	14,300 (±1,600)	647 (±132)	32,100 (±6,700)

^a Values are mean ± standard deviation.

^b TOC = total organic carbon.

^c DP = Dana Point (CA, USA) sediment.

^d ND = not detected.

^e Five replicates were analyzed.

^f SDB = San Diego Bay (CA, USA) sediment.

^g R = reference.

^h A, B, and C represent New Bedford Harbor (MA, USA) Sediment diluted with various amounts of reference sediment.

from OC-13 were used as a reference (R). Two exposure experiments were conducted: Exposures including groups A, B, and R were started in July 1995, and those including group C were started in August 1995. The duration of the experiments was 42 (groups A, B, and R) or 43 d (C), with no intermediate sampling. Three sets of 15 animals were processed at the start of the first experiment and another set of 15 animals at the start of the second experiment to produce gonad weight and PCB concentration data. Sea urchin growth rates and embryo sensitivity to copper were determined using the same methods as described for the kinetic experiment. Sediment and interstitial water samples were collected at the beginning and end of each experiment and analyzed for PCB congeners.

Sample treatment

Sediment extraction. Sediments were extracted using procedures detailed elsewhere [12] with minor modifications. Briefly, a weighed sediment sample spiked with surrogate standards was extracted three times (16, 6, and 16 h) with methylene chloride using a roller table. The combined extract was dried with 10 g of anhydrous sodium sulfate, concentrated to approximately 3 ml using a rotary evaporator at 30°C and 650 mmHg vacuum pressure, and solvent exchanged to hexane. Activated copper granules were added to the extract to remove sulfur (overnight). The extract was taken into a vial and concentrated to approximately 1 ml under a gentle N₂ stream, ready for column cleanup/fractionation.

Gonad extraction. A thawed gonad sample was homogenized thoroughly with a glass rod and weighed in a glass beaker. Appropriate amounts of surrogate standards were spiked into the sample, followed by addition of 20 ml of acetonitrile to the beaker. The mixture (and rinsates of distilled water and hexane) was transferred to a centrifuge bottle and homogenized using a Polytron high-speed homogenizer

(Brinkmann Instruments, Wesbury, NY, USA) for 30 s at a speed setting of 6. This was repeated twice with addition of 10 ml of hexane and distilled water, respectively. The bottle was capped and centrifuged for 10 min at approximately 1,000 g. The solvent layer was collected using a glass pipette and transferred to a 125-ml flat-bottom flask through a glass funnel plugged with glass wool and anhydrous sodium sulfate. Ten milliliters of hexane were added to the centrifuge bottle, which was shaken vigorously for 2 min. The bottle was centrifuged again as described previously, and the solvent was transferred to the flat-bottom flask. This was repeated once after another 10 ml of hexane were added to the bottle. The extract was concentrated to approximately 1 ml using the same procedure as for the sediment extracts.

Lipid measurements. About 3 to 5 µl of the extract were transferred using a 10-µl microsyringe to an aluminum boat placed in a microbalance. Solvent was allowed to evaporate until a constant weight was reached. The weight difference was defined as the lipid content.

Column cleanup and fractionation. An appropriate portion (depending on the lipid content) of the extract was applied to a 1:2 alumina/silica gel glass column. The first fraction, containing aliphatic hydrocarbons, was eluted with three washes of dry hexane (5 ml each) and discarded. The second fraction, containing PCBs and other aromatic hydrocarbons, was eluted by 5 ml of dry hexane and 30 ml of a 30/70 mixture of methylene chloride and hexane. The extract from this fraction was transferred to a TurboVap 500 (Zymark, Hopkinton, MA, USA) concentration tube and concentrated to 1 ml. Desired amounts of internal standards were added to the extract.

Instrumental analyses

Total organic carbon measurements. The method for measuring total organic carbon (TOC) has been given previously

[13]. Briefly, an aliquot (~30 mg) of dry sediment was weighed in a silver boat and exposed to concentrated hydrochloric acid vapors for at least 18 h to remove inorganic carbon. The acidified sample was dried at 60°C overnight and crimped in a tin boat. The measurement was made using a CHN EA 1108 elemental analyzer (Carlo Erba Instruments, Milan, Italy).

Congener-specific measurements of PCBs. Quantitation of PCB congeners was done using a calibration standard comprised of Aroclors 1242, 1248, 1254, and 1260 (1:1:1:1, wt). We found 90 detectable domains in this mixture from gas chromatography (GC)/electron capture detector (ECD) analysis; 64 are singly eluting congeners, and 28 are multicomponent peaks. This calibration standard was characterized with a set of 120 PCB congeners prior to use. Specifically, these congeners were prepared in four groups and analyzed separately using GC/ECD and GC/mass spectrometry (MS). The assignment of PCB peaks was based on a combination of information in the literature [14,15] and confirmatory mass spectral analyses. The relative response factors of individual PCB congeners were obtained using GC/ECD and used to determine the composition of the Aroclor mixture. The detection limit was 10 ng/g based on 1 g of dry sediment or wet gonad sample for each PCB congener.

