Bioaccumulation and Toxicity of Polychlorinated Biphenyls in Sea Urchins Exposed to Contaminated Sediments
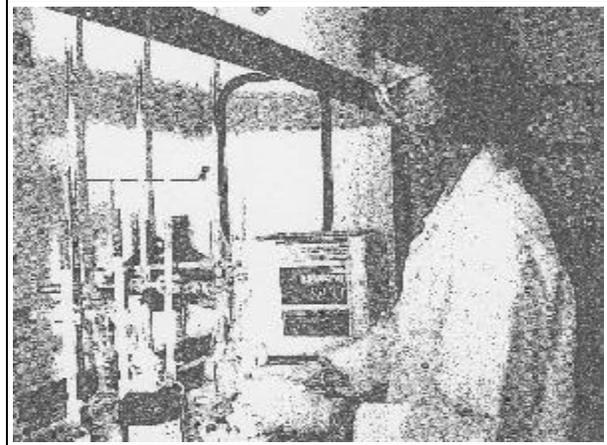
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ABSTRACT

Polychlorinated biphenyl (PCB) bioaccumulation data for the white sea urchin, *Lytechinus pictus*, are scarce, which impedes an effective assessment of sediment toxicity associated with marine species. The present study was undertaken to (1) characterize the uptake patterns of PCB congeners in *L. pictus*, (2) acquire bioaccumulation and toxicity data to establish the relationship between the body PCB burden and toxic effects in marine invertebrates, and (3) compare the PCB bioaccumulation patterns in *L. pictus* with equilibrium partitioning predictions and empirical relationships obtained for other marine species.

Uptake of most PCBs by *L. pictus* approached steady state after 35 d of exposure; it also occurred at a slower rate than that by other marine species such as infaunal bivalves, polychaetes, and amphipods. This variability in uptake rate was most likely attributable to the life history characteristics of *L. pictus*. As an epibenthic organism, *L. pictus* may not accumulate dissolved contaminants across the body wall as effectively as infaunal organisms. Survival of *L. pictus* was statistically identical after exposure to field and amended sediments, with PCB concentrations varying more than three orders of magnitude. The growth measures (diameter, weight, and gonad weight) were significantly reduced in *L. pictus* exposed to the San Diego Bay (SDB) sediment, whereas they were relatively unaffected after exposure to amended sediments (which contained much higher PCB concentrations than the SDB sediment) prepared from a New Bedford Harbor sediment. The substantially higher polycyclic aromatic hydrocarbon (PAH)/PCB concentration ratio (14) in the SDB sediment compared to that (0.0045) in the amended sediments suggested that the toxic effects as measured by the growth rates in *L. pictus* could possibly be more attributable to PAHs than PCBs.

The PCB bioaccumulation patterns in *L. pictus* showed significant deviations from equilibrium partitioning predictions, but were consistent with empirical modeling derived from a variety of marine species and contaminants. A significant dependence of measured biota sediment accumulation factors (BSAF) on K_{ow} was measured, further indicating that equilibrium partitioning of PCBs is not usually achieved among biota lipid, sediment organic carbon, and water.

INTRODUCTION

Polychlorinated biphenyls, like many other organic contaminants, are widely distributed in sediments throughout the Southern California Bight (SCB), yet there is little information to accurately assess their impacts on marine organisms. Limited toxicity testing has demonstrated reduced growth and PCB accumulation in the white sea urchin, *L. pictus*, at sites having elevated PCB concentrations (Anderson *et al*. 1988, SCCWRP 1995a). 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 examined to establish a relationship between contaminant body burden and toxicity. Regulatory and monitoring programs rely upon such bioaccumulation models for purposes such as establishing sediment quality criteria for human health (Weiss 1996) and determining the suitability of dredged materials for ocean disposal. Critical factors in these assessments are feasibility and accuracy of the bioaccumulation data.

A popular approach is to describe bioaccumulation phenomena using equilibrium partitioning assumptions (Mackay 1982; Bierman, Jr. 1990; Di Toro *et al.* 1991). It is generally accepted that lipid in biota and total organic carbon (TOC) in sediments (or soil) are relevant compartments for such partitioning. When a system consisting of sediment, biota, and water is considered, the following partition coefficients can be defined under equilibrium conditions:

$$K_{oc} = C_{oc}/C_w \quad (1)$$
$$BCF = C_l/C_w \quad (2)$$

where C_{oc}, C_w, and C_l are compound concentrations in sediment (normalized to TOC), water, and biota (normalized to lipid); K_{oc} is 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 obtain. In cases where sediment interstitial water is substantially different from surrounding water which biota aspirate, C_w is vaguely defined. A more practical and useful parameter, the biota-sediment accumulation factor (BSAF), can be derived from combining Eqs. (1) and (2):

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

Under strictly equilibrium conditions, BSAF equals unity, representing an equal partitioning of a chemical between the biota lipid and sediment TOC (Bierman, Jr. 1990). Although using equilibrium partitioning theory to determine BSAFs provides a simple method to relate bioaccumulation to sediment contamination, its validity remains questionable. 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 equilibrium partitioning. Bioaccumulation studies have been conducted with a variety of infaunal species (Lake *et al.* 1990) 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 report was conducted to investigate two aspects of PCB bioaccumulation in marine invertebrates. The first objective was to characterize the uptake and 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. The second objective was to evaluate the patterns of PCB bioaccumulation in *L. pictus* exposed to various levels of sediment contamination against equilibrium partitioning predictions and empirical relationships obtained by other researchers.