Instrument parameters. Quantitative measurements were conducted using a Hewlett-Packard (Palo Alto, CA, USA) 5890 Series II GC with a ⁶³Ni ECD and a 60-m × 0.25-mm-i.d. (0.25-μm film thickness) DB-5 column (J&W Scientific, Folsom, CA, USA). Split/splitless injection of a 1-μl sample was performed by an HP 7673 autosampler with a 1-min solvent split time. The column temperature was programmed from 90 to 180°C at 6°C/min and further increased to 290°C at 1°C/min. Carrier gas was high-purity helium with a flow rate of 2 ml/min at 90°C. Makeup gas was ultra-high-purity nitrogen at 30 ml/min. The injector and detector temperatures were both maintained at 280°C. Data were acquired and processed using a Perkin-Elmer (Wellesley, MA, USA) Nelson Turbochrom 3.3 data system running on an IBM compatible PC and Perkin-Elmer Nelson 900 series interface unit.

Confirmation of peak identification was done on selected samples using an HP 5890 Series II GC/5970 mass selective detector with a 60-m × 0.25-mm-i.d. (0.25-μm film thickness) DB-5 column and operated at the electron impact mode. Mass spectra were acquired using the selective ion monitoring technique; three fragmentation ions from each PCB isomer group were chosen for mass scanning. Because of the complicated chromatographic features associated with the PCB components found in the Aroclor standards and samples, a few small windows had to be set up, each covering PCB congeners belonging to one to three isomer groups. The dwell time was 100 ms for windows covering one isomer group and 50 ms for windows covering more than one isomer group.

Data analysis

In the kinetic exposure study, uptake of the 10 most abundant PCB congeners or domains (PCB 52/69, 97, 99, 101, 110/77/154, 118, 138, 149, 151/82, and 187) was analyzed with a first-order kinetics model [16]. In an environment where sea urchins are exposed to PCBs in sediment, the change in gonad concentration with time can be described by

$$\frac{dC_g(t)}{dt} = k_s C_s - k_e C_g(t) \quad (4)$$

where $C_g(t)$ = gonad PCB concentration (ng/g wet gonad wt) at time t , C_s = sediment PCB concentration (ng/g dry sediment wt) assumed constant during the entire kinetic exposure experiment, k_s = uptake rate coefficient, k_e = elimination rate coefficient, and t = exposure time. Equation 4 can be integrated into

$$C_g(t) = C_s \left(\frac{k_s}{k_e} \right) (1 - e^{-k_e t}) \quad (5)$$

The gonad residue at truly steady state ($t \rightarrow \infty$) is given by $C_g(\infty) = C_s(k_s/k_e)$. Practically, the time (t_{ss}) required to reach 95% of steady state has been used to represent steady-state exposure time. From Equation 5, t_{ss} can be expressed as

$$t_{ss} = -\frac{\ln(1 - f)}{k_e} \quad (6)$$

where $f = C_g(t_{ss})/C_g(\infty)$ and 0.95 by definition. The ratio of gonad residue at time t to that at steady state is related to k_e by

$$\frac{C_g(t)}{C_g(\infty)} = 1 - e^{-k_e t} \quad (7)$$

Kinetic exposure data ($C_g(t)$, C_s , and t) for individual PCBs were fitted with Equation 5 using an iterative, nonlinear, least-squares fitting procedure provided by SigmaStat® Version 2.03 (Jandel Scientific Software, San Rafael, CA, USA). The PCB concentrations below detection limits were replaced by zeros and analyzed with and without their inclusion.

Total PCB and growth differences between sea urchins exposed to DP and SDB sediments were assessed using two-sample t tests. Differences across time within sediment type were assessed with two sample t tests (for total PCB and gonad wt) and paired t tests (for diameter and total body wt). Statistical significance levels for hypotheses testing were set at 0.05; however, Type I error levels for growth parameters were Bonferroni adjusted to provide simultaneous inference for all three growth measures.

To investigate effects of exposure levels on growth and sensitivity for the equilibrium experiments, sea urchins exposed to sediments A, B, and C were compared to R using Dunnett's multiple comparison procedure. An additional Bonferroni adjustment was made for the three growth parameters. The median lethal concentration (LC50) values for embryo exposures to copper were calculated using probit analysis and the Toxstat® statistical package (University of Wyoming, Laramie, WY, USA). Analyses of differences within sediments mirrored those for the kinetic exposure study.

Dependence of BSAF on K_{ow} for each exposure level was examined by analyzing profile summaries for the linear relationship between log BSAF and log K_{ow} [17]. Specifically, for each replicate a line was fit using least-squares regression of log BSAF on log K_{ow} . Slopes for each replicate then provided an independent summary measure of linear trend that could be used to test for zero slope within each exposure level using a one-sample t test. A Bonferroni adjustment was made to account for the four sediment exposure comparisons. As with the kinetic exposure experiments, determination of significance for all statistical tests were made at an overall Type I error rate of 0.05.

RESULTS

Kinetic exposure

Total PCB concentrations in SDB sediment changed little during the experiment, varying between 522 and 592 ng/g dry

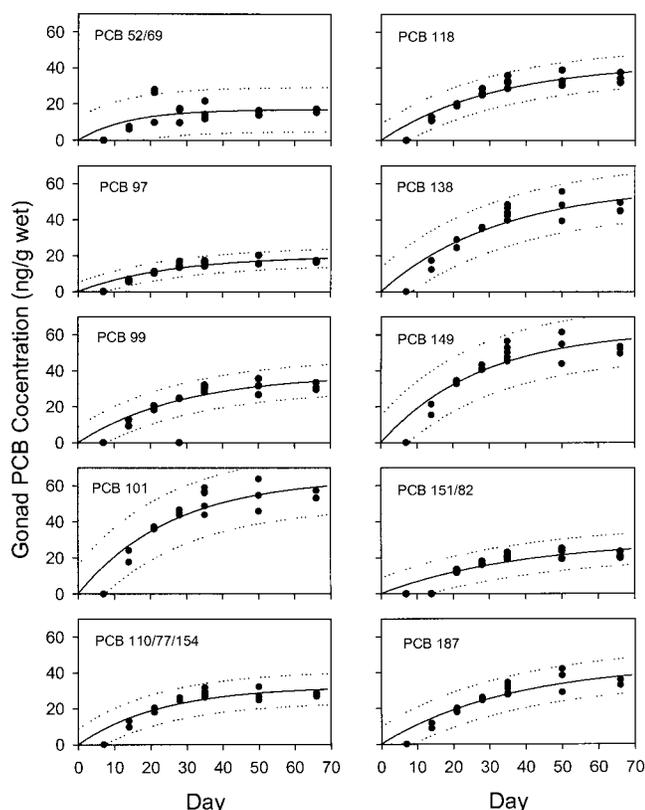


Fig. 2. First-order modeling of uptake of 10 most abundant polychlorinated biphenyl (PCB) congeners in sea urchin gonads exposed to San Diego Bay (CA, USA) sediment. Legend: ● = measured gonad PCB concentration (ng/g wet); — = predicted first-order uptake curve; --- = 95% confidence interval.

sediment weight for the initial and day 35 samples, respectively (Table 1). The average PCB congener composition in SDB sediment was also similar throughout the experiment (data not shown). This result justified treating C_s in Equation 5 constant throughout the entire kinetic exposure experiment. The DP samples contained very low PCB contents with individual PCB congeners not detected on day 0 and total PCB concentration being 1.1 ng/g dry weight on day 35. Total PCB concentration in sea urchins exposed to SDB samples increased rapidly during the first half of the experiment, attaining 76% (22.1 $\mu\text{g/g}$ lipid; Table 1) of the final concentration (28.9 $\mu\text{g/g}$ lipid) by day 35. As expected, sea urchins exposed to SDB sediment

for 35 d accumulated significantly higher PCB concentrations (one-sided p value < 0.0001) than those exposed to DP samples (Table 1).

Results from the first-order kinetics modeling with Equation 5 are depicted in Figure 2. In this exercise, the concentration (C_s) of a specific PCB congener or domain was an average of the day 0 and day 35 measurements. Numerical results from model fitting and subsequent calculations with Equations 6 and 7 are presented in Table 2.

One common feature associated with the uptake kinetics data for the selected PCB congeners is that the gonad PCB concentrations remained below detection limits (~ 5 ng/g) after 7 d of exposure (Fig. 2). Because no meaningful differences were observed for parameter estimates with or without these data points, nondetects for day 7 were included in the model fitting. However, confidence intervals for the linear regressions were widened slightly, providing a more conservative estimate of confidence. Kinetic exposure data for all but one PCB domain (PCB 52/69) were well fitted with the first-order uptake model with R^2 ranging from 0.77 to 0.86 (Table 2). The low R^2 value (0.35; Table 2) for PCB 52/69 appears at least partially due to the large variability in the replicate measurements for the day 21 samples (Fig. 2). One of the concentrations of PCB 99 for the day 28 samples was below detection limit (Fig. 2). We attributed this to analytical artifacts, and the data point was regarded as an outlier and not included in curve fitting.

The uptake rate coefficient (k_s) and elimination rate coefficient (k_e) appeared to increase and decrease, respectively, with the hydrophobicity of PCBs (Table 2). The predicted steady-state time (t_{ss}) for the selected PCBs ranged from 42 to 118 d (Table 2). The predicted ratios of gonad residues at days 35 and 42 compared to steady-state residues were all less than the practically accepted criteria (0.95) except for PCB 52/69 at day 42 (Table 2).