MATERIALS AND METHODS

Experimental Design

This project consisted of two sets of laboratory experiments. 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 various time intervals to study the pattern of PCB uptake and toxicity. In the second set of experiments (dose-response 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.

Kinetic Exposure

Sediment Collection and Preparation

Sediments were collected from a site in San Diego Bay near the NASSCO shipyard (Station SDB) with elevated PCB and PAH concentrations and also from a reference station near Dana Point (DP) in Orange County (Figure 1). Both sites were sampled on December 6 and 7, 1994, using a modified 0.1 m² Van Veen grab (Stubbs *et al.* 1987). Sediment samples 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 reference area. The animals were acclimated to laboratory conditions on DP sediments for about three weeks before the experiment was initiated.

FIGURE 1. **Map of sampling locations in southern California.**
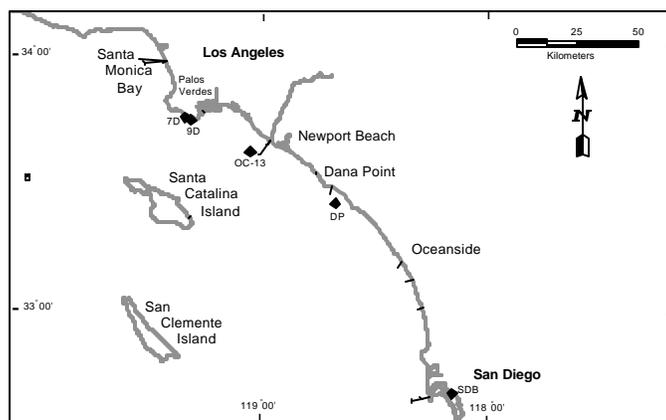
Sea Urchin Exposure

The SDB and DP sediments were homogenized and distributed into replicate polyethylene chambers (29×26×14 cm) at a depth of approximately 3 cm. Triplicate sediment samples were also collected and frozen (-20 °C) for chemical analysis at a later date. Seven DP (reference) and 21 SDB sediment replicates were prepared in order to accommodate sampling at various intervals during the experiment.

Fifteen sea urchins were added to each tub. Each animal was weighed and digitally photographed to determine initial size. 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 microencapsulated food at a rate of 0.36 g/tub every other day. The maximum exposure time was 66 d, with replicates being sampled at intermediate timepoints of 7, 14, 21, 28, 35, and 50 d. 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 were 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 were determined for each individual: changes in diameter, total body wet weight, and gonad wet weight. 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. For example, data for the smallest diameter sea urchin at the start were matched with data for the smallest individual at the end. 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 portion of the individuals sampled at 35 d was induced to spawn by injection of potassium chloride. The eggs were fertilized, and the developing embryos were exposed to copper using methods modified from Chapman *et al.* (1995). 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. The copper concentration producing 50% abnormal embryos (EC50) was determined by probit analysis.

Dose-Response Exposures

Sample Collection and Preparation

Highly contaminated sediment from New Bedford Harbor, Massachusetts, was used to prepare amended sediments. A preliminary analysis indicated that this sediment had a total PCB concentration of 3,800 µg/g (dry weight basis, which was used for all concentrations mentioned below). This sediment was mixed with varying amounts of relatively uncontaminated sediment from two locations (DP and OC-13, a station offshore Newport Beach, Figure 1) to produce a range of PCB concentrations that spanned the lower exposure levels present in the SCB. Three sediment dose levels were prepared: A, B, and C (containing approximately 4, 10, and 110 µg/g of total PCBs, respectively). The amended sediments were allowed to equilibrate at 15 °C for approximately four weeks prior to use in sea urchin 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 from OC-13 were used as a reference (R). Five sets of fifteen animals were processed at the start of the experiment to produce initial gonad weight and PCB concentration data.

Two experiments were conducted. The first experiment, which was started in July 1995, included exposure Groups A, B, and R. Sea urchins were exposed to the highest PCB dose (Group C) in a separate experiment, which was started in August 1995. The duration of the experiments was 42 (Groups A, B, and R) or 43 d (Group C), with no intermediate sampling. Sea urchin growth rates and embryo sensitivity to copper were determined using the same methods described for the kinetic experiment.

Sediment and interstitial water samples were collected at the beginning and end of each experiment and were analyzed for PCB congeners.

Sample Treatment

Sediment Extraction

Sediments were extracted using the procedures detailed elsewhere (SCCWRP 1995b) 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 ~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 ~1 mL under a gentle N_2 stream, ready for column clean-up/fractionation.

Tissue Extraction

A thawed sea urchin tissue sample was homogenized thoroughly with a glass rod and weighed in a glass beaker. Appropriate amounts of surrogate standards were spiked into the tissues, followed by addition of 20 mL of acetonitrile to the beaker. The mixture, which contained rinsates of distilled water and hexane, was transferred to a centrifuge bottle and was homogenized using a Polytron high-speed homogenizer (Brinkmann Instruments, Westbury, New York) for 30 seconds at a speed setting of 6. This procedure was repeated twice with the addition of 10 mL of hexane and distilled water, respectively. The bottle was capped and centrifuged for 10 min at ~1000×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 above, and the solvent was transferred to the flat-bottom flask. This procedure was repeated once after another 10 mL of hexane was added to the bottle. The extract was concentrated to ~1 mL using the same procedure used for the sediment extracts.

Lipid Measurements

About 3 to 5 µL of the extract was 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 Clean-up 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 Zymark TurboVap 500 (Zymark Corporation, Hopkinton, Massachusetts) concentration tube and concentrated to 1 mL. Required amounts of internal standards were added to the extract.

Instrumental Analyses

Total Organic Carbon Measurements

The method for measuring TOC has been given previously (SCCWRP 1994). To summarize, 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 Carlo Erba 1108 CHN Elemental Analyzer.

Congener-Specific Measurements of PCBs

Quantitation of PCB congeners was accomplished 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 (gas chromatograph/electron capture detector (GC/ECD) results); 64 are singly eluting congeners and 28 are multi-component 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 gas chromatograph/mass spectrometer (GC/MS). The assignment of PCB peaks was based on a combination of information in the literature (Mullin *et al.* 1984, Schulz *et al.* 1989) and confirmatory mass spectral analyses. The relative response factors of individual PCB congeners were obtained using GC/ECD and were 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 tissue sample for each PCB congener.

Instrument Parameters

Quantitative measurements were conducted using a Hewlett Packard (HP) 5890 Series II GC with a ^{63}Ni electron capture detector and a 60 m × 0.25 mm i.d. (0.25 µm film thickness) DB-5 column (J&W Scientific, Folsom, California). 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 °C 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. Make-up 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 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 accomplished 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. Due to the complicated chromatographic features associated with the PCB components found in the Aroclor standards and samples, several small windows had to be set up, each covering PCB congeners belonging to one to three isomer groups. The dwell time was 100 msec for windows covering one isomer group and 50 msec for windows covering more than one isomer group.

RESULTS

Kinetic Exposure

Total PCB concentrations in SDB sediment changed little during the experiment, varying between 522 and 592 ng/g dry 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). The DP sediment 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 concentrations in sea urchins exposed to SDB sediment increased rapidly during the first half of the experiment, attaining 76% (22.1 µg/g lipid; Table 1) of the final concentration (28.9 µg/g lipid) by Day 35 (Figure 2). As expected, sea urchins exposed to SDB sediment accumulated much higher PCB concentrations than those exposed to DP sediment.

A similar bioaccumulation pattern was shown for the 10 most abundant PCB congeners detected (Figure 3). Most congener concentrations changed little after 35 d of uptake, indicating that 35-d exposure was generally sufficient to approximate steady state concentrations in sea urchin gonads.

Biological responses (survival, growth, and embryo sensitivity) were assessed at the 35 d timepoint in order to provide data comparable to previous tests with *L. pictus*. Survival was not significantly affected by exposure to either SDB or DP sediment; only one animal died during the 35-d time period. All three measures of growth were reduced significantly in sea urchins exposed to SDB sediment, however (Table 2). Changes in body diameter and gonad weight accounted for only 55% of the growth measured for individuals exposed to DP sediment, while changes in body weight accounted for only 22% of the DP value.

TABLE 1. Total PCB concentrations in sediment, sediment interstitial water, and sea urchin tissue samples collected before and after exposure experiments.

Group		Interstitial Water	Sediment			Sea Urchin Tissues		
		ng/ml	TOC, Percent Dry	µg/g Dry	mg/g TOC	Lipid, Percent Wet	µg/g Wet	mg/g Lipid
Kinetic Exposure								
SDB	Day 0 (n=3)	nd	1.62	0.522 (±0.011)	0.0322 (±0.0024)	2.40 (±0.53)	nd	nd
	Day 35 (n=3)	nd	1.44 (±0.15)	0.592 (±0.233)	0.0421 (±0.0204)	2.22 (±0.12)[a]	0.490 (±0.056)[a]	0.0221 (±0.0019)[a]
DP	Day 0 (n=3)	nd	1.49 (±0.58)	nd	nd			
	Day 35 (n=3)	nd	0.802 (±0.026)	0.0011 (±0.0002)	0.00014 (±0.00002)	2.34 (±0.25)[a]	0.025 (±0.034)[a]	0.0012 (±0.0016)[a]
Equilibrium Exposure 1								
R[b]	Day 0 (n=3)	nd	1.12 (±0.03)	nd	nd	1.11 (±0.09)[c]	nd	nd
	Day 42 (n=4)	nd	0.325 (±0.004)	nd	nd	2.49 (±0.43)[a]	0.050 (±0.026)	0.0021 (±0.0010)
A	Day 0 (n=3)	nd	0.324 (±0.008)	3.87 (±0.81)	1.20 (±0.27)			
	Day 42 (n=4)	nd	0.331 (±0.009)	3.73 (±0.25)	1.13 (±0.07)	2.65 (±0.45)	49.4 (±7.0)	1.91 (±0.48)
B	Day 0 (n=2)	5.1 (±5.3)	0.332 (±0.002)	10.8 (±1.7)	3.26 (±0.53)			
	Day 42 (n=5)	nd	0.339 (±0.006)	10.3 (±1.2)	3.03 (±0.37)	2.82 (±0.18)	127 (±16)	4.49 (±0.42)
Equilibrium Exposure 2								
C	Day 0 (n=3)	45 (±15)	0.696 (±0.063)	113.9 (±8.3)	16.5 (±2.5)			
	Day 43 (n=5)	22 (±10)	0.753 (±0.055)	107.2 (±5.3)	14.3 (±1.6)	2.02 (±0.26)	647 (±132)	32.1 (±6.7)