Biological responses (survival, growth, and embryo sensitivity) were assessed at the 35-d time point in order to provide data comparable to previous tests with *L. pictus*. Survival was not affected by exposure to either SDB or DP sediment; only one animal died during the 35-d time period. Growth rates among sea urchins exposed to SDB sediment were lower than among those exposed to DP sediment. In fact, all three growth parameters were found to be significantly different between the two groups with p values for change in diameter, total body weight, and gonad weight being 0.006, <0.0001, and 0.004, respectively (Fig. 3). Mean change in body diameter and gonad

Table 2. Fitted variables from first-order kinetics modeling of polychlorinated biphenyl (PCB) uptake in sea urchins exposed to SDB sediment^a

PCB congener	Log K_{ow} ^b	C_s (ng/g)	R^2	$k_s \pm 95\%$ CI (per day)	$k_e \pm 95\%$ CI (per day)	t_{ss} (d)	$C_g(t)/C_g(\infty)$	
							$t = 35$	$t = 42$
52, 69	5.84	25.05	0.35	0.049 \pm 0.042	0.072 \pm 0.080	42	0.92	0.95
97	6.29	13.4	0.82	0.057 \pm 0.017	0.038 \pm 0.019	78	0.74	0.80
99	6.39	20.9	0.85	0.063 \pm 0.018	0.035 \pm 0.017	87	0.70	0.77
101	6.38	46.6	0.83	0.053 \pm 0.015	0.039 \pm 0.019	78	0.74	0.80
110, 77, 154	6.48	39.1	0.81	0.036 \pm 0.011	0.044 \pm 0.021	69	0.78	0.84
118	6.74	27.8	0.86	0.048 \pm 0.013	0.031 \pm 0.015	96	0.67	0.73
138	6.83	25.0	0.84	0.072 \pm 0.021	0.030 \pm 0.016	99	0.65	0.72
149	6.67	30.6	0.84	0.074 \pm 0.021	0.036 \pm 0.018	83	0.72	0.78
151, 82	6.64	9.0	0.77	0.085 \pm 0.033	0.025 \pm 0.021	118	0.59	0.65
187	7.17	11.1	0.85	0.112 \pm 0.031	0.028 \pm 0.015	109	0.62	0.69

^a Kinetic exposure data ($C_g(t)$, C_s , and t) were fitted with Equation 5 to obtain $k_s \pm 95\%$ CI and $k_e \pm 95\%$ CI. Both t_{ss} and $C_g(t)/C_g(\infty)$ were calculated with Equations 6 and 7, respectively. CI = confidence interval; SDB = San Diego Bay (CA, USA).

^b In the case of more than one congener listed, the log K_{ow} value of the first congener is presented.

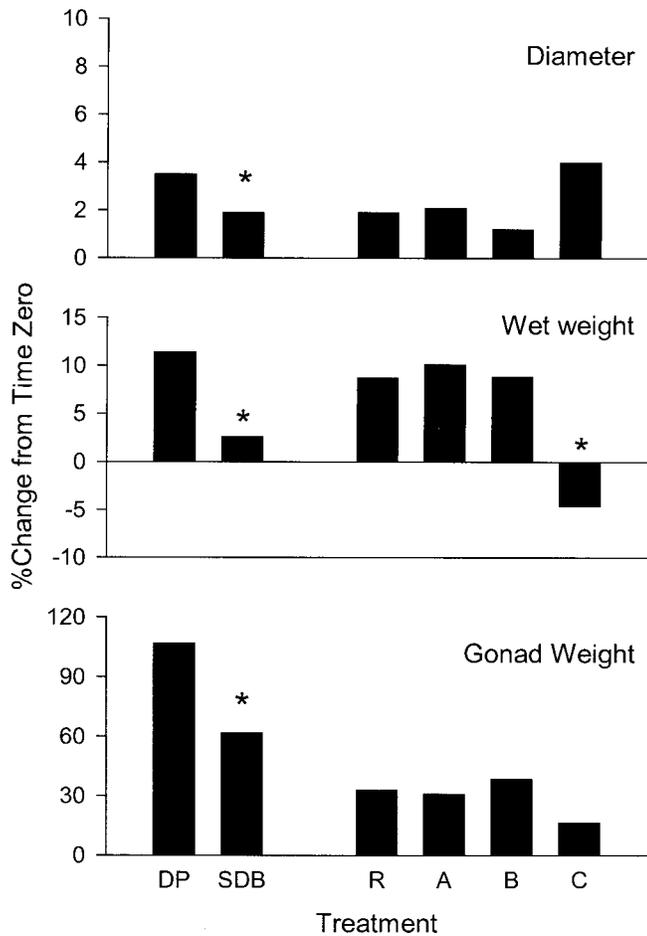


Fig. 3. Percentage change in growth parameters of *Lytechinus pictus* on exposure to various sediments. Bars marked with an asterisk are significantly reduced from the reference stations ($p < 0.05$). DP = Dana Point (CA, USA) sediment; SDB = San Diego Bay (CA, USA) sediment; R = reference; A, B, and C represent New Bedford Harbor (MA, USA) sediment diluted with various amounts of reference sediment.

weight for the sea urchins exposed to SDB sediment was estimated to be around 55% of the growth measured for individuals exposed to DP sediment, while mean change in body weight was only about 22% of the DP value (Table 3). As expected, changes in body size and weight increased significantly over a 35-d period for sea urchins exposed to DP with one-sided p values for diameter, total body weight, and gonad weight being 0.012, 0.005, and <0.0001 , respectively.

Sea urchins were successfully spawned in most of the exposure chambers sampled at day 35. The resulting embryos from both the SDB and DP exposure groups had a similar sensitivity to the standard toxicant (copper), as indicated by the median effective concentrations (EC50s) of 23 and 24 $\mu\text{g/L}$ (Table 3). These data indicate that embryo health was similar between the two exposure groups.

Equilibrium exposure

The three amended sediments spanned a wide range of PCB concentrations (~ 0 to ~ 110 $\mu\text{g/g}$ dry sediment wt; Table 1). In all cases, the measured concentrations were similar before and after the exposure experiments, indicating no substantial losses of PCBs. The precision of the measurements was generally good, with relative standard deviation $\leq 21\%$ among replicates.

Concentrations of total PCBs for most interstitial water samples were below detection limits (Table 1). As expected, the highest PCB concentration was found in the interstitial water extracted from the most contaminated sediment (C). Sample C had total PCB concentrations of 45 and 22 ng/ml at day 0 and day 43, respectively. Sample B at day 0 also contained a small amount of PCBs (5.1 ± 5.3 ng/ml), while sample A contained no detectable PCBs. The interstitial water concentration was quite variable between measurements (relative standard deviation $> 100\%$).

Sea urchin gonad PCB concentration increased by three to four orders of magnitude relative to the reference group (Table 1). The PCB bioaccumulation followed a pattern similar to the sediment concentration, with the highest values (647 $\mu\text{g/g}$ wet

Table 3. Toxicity test responses for *Lytechinus pictus* exposed to sediments in the laboratory for 35 d (kinetic exposure) or 42 d (equilibrium exposure)^a

Sample	Adults							Embryos
	Survival (%)	Diameter (mm)		Wet weight (g)		Gonad weight (g)		Copper LC50 ^b ($\mu\text{g/L}$)
		Initial	Final	Initial	Final	Initial	Final	
Kinetic exposure								
DP ^c	100 \pm 0	19.2 \pm 0.2	19.7 \pm 0.5	2.68 \pm 0.10	2.93 \pm 0.20	0.09 \pm 0.003	0.18 \pm 0.04	24 \pm 3
SDB ^d	99 \pm 0	19.1 \pm 0.7	19.4 \pm 0.6	2.62 \pm 0.08	2.70 \pm 0.19	0.09 \pm 0.003	0.15 \pm 0.024	23 \pm 1
Equilibrium exposure 1								
R ^e	99 \pm 3	22.0 \pm 0.6	22.4 \pm 0.4	3.56 \pm 0.20	3.87 \pm 0.19	0.17 \pm 0.03	0.22 \pm 0.026	42 \pm 7
A ^f	99 \pm 3	21.7 \pm 0.7	22.1 \pm 0.5	3.40 \pm 0.21	3.75 \pm 0.21	0.17 \pm 0.03	0.22 \pm 0.070	38 \pm 5
B ^f	100 \pm 0	22.1 \pm 0.6	22.4 \pm 0.6	3.55 \pm 0.25	3.86 \pm 0.28	0.17 \pm 0.03	0.23 \pm 0.023	39 \pm 4
Equilibrium exposure 2								
C ^f	89 \pm 14	21.7 \pm 0.2	22.5 \pm 0.4	3.46 \pm 0.22	3.30 \pm 0.29	0.13 \pm NA ^g	0.15 \pm 0.038	22 \pm 4 ^h

^a Values are mean \pm standard deviation with $n = 5$ except for DP ($n = 4$).

^b LC50 = median lethal concentration.

^c DP = Dana Point (CA, USA) sediment.

^d SDB = San Diego Bay (CA, USA) sediment.

^e R = reference sediment.

^f A, B, and C represent New Bedford Harbor (MA, USA) sediment diluted with various amounts of reference sediment.

^g NA = not applicable ($n = 1$).

^h Significantly lower than the R reference sample ($p < 0.05$).

Table 4. Measured biota-sediment accumulation factors (BSAFs) in sea urchins exposed to polychlorinated biphenyl (PCB) contaminated sediments

PCB congener ^a	BSAF ^b				Log K_{ow}
	A	B	C	SDB	
4 (10)	— ^c	0.42	0.68	—	4.65
6	—	0.55	0.81	—	5.06
18	1.32	1.29	2.08	—	5.24
19	—	0.46	1.21	—	5.02
22	1.56	1.32	2.17	—	5.58
25	1.68	1.61	2.42	—	5.67
26	1.67	1.53	2.51	—	5.66
31	2.17	2.12	2.68	—	5.67
41	2.07	1.74	2.43	—	5.69
44	1.94	1.62	2.53	0.15	5.75
45	—	1.55	2.21	—	5.53
49	2.47	2.00	2.94	0.87	5.85
51	—	1.57	—	—	5.63
52 (69)	2.49	2.00	2.95	0.36	5.84
70	3.26	1.76	2.90	—	6.20
74	3.12	2.14	3.01	—	6.20
84	—	1.54	2.46	—	6.04
85	—	—	—	0.78	6.30
87 (81)	—	—	—	0.50	6.29
91	2.17	1.73	2.61	—	6.13
97	2.92	2.16	3.05	0.77	6.29
99	2.68	2.28	3.05	0.93	6.39
101 (90)	2.46	1.99	2.86	0.72	6.38
105	—	—	—	0.67	6.65
107	—	—	2.35	—	6.71
110 (77, 154)	1.91	1.49	2.27	0.47	6.48
118	3.41	2.56	2.40	0.73	6.74
119	—	—	2.94	3.91	6.58
135	—	—	2.03	0.74	6.64
136	—	—	2.49	—	6.22
138	—	2.04	1.97	1.12	6.83
149	2.40	2.11	2.13	1.05	6.67
151 (82)	—	2.44	2.23	1.48	6.64
183	—	—	—	0.67	7.20
187	—	—	—	1.77	7.17
Mean \pm SD ^d	2.32 \pm 0.14	1.68 \pm 0.10	2.37 \pm 0.11	0.98 \pm 0.19	