[a]Five replicates were analyzed.
[b]Collected from OC-13 (Figure 1).
[c]Four replicates were analyzed.
nd = Not detected.

FIGURE 2. Uptake of total PCBs in sea urchin tissues exposed to San Diego Bay (SDB) and Dana Point (DP) sediments.
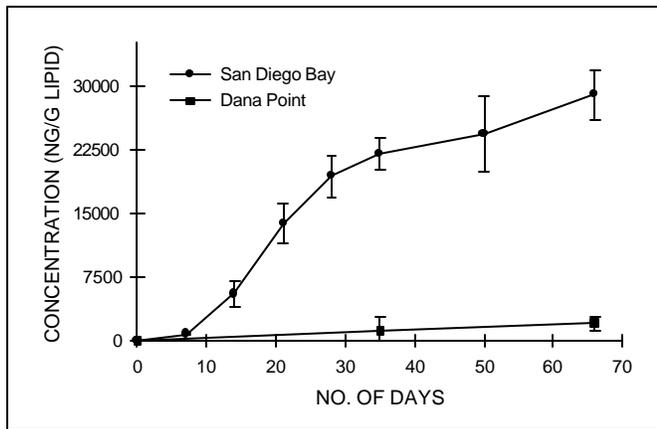

FIGURE 3. Uptake of 10 most abundant PCB congeners in sea urchin tissues exposed to San Diego Bay (SDB) sediment.
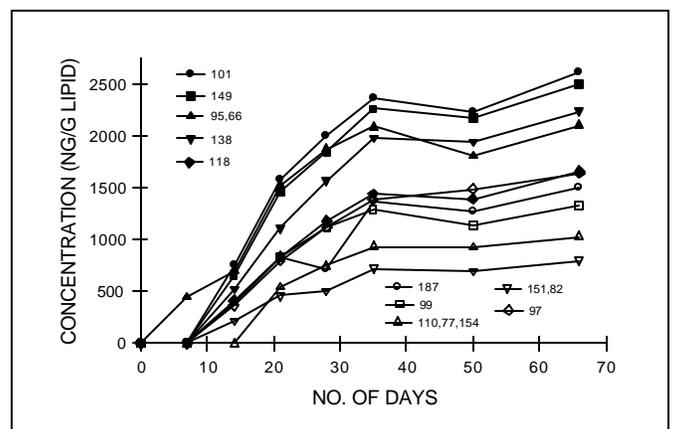

TABLE 2. Toxicity test responses for *Lytechinus pictus* exposed to sediments in the laboratory for 35 days (kinetic exposure) or 42 days (equilibrium exposure). Values are mean ± standard deviation. n=5 except for DP LC50 (n=4).

| Group | Adults | | | | Embryos |
	Survival (percent)	Diameter Change (mm)	Weight Change (g)	Gonad Weight Change (g)	Copper LC50 (µg/L)
Kinetic Exposure					
DP	100 ± 0	0.67 ± 0.17	0.31 ± 0.06	0.09 ± 0.01	24 ± 3
SDB	99 ± 0	0.37 ± 0.25[a]	0.07 ± 0.08[a]	0.05 ± 0.02[a]	23 ± 1
Equilibrium Exposure 1					
R	99 ± 3	0.41 ± 0.32	0.31 ± 0.04	0.05 ± 0.03	42 ± 7
A	99 ± 3	0.45 ± 0.29	0.34 ± 0.01	0.05 ± 0.07	38 ± 5
B	100 ± 0	0.27 ± 0.14	0.31 ± 0.04	0.06 ± 0.02	39 ± 4
Equilibrium Exposure 2					
C	89 ± 14	0.86 ± 0.36	-0.16 ± 0.22	0.02 ± 0.04	22 ± 4[b]

[a]Significantly lower than the DP reference sample (p<0.05).
[b]Significantly lower than the R reference sample (p<0.05).

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 EC50s of 23 and 24 µg/L. These data indicate that embryo health was similar between the two exposure groups.

Dose-Response Exposures
The three amended sediment PCB concentrations spanned a wide range (~0 to ~110 µg/g dry sediment weight, 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 coefficients of variation ≤21% between replicates.