^a The numbers in parentheses are congeners that may coelute with the main target PCB congeners.

^b A, B, and C represent New Bedford Harbor (MA, USA) sediment diluted with various amounts of reference sediment, and SDB = San Diego Bay (CA, USA) sediment.

^c The concentration of PCBs in either sediment or sea urchin gonad was not detectable.

^d SE = standard error.

gonad wt) measured in gonad samples from the highest exposure group (C).

Exposure to PCBs produced no effect on survival for any of the sediment groups (Table 3). Only one significant difference among the adult sea urchin growth parameters was observed (Fig. 3), that being a smaller increase in wet weight for group C than for group R ($p < 0.0001$). Of the three exposures, only group B reported a smaller change in mean diameter than the reference group; however, variability was high, and the difference was not statistically significant (Fig. 3). For the reference group, a significant increase in growth across the 35 d was observed only for the wet-weight parameter ($p < 0.0001$).

Sea urchin embryo EC50s for copper in groups A and B were not significantly different from those in group R. However, group C embryos were significantly ($p < 0.0001$) more sensitive to copper, with an EC50 of 22 $\mu\text{g/L}$ (Table 3). The group C EC50 was similar to EC50s measured in the kinetic exposure using DP and SDB sediments, however, indicating that EC50 values are within the typical range of variation for *L. pictus*.

Bioaccumulation factors

Table 4 presents the measured BSAFs for samples A, B, C, and SDB as well as related log K_{ow} values [18]. To avoid ambiguity, only the singly eluting PCB congeners or coeluting congeners with one congener much more abundant ($>5:1$) than the other(s) (based on the results of Schulz et al. [15]) were considered. Mean BSAFs were similar within the amended sediments (A, B, and C), ranging from 1.7 to 2.4. Within a congener, higher BSAF values were usually obtained for group C samples. More polar congeners, with log $K_{ow} \leq 5.24$, tended to have smaller BSAF values (Table 4).

A marked difference in BSAF values was present between the SDB and amended sediment groups. In almost every case where a comparison could be made for a single congener, the SDB accumulation factor was less than half that obtained for sea urchins exposed to amended sediments (Table 4). The mean BSAF for SDB was 0.98 ± 0.38 .

The linear regression r^2 values for individual log BSAF and log K_{ow} relationships ranged from 0.44 to 0.66 for group A, 0.40 to 0.76 for group B, 0.54 to 0.80 for group C, and 0.15

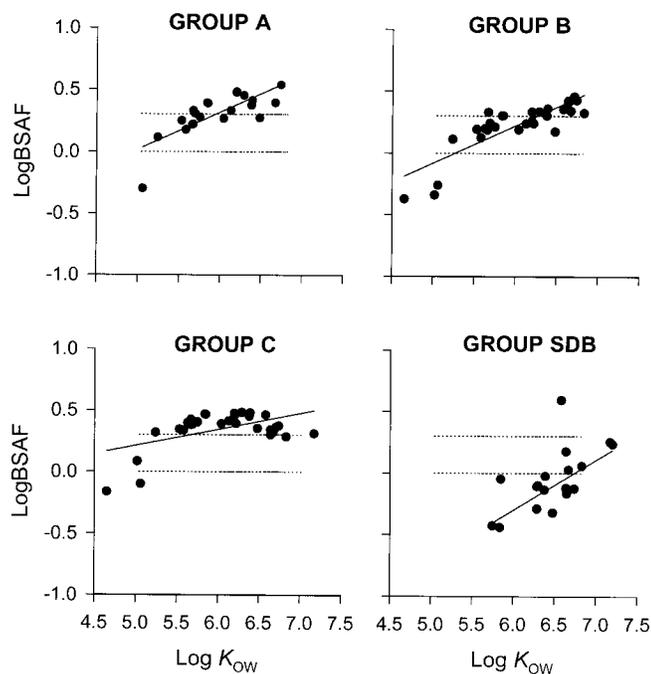


Fig. 4. Variation of measured log biota-sediment accumulation factors (BSAFs) with $\log K_{ow}$ in exposure groups A, B, C, and San Diego Bay (CA, USA) (SDB). The solid line indicates the linear regression, and the dotted lines show the range of equilibrium partitioning predictions.

to 0.40 for group SDB. Analyses of the slopes from the linear regressions showed that only group C had a slope statistically significantly different from zero, although tests for all sediment exposures yielded p values less than 0.03 (Bonferroni adjustment for the four comparisons requires a p value less than $0.05/4 = 0.012$ for statistical significance). Therefore, some evidence exists of dependence of BSAF on K_{ow} despite our inability to detect it statistically after adjusting for the multiple comparisons. As a general description of the overall trend exhibited by these data, we fit a linear regression model (Fig. 4) to the mean log BSAFs taken across all replicates (Table 4). The resulting regression equations are expressed here:

$$\text{A} \quad \log \frac{C_1}{C_{oc}} = 0.30 \log K_{ow} - 1.48 \quad (r^2 = 0.59) \quad (8)$$

$$\text{B} \quad \log \frac{C_1}{C_{oc}} = 0.30 \log K_{ow} - 1.59 \quad (r^2 = 0.70) \quad (9)$$

$$\text{C} \quad \log \frac{C_1}{C_{oc}} = 0.13 \log K_{ow} - 1.45 \quad (r^2 = 0.27) \quad (10)$$

$$\text{SDB} \quad \log \frac{C_1}{C_{oc}} = 0.40 \log K_{ow} - 2.66 \quad (r^2 = 0.40) \quad (11)$$

DISCUSSION

PCB uptake kinetics

The PCB uptake rate coefficients for *L. pictus* exposed to SDB sediment (Table 2) were about an order of magnitude smaller than those for the deposit-feeding clam *Macoma nasuta* exposed to a spiked sediment [16], but the elimination rate coefficients were similar for these species. As a consequence, *M. nasuta* had approximately an order of magnitude higher sediment bioaccumulation factors (obtained by k_s/k_e)

than *L. pictus*. However, the predicted steady-state times (t_{ss}) were similar for these two species (Table 2 and [16]), which are in general substantially higher than those observed experimentally from previous studies for infaunal bivalves, polychaetes, and amphipods [19,20]. It was reported that 80 to 100% of steady-state PCB concentrations were usually attained after 28 to 42 d of exposure [19,20].

If results from the first-order uptake modeling efforts (Table 2 and [16]) are any indication, longer exposure time than 28 to 42 d may be required to reach steady state with marine invertebrates exposed to PCBs. Apparently, this discrepancy may be associated with a number of factors, such as sediment preparation, type of animals used, and most important the ability of the first-order uptake model to truly describe the uptake kinetics under complex circumstances. It points to the need to exercise caution when determining steady state experimentally. Nevertheless, uptake kinetics modeling supplies valuable information to determine appropriate experimental times for equilibrium exposure as well as to estimate sediment bioaccumulation factors.

Biological effects of PCB bioaccumulation

Results of the equilibrium exposures are useful for assessing the potential effects of PCB bioaccumulation in *L. pictus*. In the first equilibrium experiment, no growth effects were evident (groups A and B; Table 3) at gonad total PCB concentrations up to 127 $\mu\text{g/g}$ (group B; Table 1). Evidence of toxicity was present in the second exposure (group C), where growth (change in total or gonad wt) was greatly reduced (Fig. 3) at a gonad PCB concentration of 647 $\mu\text{g/g}$ (Table 1). A threshold for PCB effects in *L. pictus* occurring within the range of 127 to 647 $\mu\text{g/g}$ is consistent with experimental data for other species. Chronic sublethal effects on the oligochaete *Lumbriculus variegatus* were observed at PCB residues of 99 to 163 $\mu\text{g/g}$ [21], and survival of the amphipod *Ampelisca abdida* was reduced at a gonad PCB concentration of 166 $\mu\text{g/g}$ [22]. Exposure of amphipods to Aroclor mixtures produced toxicity at 100 to 550 $\mu\text{g/g}$ wet weight total PCB [23], while toxicity to various fish species has been observed at PCB body burdens of 40 to 640 $\mu\text{g/g}$ [23,24]. The toxicity results for *L. pictus* reported here are consistent with a narcosis mode of action, which is predicted to produce toxic effects at body residues of approximately 500 $\mu\text{g/g}$ [25].

Examination of bioaccumulation data from the kinetic exposure and previous studies using the *L. pictus* growth test indicates that PCBs are not likely to be a major factor in the toxicity of most Southern California sediments to this organism. In the kinetic exposure, reduced growth was present at gonad PCB concentrations (0.5 $\mu\text{g/g}$, group SDB; Table 1) far below concentrations shown to have no effect on sea urchin growth in the amended sediment experiments. Previous studies have measured reduced *L. pictus* growth following exposure to sediments from a different location in San Diego Bay as well as from sewage-contaminated sites off Palos Verdes and in Santa Monica Bay (CA, USA) [1,2]. Gonad total PCB concentrations associated with reduced growth in these studies were 2.6 to 8.1 $\mu\text{g/g}$, also far below levels attained in the amended sediment experiments without apparent detrimental effects. The growth effects measured in the kinetic study appeared to be due to other contaminants.