Most interstitial water samples contained nondetectable PCBs (Table 1). As expected, the highest PCB concentra-

tion was found in the interstitial water extracted from the most contaminated sediment (Group C). Samples in Group C had concentrations of 45 and 22 ng/mL at Day 0 and Day 43, respectively. The samples in Group B at Day 0 also contained a small amount of PCBs (5.1±5.3 ng/mL). The interstitial water concentration was quite variable between measurements.

Sea urchin gonad PCB concentrations increased dramatically (by 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 µg/g wet tissue weight) measured in sea urchin tissues from the highest exposure group (Group C).

Exposure to PCB in either the first (Groups A and B) or second experiment (Group C) did not produce significant reductions in adult sea urchin growth (Table 2). Mean diameter change in Group B and body weight change in

Group C sea urchins were much less than in reference animals, but variability was high and the differences were not statistically significant.

Sea urchin embryo EC50s for copper were similar between Groups R, A, and B (38 to 42 µg/L), and not significantly different. Group C embryos were significantly more sensitive to copper, with an EC50 of 22 µg/L (Table 2). The Group C EC50 was similar to EC50s measured in the kinetic exposure using DP and SDB samples, however, indicating that this value is within the typical range of variation for *L. pictus*.

Bioaccumulation Factors

Table 3 presents the measured BSAFs for samples in Groups A, B, C, and SDB, as well as related K_{ow} values (Hawker and Connell 1988). To avoid ambiguity, only the singly eluting PCB congeners or co-eluting congeners with one congener much more abundant (>5:1) than the others (based on the results of Schulz *et al.* 1989) were considered. Mean BSAFs were similar within the amended sediments (Groups A, B, and C), ranging from 1.7 to 2.4. Within a congener, higher BSAF values were usually obtained for Group C samples. Smaller BSAF values tended to be associated with more polar congeners, those with $logK_{ow} \leq 5.24$ (Table 3).

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 3). The mean BSAF for SDB was 0.98.

A significant linear regression relationship was present between the congener-specific logBSAF and $logK_{ow}$ values for each exposure group (Figure 4). The regressions for Groups A, B, and C were highly significant (p<0.01) and accounted for 42 to 65% of the variation in the data (indicated by the coefficient of determination, r^2). A significant (p<0.05) regression was also obtained for the SDB exposure group, although the correlation was not as strong. The regression equations are expressed below:

A	$logC_i/C_{oc} = 0.20logK_{ow} - 0.86$	$(r^2=0.54)$ (4)
B	$logC_i/C_{oc} = 0.29logK_{ow} - 1.55$	$(r^2=0.65)$ (5)
C	$logC_i/C_{oc} = 0.19logK_{ow} - 0.77$	$(r^2=0.42)$ (6)
SDB	$logC_i/C_{oc} = 0.41logK_{ow} - 2.74$	$(r^2=0.31)$ (7)

TABLE 3. Measured biota sediment accumulation factors (C_i/C_{oc}) in sea urchins exposed to PCB contaminated sediments.

PCB Congener	C_i/C_{oc}				$logK_{ow}$
	A	B	C	SDB	
4 (10)	—[a]	0.42	0.68	—	4.65
6	—	0.55	0.81	—	5.06
8	—	—	—	—	5.07
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±95% CI	2.32±0.28	1.68±0.21	2.37±0.22	0.98±0.38	

[a]The concentration of PCBs in either sediment or sea urchin tissue was not detectable.

DISCUSSION

PCB Uptake Kinetics

The PCB uptake by *L. pictus* appears to occur at a slower rate than in some other marine invertebrates. More than 66 d are required for some congeners to attain steady state concentrations in *L. pictus* gonad (Figure 3), whereas experiments with infaunal bivalves, polychaetes, and amphipods report the attainment of 80 to 100% of steady state PCB concentrations after 28 to 42 d of exposure in most cases (Lee II *et al.* 1989 and Bose *et al.* 1995). Life history characteristics may account for some differences between species as *L. pictus* is epibenthic and may not accumulate dissolved contaminants across the body wall as effectively as infaunal organisms which live within the sediment.
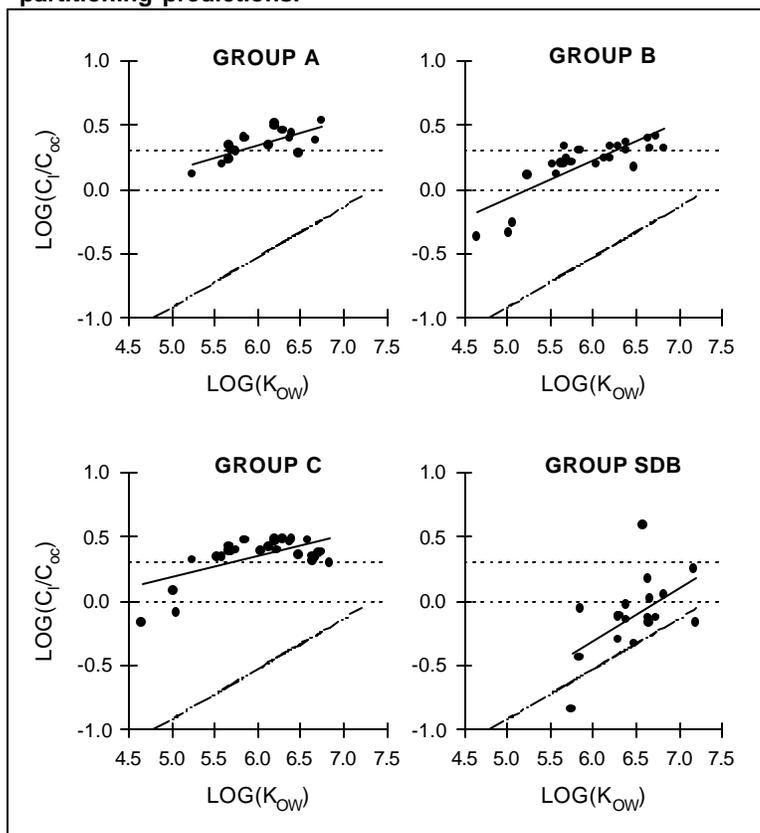
Biological Effects of PCB
Bioaccumulation

Results of the dose-response experiments provide data useful for assessing the potential effects of PCB bioaccumulation in *L. pictus*. In the first dose-response experiment, no growth effects were evident (Groups A and B, Table 2) at gonad total PCB concentrations up to 127 µg/g (Group B, Table 1). Evidence of toxicity was present in the second exposure (Group C), where growth (change in total or gonad weight) was greatly reduced (Table 2) at a gonad PCB concentration of 647 µg/g (Table 1). The presence of toxicity was ambiguous, however, as a concurrent reference group was not tested and high variability reduced the statistical power of the test.

FIGURE 4. Variation of measured C_l/C_{oc} with K_{ow} in exposure Groups A, B, C, and SDB. The solid line indicates the linear regression, the broken line is an empirical modeling expressed in Eq. (10), and the dotted lines show the range of equilibrium partitioning predictions.

concentrations of PCBs in these sediments were 0.52 (Table 1) and 3,800 µg/g, respectively. Therefore, the ratios of PAHs/PCBs were 14 and 0.0045, respectively, in these sediments. The substantially higher PAH concentration in the SDB sediment compared to the amended sediments may have contributed to the growth effects observed in *L. pictus* exposed to the SDB sediment.

Few data relating toxicity to PCB bioaccumulation are available for comparison, but it appears that the relatively high body burdens needed to elicit toxicity in *L. pictus* are within the range reported for other species. Exposure of amphipods to Aroclor mixtures produced toxicity at 100 to 550 µg/g wet weight total PCB (Borgmann *et al.* 1990), while toxicity to various fish species has been observed at PCB body burdens of 40 to 640 µg/g (cited in Borgmann *et al.* 1990, Monosson *et al.* 1994).

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 µg/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 (Anderson *et al.* 1988, SCCWRP 1995a). Gonad total PCB concentrations associated with reduced growth in these studies were 2.6 to 8.1 µg/g, also far below levels attained in the amended sediment experiments without apparent detrimental effects. The growth effects measured in the kinetic study appear to be attributable to other contaminants.

Concentrations of PAHs were also measured in the original SDB (7.3±0.5 µg/g, n=3) and New Bedford Harbor (17.0±1.8 µg/g, n=4) sediments. By comparison, the

No adverse health effects were produced on sea urchin embryos by the high gonad PCB concentrations observed in the dose-response exposure experiments. This result is consistent with data from embryo toxicity tests using PCB-spiked water (Schweitzer and Bay, this annual report), where tissue tetrachlorobiphenyl concentrations of 10,000 µg/g were required to produce abnormal embryo development. Studies with fish indicate that embryos and developing larvae are adversely affected by relatively low PCB concentrations of 0.12 to 12 µg/g (Monosson *et al.* 1994, von Westernhagen *et al.* 1981). The large variation in sensitivity between developing fish and sea urchins may be related to differences in metabolic pathways. The relatively high sensitivity reported in these fish studies may also be an artifact of the research methods used. Von Westernhagen *et al.* (1981) used field-exposed fish to derive their dose-response relationship, so that effects may have been due to

other contaminants in addition to PCBs (analogous to our hypothesis for SDB). The PCB effects observed by Monosson *et al.* (1994) were determined for a single congener (3,3',4,4' tetrachlorobiphenyl), which is thought to be of greater toxicity than the mixture of congeners present in our sea urchin exposures.

Additional research is needed to better understand the biological effects of PCBs on marine organisms. The experiments described in this report were conducted using sediment 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 (Schweitzer and Bay, this report). Additional synoptic toxicity and bioaccumulation experiments using single congeners are needed to clarify structure-activity relationships for marine organisms, which will facilitate the use of data from diverse locations and species.

PCB Bioaccumulation Patterns in L. pictus

The equilibrium partitioning theory predicts BSAFs of 1 to 2 (Morrison *et al.* 1996), whereas a wide range of BSAF values has been obtained experimentally. The PCB BSAFs can vary by an order of magnitude between experiments conducted with the same species (Lake *et al.* 1990), 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* (Bose *et al.* 1995). The variations in *L. pictus* BSAFs that were observed between the SDB and amended sediments may also have been due to differences in exposure times (35 vs. 42 d) and sediment types between experiments.