The reduced growth of sea urchins exposed to SDB sediment in the kinetic experiments may have been due to PAH compounds rather than PCBs. The concentration of total PAHs

in undiluted New Bedford Harbor sediment used to prepare amended sediments A, B, and C was $17.0 \pm 1.8 \mu\text{g/g}$ ($n = 4$). On preparation, sediments A, B, and C contained 0.05, 0.15, and $2.4 \mu\text{g/g}$ of total PAHs, respectively. These values are all below the ERL sediment quality guideline of $4.0 \mu\text{g/g}$ for total PAHs [26], suggesting that toxic effects are unlikely at these concentrations. The PAH concentration in SDB sediment was $7.3 \pm 0.5 \mu\text{g/g}$ ($n = 3$), above the effects range low, and may have contributed to the growth effects in *L. pictus* exposed to SDB sediment.

Additional research is needed to better understand the biological effects of PCBs on marine organisms. The experiments described in this report were conducted using sediments containing a complex mixture of PCB congeners and other contaminants. The biological activity of individual congeners varies depending on structure, although this relationship is not understood for marine invertebrates [27]. Additional synoptic toxicity and bioaccumulation experiments using single congeners are needed to clarify structure–activity relationships for marine organisms that will facilitate the use of data from diverse locations and species.

PCB bioaccumulation patterns in *L. pictus*

The EPT predicts BSAFs of 1 to 2 [28], whereas a wide range of BSAF values have been obtained experimentally. The PCB BSAFs can vary by an order of magnitude between experiments conducted with the same species [9], which have been attributed to variations in test methods and the type of sediment examined. The BSAFs measured in the present study fall within the range of values reported by others. For instance, in a spiked sediment study using 13 PCB congeners, mean BSAFs of 1.2 to 2.8 were measured for the surface-deposit-feeding clam *Macoma nasuta* [20].

The variations in *L. pictus* BSAFs that we observed between SDB and amended sediments (Table 4) may have been due to differences in sediment type or laboratory handling between experiments. Both the nature of the organic carbon in sediments and variations in sediment manipulation or equilibration in the laboratory have been shown to influence the uptake kinetics of nonpolar organics [29,30]. Differences in the sediment organic matter type are likely in this study since the SDB and NBH sediments were obtained from very different environments. Enhanced PCB bioavailability in the equilibrium exposures may have been produced as a result of the extensive sediment mixing and dilution required to prepare the treatments. Mixing of sediment as slurries and relatively short equilibration times (<60 d) have been shown to increase the bioavailability of less soluble PAHs [29]. The consistently larger uptake rate coefficients for PCBs associated with spiked sediment [16] than those obtained from the present study (Table 2) also suggest that spiked sediments with insufficient aging tend to enhance bioaccumulation into marine invertebrates.

In our study, some evidence exists to support dependence of BSAF on K_{ow} in both the kinetic and the equilibrium exposure studies (Fig. 4), suggesting that factors other than equilibrium partitioning between lipid, aqueous, and sediment carbon phases may be important here. The BSAF varied by more than a factor of five between congeners (Table 4), with 27 to 70% of this variation related to the octanol–water partition coefficient (Eqns. 8–11). While, strictly speaking, statistical significance was not attained for most slope values, p values were marginally close to adjusted significance levels. In addition, some evidence of a nonlinear (parabolic) relationship

was apparent, particularly in groups B and C. This could also have contributed to the resulting nonstatistical significance of the means. A similar parabolic relationship has been found by others and is attributed to disequilibria between sediment and organism and related to factors such as bioaccumulation from the diet, biomagnification, variations in congener lipid solubility, and slow desorption of high- and low- K_{ow} compounds sorbed on sediment [5,28,31–33]. Our intention, however, was not to fit the best predictive model but rather to provide a general description of linear trend.

In a previous study, sediments and *L. pictus* were collected from contaminated locations (stations 7D and 9D) on the Palos Verdes Shelf (Fig. 1) and analyzed for 11 PCB congeners [34; <http://www.sccwrp.org/org/pubs/techrpt/htm>]. The mean BSAF for these congeners was 3.0, similar to those measured in the equilibrium exposures (Table 4). Regression analyses on the means obtained from the field data yield

$$7D \quad \log \frac{C_1}{C_{oc}} = 0.35 \log K_{ow} - 1.67 \quad (r^2 = 0.46)$$

$$9D \quad \log \frac{C_1}{C_{oc}} = 0.38 \log K_{ow} - 2.15 \quad (r^2 = 0.33)$$

These relationships essentially overlap with those expressed in Equations 8 through 11, indicating that the bioaccumulation processes in the present laboratory exposure experiments are similar to those occurring in the marine environment. The use of amended sediments in laboratory exposures therefore is valuable for the study of PCB bioaccumulation processes as long as the effects from sample preparation are well characterized. The variability in BSAFs among the different sediment types used in this study were not predicted by the EPT model, suggesting that important differences existed among the sediments that were not adequately described by conventional measures of TOC and K_{ow} . Care should be exercised in the selection of laboratory sediments and interpretation of bioaccumulation experiments until these additional factors influencing PCB bioavailability are characterized.

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