In our study, a significant dependence of BSAF on K_{ow} was observed in both the kinetic and dose-response exposures (Figure 4), further evidence that factors other than equilibrium partitioning among lipid, aqueous, and sediment carbon phases are important. The BSAF varied by more than a factor of 5 between congeners (Table 3), with 31 to 65% of this variation related to the octanol-water partition coefficient (Eqs. (4) to (7)). Although a significant linear relationship was present between the log transformed data, evidence of a nonlinear (parabolic) relationship was present in some groups (e.g., Groups B and C). A similar parabolic relationship has been found by others and is attributed to disequilibria between sediment and organism, related to factors such as bioaccumulation from the diet, biomagnification, and variations in congener lipid solubility (Bierman, Jr. 1990; Chessells *et al.* 1992; Morrison *et al.* 1996).

The BSAF in sea urchins from the present study can be compared to more general empirical results by others. Kenaga and Goring (1980) proposed the following equations:

$$logBCF = 0.935logK_{ow} - 1.495 \qquad (8)$$
$$logK_{oc} = 0.544logK_{ow} + 1.377 \qquad (9)$$

Eq. (8) is similar to a model ($logBCF = logK_{ow} - 1.32$) derived by Mackay (1982), whereas Eq. (9) is substantially different from a relationship between K_{oc} and K_{ow} ($logK_{oc} = 0.983logK_{ow} + 0.00028$) suggested by Di Toro (1985). We believe that Eq. (9) probably describes more practical steady state conditions. For example, Bergen *et al.* (1993) measured selected PCB congeners in the seawater of New Bedford Harbor and obtained a relationship between Kp ($=K_{oc} \times \%TOC$) and K_{ow} as: $logK_p = 0.640logK_{ow} + 1.074$ ($r^2=0.52$, summer) and $logK_p = 0.733logK_{ow} + 1.125$ ($r^2=0.52$, winter). The slopes of these two regressions are substantially smaller than unity. Combination of Eqs. (8) and (9) yields

$$logBSAF = 0.391logK_{ow} - 2.872 \qquad (10)$$

Our observed C_l/C_{oc} vs. K_{ow} relationships showed a slight difference from the empirical modeling (Figure 4). The discrepancy appears to be dependent more on the sample type than on the sediment concentration. Sediments in Groups A, B, and C were prepared by mixing the same highly contaminated sediments from New Bedford Harbor with relatively clean sediments from DP and OC-13. On the other hand, sediments in Group SDB were used without any modification.

In a previous study, sediments and *L. pictus* were collected from contaminated locations (Stations 7D and 9D) on the Palos Verdes Shelf (Figure 1) and analyzed for 11 PCB congeners (Bay *et al.* 1994). The mean BSAF for these congeners was 3.0, similar to those measured in the dose-response exposures (Table 3). Regression analyses on the field data yield:

7D $\qquad logC_l/C_{oc} = 0.35logK_{ow} - 1.67$ ($r^2=0.46$)
9D $\qquad logC_l/C_{oc} = 0.38logK_{ow} - 2.15$ ($r^2=0.33$)

These relationships essentially overlap with those expressed in Eqs. (4) to (7), indicating that the bioaccumulation processes in the present laboratory exposure experiments closely resembled those occurring in the marine environment. The use of amended sediments in laboratory exposures therefore is valuable in the assessment of PCB bioaccumulation.

LITERATURE CITED

Anderson, J.W., S.M. Bay and B.E. Thompson. 1988. Characteristics and effects of contaminated sediments from southern California. Prepared for California State Water Resources Control Board, Contract # 6-214-250-0. Sacramento, CA. 120 p.

Bay, S., D. Greenstein, J. Brown and A. Jirik. 1994. Investigation of toxicity in Palos Verdes sediments. Prepared for Santa Monica Bay Restoration Project. 103 p.

Bergen, B.J., W.G. Nelson and R.J. Pruell. 1993. Partitioning of polchlorinated biphenyl congeners in the seawater of New Bedford Harbor, Massachusetts. *Environmental Science and Technology* 27:938-942.

Bierman, V.J., Jr. 1990. Equilibrium partitioning and biomagnification of organic chemicals in benthic animals. *Environmental Science and Technology* 24:1407-1412.

Borgmann, U., W.P. Norwood and K.M. Ralph. 1990. Chronic toxicity and bioaccumulation of 2,5,2',5'- and 3,4,3',4'-tetrachlorobiphenyl and Aroclor 1242 in the amphipod Hyalella azteca. *Archives of Environmental Contamination and Toxicology* 19:558-564.

Bose, B.L., M. Winsor, J. Lee II, S. Echols, J. Pelletier and R. Randall. 1995. PCB congeners and hexachlorobenzene biota sediment accumulation factors for *Macoma nasuta* exposed to sediments with different total organic carbon contents. *Environmental Toxicology and Chemistry* 14:303-310.

Chapman, G.A., D.L. Denton and J.M. Lazorchak. 1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. EPA 600/R-95/136. National Exposure Research Laboratory, U.S. Environmental Protection Agency. Cincinnati, OH.

Chessells, M., C.W. Hawker and D.W. Connell. 1992. Influence of solubility in lipid on bioconcentration of hydrophobic compounds. *Ecotoxicology and Environmental Safety* 23:260-273.

Di Toro, M.D., C.S. Zarba, D.J. Hansen, W.J. Berry, R.C. Swartz, C.E. Cowan, S.P. Pavlou, H.E. Allen, N.A. Thomas and P.R. Paquin. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. *Environmental Toxicology and Chemistry* 10:1541-1583.

Hawker, D.W. and D.W. Connell. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environmental Science and Technology* 22:382-387.

Kenaga, E.E. and C.A. Goring. 1980. Relationship between water solubility, soil sorption, octanol-water partitioning, and concentration of chemicals in biota. pp. 78-115 *in*: J.G. Eaton, P.R. Parrish and A.C. Hendricks (eds.), *Aquatic Toxicology*. American Society for Testing and Materials, Special Technical Publication 707. New Orleans, LA.

Lake, J.L., N.I. Rubinstein, H. Lee II, C.A. Lake, J. Heltshe and S. Pavignano. 1990. Equilibrium partitioning and bioaccumulation of sediment-associated contaminants by infaunal organisms. *Environmental Toxicology and Chemistry* 9:1095-1106.

Lee II, H., B.L. Bose, J. Pelletier, M. Winsor, D.T. Specht and R.C. Randall. 1989. Guidance manual: Bedded sediment bioaccumulation tests. EPA 600/x-89/302. U.S. Environmental Protection Agency, ERL-N. Newport, OR.

Mackay, D. 1982. Correlation of bioconcentration factors. *Environmental Science and Technology* 16:274-178.

Monosson, E., W.J. Fleming and C.V. Sullivan. 1994. Effects of the planar PCB 3,3',4,4'-tetrachlorobiphenyl (TCB) on ovarian development, plasma levels of sex steroid hormones and vitellogenin, and progeny survival in the white perch (*Morone americana*). *Aquatic Toxicology* 29:1-19.

Morrison, H.A., F.A.P.C. Gobas, R. Lazar and G.D. Haffner. 1996. Development and verification of a bioaccumulation model for organic contaminants in benthic invertebrates. *Environmental Science and Technology* 30:3377-3384.

Mullin, M.D., C.M. Pochini, S. McCrindle, M. Romkes, S.H. Safe and L.M. Safe. 1984. High resolution PCB analysis: Synthesis and chromatographic properties of all 209 PCB congeners. *Environmental Science and Technology* 18:468-478.

SCCWRP. See Southern California Coastal Water Research Project.

Schulz, D.E., G. Petrick and J.C. Duinker. 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. *Environmental Science and Technology* 23:852-859.

Southern California Coastal Water Research Project. 1994. Preliminary study of seasonal variation of carbon and nitrogen in sediments off Point Loma. pp. 91-98 *in*: J.N. Cross (ed.),
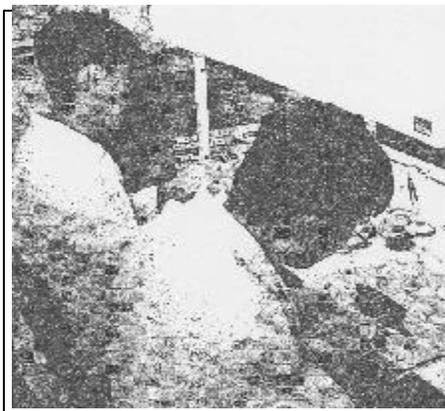
Southern California Coastal Water Research Project Annual Report 1992-93. Westminster, CA.

Southern California Coastal Water Research Project. 1995a. Toxicity of sediments on the Palos Verdes Shelf. pp. 79-90 *in*: J.N. Cross, C. Francisco and D. Hallock (eds.), Southern California Coastal Water Research Project Annual Report 1993-94. Westminster, CA.

Southern California Coastal Water Research Project. 1995b. Post-depositional distribution of organic contaminants near the Hyperion 7-mile outfall in Santa Monica Bay. pp. 43-54 *in*: J.N. Cross, C. Francisco and D. Hallock (eds.), Southern California Coastal Water Research Project Annual Report 1993-94. Westminster, CA.

Stubbs, H.H., D.W. Diehl and G.P. Hershelman. 1987. A Van Veen grab sampling method. Contribution No. C-204. Southern California Coastal Water Research Project. Long Beach, CA. 4 p.

von Westernhagen, H., H. Rosenthal, V. Dethlefsen, W. Ernst, U. Harms and P.-D. Hansen. 1981. Bioaccumulating substances and reproductive success in baltic flounder *Platichthys flesus. Aquatic Toxicology* 1:85-99.

Weiss, L.B. 1996. Development of health-based criteria for sediments in Puget Sound, Washington. U.S. Environmental Protection Agency, National Sediment Bioaccumulation Conference, September 11-13. Bethesda, MD.

ACKNOWLEDGMENTS